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# TWO TRITERPENE SAPONINS FROM *ARENARIA FILICAULIS*

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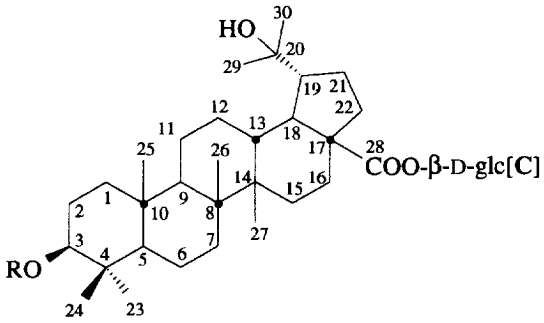
**Key Word Index**—*Arenaria filicaulis*; Caryophyllacae; triterpene; saponin; stereochemistry, NMR; ROESY; TOCSY; HMBC.

**Abstract**—Two novel triterpenoid saponins, Snatzkein C, (3β,20-dihydroxylupan-28-oic acid 3-*O*-[β-D-galactopyranosyl-(1 → 2)-β-D-glucopyranosyl]-28-*O*-β-D-glucopyranoside) and SnatzkeinD, (3β,20-dihydroxylupan-28-oic acid 3-*O*-[β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]-28-*O*-β-D-glucopyranoside), have been isolated from *Arenaria filicaulis*. Their structure and conformational behaviour were elucidated by one- and two-dimensional $^1$H NMR and $^{13}$C NMR spectroscopy. © 1998 Elsevier Science Ltd. All rights reserved

## INTRODUCTION

The pharmacodynamic activities [1, 2] of triterpenoid saponins prompted us to investigate their natural occurrence in the family Caryophyllaceae [3–6]. The rhizomes of *Arenaria filicaulis* Boiss. (syn. *Gypsophila filicaulis* (Boiss.) Borm.) have a considerable use in ethnic medicine, notably in Syria and China, for treatment of bladder illness, diuretic, laxative and as a sweetener. We have previously reported the isolation of two novel saponins, Snatzkein A and B [7]. In this paper we describe the isolation, structure elucidation, the conformational behaviour and the complete $^1$H NMR and $^{13}$C NMR assignments of two more compounds, Snatzkein C (**1**) and D (**2**), with a lupane skeleton (Scheme 1).




**1**: R = β-D-gal[**B**]-(1→2)- β-D-glc[**A**] –

**2**: R = β-D-glc[**B**]-(1→2)- β-D-glc[**A**] –

Scheme 1.

## RESULTS AND DISCUSSION

The structures of **1** and **2** were determined by extensive one- and two-dimensional NMR investigation utilizing the advantage of gradient selection and linear prediction. The strategy employed for signal and structure assignment has been described by us previously for the related saponins Snatzkein A and B [7]. Due to severe signal overlap, even at 500 MHz, we recorded $^1$H NMR spectrum and a ROESY spectrum of **1** at 750 MHz. We achieved a complete signal assignment not only for the carbons but also for the proton signals (Table 1).

The $^1$H and $^{13}$C chemical shifts and $J(^1H, ^1H)$ couplings of **1** and **2** are given in Table 1. Through-bond atom connectivities were obtained from COSY, TOCSY, HMQC, HMBC (Table 2) and HMQC-TOCSY spectra, whereas through-space connectivities were gathered from ROESY experiments (Table 3).

The 3β,20-dihydroxylupan-28-oic acid skeleton has been described recently by Tsichritzis and Jakupovic [8] but from another plant, a Southern African

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Table 1. $^{1}H$ NMR and $^{13}C$ NMR chemical shifts of compounds **1** and **2**

| | $^{1}H$ | **1** | $^{13}C$ | $^{1}H$ | **2** | $^{13}C$ |
|---|---|---|---|---|---|---|
| 1 | α0.72 | | 38.9 | α0.71 | | 39.0 |
| | β1.42 | | | β1.44 | | |
| 2 | α2.17 | | 26.6 | α2.18 | | 26.6 |
| | β1.77 | | | β1.79 | | |
| 3 | α3.24 | (11.7; 4.5) | 88.8 | α3.23 | (9.7; 4.5) | 89.0 |
| 4 | — | | 39.5 | — | | 39.5 |
| 5 | α0.61 | (12.0; 1.6) | 55.7 | α0.61 | (11.8; 1.4) | 55.7 |
| 6 | α1.39 | | 18.3 | α1.39 | | 18.3 |
| | β1.22 | | | β1.23 | | |
| 7 | α1.31 | | 34.9 | α1.32 | | 34.9 |
| | β1.31 | | | β1.32 | | |
| 8 | — | | 41.6 | — | | 41.6 |
| 9 | α1.24 | | 50.8 | α1.23 | | 50.9 |
| 10 | — | | 36.8 | — | | 36.9 |
| 11 | α1.42 | | 21.7 | α1.42 | | 21.8 |
| | β1.24 | | | β1.25 | | |
| 12 | α1.61 | | 29.5 | α1.61 | | 29.6 |
| | β2.11 | | | β2.12 | | |
| 13 | β2.77 | (13.0; 11.2; 3.4) | 38.5 | β2.76 | (12.0; 11.1; 3.4) | 38.6 |
| 14 | — | | 43.5 | — | | 43.5 |
| 15 | α1.24 | | 30.5 | α1.23 | | 30.5 |
| | β2.17 | | | β2.17 | | |
| 16 | α1.54 | | 32.4 | α1.55 | | 32.4 |
| | β2.68 | | | β2.67 | | |
| 17 | — | | 59.5 | — | | 59.5 |
| 18 | α1.94 | (11.2; 8.6) | 49.1 | α1.95 | (11.1; 8.6) | 49.2 |
| 19 | β2.61 | (10.4; 8.2; 2.1) | 49.8 | β2.61 | (9.6; 9.6; 2.0) | 49.8 |
| 20 | — | | 72.1 | — | | 72.1 |
| 21 | α1.69 | | 29.0 | α1.70 | | 29.0 |
| | β2.16 | | | β2.17 | | |
| 22 | α1.60 | | 36.6 | α1.60 | | 37.0 |
| | β2.05 | (11.8; 6.8; ~0) | | β2.05 | (11.8; 6.7; ~0) | |
| 23 | α1.24 | | 27.9 | α1.21 | | 27.9 |
| 24 | β1.02 | | 16.4 | β1.03 | | 16.5 |
| 25 | β0.71 | | 16.3 | β0.72 | | 16.3 |
| 26 | β1.15 | | 16.7 | β1.15 | | 16.7 |
| 27 | α1.10 | | 15.1 | α1.09 | | 15.2 |
| 28 | — | | 175.4 | — | | 175.2 |
| 29 | 1.30 | | 26.8 | 1.30 | | 26.9 |
| 30 | 1.37 | | 31.4 | 1.37 | | 31.5 |
| A1 | 4.87 | (7.6) | 104.8 | 4.86 | (7.7) | 104.9 |
| A2 | 4.15 | (9.0) | 84.3 | 4.20 | (10.2) | 83.2 |
| A3 | 4.25 | (9.0) | 78.2 | 4.27 | (10.0) | 77.9 |
| A4 | 4.12 | (9.5) | 71.5 | 4.09 | (10.0) | 71.5 |
| A5 | 3.87 | | 77.8 | 3.89 | | 78.2 |
| A6 | 4.28 | (5.7) | 62.7 | a4.29 | (2.3) | 62.7 |
| — | 4.52 | (2.5) | | b4.51 | | |
| B1 | 5.15 | (7.6) | 107.0 | 5.32 | (7.7) | 105.8 |
| B2 | 4.57 | (9.8) | 74.6 | 4.07 | (9.0) | 76.7 |
| B3 | 4.10 | (1.0) | 74.8 | 4.20 | (10.2) | 77.8 |
| B4 | 4.66 | (2.4) | 69.3 | 4.28 | (10.0) | 71.5 |
| B5 | 4.01 | | 76.8 | 3.87 | | 78.1 |
| B6 | 4.34 | (4.5) | 61.1 | a4.35 | | 62.1 |
| | 4.55 | (7.6) | | b4.41 | | |
| C1 | 6.39 | (8.1) | 95.2 | 6.38 | (8.2) | 95.2 |
| C2 | 4.17 | (9.0) | 74.0 | 4.16 | (8.7) | 74.1 |
| C3 | 4.26 | (9.0) | 78.8 | 4.27 | (9.5) | 78.8 |
| C4 | 4.32 | (9.5) | 70.9 | 4.33 | (9.5) | 71.0 |
| C5 | 4.02 | | 79.2 | 4.01 | | 79.2 |
| C6 | a4.36 | (4.5) | 61.0 | a ~ 4.41 | | 62.6 |
| | b4.41 | (2.5) | | b ~ 4.41 | | |

Table 2. Characteristic $^{13}C$–$^{1}H$ long-range correlations observed by HMBC measurements [$J(^{13}C,^{1}H) = 7$ Hz]

| $^{1}H$ | **1** $^{13}C$ | **2** $^{13}C$ |
|---|---|---|
| 3α | 4; 23; 24; A1 | 4; 23; 24; A1 |
| 5α | 4; 10; 24 | 4; 6; 10; 24 |
| 16α | 15; 28 | 15; 28 |
| 16β | 14 | 14 |
| 18α | 13; 17; 19; 20; 28 | 13; 19; 28 |
| 19β | 18; 20 | 13; 18; 20 |
| 21α | 17; 20 | 17; 20 |
| 22α | 17; 21; 28 | 17; 21; 28 |
| 22β | 17; 18; 19; 28 | 18; 19; 28 |
| 23 | 4; 5; 24 | 3; 4; 5; 24 |
| 24 | 4; 5; 23 | 3; 4; 5; 23 |
| 25 | 1; 5; 9; 10 | 1; 5; 10 |
| 26 | 7; 8; 9; 14 | 7; 8; 9; 14 |
| 27 | 8; 13; 14; 15 | 8; 13; 14; 15 |
| 29 | 19; 20; 30 | 19; 20; 30 |
| 30 | 19; 20; 29 | 19; 20; 29 |
| A1 | | 3 |
| A2 | B1 | B1 |
| B1 | A2 | A2 |
| C1 | 28 | 28 |

*Relhania* species. Glycosides seem to be unknown. The *trans*-annulated five-membered ring adopts an envelop conformation where C-17 is the out-of-plane atom of the envelope. This is evident from $J$(H-21α,H-22β) ≈ 0 Hz and $J$(H-19β,H-21α) = 2.1/2.0 Hz (**1** and **2**, respectively) couplings and correlates well with our previous observation for Snatzkein A and B [7].

The connectivities of the monosaccharide units were established on the basis of HMBC cross peaks (Table 2) indicating long-range $^{13}C$-$^{1}H$ couplings. The anomeric proton with the largest chemical shift (H-C1: $\delta = 6.39$ and 6.38 for **1** and **2**, respectively) belongs to an ester glucose (C-28 attachment). Correlation between C-3 and the anomeric proton H-A1, as well as carbon C-A1 and H-3β, proves the position of glucose A at C-3 of the aglycone. Finally, there are both types of $^{3}J(^{13}C,^{1}H)$ couplings between the anomeric CH fragment of sugar B (galactose in **1** and glucose in **2**) and the CH-fragment A2 (Table 2).

ROESY cross peaks (Table 3) provide some evidence for conformational preferences. It turns out that the disaccharide part adopts a conformation as shown in Fig. 1(a); it should be noted that the two correlation peaks connecting H-A1 with H-2α and H-23 indicate that some swinging around the C-3—O bond is taking place. The orientation of the two monosaccharide subunits **A** and **B** is more or less restricted to the arrangement depicted in Fig. 1(b). This corresponds nicely to similar compounds which we published recently [4]. It should be mentioned, however, that we found a cross peak connecting the protons H-A1 and H-B3 indicating a partial mobility around the interglycosidic C—O—C bond.

Finally, the anomeric proton signal of glucose **C** (H-C1) shows only one single cross peak with that of H-26, showing that this monosaccharide is turned over the β-side of the aglycone, as demonstrated in Fig. 1(c). Again, this is in accordance with our earlier report for another ester glycoside [6]. All conformations are in agreement with the *exo*-anomeric effect [9, 10].

Table 3. Characteristic $^{1}H$–$^{1}H$ proximities obtained by ROESY experiments

| | **1** | **2** |
|---|---|---|
| 3α | 1α; 2α; 5α; 23α; A1 | 1α; 2α; 5α; 23α; A1 |
| 5α | 1α; 3α; 6α; 7α; 9α; 23 | 1α; 3α; 6α; 7α; 9α; 23 |
| 12β | 11α; 11β; 12α; 13β; 19β; 30 | 11α; 11β; 12α; 13β; 19β; 30 |
| 13β | 11β; 12β; 19β; 26 | 11β; 12β; 19β; 26 |
| 16α | 16β; 18α; 27 | 16β; 18α; 27 |
| 16β | 15α; 15β; 16α | 15α; 15β; 16α; 22β |
| 18α | 12α; 16α; 19β; 22α; 27; 29; 30 | 12α; 16α; 19β; 22α; 27; 29; 30 |
| 19β | 12β; 13β; 18α; 21β; 22β; 29; 30 | 12β; 13β; 18α; 21β; 22β; 29; 30 |
| 21α | 21β; 22β; 29; 30 | 21β; 22β; 29; 30 |
| 22β | 19β; 21α; 22α | 16β; 19β; 21α; 22α |
| 23 | 3α; 5α; 24; A1 | 3α; 5α; 24; A1 |
| 24 | 2β; 23; 25 | 2β; 23; 25; B1; B5; B6b |
| 25 | 1β; 2β; 6β; 11β; 24; 26 | 1β; 2β; 6β; 11β; 24; 26 |
| 26 | 7β; 13β; 15β; 25; C1 | 7β; 13β; 15β; 25; C1 |
| 27 | 7α; 9α; 12α; 16α; 18α | 7α; 9α; 12α; 16α; 18α |
| 29 | 12α; 18α; 19β; 21α; 30 | 12α; 18α; 19β; 21α; 30 |
| 30 | 12α; 18α; 19β; 21α; 29 | 12α; 18α; 19β; 21α; 29 |
| A1 | 3; 23; B3 | 2α; 3; 23; B3 |
| B1 | 24; A2; A3 | 24; A2; A3 |
| C1 | 26 | 26 |

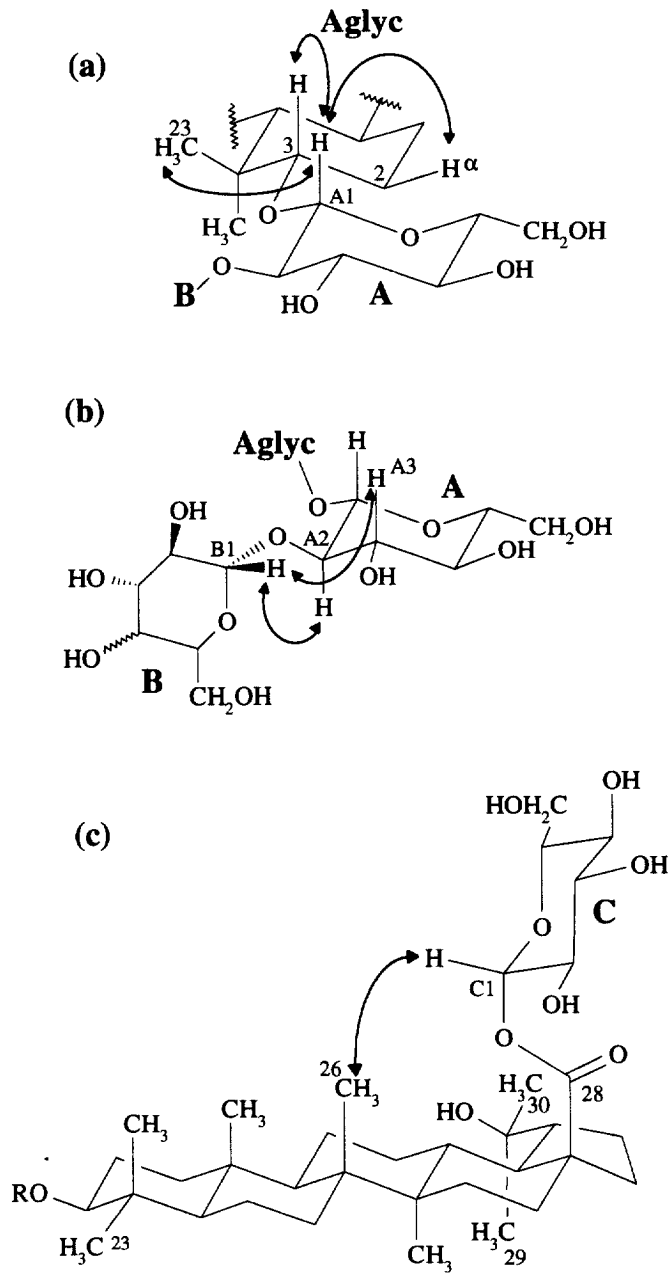


Fig. 1. Conformational preferences: (a) monosaccharide **A** and aglycone; (b) monosaccharides **A** and **B**; (c) monosaccharide **C** and aglycone. Arrows indicate steric proximities obtained by ROESY experiments.

We observed characteristic differences of the chemical shifts of C-29 and C-30 signals ($\Delta\delta$ = 5.4 and 5.6, respectively). This can be explained by the well-known *γ-gauche* effect. There are two carbon atoms in *gauche* arrangement with respect to C-29, namely C-18 and C-21, whereas there is only one for C-30, namely C-21. Thus, a diastereotopic differentiation was possible, the methyl group C-29 is pro-*R* while C-30 is pro-*S*. In the preferred conformation of the carbinol substituent around the C-19/C-20 bond, the hydroxy group is directed towards C-13.

## EXPERIMENTAL

### *General*

NMR spectra were recorded in pyridine-$d_5$ at room temp. using DRX-500 and DMX-750 spectrometers.

Chemical shifts are given on the $\delta$-scale and were referenced to the solvent (C-$\beta$: $\delta = 123.4$ and H-$\beta$: $\delta = 7.17$). In the 1D and 2D NMR experiments pulse programs were taken from the Bruker software library. The HMBC measurements were optimized for 7 Hz long-range $J$(C,H) couplings, whereas the ROESY spectra were run with spinlock 250 ms. In case of TOCSY experiments mixing time of 80 ms were applied. Mps: uncorr. IR spectra in KBr. Column chromatography was performed using silica gel; TLC with silica gel 60 $F_{254}$ plates. Elemental analysis have not yet been performed in order to save the material for biological studies.

### *Isolation*

*Arenaria filicaulis* (Boiss.) has been collected from the plains and areas around Damascus (Syria) and was identified by Prof. A. Elkhatib, Damascus University. A voucher specimen is kept in the herbarium of Damascus University. The dried powdered rhizomes of the plant (3 kg) were exhaustively extracted by MeOH which was finally distilled *in vacuo*. The residue was dissolved in $H_2O$ and successively extracted by $Et_2O$ and *n*-BuOH. The *n*-BuOH extract was dried off and the residue (48 g) was applied over silica gel column and washed by a solvent composed of $CHCl_3$, MeOH and $H_2O$ (100:10:1). The polarity of the solvent was increased by reduction of the $CHCl_3$ quantity. When the composition of the solvent reached 20:10:1, a fraction (910 mg) containing a major compound was collected. Purification was achieved by medium pressure reversed phase CC ($RP_8$, 42% MeOH). The product was finally filtered through Sephadex LH 20 (MeOH) to give 45 mg of pure **2**, $R_f = 0.26$ using $CHCl_3$, MeOH and $H_2O$ (18:8:1). Another fr. 510 mg was eluted by the same polarity (20:10:1) which was separated firstly over silica gel CC by $CHCl_3$ and MeOH (3:1). The compound was purified using a small column ($RP_8$, 38% MeOH), and the product was finally filtered through Sephadex LH-20 (MeOH) to give 25 mg of pure **1**, $R_f = 0.24$ using $CHCl_3$, MeOH and $H_2O$ (18:8:1).

3$\beta$,20-*Dihydroxylupan*-28-*oic acid* 3-*O*-[$\beta$-D-galactopyranosyl-(1 → 2)-$\beta$-D-glucopyranosyl]-28-O-$\beta$-D-glucopyranoside (**1**). Mp 234–235°, $[\alpha]_D^{20} = -52.1$ (MeOH; *c* 0.46); $\nu_{max}^{KBr}$ $cm^{-1}$: 3325 (OH), 1750 (C=O), 1072 (C—O); FAB-MS, (molecular weight: $C_{48}H_{80}O_{19}$) *m/z*: 984 [aglycone + 3 hexoses + Na + H]$^+$.

3$\beta$,20-*Dihydroxylupan*-28-*oic acid* 3-*O*-[$\beta$-D-glucopyranosyl-(1 → 2)-$\beta$-D-glucopyranosyl]-28-O-$\beta$-D-glucopyranoside (**2**). Mp 223–225°, $[\alpha]_D^{20} = -27.7$ (MeOH; $c = 0.36$); $\nu_{max}^{KBr}$ $cm^{-1}$: 3225 (OH), 1740 (C=O), 1068 (C—O); FAB-MS (molecular weight: $C_{48}H_{80}O_{19}$) *m/z*: 984 [aglycone + 3 hexoses + Na + H]$^+$, 804 [aglycone + 2 hexoses − OH + Na + H]$^+$.

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