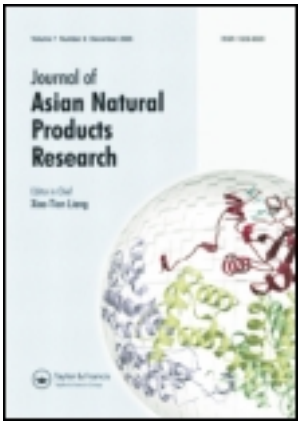

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Jian-Guang Luo [a], Wei Nie [a] & Ling-Yi Kong [a]

[a] Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing, 210009, China

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Three new sulfated triterpenoids from the roots of *Gypsophila pacifica*

Jian-Guang Luo, Wei Nie and Ling-Yi Kong*

Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing 210009, China

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Three new sulfated triterpenoids (**1**–**3**), along with one known compound (**4**), were isolated from the roots of *Gypsophila pacifica* Kom. The structures of the new compounds were established as 3β-*O*-sulfate gypsogenin 28-*O*-β-D-glucopyranosyl ester (**1**), 3β-*O*-sulfate gypsogenin (**2**), and 3β-*O*-sulfate quillaic acid (**3**) on the basis of 1D, 2D NMR, and HR-ESI-MS methods.

Keywords: *Gypsophila pacifica*; Caryophyllaceae; sulfated triterpenoids

1. Introduction

The genus *Gypsophila*, which belongs to the family Caryophyllaceae, comprises more than 150 species distributed throughout the world. Some of these species have long been used as pharmaceutical and ornamental plants [1]. In northern China, 18 species and a variety are reported. Xinjiang is the center of distribution and differentiation with the number of species gradually decreasing eastwards [2].

Gypsophila pacifica Kom., a small perennial herb, is a typical representative of the genus *Gypsophila*, widely distributed in the northeast regions of China. Its roots have been used as a substitute for the traditional Chinese medicine Yin-Chai-Hu (roots of *Stellaria dichotoma* L. var. *lanceolata* Bge) to treat fever, consumptive disease, and infantile malnutrition syndrome [3]. In previous chemical studies, triterpenoid saponins were reported from *G. pacifica* [4,5]. Our continuing research for constituents from its roots resulted in the isolation of four sulfated triterpenoids **1**–**4** (Figure 1), of which **1**–**3** are new compounds. Herein, we describe the isolation and structural elucidation of the new compounds.

2. Results and discussion

Compound **1**, obtained as a white amorphous powder, was assigned to the molecular formula $C_{36}H_{56}O_{12}S$ as determined from HR-ESI-MS (*m/z*: 711.3433 $[M - H]^-$). The ESI-MS of **1** exhibited the $[M - H]^-$ ion (*m/z* 711), $[M + NH_4]^+$ ion (*m/z* 730), and $[M + Na]^+$ ion (*m/z* 735). The IR spectrum with absorption bands at 1229 cm^{-1} indicated the presence of a sulfate group in **1** [6]. Acid hydrolysis of **1** afforded D-glucose as component sugar and aglycone, followed by treatment with $BaCl_2$, gave white precipitates, thus demonstrating the existence of sulfate residues [7]. The ^{13}C NMR spectral data, summarized in Table 1, showed 36 carbon resonances, indicating the presence of a sugar moiety with a triterpenoid aglycone. The ^1H NMR spectrum (Table 1) indicated the presence of six angular CH_3 groups (δ_H 0.80, 0.88, 0.91, 1.05, 1.21, and 1.24), a vinyl H-atom at δ_H 5.39 (br s), and an

*Corresponding author. Email: cpu_lykong@126.com

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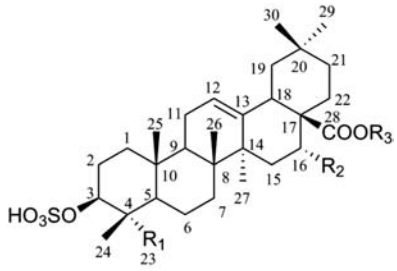

	R_1	R_2	R_3
1	CHO	H	Glc
2	CHO	H	H
3	CHO	OH	H
4	CH_3	H	H

Figure 1. Structures of **1**–**4**.

aldehyde proton at δ_H 9.80 (s). Moreover, an anomeric proton signal at δ_H 6.30 (d, 8.2) and an oxygenated methylene proton signal at δ_H 5.14 (1H, dd, 4.0, 12.5) showed the β-configuration of sugar moiety and 3-OH, respectively. After assignments of the NMR (Table 1) data on the basis of HSQC and HMBC experiments (Figure 2), the aglycone in **1** was determined as gypsogenin [8], and the carbon signals appeared at δ_C 95.6, 73.9, 78.2, 70.8, 78.7, and 62.0 were assigned to C-1′–C-6′ of D-glucose. When comparing the data of **1** (Table 1) with those of gypsogenin, the resonances for C-3 and C-28 were shifted from δ_C 71.9 to 79.2, and from δ_C 178.4 to 176.3, respectively, which revealed that the OH group at C-3 and COOH group at C-28 was substituted. The HMBC correlations of the proton at δ_H 6.30 (H-1 of glucose) with the carbon at δ_C 176.3 (C-28 of aglycone) confirmed that the sugar residue was attached to C-28 of aglycone (Figure 2). Since HR-ESI-MS, IR, and acid hydrolysis of **1** suggested the presence of a sulfate group in the molecule, the position of the sulfate group was assigned to C-3 on the basis of the downfield chemical shifts of H-3 ($\Delta\delta + 1.10$) and C-3 ($\Delta\delta + 7.3$), which are consistent with the presence of a sulfate group [7–9]. Thus, the structure of this new compound was established as 3β-*O*-sulfate gypsogenin 28-*O*-β-D-glucopyranosyl ester.

Compound **2** was isolated as a white amorphous powder. The negative-ion HR-ESI-MS of **2** exhibited a pseudo-molecular ion at *m/z* 549.2905 $[M - H]^-$ (calcd for $C_{30}H_{45}O_7S$, 549.2891), consistent with a molecular formula of $C_{30}H_{46}O_7S$, 162 mass units less than **1**. The ^1H and ^{13}C NMR spectra of **2** (Table 1) resembled to those of **1**, except for the absence of glucosyl signals and downfield shifts of C-28 from δ_C 176.3 to 182.0, which revealed that C-28 of **2** was non-substituted. Combining the evidence of HSQC and HMBC experiments, the structure of **2** was elucidated to be 3β-*O*-sulfate gypsogenin.

Compound **3** had a molecular formula of $C_{30}H_{46}O_7S$ as established by HR-ESI-MS (*m/z*: 565.2819 $[M - H]^-$). The IR spectrum showed the presence of OH (3435 cm^{-1}), CO (1704 cm^{-1}), and —SO_3 (1231 cm^{-1}) functions. These suggested that **3** also possessed a sulfate group in the molecule. The ^1H and ^{13}C NMR spectra of compound **3** were similar to those of compound **2**, except for the presence of 16-OH in **3**. This was evidenced by carbon shifts at δ_C 36.6 (C-15) and δ_C 75.3 (C-16) of **3** with respect to the corresponding values of δ_C 28.8 and 24.1 in **2**. The appearance of H-16 as a broad singlet at δ_H 5.31 indicated that 16-OH was α-oriented. The above detailed NMR (Table 1) analysis identified the aglycone as quillaic acid [10], which was further confirmed by the HSQC and HMBC experiments. Concerning the sulfate group, the downfield shifts of the H-3 ($\Delta\delta + 1.1$) and C-3 ($\Delta\delta + 9.6$) signals of **3**, compared with a non-substituted moiety, indicated that the

Table 1. ^{1}H (500 MHz) and ^{13}C NMR(125 MHz) spectral data for **1**–**3**[a].

	1[b]		**2**[c]		**3**[c]	
No.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	38.6	1.44 (m), 0.94 (m)	39.1	1.41 (m), 0.90 (m)	39.2	0.84 (m), 1.34 (m)
2	28.4	2.28 (m)	28.8	2.23 (m)	30.3	2.08 (m)
3	79.2	5.14 (dd, 4.0, 12.5)	80.4	4.84 (dd, 4.0, 12.0)	80.2	4.85 (dd, 4.2, 12.0)
4	55.1	–	55.9	–	55.9	–
5	47.6	1.51 (m)	48.6	1.49 (m)	47.6	1.41 (m)
6	20.4	1.58 (m), 1.34 (m)	21.4	1.53 (m), 1.24 (m)	21.3	1.38 (m), 1.14 (m)
7	33.0	1.68 (m)	33.8	1.62 (m)	33.4	1.60 (m)
8	39.9	–	40.9	–	41.0	–
9	47.1	1.76 (m)	48.2	1.70 (m)	48.2	1.66 (m)
10	35.7	–	36.9	–	36.9	–
11	23.2	2.07 (m), 1.95 (m)	24.0	2.01 (m), 1.86 (m)	24.9	2.07 (m), 1.95 (m)
12	122.3	5.39 (br s)	123.4	5.35 (br s)	123.1	5.31 (br s)
13	144.0	–	145.3	–	145.2	–
14	42.0	–	43.0	–	41.0	–
15	28.0	2.29 (m), 1.68 (m)	28.8	2.19 (m), 1.62 (m)	36.6	2.09 (m), 1.48 (m)
16	23.8	2.07 (m), 0.90 (m)	24.1	2.02 (m), 0.84 (m)	75.3	5.31 (br s)
17	46.8	–	47.6	–	47.2	–
18	41.5	3.30 (m)	42.8	3.21 (m)	42.8	3.00 (m)
19	46.0	1.79 (m), 1.25 (m)	47.2	1.72 (m), 1.19 (m)	47.6	1.25 (m), 1.69 (m)
20	30.6	–	31.6	–	30.7	–
21	33.8	1.32 (m), 1.14 (m)	34.9	1.28 (m), 1.18 (m)	36.2	1.14 (m), 1.22 (m)
22	32.3	2.04 (m), 1.75 (m)	33.2	1.96 (m), 1.69 (m)	31.4	1.75 (m), 2.04 (m)
23	205.4	9.80 (s)	206.9	9.31 (s)	206.8	9.32 (s)
24	9.7	1.24 (s)	9.8	1.20 (s)	9.9	1.41 (s)
25	15.9	0.80 (s)	16.1	0.82 (s)	16.2	0.81 (s)
26	17.1	1.05 (s)	17.7	1.01 (s)	17.8	1.02 (s)
27	26.0	1.21 (s)	26.4	1.15 (s)	27.3	1.71 (s)
28	176.3	–	182.0	–	176.0	–
29	33.0	0.91 (s)	33.5	0.94 (s)	33.5	0.87 (s)
30	23.8	0.88 (s)	24.0	0.91 (s)	24.4	0.97 (s)
Glc						
1′	95.6	6.30 (d, 8.2)				
2′	73.9	4.20 (m)				
3′	78.2	4.27 (m)				
4′	70.8	4.35 (m)				
5′	78.7	4.03 (m)				
6′	62.0	4.41 (m), 4.48 (m)				

Notes: [a]Assignments were made on the basis of HSQC and HMBC experiments.
[b]Measured in C_5D_5N.
[c]Measured in CD_3OD.

sulfate group was in position C-3 of the aglycone. Accordingly, the structure of new compound was established as 3β-*O*-sulfate quillaic acid.

Compound **4** was identified as 3β-*O*-sulfate oleanolic acid by direct comparison of their spectral analysis with the literature [11].

The appearance of sulfated or sulfur-containing triterpenoids is quite rare. From the species of the Caryophyllaceae family, only eight compounds of this type are reported [6,7,12–14]. It is noteworthy to mention that four sulfated triterpenoids were simultaneously isolated from the roots of *G. pacifica* in our present study.

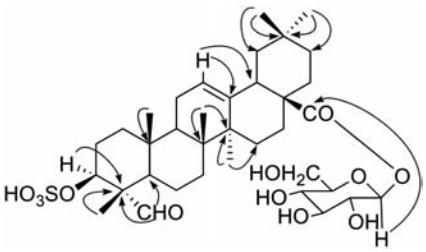

Figure 2. Key HMBC correlations for compound **1**.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a JASCO P-1020 polarimeter. IR (KBr disks) spectra were recorded by Bruker Tensor 27 spectrometer. 1D and 2D NMR spectra were recorded at ACF-500 NMR instrument (^{1}H: 500 MHz, ^{13}C: 125 MHz), with TMS as an internal standard. Mass spectra were obtained on an MS Agilent 1100 Series LC/MSD Trap mass spectrometer (ESI-MS) and a Micro Q-TOF MS (HR-ESI-MS), respectively. TLC was performed on a precoated silica gel G (Qingdao Haiyang Chemical Co. Ltd, Qingdao, China), and detection was achieved by 15% H_2SO_4–EtOH for triterpenoids. Silica gel H (Qingdao Haiyang Chemical Co. Ltd) and Sephadex LH-20 (Pharmacia, Amersham Biosciences, Uppsala, Sweden) were used for column chromatography.

3.2 Plant material

The roots of *G. pacifica* were collected from Xifeng region, Liaoning Province, China, in October 2005. The botanical origin of material was identified by Prof. Minjian Qin, Research Department of Traditional Chinese Medicinal Resources, China Pharmaceutical University, and the voucher specimens (No. 051020) have been deposited at the Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing, China.

3.3 Extraction and isolation

The roots of *G. pacifica* (8.9 kg) were ground into powders and then extracted with 70% aqueous EtOH (v/v) three times (10 l, 2 h each) under reflux. After evaporation, the residue was suspended in water and partitioned by EtOAc, *n*-BuOH, and water. The EtOAc portion (60 g) was fractionated by silica gel column (100–200 mesh), which was eluted with $CHCl_3$/MeOH with gradually increasing polarity to give five fractions (fractions 1–5); fraction 3 (500 mg, $CHCl_3$/MeOH, 10:1, v/v) was further subjected to repeated silica gel column with $CHCl_3$/MeOH gradually to yield pure **2** (105 mg), **3** (7 mg), and **4** (4 mg), respectively. The *n*-BuOH-soluble portion (268 g) was fractionated by MCI gel, which was eluted with MeOH/H_2O (0:100, 30:70, 50:50, 70:30, and 100:0, v/v) to give five fractions (fractions 1–5); fraction 5 (180 mg, MeOH/H_2O, 100:0, v/v) was further purified by repeated silica gel column ($CHCl_3$/MeOH, 3:1, v/v) to afford pure **1** (28 mg).

3.3.1 3β-O-Sulfate gypsogenin 28-O-β-D-glucopyranosyl ester (*1*)

A white amorphous powder (MeOH); $[\alpha]_D^{25}$ + 5.95 (*c* 0.01; MeOH); IR (KBr) ν_{max}: 3418, 2948, 1735, 1639, 1229, 1073 cm^{-1}. ^{1}H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N) spectral data, see Table 1. ESI-MS *m/z*: 711 $[M - H]^-$, 730 $[M + NH_4]^+$, 735 $[M + Na]^+$; HR-ESI-MS *m/z*: 711.3433 $[M - H]^-$ (calcd for $C_{36}H_{55}O_{12}S$, 711.3420).

3.3.2 3β-O-Sulfate gypsogenin (*2*)

A white amorphous powder (MeOH); $[\alpha]_D^{25}$ + 42.03 (*c* 0.08; MeOH); IR (KBr) ν_{max}: 3442, 2948, 1690, 1638, 1244, 1078 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) spectral data, see Table 1. ESI-MS *m/z*: 549

$[M - H]^-$, 568 $[M + NH_4]^+$, 573 $[M + Na]^+$; HR-ESI-MS *m/z*: 549.2905 $[M - H]^-$ (calcd for $C_{30}H_{45}O_7S$, 549.2891).

3.3.3 3β-O-Sulfate quillaic acid (*3*)

A white amorphous powder (MeOH); $[\alpha]_D^{25} + 10.81$ (*c* 0.09; MeOH); IR (KBr) ν_{max}: 3435, 2945, 1704, 1637, 1231, 1075 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) spectral data, see Table 1. ESI-MS *m/z*: 565 $[M - H]^-$, 584 $[M + NH_4]^+$, 591 $[M + Na]^+$; HR-ESI-MS *m/z*: 565.2819 $[M - H]^-$ (calcd for $C_{30}H_{45}O_8S$, 565.2841).

3.3.4 Acid hydrolysis of *1*

Compound **1** (5 mg) dissolved in 0.5 ml of 2 N HCl was refluxed for 1 h. The reaction mixture was neutralized and extracted with EtOAc to obtain aglycone. The aglycone was identified as gypsogenin by TLC ($CHCl_3$–CH_3OH, 8:2, v/v) in comparison with authentic sample. An aliquot of aqueous layer was treated with 70% $BaCl_2$ to give a white precipitate ($BaSO_4$), which revealed the presence of sulfate groups in **1**. The remainder of the aqueous layer was concentrated under reduced pressure. The residue was examined by TLC with *n*-BuOH–HOAc–H_2O (4:1:5, v/v/v, upper layer) as the development and compared with the authentic D-glucose.

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