# 3-*O*-Sulfo-triterpenoid Saponins from *Gypsophila trichotoma* Wend

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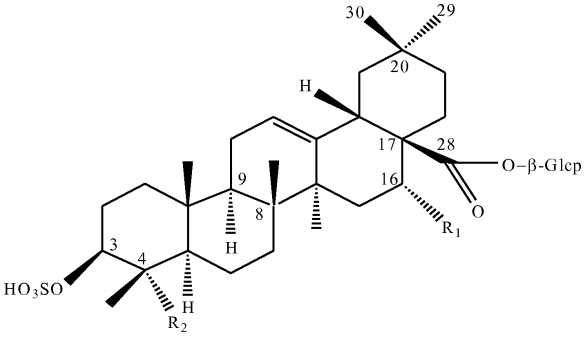
Three new sulfated oleanane-type saponins were isolated from the roots of *Gypsophila trichotoma* Wend. (Caryophyllaceae). Their structures were established as 3-*O*-sulfoechinocystic acid 28-$\beta$-glucopyranosyl ester (**1**), 3-*O*-sulfooleanolic acid 28-$\beta$-glucopyranosyl ester (**2**) and 3-*O*-sulfoquillaic acid 28-$\beta$-glucopyranosyl ester (**3**) on the basis of chemical and spectral evidences.

*Key words:* Caryophyllaceae, *Gypsophila trichotoma*, Oleanane-type Saponins

## Introduction

*Gypsophila trichotoma* Wend. (Caryophyllaceae) is a perennial herbaceous plant, growing in Southeast Europe, Southwest Asia, Kazakhstan, West Mongolia, Russia and Turkmenistan. The species is spread in Bulgaria along the Black Sea coast [1]. Previous researchers reported the isolation of six triterpene glucosides with gypsogenin as an aglycon from the plant [2 – 6]. In a continuation of our phytochemical investigation on *Gypsophila trichotoma* [7], we describe the isolation and structure elucidation of three new sulfated oleanane-type saponins (**1** – **3**).




**1**: $R_1$ = OH, $R_2$ = $CH_3$
**2**: $R_1$ = H, $R_2$ = $CH_3$
**3**: $R_1$ = OH, $R_2$ = CHO

## Results and Discussion

Purification of the 1-BuOH-soluble extract of the roots of *G. trichotoma* by repeated column chromatography over Sephadex LH-20, silica gel and RP-18 material, followed by preparative TLC, yielded three saponins **1**, **2** and **3**.

HRMS ((–)-ESI) of compound **1** showed a quasi-molecular ion peak $[M–H]^-$ at *m/z* = 713.3575 (calcd. 713.3571) corresponding to the formula $C_{36}H_{57}O_{12}S$ and a fragment ion at *m/z* = 551.2947 $[(M–H)–162]^-$ indicating the loss of a hexosyl unit. EIMS of **1** showed significant fragments at *m/z* = 408 $[(M–H)–162–H_2SO_3–H_2O–CO_2]^+$ and *m/z* = 392 $[(M–H)–162–H_2SO_4–H_2O–CO_2]^+$. These peaks were recorded in the HRMS (EI) at 408.3389 (calcd. 408.339 for $C_{29}H_{44}O$) and 392.4401 (calcd. 392.3443 for $C_{29}H_{44}$).

The $^1$H NMR spectrum of **1** showed signals for seven tertiary methyl groups at $\delta_H$ = 0.79, 0.83, 0.88, 0.95, 0.97, 1.04, and 1.37, an olefinic proton resonance at $\delta$ = 5.31 (t, *J* = 3.2 Hz), a broad one-proton double doublet at $\delta$ = 2.99 (br dd, *J* = 14.3, 4.0 Hz) and two oxygenated methine signals at $\delta$ = 3.94 (dd, *J* = 11.8, 4.4 Hz) and 4.53 (brs).

$^1$H and $^{13}$C NMR data clearly indicated the presence of one glucose moiety. The signal for the anomeric proton at $\delta$ = 5.34 (d, *J* = 8.0 Hz, H-1′) revealed the $\beta$-configuration of the glucose unit. Further evidence was obtained by hydrolysis and co-TLC with an authentic sample. In the HMBC spectrum a correlation was observed between the anomeric proton and an ester carbon at $\delta$ = 177.27 due to C-28 of the aglycone moiety. The presence of an additional secondary hydroxyl group was indicated by a carbon signal at $\delta$ =

74.91 and a proton signal at $\delta$ = 4.53 (1H, brs). Allocation of this proton was clearly established by HMBC experiments, which showed correlations of H-16 ($\delta$ = 4.53) with three carbon signals at $\delta$ = 50.04 (C-17), 42.66 (C-14), and 42.13 (C-18). These results confirm the position of the secondary hydroxyl group at C-16. The small *J* value of H-16 (triplet-like brs) characteristic of an equatorial proton indicated the axial orientation of the 16-hydroxy group [8, 9].

The $^{13}$C NMR and DEPT spectra revealed an olefinic methine carbon resonance at $\delta$ = 123.58 (C-12), whereas seven quaternary carbon resonances at 144.66, 50.04, 42.66, 40.83, 39.53, 38.05, and 31.27 ppm could be assigned to C-13, C-17, C-14, C-8, C-4, C-10, and C-20, respectively (Table 1).

The *O*-sulfo unit had to be attached to the carbon atom at $\delta$c = 87.66 due to the characteristic downfield shift [10]. The location of this carbon was established through long-range HMBC correlations with $H_3$-24 ($\delta$ = 0.83) and $H_3$-23 ($\delta$ = 1.04), and couplings between C-5 ($\delta$c = 57.33) and $H_3$-25 ($\delta$ = 0.97) as well as $H_3$-23 and $H_3$-24, revealing it to be C-3.

Other significant HMBC correlations were observed for two tertiary methyl signals, $\delta_H$ = 1.37 ($H_3$-27) and 0.79 ($H_3$-26), and for one proton signal at $\delta$ = 2.99 (H-18) with the quaternary C-14 ($\delta$c = 42.66). In addition, cross-peaks were observed between the methine proton H-18 ($\delta$ = 2.99) and four carbon signals at $\delta$c = 177.27 (C-28), 144.66 (C-13), 123.58 (C-12), and 24.50 (C-11).

The data presented here demonstrate unambiguously that **1** is an olean-12-ene triterpenoid aglycone. On the basis of these results, **1** was identified as 3-*O*-sulfoechinocystic acid 28-$\beta$-glucopyranosyl ester. This compound was already obtained as a hydrolysis product of a saponin mixture from *Bupleurum rotundifolium* [11]. Nevertheless, it has not been reported as a natural product up to now, and its NMR data were not yet completely published.

The HRMS ((–)-ESI) of **2** exhibited a quasimolecular ion peak at *m/z* = 697.3582 (calcd. 697.3613), consistent with the molecular formula $C_{36}H_{57}O_{11}S$ and a fragment peak at *m/z* = 535.3088 $[(M–H)–162]^-$ generated by the loss of a glucosyl moiety. The molecular weight of compound **2** is thus 16 mass units less than that of **1**.

Its $^{13}$C NMR spectrum was similar to that of **1** but featured an additional aliphatic methylene unit ($\delta$ = 23.74) and one oxymethine carbon atom less ($\delta$ = 74.91 for **1**).

Table 1. $^{13}$C NMR spectral data for compounds **1** – **3** (150.9 MHz, $CD_3OD$).

| Position | **1** | **2** | **3** | Position | **1** | **2** | **3** |
|---|---|---|---|---|---|---|---|
| 1 | 39.75 | 39.67 | 39.28 | 2 | 25.27 | 25.23 | 24.40 |
| 3 | 87.66 | 87.60 | 80.13 | 4 | 39.53 | 39.51 | 55.91 |
| 5 | 57.33 | 57.21 | 48.57 | 6 | 19.49 | 19.47 | 21.38 |
| 7 | 34.19 | 33.91 | 33.44 | 8 | 40.83 | 40.71 | 41.12 |
| 9 | 48.13 | 48.42 | 48.03 | 10 | 38.05 | 38.04 | 36.90 |
| 11 | 24.50 | 24.56 | 24.44 | 12 | 123.58 | 123.71 | 123.31 |
| 13 | 144.66 | 144.87 | 144.81 | 14 | 42.66 | 42.93 | 42.74 |
| 15 | 36.28 | 28.90 | 36.22 | 16 | 74.91 | 23.74 | 74.87 |
| 17 | 50.04 | 48.03 | 49.96 | 18 | 42.13 | 42.60 | 42.10 |
| 19 | 47.76 | 47.19 | 47.75 | 20 | 31.27 | 31.54 | 31.29 |
| 21 | 36.45 | 34.90 | 36.45 | 22 | 32.52 | 33.15 | 31.82 |
| 23 | 28.81 | 28.80 | 206.87 | 24 | 16.96 | 16.94 | 9.85 |
| 25 | 16.12 | 15.99 | 16.26 | 26 | 17.78 | 17.72 | 17.74 |
| 27 | 27.26 | 26.28 | 27.27 | 28 | 177.27 | 178.08 | 177.25 |
| 29 | 33.34 | 33.47 | 33.34 | 30 | 25.02 | 23.95 | 24.95 |
| 28-$\beta$-glcp | | | | | | | |
| 1′ | 95.75 | 95.72 | 95.71 | 2′ | 74.01 | 73.93 | 74.00 |
| 3′ | 78.31 | 78.32 | 78.32 | 4′ | 71.12 | 71.11 | 71.08 |
| 5′ | 78.74 | 78.71 | 78.75 | 6′ | 62.43 | 62.41 | 62.39 |

The structure of **2** was deduced from DEPT analysis and HETCOR, COSY and HMBC experiments. Thus, we conclude that **2** is 3-*O*-sulfooleanolic acid 28-$\beta$-glucopyranosyl ester, which also has been described as a hydrolysis product by Akai *et al*. [11].

The HRMS ((–)-ESI) of **3** displayed a quasimolecular ion peak $[M–H]^-$ at *m/z* = 727.3366 (calcd. 727.3360), suggesting a molecular formula of $C_{36}H_{55}O_{13}S$. Another significant fragment ion was observed at *m/z* = 503.2729, arising from the loss of a glucosyl residue, followed by successive loss of one molecule of $H_2O$ and of $CO_2$ from the ion $[M–H]^-$. The $^1$H and $^{13}$C NMR spectra again were quite similar to those of **1**, but the difference of 14 mass units between **1** and **3** and the appearance of a one-proton singlet at $\delta$ = 9.31 and a carbonyl carbon at $\delta$ = 206.87 in the $^1$H and $^{13}$C NMR spectra of **3**, respectively, suggested the presence of an aldehyde group.

The $^{13}$C NMR signals of the aglycone of compound **3** show downfield shifts for C-4 ($\delta$ = 55.91) and C-6 ($\delta$ = 21.38) compared to $\delta$ = 39.53 and $\delta$ = 19.49 in **1** and $\delta$ = 39.51 and $\delta$ = 19.47 in **2**, respectively (Table 1). Cross peaks in the HMBC spectra between the methyl singlet ($\delta$ = 1.04, $H_3$-24) and the oxygenated methine carbon resonance at $\delta$ = 80.13 (C-3) and the carbonyl carbon resonance at $\delta$ = 206.87 (C-23) confirm the location of the aldehyde group at C-4, thus hinting to quillaic acid as the aglycone moiety. The considerable upfield shift of the methyl carbon (C-24, $\delta_C$ = 9.85) is consistent with this assumption [12].

Furthermore, the NOESY spectrum showed correlations between $H_3$-25 and the axial methyl group $H_3$-24, thus proving the equatorial orientation of the aldehyde group at C-4.

Compound **3** was, therefore, deduced as 3-*O*-sulfoquillaic acid 28-$\beta$-glucopyranosyl ester, which represents a new natural product.

## Experimental Section

*General*

Optical rotations were measured with a Perkin-Elmer 343 polarimeter. $^1$H NMR (600 MHz) and $^{13}$C NMR (150.9 MHz) spectra were recorded on a Bruker DRX-600 spectrometer in $CD_3OD$. HRMS (ESI) was carried out on an ESI-MSD spectrometer (Fa. Agilent Technologies). MS (EI) and HRMS (EI) spectra were acquired with Varian MATCH7 and Finnigan MAT711 spectrometers, respectively.

TLC was carried out on silica gel 60 $F_{254}$ (0.24 mm thick, Merck) plates, using the solvent systems 1-BuOH-AcOH-$H_2O$ (4 : 1 : 1) and $CHCl_3$-MeOH (9 : 1). The spots were visualized by spraying with anisaldehyde/conc. $H_2SO_4$ (for saponins and sapogenins) or thymol EtOH/conc. $H_2SO_4$solution (for sugars), followed by heating at 110 °C. Sephadex LH-20, silica gel 60 (70 – 230 mesh, Merck) and RP-18 silica gel were used for CC. Prep. TLC was performed on silica gel plates (silica gel 60, 0.5 mm thick, Merck).

*Plant material*

The roots of the plant were collected in June 2005 at the Black Sea coast, locality "Zelenka", near the village of Balgarevo, Bulgaria. The voucher specimen (SO 103887) was deposited in the Herbarium of Sofia University, Bulgaria.

*Extraction and isolation*

The dried roots (800 g) were exhaustively extracted with 80 % MeOH, and the extract was evaporated under reduced pressure to afford the aqueous residue which was partitioned between $CH_2Cl_2$, 1-BuOH and water. The 1-BuOH extract was evaporated to dryness and chromatographed on a Sephadex LH-20 column, eluting with MeOH to give two main fractions, rich of saponins (Fr. I and II). Fr. I was further purified by flash chromatography over RP-18 silica (eluted with AcCN-$H_2O$, 9 : 1) and prep. TLC ($CHCl_3$-MeOH, 9 : 1) to give **2** (15 mg). Fr. II was separated by flash chromatography over silica gel, eluting with $CHCl_3$-MeOH-$H_2O$ (18 : 11 : 2), and finally purified by prep. TLC (BuOH-AcOH-$H_2O$, 4 : 1 : 5) to afford **1** (20 mg) and **3** (10 mg).

*3-O-Sulfoechinocystic acid 28-β-glucopyranosyl ester (**1**)*

White amorphous powder. – $[\alpha]_D^{20}$ = + 6° (*c* = 0.98, MeOH). – $^1$H NMR (600 MHz, $CD_3OD$): $\delta$ = 0.79 (3 H, s, $H_3$-26), 0.83 (3 H, s, $H_3$-24), 0.88 (3 H, s, $H_3$-29), 0.95 (3 H, s, $H_3$-30), 0.97 (3 H, s, $H_3$-25), 1.04 (3 H, s, $H_3$-23), 1.06 (1 H, m, H-19a), 1.37 (3 H, s, $H_3$-27), 1.73 (1 H, m, H-2a), 1.90 (1 H, m, H-11a), 2.10 (1 H, m, H-2b), 2.29 (1 H, t-like, *J* = 13.6 Hz, H-19b), 2.99 (1 H, br dd, *J* = 14.3, 4.0 Hz, H-18$\beta$), 3.27 – 3.34 (4 H, H-2′, H-3′, H-4′, H-5′), 3.67 (1 H, dd, *J* = 11.9, 4.9 Hz, H-6′a), 3.81 (1 H, br d, *J* = 11.9 Hz, H-6′b), 3.94 (1 H, dd, *J* = 11.8, 4.4 Hz, H-3$\alpha$), 4.53 (1 H, br s, H-16$\beta$), 5.31 (1 H, t, *J* = 3.2 Hz, H-12), 5.34 (1 H, d, *J* = 8.0 Hz, H-1′). For $^{13}$C NMR data see Table 1. – FABMS (negative): *m/z* = 713 $[M–H]^-$, 551 $[(M–H)–162]^-$. – HRMS ((–)-ESI): *m/z* = 713.3575 (calcd. 713.3571 for $C_{36}H_{57}O_{12}S$, $[M–H]^-$). – MS (EI): *m/z* = 408 $[(M–H)–162–H_2SO_3–H_2O–CO_2]^+$, 392 $[(M–H)–162–H_2SO_4–H_2O–CO_2]^+$. – HRMS (EI): *m/z* = 408.3389 (calcd. 408.3392 for $C_{29}H_{44}O$), 392.4401 (calcd. 392.3443 for $C_{29}H_{44}$).

*3-O-Sulfooleanolic acid 28-β-glucopyranosyl ester (**2**)*

White amorphous powder. – $[\alpha]_D^{20}$ = + 25° (*c* = 0.26, MeOH). – $^1$H NMR (600 MHz, $CD_3OD$): $\delta$ = 0.80 (3 H, s, $H_3$-26), 0.83 (3 H, s, $H_3$-24), 0.91 (3 H, s, $H_3$-29), 0.93 (3 H, s, $H_3$-30), 0.97 (3 H, s, $H_3$-25), 1.03 (3 H, s, $H_3$-23), 1.14 (1 H, m, H-19a), 1.16 (3 H, s, $H_3$-27), 1.71 (1 H, m, H-2a), 1.73 (1 H, m, H-19b), 1.90 (1 H, m, H-11a), 2.07 (1 H, m, H-2b), 2.85 (1 H, br dd, *J* = 14.0, 4.0 Hz, H-18$\beta$), 3.30 – 3.35 (4 H, H-2′, H-3′, H-4′, H-5′), 3.67 (1 H, dd, *J* = 12.0, 5.0 Hz, H-6′a), 3.81 (1 H, br d, *J* = 12.0 Hz, H-6′b), 3.92 (1 H, dd, *J* = 11.8, 4.4 Hz, H-3$\alpha$), 5.25 (1 H, t, *J* = 3.0 Hz, H-12), 5.38 (1 H, d, *J* = 8.0 Hz, H-1′). For $^{13}$C NMR data see Table 1. – HRMS ((–)-ESI): *m/z* = 697.3582 (calcd. 697.3613 for $C_{36}H_{57}O_{11}S$, $[M–H]^-$), 535.3088, $[(M–H)–162]^-$.

*3-O-Sulfoquillaic acid 28-β-glucopyranosyl ester (**3**)*

White amorphous powder. – $^1$H NMR (600 MHz, $CD_3OD$): $\delta$ = 0.79 (3 H, s, $H_3$-26), 0.88 (3 H, s, $H_3$-29), 0.95 (3 H, s, $H_3$-30), 1.02 (3 H, s, $H_3$-25), 1.04 (3 H, s, $H_3$-24), 1.05 (1 H, m, H-19a), 1.40 (3 H, s, $H_3$-27), 1.90 (1 H, m, H-11a), 2.29 (1 H, t-like, *J* = 13.5 Hz, H-19b), 2.99 (1 H, br dd, *J* = 14.2, 4.0 Hz, H-18$\beta$), 3.28 – 3.33 (4 H, H-2′, H-3′, H-4′, H-5′), 3.67 (1 H, dd, *J* = 11.9, 4.8 Hz, H-6′a), 3.81 (1 H, br d, *J* = 11.9 Hz, H-6′b), 4.51 (1 H, dd, *J* = 11.6, 4.3 Hz, H-3$\alpha$), 4.53 (1 H, br s, H-16$\beta$), 5.33 (1 H, t, *J* = 3.5 Hz, H-12), 5.34 (1 H, d, *J* = 8.0 Hz, H-1′), 9.31 (1 H, s, H-23). For $^{13}$C NMR data see Table 1. – HRMS ((–)-ESI): *m/z* = 727.3366 (calcd. 727.3360 for $C_{36}H_{55}O_{13}S$, $[M–H]^-$), 503.2729 $[(M–H)–162]^-$.

*Acid hydrolysis*

Compounds **1** – **3** (2 mg each) were refluxed with 7 % methanolic HCl (5 mL) for 3 h. The MeOH was

evaporated, the mixture was diluted with $H_2O$, and the hydrolysate was partitioned between EtOAc and $H_2O$. The aqueous layer was neutralized with $Ag_2CO_3$, filtered, evaporated and tested for carbohydrates by co-TLC with authentic samples using EtOAc-MeOH-HOAc-$H_2O$ (12 : 3 : 3 : 2). Only one spot corresponding to D-glucose was obtained.

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