Saponins from *Zygophyllum gaetulum*

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Received May 12, 1997[⊗]

Three new bisdesmosidic triterpene saponins, zygophylosides I (**1**), L (**2**) and M (**3**), and three known quinovic acid glycosides were isolated from *Zygophyllum gaetulum*. The new compounds were determined to be 3*β*-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→2)-*β*-D-glucopyranosylurs-20(21)-en-28-oic acid 28-*O*-[*β*-D-2-*O*-sulfonylglucopyranosyl] ester (**1**), 3*β*-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→2)-*β*-D-glucopyranosyl]urs-20(21)-en-28-oic acid 28-*O*-[*β*-D-glucopyranosyl] ester (**2**), and 3*β*-*O*-*β*-D-quinovopyranosyl-27-nor-olean-12-en-28-oic acid 28-*O*-*β*-D-glucopyranosyl ester (**3**) by use of 1D and 2D NMR techniques.

In the course of our research program on African medicinal plants, we investigated *Zygophyllum gaetulum* Emb. & Maire (Zygophyllaceae), vernacular name "aggaya", a species endemic in the Moroccan Sahara. The aqueous extracts of the aerial parts are used in the indigenous system of medicine as an antispasmodic, antieczema, and antidiabetic drug, and as a remedy for stomach and liver pain.[1] No previous investigation is reported on this species. In this paper we describe the isolation and elucidation of three new bisdesmosidic saponins, compounds **1** (zygophyloside I), **2** (zygophyloside L), and **3** (zygophyloside M). The related known glycosides, 3*β*-*O*-*β*-D-glucopyranosylquinovic acid 28-*O*-*β*-D-glucopyranosyl ester,[2] zygophyloside G,[3] and zygophyloside E[4] were also isolated. The presence of triterpenes of the ursane series and of $-SO_3H$ moiety in the metabolites from *Zygophyllum* species has already been reported[3−6] and seems to be of chemotaxonomic significance.

Six saponins were isolated and purified by Sephadex LH-20 column chromatography and reversed-phase HPLC from the MeOH extract of the aerial part of *Z. gaetulum*. Zygophylosides I (**1**) ($C_{53}H_{86}O_{24}S$) and M (**2**) ($C_{53}H_{86}O_{21}$) gave quasi-molecular ion peaks at *m*/*z* 1137 $[M - H]^-$ and *m*/*z* 1057 $[M - H]^-$, respectively, and prominent peaks due to the loss of a hexose for **2** and a hexose plus $-SO_3$ for **1** in their negative FABMS. The NMR spectral data of saponins **1** and **2** (Tables1−3) revealed the feature of a triterpenic acid with a double bond and four sugar units, one of which was linked to a carboxyl group (δ_H 5.42, δ_C 92.83 in **1**) via ester linkage.[2] The remaining sugars were attached to the oxygen at C-3.

The ^{1}H-NMR spectra of **1** and **2** displayed signals for six tertiary Me groups (δ 0.85, 0.90, 1.00, 1.01, 1.08, and 1.63) and one secondary Me group (δ 1.04, d, J = 6.5 Hz) in the aglycon moiety, indicating a pentacyclic triterpene skeleton of the ursane or taraxastane series;[7−10] one of the singlet Me signals appeared at comparatively lowfield (δ 1.63), suggesting that it could be attached with an olefinic carbon. In addition it was possible to observe an olefinic proton signal at δ 5.27 (1H, dd, J = 6.0 and 1.5 Hz) and a signal typical of H-3ax (δ 3.20, dd, J = 11.1, 4.5 Hz) due to the presence of *β*-OH group at C-3 position (Table 1).

Full assignments of the proton and carbon signals of the aglycon part of **1** were secured by ^{1}H−^{1}H DQF−COSY[11] and HSQC[12] spectra. A combination of COSY and HSQC experiments showed the following connectivities C-3−C-2−C-1 for ring A, C-5−C-6−C-7 for ring B, C-16−C-15 for ring D, and C-13−C-12−C-11−C-9 starting from the well-resolved signals at δ 3.20 (H-3), 0.75 (H-5), 2.31 (H-16eq), and 2.42 (H-13). The proton and carbon signals due to the A, B, C, and D rings indicated a 3-*β*-hydroxy-12,13-dihydro-ursane skeleton carrying a −COOR group at C-28 and glycosylated at C-3.[7−10] The position of the double bond on ring E at C-20(21) was established by the following evidence: there was only one proton (δ_H 5.27) connected to the olefinic carbons (δ_C 142.00 and 118.26), supporting the structure CH═C. The CH_3 signal at δ_H 1.63 (s, Me-30) indicated a vinylic Me, the other (Me-29) at δ 1.04 (d, J = 6.5 Hz) appeared shifted downfield by ca. +0.08 ppm with respect to the doublet Me signals typical of ursolic acid skeleton,[2] due to the presence of the double bond. The COSY spectrum indicated that the olefinic proton was connected to a methylene group at 1.82 (dd, J = 12.0, 1.5 Hz) and 2.36 (dd, J = 12.0, 6.0 Hz), which correlated by HSQC to a CH_2 signal at δ_C 37.89, ascribable to position C-22. The above-mentioned properties left only position C-20(21) for the vinylic double

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[⊗] Abstract published in *Advance ACS Abstracts*, December 15, 1997.

S0163-3864(97)00245-0 CCC: $15.00
Published on Web 01/23/1998

Table 1. ^{13}C-NMR and ^{1}H-NMR Assignments and ^{13}C−^{1}H Long-range Correlations of Aglycon of Compound **1** by ^{1}H−^{1}H COSY, HSQC, and HMBC Experiments in CD_3OD

carbons	δ_C	DEPT	$\delta_{H\,(J_{HH}\text{ in Hz})}$	cross peaks (δ_C) in HMBC spectrum
1	40.09	CH_2	1.17 m, 1.88 m	
2	27.06	CH_2	1.08 m, 1.80 m	
3	91.47	CH	3.20 dd (11.1, 4.5)	16.73 (C-23), 28.31 (C-24), 4.49 (Glc-1′)
4	40.34	C		
5	57.16	CH	0.75 m	16.73 (C-23), 19.16 (C-6), 33.08 (C-7)
6	19.16	CH_2	1.35 m, 1.55 m	
7	33.08	CH_2	1.36 m, 1.53 m	
8	42.06	C		
9	51.99	CH	1.37 m	
10	38.08	C		
11	22.60	CH_2	1.73 m, 1.95 m	
12	30.29	CH_2	1.22 m, 1.32 m	
13	40.21	CH	2.42 dt (12.0, 3.4)	
14	42.80	C		
15	28.61	CH_2	1.05 m 1.11 m	
16	33.02	CH_2	1.36 ddd (13.0, 12.0, 5.0) 2.31 ddd (12.0, 5.0, 3.1)	
17	50.57	C		
18	50.26	CH	1.23 br s	
19	38.31	CH	2.16 dd (11.5, 6.5)	
20	l42.0	C		
21	118.26	CH	5.27 dd (6.0, 1.5)	
22	37.89	CH_2	1.82 dd (12.0,1.5) 2.36 dd (12.0, 6.0)	50.26 (C-18), 118.26 (C-21), 142.0 (C-20)
23	16.73	CH_3	0.85 s	27.06 (C-2), 40.34(C-4), 57.16 (C-5)
24	28.31	CH_3	1.08 s	16.73 (C-23), 40.34 (C-4), 57.16 (C-5)
25	16.40	CH_3	1.01 s	40.34 (C-4), 51.99 (C-9)
26	16.91	CH_3	0.90 s	38.08 (C-10), 40.21 (C-13), 57.16 (C-5)
27	15.24	CH_3	1.00 s	28.61 (C-15), 33.08 (C-16), 42.06 (C-8), 42.80 (C-14)
28	176.33	C		
29	23.84	CH_3	1.04 d (6.5)	50.26 (C-18), 142.0 (C-20)
30	21.98	CH_3	1.63 s	38.31 (C-19), 118.26 (C-21), 142.0 (C-20)

Table 2. ^{13}C-NMR and ^{1}-H NMR Assignments of Aglycon of Compound **2** by ^{1}H−^{1}H COSY and HSQC Experiments in CD_3OD

carbons	δ_C	DEPT	$\delta_{H\,(J_{HH}\text{ in Hz})}$
1	39.84	CH_2	1.02 m, 1.72 m
2	28.00	CH_2	1.05 m, 1.78 m
3	90.54	CH	3.20 dd (11.2, 4.5)
4	40.35	C	
5	56.83	CH	0.75 m
6	19.89	CH_2	1.10 m, 1.55 m
7	32.74	CH_2	1.32 m, 1.50 m
8	43.0	C	
9	51.71	CH	1.40 m
10	38.01	C	
11	23.57	CH_2	1.35 m, 1.92 m
12	30.68	CH_2	1.27 m, 1.33 m
13	40.0	CH	2.41 dt (12.2, 3.4)
14	42.90	C	
15	28.07	CH_2	1.05 m, 1.27 m
16	33.37	CH_2	1.45 br 2.10 ddd (12.2, 5.2, 3.2)
17	50.64	C	
18	50.11	CH	1.26 br
19	38.13	CH	2.15 dd (11.5, 6.5)
20	141.98	C	
21	117.94	CH	5.27 dd (6.0, 1.5)
22	38.04	CH_2	1.87 dd (12.2, 1.5), 2.32 dd (12.2, 6.2)
23	16.38	CH_3	0.85 s
24	28.07	CH_3	1.08 s
25	16.28	CH_3	1.00 s
26	16.55	CH_3	0.90 s
27	14.95	CH_3	1.03 s
28	176.55	C	
29	23.58	CH_3	1.04 d (6.5)
30	21.68	CH_3	1.63 s

bond. The value of the coupling constant between H-18 and H-19 (J = 11.5 Hz) indicated that both protons were in an axial position; therefore, the Me at C-19 should be in β-position and rings D/E should have a cis connection.

This interpretation was unambiguously confirmed by the HMBC[13] (8 Hz) spectrum of **1**, which showed significant cross peaks, due to $^{2}J_{C-H}$ and $^{3}J_{C-H}$ correlations, between H-22ax (δ 2.36) and C-21, C-20 and C-18, between the protons of Me-29 (δ 1.04) and C-18 and C-20, and between the protons of Me-30 (δ 1.63) and C-19, C-20, and C-21. Similar correlations between H-3 (δ 3.20) and Me-23, Me-24 and C-1 of a glucose unit led us to confirm the proposed structure.

The stereochemistry at C-17,18 (cis linkage of D/E rings) was authenticated by the ROESY[14] spectrum, which showed key correlation peaks between H-19 (δ 2.16) and the signals at δ 1.01 (Me-27) and 1.63 (Me-30), between H-13 (δ 2.42) and Me-25 (δ 1.00), H-18 (δ 1.23) and H-16eq (δ 2.31), as well as between H-21 (δ 5.27), H-22ax (δ 2.36), and Me-30 (δ 1.63) signals. In the triterpenoids of the taraxastane series where the D/E ring junction is trans, no such interactions could be observed.[9,10] Other diagnostic ROEs were recorded between H-5α and H-3α and between H-1′ of the glucose and both H-2′ of the glucose and H-3 of the aglycon. Therefore, the structure of the aglycon of saponin **1** is 3β-urs-20(21)-en-28-oic acid. To the best of our knowledge this is the first report of a naturally occurring pentacyclic triterpene of the ursane series with a double bond at C-20(21) and a −COOH at C-28; however, Δ^{12} or Δ^{18} or $\Delta^{12,18}$ or $\Delta^{18,20}$ ursanes have been previously described.[15] Its isolation from *Z. gaetulum* may be taxonomically significant.

Four anomeric signals at δ_H 4.49, 5.00, 5.05, and 5.52 were easily identified in the ^{1}H spectrum of **1**. They correlated to carbons at δ_C 105.65, 101.41, 102.15, and 92.83, respectively, in the HSQC spectrum. The chemical shift of the fourth signal showed that this sugar moiety was attached to C-28 of the aglycon by an ester

Table 3. ^{13}C-NMR and ^{1}H-NMR Data[a] of Sugar Moiety of Compounds **1** and **2** in CD_3OD

	1		**2**	
position[c]	δ_C	δ_H (J_{HH} in Hz)[b]	δ_C	δ_H (J_{HH} in Hz)[b]
Glc-1′	105.65	4.49 d (7.8)	105.47	4.48 d (7.5)
Glc-2′	79.46	3.42 dd (9.0, 7.8)	79.57	3.43 dd (9.0, 7.5)
Glc-3′	76.56	3.63 t (9.0)	76.47	3.60 t (9.0)
Gl-4′	73.38	3.52 t (9.0)	73.52	3.51 t (9.0)
Glc-5′	76.64	3.64 ddd (9.0, 4.5, 3.0)	77.71	3.60 ddd (9.0, 4.5, 3.5)
Glc-6′	61.88	3.70 dd (12.0, 4.5)	62.13	3.72 dd (12.0, 4.5)
		3.85 dd (12.0, 3.0)		3.84 dd (12.0, 3.5)
Ara-1″	101.41	5.00 d (5.2)	101.17	5.03 d (5.2)
Ara-2″	75.54	3.92 dd (5.2, 8.5)	75.69	3.92 dd (5.2, 8.5)
Ara-3″	72.36	3.82 dd (8.5, 3.0)	72.32	3.83 dd (8.5, 3.0)
Ara-4″	67.43	3.80 m	67.41	3.82 m
Ara-5″	63.37	3.45 dd (12.0, 3.0)	63.30	3.45 dd (12.0, 2.5)
		3.95 dd (12.0, 2.0)		3.97 dd (12.0, 2.00)
Rha-1‴	102.15	5.05 d (1.5)	101.97	5.05 d (1.5)
Rha-2‴	72.09	3.88 dd (1.5, 2.0)	72.12	3.88 dd (1.5, 2.5)
Rha-3‴	71.82	3.71 dd (2.0, 8.5)	71.91	3.71 dd (2.5, 8.6)
Rha-4‴	73.65	3.45 t (8.5)	73.67	3.41 t (8.6)
Rha-5‴	69.86	3.87 dd (8.5, 6.6)	69.94	3.88 dd (8.6, 6.5)
Rha-6‴	17.96	1.30 d (6.6)	18.00	1.30 d (6.5)
Glc-1⁗	92.83	5.52 d (7.6)	94.89	5.47 d (7.5)
Glc-2⁗	79.52	4.20 dd (9.0, 7.5)	73.98	3.30 dd (9.5, 7.5)
Glc-3⁗	77.30	3.76 t (9.0)	78.07	3.43 t (9.5)
Glc-4⁗	70.74	3.53 t (9.0)	70.98	3.57 t (9.5)
Glc-5⁗	78.04	3.40 m	78.33	3.37 m
Glc-6⁗	61.88	3.73 dd (12.2, 5.0)	62.13	3.70 dd (12.2, 4.5)
		3.84 dd (12.2, 3.5)		3.85 dd (12.2, 3.5)

[a] Assignments confirmed by 1D TOCSY and 2D COSY, HSQC, HMBC experiments. [b] ^{1}H−^{1}H coupling constants in the sugar spin−spin were measured from TOCSY and COSY spectra in Hz. [c] Glc = β-D-glucopyranosyl, Ara = α-L-arabinopyranosyl, Rha = α-L-rhamnopyranosyl.

bond. The sugars were determined to be a trisaccharide chain formed by glucose, arabinose, and rhamnose linked to C-3 of the aglycon, and a 2-*O*-sulfonyl-glucose at C-28 by use of monodimensional TOCSY,[16] 2D DQF−COSY, and HSQC experiments. Even at highfield (600 MHz) the 1D sugar spectral region of **1** was complex as most of the shifts were overlapped and found between δ 4.20 and 3.30. The isolated anomeric proton signals resonating at an uncrowded region of the spectrum (between δ 4.49 and 5.52) have been the starting point for the 1D TOCSY experiments. Because of the selectivity of the multistep coherence transfer, the 1D TOCSY subspectra of the single monosaccharide unit could be extracted from the crowded overlapping region. Each subspectrum could be attributed to one set of coupled protons such as H−C (1) to H−C (4) (for arabinose) or H−C (6) (for glucose and rhamnose) of an individual monosaccharide. The 1D TOCSY subspectra of the four monosaccharide units could be interpreted and, at the same time, the type of sugar, its configuration, and conformation assigned by the 2D COSY spectrum, as summarized in Table 3. The HSQC spectrum led to establish the position of the interglycosydic linkages by comparison of observed resonances with those of the corresponding methylpyranosides and accounting for the known effects of glycosydation.[17] Once the proton and carbon spectra had been completely assigned, an unambiguous determination of the sequence and linkage sites was obtained from the long-range C−H correlations (HMBC spectrum) and from the ROEs in the 2D ROESY spectrum.

The esterified sugar substituent at C-28 was identified from the following evidence: a 1D TOCSY subspectrum obtained by irradiating at δ 5.52 showed a set of coupled protons at δ 4.20, 3.76, 3.53, 3.40 (all CH), and 3.73 and 3.84 (CH_2) assigned as H-1⁗ to H_2-6⁗ of a 2-*O*-esterified glucopyranose by the COSY spectrum. Analysis of the correlated ^{13}C-NMR signals in the HSQC spectrum led to the identification of a 2-substituted glucopyranose linked at C-28 by an ester bond. The characteristic downfield shift[3,4] of H-2⁗ (δ 4.20) and C-2⁗ (δ 79.52) indicated the location of the $-SO_3H$ group to this position. The cross peak of the ^{3}J long-range coupling between H-1⁗ of the 2-*O*-sulfonylglucopyranose and C-28 of the aglycon gave definitive evidence for the position of this sugar moiety. The presence of the $-SO_3H$ group was confirmed by solvolysis of **1**, which yielded compound **2** (see Experimental Section). The other three monosaccharide units were connected together, and one showed correlation (HMBC) with C-3 of the aglycon. The absence of any glycosylation shift for the carbon resonances of the rhamnopyranose suggested this sugar to be the terminal unit, while glycosylation shifts were observed for C-2 (ca. 6 ppm) of the arabinopyranose and glucopyranose units. The ^{1}H-NMR as well as ^{13}C-NMR data of key carbons (C-2, C-3, and C-5), indicated a β-configuration at the anomeric positions of the two D-glucopyranosyl units (J_{H1-H2} = 7.5 Hz) and an α-configuration for the L-rhamnopyranose (J_{H1-H2} = 1.5 Hz). L-Arabinose in the pyranose form was evident from ^{13}C-NMR data; however, the value of its J_{H1-H2} coupling constant (5.2 Hz), midway between that reported for methyl-α-arabinopyranoside (4 Hz) and methyl-β-arabinopyranoside (8 Hz), was not diagnostic owing to the high conformational mobility of arabinopyranosides ($^{4}C_1 \leftrightarrow {}^{1}C_4$). As we also reported in previous work,[18,19] evidence of α-L-arabinopyranoside was obtained from ROESY spectrum, which showed NOEs from C-1″ to C-2″, C-3″, and C-5″ indicative of α-L-arabinopyranoside in rapid conformational exchange from $^{4}C_1 \leftrightarrow {}^{1}C_4$ conformation.

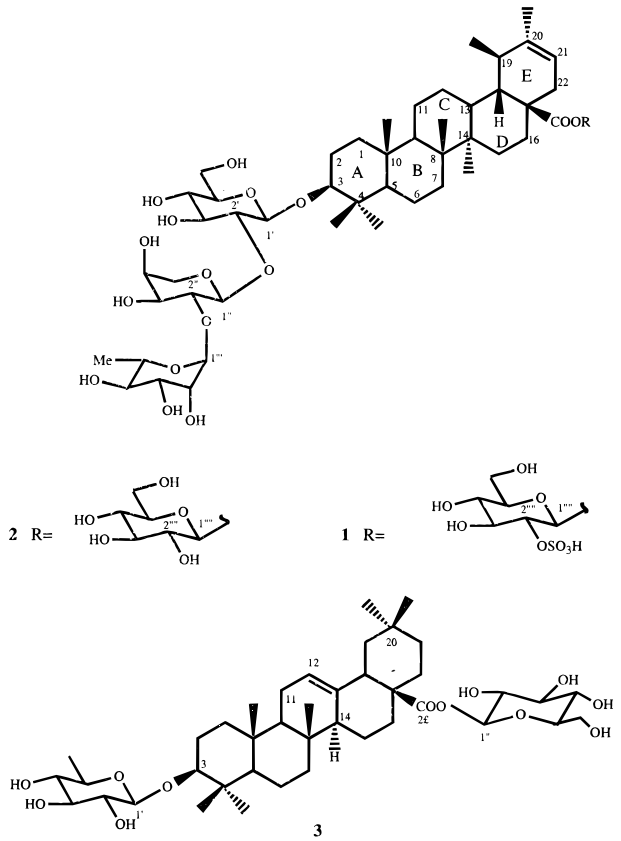

Figure 1.

Key correlation peaks in the HMBC spectrum between H-1 of the glucose unit at δ 4.49 and C-3 (δ_C 91.47) of the aglycon and between H-2″ of arabinose (δ_H 3.92) and C-1‴ of the terminal rhamnose unit (δ_C 102.15) allowed the sequence of the trisaccharide chain at C-3 to be determined as shown in Figure 1. Thus **1** is 3*β*-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→2)-*β*-D-glucopyranosylurs-20(21)-en-28-oic acid 28-*0*-[*β*-D-2-*O*-sulfonylglucopyranosyl] ester.

The NMR data indicated the same aglycon and sugar chain at C-3 for both compounds **1** and **2**. The ^{1}H-NMR spectrum of **2** was very similar to that of **1** except for the chemical shift of H-2⁗ signal (δ 3.30 in **2**, 4.20 in **1**) of the glucose moiety at C-28. The carbon resonances of C-1⁗ (+2.06 ppm), C-2⁗ (−5.54 ppm), and C-3⁗ (+0.77 ppm) of the glucose moiety at C-28 indicated the loss of the sulfate group at C-2⁗. It is interesting to note that the loss of the $-SO_3H$ group at C-2⁗ induced an upfield shift of the resonance of the C-16eq proton (−0.21 ppm) and a downfield shift of Me-27 (+0.03 ppm) on the aglycon of compound **2** with respect to compound **1**. From the above data, **2** was determined to be 3*β*-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→2)-*β*-D-glucopyranosylurs-20(21)-en-28-oic acid 28-*O*-[*β*-D-glucopyranosyl] ester.

The negative FABMS spectrum of **3** showed a quasi-molecular anion at *m*/*z* 749 $[M - H]^-$ and two main peaks due to the loss of a hexose and a deoxyhexose unit. The ^{1}H-NMR spectrum in the aglycon part showed the presence of six singlet Me signals at δ 0.86, 0.90, 0.91, 0.95, 0.99, and 1.03, indicating a nor-oleane pentacyclic skeleton. An olefinic hydrogen resonating at comparatively lowfield (δ 5.68 br m) and the typical signal of an oxymethine at C-3 (δ 3.21, dd, J = 12.0, 4.5 Hz) were also present. The ^{13}C and DEPT ^{13}C-NMR spectra showed 41 signals. The 29 ^{13}C-NMR resonances of the aglycon were recognized by subtracting the 12 sugar carbon resonances from the total spectrum. Some of them are peculiar for a 3-*β*-glycosylated olean-28-oic acid.[20,21] The absence of the signal ascribable to Me-27 typically resonating at ca. δ_C 26.0 in the oleane skeleton, the olefinic resonances at δ_C 127.9 (CH) shifted to downfield by ca. 5 ppm and at δ_C 140.0 (C) shifted to upfield by ca. 3 ppm with respect to oleanolic acid,[20,21] as well as the presence of a CH resonating at 55.5 (C-14), indicated a 27-nor-olean-12-en-28-oic acid aglycon. The NMR spectra of **3** in the sugar region exhibited an ester-bonded anomeric signal at δ_H 5.42 (d, J = 7.5 Hz) and δ_C 95.7 and a second ether-linked anomeric signal at δ_H 4.31 (d, J = 7.9 Hz) and δ_C 106.6. Starting from these well-resolved anomeric proton signals the other proton sugar signals could be assigned by 1D TOCSY and 2D DQF−COSY spectra. In the 1D TOCSY spectrum of compound **3**, H-1 signal at δ 5.42 showed connectivities to four methines and a methylene; the COSY spectrum established the proton sequence within this monosaccharide as H-1 (δ 5.42), H-2 (δ 3.28), H-3 (δ 3.43), H-4 (δ 3.57), H-5 (δ 3.37), H_2-6 (δ 3.70 and 3.85). Similar observations on the other sugar residue and analysis of the ^{13}C and DEPT ^{13}C-NMR data (see Experimental Section) led to the identification of *β*-D-quinovopyranose linked at C-3 and *β*-D-glucopyranose linked at C-28 of the aglycon. Thus, **3** was the novel compound 3*β*-*O*-*β*-D-quinovopyranosyl-27-nor-olean-12-en-28-oic acid 28-*O*-*β*-D-glucopyranosyl ester.

The remaining saponins were identified by comparison of their NMR spectral data with those from the literature to be 3*β*-*O*-*β*-D-glucopyranosyl-quinovic acid 28-*O*-*β*-D-glucopyranosyl ester previously isolated from *Guettarda platypoda*,[2] 3*β*-*O*-[*β*-D-2-*O*-sulfonylglucopyranosyl]-quinovic acid 28-*O*-[*β*-D-glucopyranosyl) ester (zygophyloside G)[3] and 3*β*-*O*-[*β*-D-2-*O*-sulfonylquinovopyranosyl]-quinovic acid 28-*O*-[*β*-D-glucopyranosyl) ester (zygophyloside E)[4] previously isolated from *Z. propinquum*.

Experimental Section

General Experimental Procedures. A Bruker DRX−600 spectrometer operating at 599.19 MHz for ^{1}H and 150.858 for ^{13}C using the UXNMR software package was used for NMR measurements in CD_3OD solutions. 2D experiments: ^{1}H−^{1}H DQF−COSY,[11] inverse-detected ^{1}H−^{13}C HSQC[12] and HMBC,[13] and ROESY[14] were obtained by employing the conventional pulse sequences as described previously.[18,19,22] The selective excitation spectra, 1D TOCSY[16] were acquired using waveform generator-based GAUSS shaped pulses, mixing times ranging from 100 to 120 ms, and a MLEV-17 spin-lock field of 10 kHz preceded by a 2.5-ms trim pulse. Optical rotations were measured on a Perkin−Elmer 141 polarimeter using a sodium lamp operating at 589 nm in 1% w/v solutions in MeOH. FABMS were recorded in a glycerol matrix in the negative ion mode on a VG ZAB instrument (XE atoms of energy of 2−6 KV). HPLC separations were performed with a Waters model 6000A pump equipped with a U6K injector and a model 401 refractive index detector.

Plant Material. *Z. gaetulum* Emb. & Maire was collected in September 1992, near Zreouila 27 km from

Goulmine, southern Morocco. The plant was identified by Dr. A. Khaoudji of the Department de Biologie, Faculté des Sciences, Rabat, Morocco. A voucher specimen of the plant is deposited at the herbarium of Laboratoire de Chimie des Plantes et de Synthèse Organique et Bioorganique, Faculté des Sciences, Rabat, Morocco.

Extraction and Isolation. Air-dried and powdered aerial parts of *Z. gaetulum* (1 kg) were sequentially extracted at room temperature with petroleum ether, $CHCl_3$, $CHCl_3$−MeOH (9:1), MeOH, and H_2O to afford 7.85, 7.03, 19.46, and 11.03 g of residue, respectively. Part of the MeOH residue (4 g) was chromatographed on a Sephadex LH-20 column (100 × 5 cm) eluting with MeOH. Fractions of 8 mL were collected and combined by TLC similarity [SiO_2 plates, *n*-BuOH−AcOH−H_2O (60:15:25) and $CHCl_3$−MeOH−H_2O (80:18:2)] into nine main fractions A−I. Fractions B (161 mg), E (520 mg), and I (329 mg) were further chromatographed by RPHPLC (*μ*-Bondapak C-18 column, 30 cm × 7.8 mm, flow rate 1.5 mL/min) with MeOH−H_2O (54:46) as solvent system. Fraction B, containing the more polar glycosides, yielded compounds **1** (t_R = 4.5 min, 30.5 mg) and **2** (t_R = 9 min, 22 mg). Fractions E and I, containing the less polar glycosides, provided compounds **3** (t_R = 21 min, 15 mg), 3*β*-*O*-*β*-D-glucopyranosyl-quinovic acid 28-*O*-*β*-D-glucopyranosyl ester (t_R = 20.5 min, 15.5 mg), zygophyloside G (t_R = 15 min, 10 mg) and zygophyloside E (t_R = 18 min, 12 mg). The known saponins were identified by comparison of their NMR spectra with literature data.[2−4]

Compound 1: $[\alpha]^{25}_D$ +15.5; negative FABMS *m*/*z* [M − H]$^-$ 1137, [(M − H)$^-$ SO_3]$^-$ 1057, [(M − H) − (SO_3 + 162)]$^-$ 895; NMR data for the aglycon moiety are reported in Table 1; for the sugar moiety, in Table 3.

Compound 2: $[\alpha]^{25}_D$ +18.1; negative FABMS *m*/*z* [M − H]$^-$ 1057, [(M − H)$^-$ 162]$^-$ 895; NMR data for the aglycon moiety are reported in Table 2; for the sugar moiety, in Table 3.

Compound 3: $[\alpha]_D^{25}$ +29.7; negative FABMS *m*/*z* [M − H]$^-$ 749, [(M − H) − 146]$^-$ 603, [(M − H) − 162]$^-$ 587, [(M − H) − (146 + 162)]$^-$ 441; ^{1}H NMR δ 0.86 (3H, s, Me-23), 0.90 (3H, s, Me-26), 0.91 (3H, s, Me-30 or 29), 0.95 (3H, s, Me-29 or 30), 0.99 (3H, s, Me-25), 1.03 (3H, s, Me-24), 1.28 (3H, d, J = 6.6 Hz, Qui-6), 3.21 (1H, dd, J = 12.0, 4.5 Hz, H-3), 3.23 (1H, dd, J = 7.9, 9.5 Hz, Qui-2), 3.28 (1H, dd, J = 9.0, 7.6 Hz, Glc-2), 3.30 (1H, t, J = 9.5 Hz, Qui-4), 3.33 (1H, dd, J = 9.2, 6.5 Hz, Qui-5), 3.37 (1H, ddd, J = 9.0, 4.0, 2.5 Hz, Glc-5), 3.40 (1H, t, J = 9.5 Hz, Qui-3), 3.43 (1H, t, J = 9.0 Hz, Glc-3), 3.57 (1H, t, J = 9.0 Hz, Glc-4), 3.70 (1H, dd, J = 12.0, 4.0 Hz, Glc-6a), 3.85 (1H, dd, J = 12.0, 2.5 Hz, Glc-6b), 4.31 (1H, d, J = 7.9 Hz, Qui-1), 5.42 (1H, d, J = 7.6 Hz, Glc-1), 5.68 (IH, br m, H-12); ^{13}C NMR δ 17.0 (Me-25), 18.1 (Qui-6), 18.4 (Me-26), 19.0 (Me-23), 19.2 (CH_2-6), 24.1 (CH_2-11), 24.38 (Me-30), 25.2 (CH_2-16), 25.4 (CH_2-15), 27.0 (CH_2-2), 28.5 (Me-24), 30.1 (C-20), 31.0 (CH_2-22), 33.6 (Me-29), 33.9 (CH_2-7), 34.0 (CH_2-21), 37.9 (C-10), 39.8 (CH_2-1), 40.1 (C-4), 40.6 (C-8), 43.0 (CH-18), 45.0 (CH_2-19), 47.8 (C-17), 48.0 (CH-9), 55.5 (CH-14), 57.8 (CH-5), 62.5 (Glc-6), 71.2 (Qui-4), 73.1 (Glc-4), 74.0 (Glc-2), 75.9 (Qui-2), 77.1 (Qui-5), 78.0 (Glc-5), 78.4 (Qui-3), 78.7 (Glc-3), 90.7 (CH-3), 95.7 (Glc-1), 106.6 (Qui-1), 127.9 (C-12), 140.0 (C-13), 176.0 (C-28).

Solvolysis of 1. A solution of **1** in a 1:1 mixture of dioxane and pyridine (0.6 mL) was heated in a stoppered reaction vial at 120 °C for 4 h. The mixture was diluted with H_2O and extracted with *n*-BuOH. The solvent was evaporated under reduced pressure and the residue analyzed by ^{1}H NMR, which gave data identical with those of compound **2**.

References and Notes

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NP970245Y