Two Sulfated Triterpenoidal Saponins from the Barks of *Zygophyllum fabago* L.

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Two new sulfated triterpenoid saponins, zygophylosides Q (1) and R (2), have been isolated from the barks of *Zygophyllum fabago* L. Their structures were elucidated as 3β,24,28,30-tetrahydro-urs-20-ene-24-*O*-sulphonyl-3-*O*-[β-D-glucopyranosyl]-30-*O*-β-D-glucopyranoside and 3β,24,28,30-tetrahydro-urs-20-ene-24-*O*-sulphonyl-3-*O*-[β-D-xylopyranosyl]-30-*O*-β-D-glucopyranoside, respectively, by spectral and chemical evidence.

Key words *Zygophyllum fabago* L.; sulfated triterpenoid saponin; zygophyloside Q; zygophyloside R

Zygophyllum fabago L. belongs to the Zygophyllaceae family and is mainly distributed in the Gansu provinces and Xinjiang autonomous region of China. It is used as antitussive, expectorant, anti-inflammatory and removing pains.[1)] In the course of our study on the chemical composition of the plant, two 27-nor-triterpenoid glycoside and two triterpenoid saponins were isolated, and both of the triterpenoid saponins contain a sulfate group linked to the β-D-xyloside moiety.[2,3)] In a continuing study of the same plant, two new triterpenoid saponins were isolated, and the sulphate group was in position of the aglycone. The structures of **1** and **2** were elucidated with the help of spectral and chemical methods.

Compound **1** was isolated as colorless powder. Its molecular formula was determined as $C_{42}H_{70}O_{17}S$ by HR-ESI-MS, showing a $[M+H]^+$ peak at *m/z* 879.43677, corresponding to eight degrees of unsaturation. The ESI mass spectrum of **1** exhibited the $[M+2Na-1]^+$ ion (*m/z* 923) and the $[M+H]^+$ ion (*m/z* 879). The IR spectrum with absorption bands at 1254 and 1209 cm^{-1} indicated the presence of a sulfate group in **1**. The configuration of the sugar unit was assigned after hydrolysis of **1** with 2 M HCl. The acid hydrolysis afforded D-glucose. The ^{13}C-NMR spectral data, summarized in Table 1, showed 42 carbon resonances, indicating the presence of two sugar moieties with a triterpenoid aglycone. The ^{1}H-NMR spectrum (Table 1) indicated the presence of four tertiary and one secondary methyl groups at δ 0.85 (3H-23), 0.72 (3H-25), 0.76 (3H-26), 0.86 (3H-27) and doublet at δ 1.11 (3H, d, J=6.0 Hz, H-29). The ^{1}H-NMR spectrum also displayed one olefinic protons at δ 5.80 (1H, d, J=7.2 Hz, H-21). Two anomeric proton signals were observed at δ 5.30 (1H, d, J=7.8 Hz, H-1′), 4.91 (1H, d, J=7.8 Hz, H-1″) showed the β-configuration of sugar moiety. The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra of the aglycone showed two olefinic carbons at δ 141.8 (C-20) and 122.3 (C-21), five methyls at δ 13.4 (C-23), 17.0 (C-25), 16.1 (C-26), 14.9 (C-27), 23.0 (C-29), three oxygenated methylenes at δ 68.8 (C-24), 58.2 (C-28), 73.4 (C-30), and one oxygenated methine at δ 82.3 (C-3). The ^{13}C-NMR signals appeared at δ 105.4, 75.8, 78.7, 71.7, 78.0, 62.7 and 105.7, 75.3, 78.5, 71.7, 78.6, 62.7 were assigned to C-1′—C-6′ and C-1″—C-6″ of two glucoses. The heteronuclear multiple bond correlation (HMBC) spectrum showed the correlations for H-21/C-17, H-21/C-19, H-21/C-30, H-24/C-3, H-24/C-5, H-28/C-16, H-28/C-22, H-30/C-18, H-30/C-20, H-29/C-20. The correlations for H-1′/C-3, H-1″/C-30 in the HMBC spectrum showed two glucose moieties were attached at C-3 and C-30 of the aglycone, respectively. The value of the coupling constant between H-18 and H-19 ($^3J_{18,19}$=12 Hz) indicated that both protons were in an axial position. This, and the nuclear Overhauser effect spectroscopy (NOESY) cross peak between H-29 and H-18 showed the *cis*-connection of the rings D and E. The relative configuration at C-23 was determined by the NOESY cross peak. The irradiations of H-23 shows NOE with H-3α and H-5α, indicating the hydroxyl group in C-24 was located at the 24β. The downfield shifts of the H-24 ($\Delta\delta$ +1.00) and C-24 ($\Delta\delta$ +5.0) signals of **1** compared with a nonsubstituted moiety indicate that the sulphate group is in position C-24 of the aglycone.[3)] The above structural elucidation of **1** was further supported by its ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY), heteronuclear multiple quantum correlation (HMQC) and HMBC data, respectively. From these results, the structure of **1** (Fig. 1) was established as 3β,24,28,30-tetrahydro-urs-20-ene-24-*O*-sulphonyl-3-*O*-[β-D-glucopyranosyl]-30-*O*-β-D-glucopyranoside.

Compound **2** was isolated as colorless powder. Its molecular formula was determined as $C_{41}H_{68}O_{16}S$ by HR-ESI-MS, showing a $[M+H]^+$ peak at *m/z* 849.42876, corresponding to

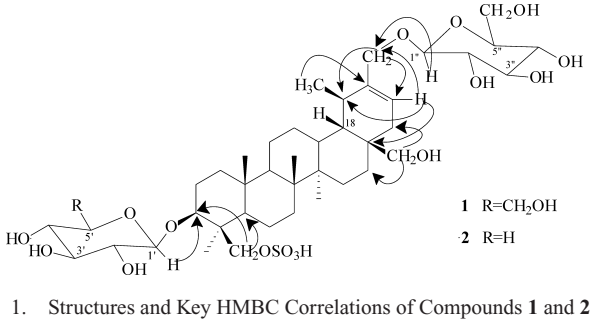

Fig. 1. Structures and Key HMBC Correlations of Compounds **1** and **2**

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Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for Compounds **1** and **2** in Pyridine-d_5

No.	**1**		**2**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	38.5	0.73 (overlap), 1.45 (overlap)	38.6	0.84 (overlap), 1.45 (overlap)
2	25.9	1.85 (m), 2.22 (m)	26.2	1.94 (m), 2.21 (m)
3	82.3	4.16 (overlap)	82.3	4.17 (overlap)
4	42.8		42.9	
5	47.6	1.46 (m)	47.6	1.52 (m)
6	17.9	1.20 (overlap), 1.71 (m)	17.9	1.21 (overlap), 1.73 (m)
7	34.2	1.14 (overlap), 1.38 (overlap)	34.2	1.16 (overlap), 1.41 (overlap)
8	41.3		41.3	
9	50.3	1.22 (overlap)	50.3	1.28 (overlap)
10	36.8		36.9	
11	21.7	0.82 (overlap), 1.28 (overlap)	21.7	0.85 (overlap), 1.32 (overlap)
12	28.0	1.10 (overlap), 1.42 (overlap)	28.0	1.12 (overlap), 1.34 (overlap)
13	38.4	1.50 (overlap)	38.4	1.60 (overlap)
14	42.3		42.3	
15	27.0	0.94 (m), 1.83 (m)	27.0	0.94 (m), 1.83 (m)
16	30.7	1.16 (overlap), 2.12 (m)	30.7	1.16 (overlap), 2.13 (m)
17	39.3		39.3	
18	48.6	1.31 (overlap)	48.5	1.32 (overlap)
19	32.0	2.31 (d, 12 Hz)	31.9	2.32 (d, 12 Hz)
20	141.8		141.8	
21	122.3	5.80 (d, 7.2 Hz)	122.3	5.80 (d, 7.2 Hz)
22	35.1	1.61 (m), 2.63 (dd, 6.6, 15.6 Hz)	35.0	1.60 (overlap), 2.63 (dd, 7.2, 15.6 Hz)
23	13.4	0.85 (s)	13.4	0.85 (s)
24	68.8	4.2 (overlap), 4.89 (overlap)	68.7	4.2 (overlap), 4.87 (overlap)
25	17.0	0.72 (s)	17.1	0.76 (s)
26	16.1	0.76 (s)	16.1	0.77 (s)
27	14.9	0.86 (s)	14.9	0.85 (s)
28	58.2	3.90 (overlap), 3.98 (overlap)	58.2	3.90 (overlap), 3.97 (overlap)
29	23.0	1.11 (d, 6 Hz)	22.9	1.10 (d, 6.6 Hz)
30	73.4	4.47 (d, 12 Hz), 4.55 (d, 12 Hz)	73.4	4.48 (d, 11.4 Hz), 4.55 (d, 11.4 Hz)
1′	105.4	5.30 (d, 7.8 Hz)	106.1	5.28 (d, 7.2 Hz)
2′	75.8	3.98 (overlap)	75.6	3.96 (overlap)
3′	78.7	4.34 (overlap)	78.5	4.22 (overlap)
4′	71.7	4.25 (overlap)	71.3	4.12 (m)
5′	78.0	3.88 (overlap)	66.9	3.67 (t, 10.8 Hz), 4.22 (overlap)
6′	62.7	4.32 (overlap), 4.42 (overlap)		
1″	105.7	4.91 (d, 7.8 Hz)	105.7	4.87 (d, 7.8 Hz)
2″	75.3	4.03 (m)	75.3	4.04 (m)
3″	78.5	4.23 (overlap)	78.6	4.22 (overlap)
4″	71.7	4.19 (overlap)	71.7	4.23 (overlap)
5″	78.6	4.22 (overlap)	78.7	3.96 (overlap)
6″	62.7	4.42 (overlap), 4.58 (m)	62.7	4.42 (dd, 4.8, 12 Hz), 4.58 (dd, 2.4,12 Hz)

eight degrees of unsaturation. The ESI mass spectrum of **2** exhibited the $[M+2Na-1]^+$ ion (m/z 893) and the $[M+H]^+$ ion (m/z 849). The IR spectrum with absorption bands at 1248 and 1205 cm^{-1} indicated the presence of a sulfate group in **2**. The configuration of the sugar unit was assigned after hydrolysis of **2** with 2 M HCl. The acid hydrolysis afforded D-xylose and D-glucose. The ^{13}C-NMR spectrum (Table 1) of **2** showed two anomeric signals which appeared at δ 106.1 and 105.7, indicating the presence of two sugar moieties. A comparison of the ^{13}C-NMR spectrum of **2** with that of **1** showed that the chemical shifts due to the aglycone in both saponins were almost the same. The anomeric proton signals were observed at δ 5.28 (1H, d, J=7.2 Hz, H-1′) and 4.87 (1H, d, J=7.8 Hz, H-1″) showed the β-configuration of sugar moieties. The correlations for H-1′/C-3, H-1″/C-30 in the HMBC spectrum showed two sugar moieties were attached at C-3 and C-30 of the aglycone, respectively. The ^{13}C-NMR signals appeared at δ 106.1, 75.6, 78.5, 71.3, 66.9 and 105.7, 75.3, 78.6, 71.7, 78.7, 62.7 were assigned to

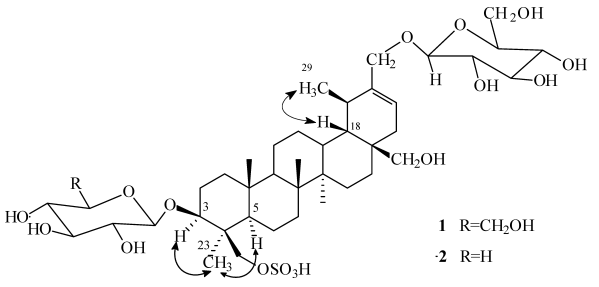

Fig. 2. The Key Correlations Observed from NOESY Spectrum of Compounds **1** and **2**

C-1′—C-5′ of xylcose and C-1″—C-6″ of glucose. The above structural elucidation of **2** was further supported by its ^{1}H–^{1}H COSY, HMQC and HMBC data. From these results, the structure of **2** (Fig. 1) was established as 3β,24,28,30-tetrahydro-urs-20-ene-24-*O*-sulphonyl-3-*O*-[β-D-xylopyranosyl]-30-*O*-β-D-glucopyranoside.

Experimental

General Melting points were determined using a Fisher Johns apparatus and are uncorrected. IR spectra were obtained in KBr disks on a Perkin-Elmer 983G spectrophotometer. NMR spectra were recorded on a INOVA 400 spectrometer. ESI-MS was recorded in a Micromass ZabSpec spectrometer. GLC was carried out on a TSQ7000 (Finnigan) GC-MS instrument. TLC employed precoated Silica gel plates (5—7 μm, Qingdao Haiyang). For column chromatography, Silica gel (H, 200—300 mesh, Qingdao Haiyang), Macroporous resin D101 (26—60 mesh, Tianjin Haiguang Chemistry Company, Tianjin, China) were used. HPLC was performed on a Waters 515 instrument equipped with a Waters UV-2996 detector. A YMC-Pack ODS-A (10×250 mm i.d.) column was used for preparative purpose.

Plant Material The barks of *Zygophyllum fabago* L. were collected from Wulumuqi, Xinjiang Autonomous Region of China in March 2004, and identified by Prof. Guo-Qiang Li of Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, China, where a voucher sample (Voucher Number: ZF200403) has been deposited.

Extraction and Isolation The air-dried, powdered barks (4 kg) of the plant material were successively extracted with 75% EtOH (each 65 l×3) under reflux. The resultant extract was combined and dried under reduced pressure to give concentrated extractives (200 g). The latter was subsequently suspended in water and partitioned successively with $CHCl_3$, EtOAc and 1-butanol. The 1-butanol part (60 g) was subjected to column chromatography by a combination of D_{101} macroporous resin, eluted gradiently with H_2O, 10% EtOH, 30% EtOH, 50% EtOH, 75% EtOH, 95% EtOH, successively. The fraction eluted with 30% EtOH (11 g) was subjected to a silica gel column with a $CHCl_3$–MeOH gradient system (1 : 0—0 : 1) , affording 20 fractions. Fraction 15 (0.8 g) was purified by repeated ODS column chromatography [MeOH–H_2O (2 : 8—1 : 0)] , and further purified by HPLC to afford compounds **1** (15 mg) and **2** (28 mg).

Compound **1**: Colorless powder; $[\alpha]_D^{20}$ +21.2 (c=0.066, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3415, 2941, 2875, 1655, 1458, 1388, 1254, 1209, 1070, 1043, 984; ESI-MS *m*/*z*: 923, 413, 301; HR-ESI-MS *m*/*z*: 879.43677 (Calcd for $C_{42}H_{71}O_{17}S$, 879.44119); ^{1}H- and ^{13}C-NMR: see Table 1.

Compound **2**: Colorless powder; $[\alpha]_D^{20}$ +46.2 (c=0.013, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3371, 2937, 2873, 1651, 1454, 1381, 1248, 1205, 1068, 1041, 985; ESI-MS *m*/*z*: 893, 413, 301; HR-ESI-MS *m*/*z*: 849.42876 (Calcd for $C_{41}H_{69}O_{16}S$, 849.43063); ^{1}H- and ^{13}C-NMR: see Table 1.

Acid Hydrolysis Compounds 1 and 2 Compounds **1** and **2** (3 mg, respectively) were hydrolyzed separately with 2 M HCl (0.5 ml) for 10 h at 95 °C. After filtration of the reaction mixture, the filtrate was evaporated under vacuum. After addition of H_2O, the acidic solution was evaporated again to remove HCl. This procedure was repeated until a neutral solution was obtained, which was finally evaporated and dried *in vacuo* to furnish a monosaccharide residue. The residue was dissolved in pyridine (1 ml), to which 2 mg of L-cysteine methyl ester hydrochloride was added. The mixture was kept at 60 °C for 2 h and evaporated under N_2 stream and dried *in vacuo*. The residue was trimethylsilylated with *N*-trimethylsilylimidazole (0.2 ml) for 2 h. The mixture was partitioned between *n*-hexane and H_2O (2 ml each), and the *n*-hexane extract was analyzed by GC-MS under the following conditions: capillary column, DB-5 (30 m×0.25 mm×0.25 μm); detection, FID; detector temperature, 280 °C; injection temperature, 250 °C; initial temperature was maintained at 100 °C for 2 min and then raised to 280 °C at the rate of 10 °C/min, and final temperature was maintained for 5 min; carrier, N_2 gas. The peak of the hydrolysate of **1** was detected at 24.87 min (D-glucose) by comparison with authentic sample. The peaks of D-xylcose (15.48 min) and D-glucose (24.87 min) were detected in the hydrolysate of **2** by the same method.

Detection of the Sulfate Group A 5—6 mg aliquot of each sample was refluxed with 10% HCl (4 ml) for 4 h and then extracted with Et_2O. An aliquot of the aqueous layer of each was treated with 70% $BaCl_2$ to give a white precipitate ($BaSO_4$).[5]

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