# A New Gypsogenin Saponin from *Arenaria filicaulis*

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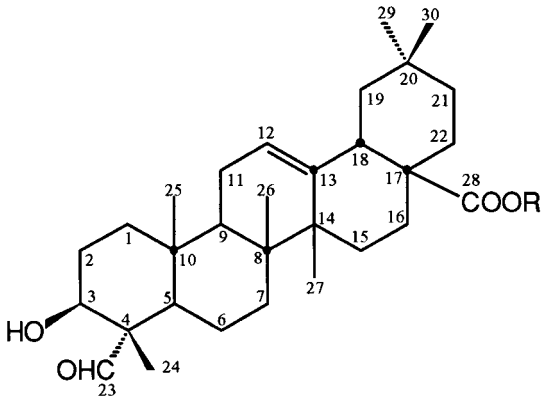
The structure elucidation of a new saponin from *Arenaria filicaulis* (Boiss.), Caryophyllaceae, Snatzkein F (**1**), containing gypsogenin with a tetrasaccharide moiety was accomplished by use of 1D and 2D NMR methods.

In our previous investigations of *Arenaria filicaulis* five novel triterpene saponins were isolated.[1−3] The rhizomes of this plant had a considerable application as a sweet diet (Halawa Tahinia) and in arabic folk medicine, they are reported to have antirheumatic, laxative, and urinary tract disinfectant activity.[4,5] In addition the plant contains some minor constituents; this work reports the isolation and identification of constituents of one of these (Snatzkein F, 3$\beta$-hydroxyolean-12-en-23-oxo-28-oic acid 28-*O*-[$\beta$-D-xylopyranosyl(1→4)-$\alpha$-L-rhamnopyranosyl(1→2)]-[$\beta$-L-arabinofuranosyl(1→3)$\beta$-D-4-*O*-acetylfucopyranoside).




Snatzkein F (**1**)

The structure of Snatzkein F was determined with the combination of several two-dimensional NMR methods. The strategy applied for signal assignment has been described by us before for the related Snatzkeins A−E.[1−3] Our unambiguously assigned $^1$H and $^{13}$C NMR data are summarized in Table 1 along with characteristic NOE contacts (ROESY) and long-range $^{13}$C,$^1$H coupling responses (HMBC). A comparison of these chemical shifts with our previous data[6] and also with a recent review on $^{13}$C NMR of triterpenoids[7] revealed that the aglycone is gypsogenin.

The $^{13}$C, DEPT, and HMQC experiments proved that the aglycone consist of six $CH_3$, ten $CH_2$, and six CH fragments. Four of the CH groups resonate in the $sp^3$ region and one of them ($\delta_{CH}$ 71.6) is attached to an OH substituent. The remaining two CH signals appear in the $sp^2$ range and refer to =CH (122.5) and HC=O (207.3) groups. Six of the eight quaternary carbon signals were observed in the $sp^3$ region, whereas the signals at 144.0 and 176.5 medicated the presence of a trisubstituted C=C double bond and an ester function, respectively.

The six methyl singlets provided a clear entry to the analysis of the HMBC spectrum (cf. Table 1) because on the basis of the cross-peaks one can read out the carbon atoms of the gypsogenin skeleton over two and three bonds. These structural subunits can be interconnected to rings by $^1$H,$^1$H COSY and HMQC−TOCSY measurements. The high performance of the latter experiment, allowing the combination of $^1$H,$^1$H and $^{13}$C,$^1$H connectivity information, was utilized. In the rows of the well-separated $^{13}$C signals the corresponding $^1$H spin network, e.g. the connectivities for example from C-1 to H-2 and H-3, from C-7 to H-6 and H-5, from C-11 to H-9 and H-12 protons, etc were observed. The H-24/H-25, H-25/H-26 and H-27/H-9 steric proximities observed in the ROESY spectrum proved the trans-type junctions of the A/B/C/D rings of the aglycone, whereas the cross-peak H-27/H-19$\alpha$ verified the cis D/E linking.

The tetrasaccharide moiety was elucidated using the same NMR methods mentioned above; the identification of the four sugar subunits was achieved utilizing the characteristic $^{13}$C chemical shifts of the individual saccharide moieties in comparison with literature data, $^1$H,$^1$H vicinal coupling constants, and NOE contacts. The tetrasaccharide is connected to the carboxylic group 28 as shown by a HMBC peak correlating C-28 and the anomeric hydrogen of sugar A (Table 2). The monosaccharide A is 4-*O*-acetylfucopyranose with an axial anomeric hydrogen (H-A1). This configuration results from the $^3J_{H-A1,H-A2}$ = 8.5 Hz coupling, and is also confirmed by a one-bond $^{13}$C,$^1$H coupling constant of 166 Hz as determined from the $^{13}$C-coupled HMQC spectrum.[8,9] A disaccharide unit attached to the C-2 atom of the 4-*O*-acetylfucose (see H-A2/C-B1 and H-B1/C-A2 HMBC cross-peaks in Table 2) consists of rhamnopyranose (sugar B; the equatorial anomeric hydrogen proven by $^1J_{CH}$ = 172 Hz), which carries a xylopyranose at its C-4. This connectivity is straightforward from the H-B4/C-D1 and H-D1/C-B4 HMBC contacts (cf. Table 2). The values of the $^3J_{H-D1,H-D2}$ = 8.5 Hz, and $^1J_{CH}$ = 159 Hz couplings reveal that the xylose has an axial anomeric hydrogen. In addition, the 4-*O*-acetylfucose bears an ara-

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**Table 1.** $^1$H and $^{13}$C Chemical Shifts, Characteristic $J_{H,H}$ Couplings in Parentheses [Hz], HMBC, and ROESY Cross-Peaks of the Aglycone

| | | $^1$H ($^3J(^1$H,$^1$H) [Hz]) | $^{13}$C | HMBC ($^{13}$C partners) | ROESY |
|---|---|---|---|---|---|
| 1 | α | 1.03 | 38.7 | – | 2α, 3α, 5α, 9α |
| | *β* | 1.57 | | – | 2α, 2*β*, 11α, 11*β*, 24, 25 |
| 2 | α | 1.86 | 27.1 | 3 | 1α, 1*β*, 3α |
| | *β* | 1.86 | | 3 | 1*β*, 24, 25 |
| 3 | α | 4.03 | 71.6 | - | 1α, 2α, 5α |
| 4 | | – | 56.3 | – | – |
| 5 | α | 1.45 | 48.0 | 4, 6, 10, 23, 24, 25 | 1α, 3α, 9α |
| 6 | α | 1.51 | 21.3 | – | – |
| | *β* | 1.17 | – | - | 5α, 6α, 7α |
| 7 | α | 1.63 | 32.7 | – | 6*β*, 27 |
| | *β* | 1.56 | | – | – |
| 8 | | – | 40.3 | – | – |
| 9 | α | 1.74 | 48.0 | 8, 10, 11, 25, 26 | 1α, 5α, 27 |
| 10 | | – | 36.2 | – | – |
| 11 | α | 1.92 | 23.8 | – | 1*β*, 12 |
| | *β* | 1.92 | | – | 1*β*, 12, 25,26 |
| 12 | | 5.43 | 122.5 | 9, 14 | 11α, 11*β*, 18*β*, 19α, 26 |
| 13 | | – | 144.0 | – | – |
| 14 | | – | 42.4 | – | – |
| 15 | α | 1.43 | 28.4 | – | 16α, 16*β* |
| | *β* | 1.98 | | 16 | 26, B5 |
| 16 | α | 2.09 (13.5,13.5,3.1) | 23.5 | 15, 17, 28 | 15α, 19α, 21α, 22α, 27 |
| | *β* | 1.88 | | – | 15α, 22*β*, B5 |
| 17 | | – | 47.2 | – | – |
| 18 | *β* | 3.09 (13.5, 4.0) | 42.1 | 12, 13, 14, 17, 28 | 12, 19*β*, 22*β*, 30 |
| 19 | α | 1.20 | 46.4 | 18, 20 | 27, 29 |
| | *β* | 1.73 | | 17, 20 | 12, 18*β*, 29, 30 |
| 20 | | – | 30.7 | – | – |
| 21 | α | 1.28 (14.3,13.8,4.2) | 34.0 | – | 16α, 22α, 29 |
| | *β* | 1.11 | | – | 22α, 22*β*, 29, 30 |
| 22 | α | 1.71 | 32.3 | 20 | 21α, 22*β* |
| | *β* | 1.97 | | – | 18*β*, 21*β*, 22α, 30 |
| 23 | α | 9.55 | 207.3 | 4, 24 | |
| 24 | *β* | 1.31 | 9.8 | 3, 4, 5, 23 | 1*β*, 2*β*, 6*β*, 25 |
| 25 | *β* | 0.90 | 15.9 | 1, 5, 9, 10 | 1*β*, 2*β*, 11*β*, 24, 26 |
| 26 | *β* | 1.05 | 17.5 | 7, 8, 9, 14 | 11*β*, 12, 25, A1 |
| 27 | α | 1.23 | 25.9 | 8, 13, 14, 15 | 7α, 7*β*, 9α, 15α, 16α, 19α |
| 28 | | – | 176.5 | – | – |
| 29 | | 0.84 | 23.7 | 19, 20, 21, 30 | 19α, 19*β*, 21α, 21*β* |
| 30 | | 0.86 | 33.1 | 19, 20, 21 30 | 18*β*, 19*β*, 21*β*,, 22*β* |

**Table 2.** $^1$H and $^{13}$C Chemical Shifts, Characteristic $J_{H,H}$ Couplings in Parentheses [Hz], and HMBC Cross-Peaks of the Tetrasaccharide Moiety

| | | $^1$H | $^3J(^1$H,$^1$H) [Hz] | $^{13}$C | HMBC ($^{13}$C partners) |
|---|---|---|---|---|---|
| A1 | | 5.99 | (8.5) | 94.4 | 28 |
| A2 | | 4.53 | (9.2) | 73.6 | A1, A3, B1 |
| A3 | | 4.25 | (3.5) | 80.8 | A2, A4, C1 |
| A4 | | 5.76 | | 73.9 | A2, A3, $H_3C$-**C**O- |
| A5 | | 3.93 | | 70.5 | A1, A4, A6 |
| A6 | | 1.16 | (6.4) | 16.5 | A4, A5 |
| B1 | | 6.00 | (1.5) | 102.1 | A2, B3, B5 |
| B2 | | 4.73 | | 71.4 | B3, B4 |
| B3 | | 4.58 | (9.0) | 72.4 | |
| B4 | | 4.30 | (9.5) | 84.7 | B3, B5, B6, D1 |
| B5 | | 4.39 | | 68.7 | B4 |
| B6 | | 1.76 | (6.1) | 18.6 | B4, B5 |
| C1 | | 5.71 | (1.6) | 111.8 | A3, C4 |
| C2 | | 4.88 | | 83.6 | |
| C3 | | 4.81 | | 78.1 | |
| C4 | | 4.70 | (7.3, 3.5, 3.5) | 85.9 | C3 |
| C5 | a | 4.16 | | 62.0 | |
| | b | 4.30 | | | |
| D1 | | 5.01 | (7.3) | 107.5 | B4 |
| D2 | | 4.02 | | 76.2 | D1, D4 |
| D3 | | 4.02 | | 78.7 | D1, D2, D4 |
| D4 | | 4.15 | | 70.9 | |
| D5 | a | 3.49 | (11.1, 10.6) | 67.5 | D1, D3, D4 |
| | b | 4.23 | (11.1, 5.4) | | D1, D3, D4 |
| **H$_3$C**-CO- | | 1.97 | | 20.8 | $H_3C$-**C**O- |
| $H_3C$-**C**O- | | | | 170.8 | |

binofuranose moiety (C), which is obvious from the H-A3/C-C1 and H-C1/C-A3 contacts observed in the HMBC spectrum. In the sugar moiety C a vicinal $^3J_{H-C1,H-C2}$ coupling constant of 1.6 Hz was observed leading to the conclusion that the protons H-C1 and H-C2 are trans-configured; in the cis-configuration there is no possibility to reach a torsion angle H-1−C-1−C-2−H-2 close to 90°. The identification of the C-1 configuration of the arabinofuranose moiety (C) was corroborated by comparison with the $^{13}$C chemical shifts of both diastereomers taken from the literature.[10,11]

As reported by us previously[3] the sugar moiety attached to the carboxylic group 28 (A) adopts a conformation in which its anomeric proton is close in space to H-26 of the aglycone as proven by NOE contacts (Table 1, cf. Figure 1, bottom). A large number of further intra- and interglycosidic NOE effects were identified and are compiled in Table 3 and are indicated by double arrows in Figure 1. They present rich evidence of restricted mobility and conformational preferences within the tetrasaccharide. If we assume that all monosaccharides possess the same absolute configurations as communicated before in several related instances for other plant species,[12−14] we can establish two favored conformations (depicted in Figure 1) displaying different orientations of the rhamnose with respect to acetylfucose and arabinose (top). The left one is the conformer, where the C-A2−O−C-B1−O(B) dihedral angle

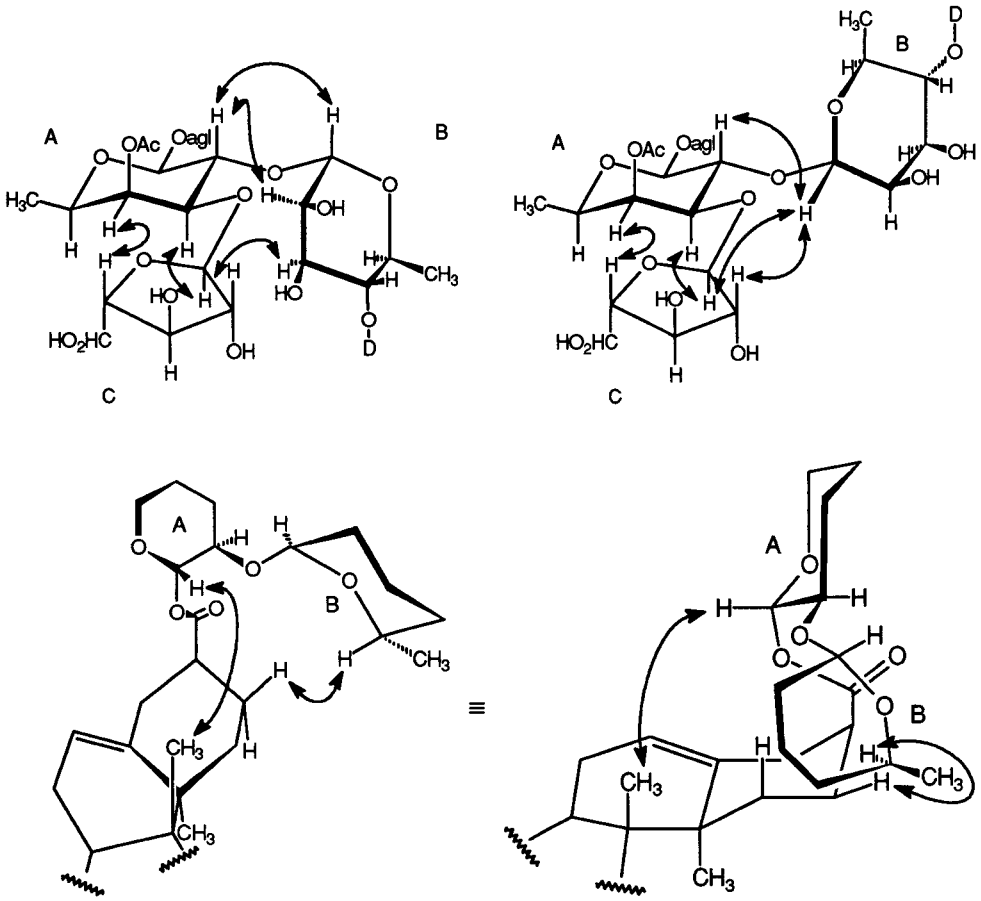

**Figure 1.** Favored conformations of the tetrasaccharide part of Snatzkein F (**1**); double arrows indicate NOE contacts (ROESY); in the lower graphical representations (same conformation but viewed from different sides) some hydroxy groups were omitted for sake of clarity.

**Table 3.** Intra- and Interglycosidic ROESY Correlations of the Tetrasaccharide Moiety

Intraglycosidic ROESY Correlations

| A | B | C | D |
|---|---|---|---|
| A1/A3, A1/A5, A3/A4, A3/A5, A4/A5, A4/A6, A5/A6, A6/C**H**$_3$CO | B1/B2, B2/B3, B3/B5, B4/B6, B5/B6 | C1/C2, C1/C3, C3/C5a, C3/C5b, C4/C5a, C4/C5b | D1/D3, D1/D4, D1/D5a, D5a/D5b |

Interglycosidic ROESY Correlations

| A/B | A/C | B/C | B/D |
|---|---|---|---|
| A2/B1, A2/B2 | A2/C1, A3/C1, A4/C4 | B1/C1, B1/C2, B2/C1, B3/C1, | B3/D1, B4/D1, B5/D1, B6/D1 |

is about 180°, the H-B3 and H-C1 protons are only in this arrangement in steric proximity. In the second conformation, the C-A2 and the oxygen atom of the rhamnose are gauche. This conformation results in the ROESY correlations H-B1/H-C1 and H-B1/H-C2, respectively. It should be noted that this interpretation supports the absolute configurations of the sugars A, B and C.

## Experimental Section

**General Experimental Procedures.** $^1$H and $^{13}$C NMR spectra (Table 1) were recorded in pyridine-$d_5$ using a Bruker DRX-500 spectrometer. The chemical shifts are given in ppm and referenced to the solvent signal. Structural and NMR signal assignment were accomplished by extensive use of DEPT, and two-dimensional DQF−COSY, gs-HMQC ($^{13}$C-coupled and decoupled), gs-HMBC, HMQC−TOCSY, ROESY experiments. Spectral parameters have been communicated before.[2] The FAB mass spectra were obtained with a Finnigan MAT 84330 mass spectrometer in *m*-nitrobenzylic alcohol as matrix. Elemental analysis was performed on a Carlo Erba model 1106 analyzer.

**Isolation.** *A. filicaulis* was collected from the plains and areas around Damascus in May 1996. The plant was identified by Prof. A. El-Khatib, Damascus University, Damascus, Syria, and a voucher specimen was kept in the herbarium of the university.

Dried powdered rhizomes (6.55 kg) were exhaustively extracted with methanol. The solvent was then totally removed at 50 °C under reduced pressure and the residue was dissolved in 750 mL of distilled water. Fats and oils were removed by extracting the aqueous solution with diethyl ether (3× with 750 mL). The defatted aqueous solution was exhaustively extracted by *n*-butanol saturated with water (5× with 750 mL). *n*-Butanol was then distilled *in vacuo* at a temperature not exceeding 50 °C. The residue (87 g) was fractionated over a silica gel column (Baker) and eluted by a solvent mixture composed of chloroform, methanol, and water with ratios changing from 80:10:1 to 10:10:1, and finally with methanol.

The fraction eluted by the solvent polarity 30:10:1 gave 290 mg of the crude saponin. The compound was purified on a reversed phase column $RP_8$ under medium pressure. Pure 3$\beta$-hydroxyolean-12-en-23-oxo-28-oic acid 28-*O*-[$\beta$-D-xylopyranosyl(1→4)-$\alpha$-L-rhamnopyranosyl(1→2)]-[$\beta$-L-arabinofuranosyl-(1→3)]-$\beta$-D-4-*O*-acetylfucopyranoside (**1**, 18 mg) was eluted by 37% methanol. It has an $R_f$ value = 0.84 using a solvent system composed of chloroform, methanol, and water (18:8:1). Mp = 275−277 °C; $[\alpha]_D$ +2.7° (*c* 0.65, MeOH).

Positive FAB mass spectrum, *m*/*z* (relative intensity) = 1091 ($M^+$ + Na, 11.3), 115 (xylosyl$^+$ − $H_2O$, 100); negative FAB mass spectrum, *m*/*z* (relative intensity) = 1067 ($M^+$ − H, 100). Eleme. anal. C 59.83%, H 8.02%; calcd for $C_{54}H_{84}O_{21}$, C 60.66%, H 7.92%.

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