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Note

Structure of a Further Triterpene Saponin from *Arenaria Filicaulis* Boiss.†

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ABSTRACT: A novel triterpenoid saponin, Snatzkein E, was isolated from *Arenaria filicaulis* Boiss. It possesses a new aglycone and a glucose moiety carrying a sulfate group at C-2′. Its structure and conformational behaviour were investigated by one- and two-dimensional ^{1}H and ^{13}C NMR spectroscopy. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; stereochemistry; conformation; triterpene; saponin; COSY; ROESY; HSQC; HMQC; HMQC-TOCSY; HMBC

INTRODUCTION

Our continuing interest in the saponins of Caryophyllaceous plants led to the isolation of a number of new saponins from the rhizomes of *Arenaria filicaulis* Boiss. (syn. *Gypsophila filicaulis* Boiss., Borm.),[1] a plant used in Syrian traditional medicine for the treatment of rheumatism, bladder illness and constipation. In this paper we describe the isolation, structure elucidation, conformational behaviour and complete ^{1}H and ^{13}C NMR assignments of a further compound of this series, named snatzkein E(**1**), which contains a novel lupane aglycone structure.

EXPERIMENTAL

Isolation

Arenaria filicaulis Boiss. was collected from the plains and areas around Damascus (Syria) and was identified by Professor A. El-Khatib, Damascus University. A voucher specimen is kept in the herbarium of the university.

The shade-dried powdered rhizomes of the plant (3 kg) were exhaustively extracted with methanol and the combined extracts were distilled *in vacuo*. The residue was dissolved in water and successively extracted with diethyl ether and *n*-butanol. The *n*-butanol extract was dried and the residue (48 g) was applied to a silica gel column (Baker) and washed with chloroform–methanol–water (100:10:1). The polarity of the solvent was increased stepwise by reduction of the proportion of chloroform until the ratio reached 10:10:1 and finally with methanol. The last fraction obtained at a ratio of 40:10:1 gave 326 mg; TLC showed a major spot. Purification was achieved by medium-pressure reversed-phase column chromatography (RP-8, 33% methanol). The product was finally filtered through Sephadex LH-20 (methanol) to give 26 mg of pure **1**, R_f = 0.39 using chloroform–methanol–water (9:4:0.5). M.P. 235 °C, $[\alpha]_D^{20} = 43.36$ (methanol, $c = 0.224$); IR (KBr), 3400 cm^{-1} (OH), 1073 cm^{-1} (C—O); positive fast atom bombardment mass spectrometry (FAB-MS), *m/z* 763 $[glucoside + SO_3 + 2Na - H]^+$, 265 $[2'\text{-}SO_2ONa\text{-}glucosyl]^+$; negative FAB-MS, *m/z* 717 $[M - 1]^-$, 80 $[SO_3]^-$, 97 $[SO_3OH]^-$.

Spectroscopy

NMR spectra were recorded in pyridine-d_5 at room temperature using Bruker Avance DRX-500 and DMX-800 spectrometers. Chemical shifts are given on the δ-scale and were referenced to the solvent (C-β,

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$\delta = 123.4$; H-β, $\delta = 7.17$). In 1D measurements (^{1}H, ^{13}C, DEPT), 64K data points were used for the FID.

The pulse programs of the following 2D experiments were taken from the Bruker software library and the parameters were as follows.

500/125 MHz gradient-selected HMQC[2] spectra. Relaxation delay $D_1 = 2.0$ s; evolution delay $D_2 = 3.45$ ms; 90° pulse, 11.5 μs for ^{1}H, 10.0 μs for ^{13}C hard pulses and 65.0 μs for ^{13}C GARP decoupling; 1K points in t_2; spectral width, 8 ppm in F_2 and 130 ppm in F_1; 256 experiments in t_1; linear prediction to 512 points; zero-filling up to 1K.

500/125 MHz and 800/200 MHz gradient-selected HSQC edited[3] spectra. Relaxation delay $D_1 = 1.5$ s; evolution delay $D_2 = 3.45$ ms; 90° pulse, 11.5 μs for ^{1}H, 10.0 μs for ^{13}C hard pulses and 65.0 μs for ^{13}C GARP decoupling; 1K points in t_2; spectral width, 8 ppm in F_2 and 130 ppm in F_1; 256 experiments in t_1; linear prediction to 512 points; zero-filling up to 1K.

500/125 MHz gradient-selected HMBC[3] spectra. Relaxation delay $D_1 = 1.5$ s; evolution delay $D_2 = 3.45$ ms; delay for evolution of long-range coupling $D_6 = 70$ ms ($J = 7$ Hz); 1K points in t_2; spectral width, 8 ppm in F_2 and 180 ppm in F_1; 256 experiments in t_1; linear prediction to 512 points; zero-filling up to 1K.

500 MHz ROESY[4] spectra. Relaxation delay $D_1 = 2.0$ s; 90° pulse for ^{1}H; spin lock, 300 ms; 1K points in t_2; spectral width, 8 ppm in both dimensions; 256 experiments in t_1; linear prediction to 512 points; zero filling up to 1K.

500 MHz gradient-selected ^{1}H, ^{1}H COSY[3] spectra. Relaxation delay $D_1 = 1.0$ s; 90° pulse for ^{1}H; 2K points in t_2; spectral width 8 ppm in both dimensions; 256 experiments in t_1, linear prediction to 512 points; zero filling up to 2K.

500/125 MHz HMQC-TOCSY[5] spectra. Relaxation delay $D_1 = 1.5$ s; evolution delay $D_2 = 3.45$ ms; 90° pulse for ^{1}H and ^{13}C; 90° pulse for ^{13}C GARP decoupling; 90° pulse for MLEV; TRIM pulse, 80 μs; 1K points in t_2; spectral width, 8 ppm in F_2 and 130 ppm in F_1; 256 experiments in t_1; linear prediction to 512 points; zero filling up to 2K.

Mass spectra. Finnigan MAT 8430 instrument in the FAB mode at 9 kV using Xe; matrix, *m*-nitrobenzyl alcohol.

RESULTS AND DISCUSSION

Signal and structural assignments

Structural determinations are based on the NMR spectral assignments, which were confirmed by DEPT, ^{1}H, ^{1}H COSY, TOCSY, HMQC, edited HSQC, HMBC, ROESY and HMQC-TOCSY experiments as described before.[1] The ^{1}H and ^{13}C chemical shifts and $J(^1\text{H},^1\text{H})$ couplings of **1** are given in Table 1 and some characteristic ^{1}H–^{1}H proximities (ROESY) and ^{13}C–^{1}H long-range correlations (HMBC) in Table 2. For illustration, a section of the HMBC spectrum of **1** is depicted in Fig. 1.

The ^{13}C NMR spectrum of **1** shows 36 signals. The sugar moiety exhibits five CH and one CH_2 fragment, whereas the aglycone part consists of 30 carbon signals, seven CH_3, ten CH_2, seven CH and six quaternary carbon atoms, appearing in the sp^3 region.

Assignment of the H-3 and C-3 signals is straightforward and can be utilized as a starting point for the determination of atom connectivities. The HMBC mea-

Table 1. ^{1}H and ^{13}C NMR chemical shifts and $J(^1\text{H},^1\text{H})$ couplings of 1

Atom		^{1}H	J(Hz)	^{13}C
1	α	0.71	12.0, 12.0, 2.8	39.0
	β	1.44		
2	α	2.14		26.5
	β	1.80		
3	α	3.29	11.6, 4.7	89.6
4				39.7
5	α	0.63	11.5, ~1	55.8
6	α	1.41		18.4
	β	1.23		
7	α	1.32		34.9
	β	1.32		
8				41.8
9	α	1.20		50.3
10				36.9
11	α	1.40		21.7
	β	1.13		
12	α	1.71		28.8
	β	2.13		
13	β	1.69		36.3
14				45.3
15	α	1.72		38.9
	β	2.19		
16	α	4.12	12.2, 5.0	78.37
17				53.5
18	α	1.81	10.5, 8.7	48.2
19	β	2.10	10.5, 10.5, 2.7	50.1
20				72.4
21	α	2.13		28.9
	β	1.54	12.5, 10.5, 10.5, 5.7	
22	α	1.40		32.8
	β	2.85	11.6, 5.7	
23	α	1.42		28.2
24	β	1.13		16.8
25	β	0.68		16.3
26	β	1.00		16.2
27	α	1.10		16.6
28	a	3.73	11.0	61.5
	b	4.54	11.0	
29		1.30		26.1
30		1.40		32.0
HO-C(20)		4.94		

Table 2 Characteristic ^{1}H–^{1}H proximities (ROESY) and ^{13}C–^{1}H long-range correlations (HMBC) of 1

			HMBC
^{1}H		ROESY ^{1}H	^{13}C
2	α	1α, 3α, 1′	
3	α	1α, 2α, 5α, 23α, 24β, 1′, 2′	4, 23, 24, 1′
5	α	3α, 6α, 7α, 9α, 23α, 24β	4, 6, 10, 24, 25
9	α	1α, 5α, 11α	8, 10, 25, 26
13	β	15β, 19β, 26β	
15	α	7α, 7β, 27α	10, 14, 16, 17, 27
	β	26β, 28b	27
16	α	15α, 18α, 22α, 27α	
18	α	16α, 27α, 29, 30	17, 19, 20, 28
19	β	28a, 29, 30	
22	β	19β, 28a	17, 18, 19
23	α	1′, 2′, 3α, 24β	3, 4, 5, 24
24	β	2β, 3α, 23α, 25β, 1′, 2′	3, 4, 5, 23
25	β	2β, 6β, 7β, 24β, 26β	1, 5, 9, 10
26	β	6β, 7β, 11β, 13β, 15β, 25β, 28b	7, 8, 9, 14
27	α	7α, 7β, 9α, 16α, 18α	8, 13, 14, 15
28	a	13β, 19β, 22β	
	b	13β, 15β, 21β, 26β	22
29		12α, 18α, 19β, 30, HO-C(20)	19, 20, 30
30		19β 29, HO-C(20)	19, 20, 29
1′		2α, 3α, 23α, 3′, 4′, 5′	3
2′		3α, 23α, 24β, 3′, 4′, 5′	1′, 3′
HO-C(20)		29, 30	29

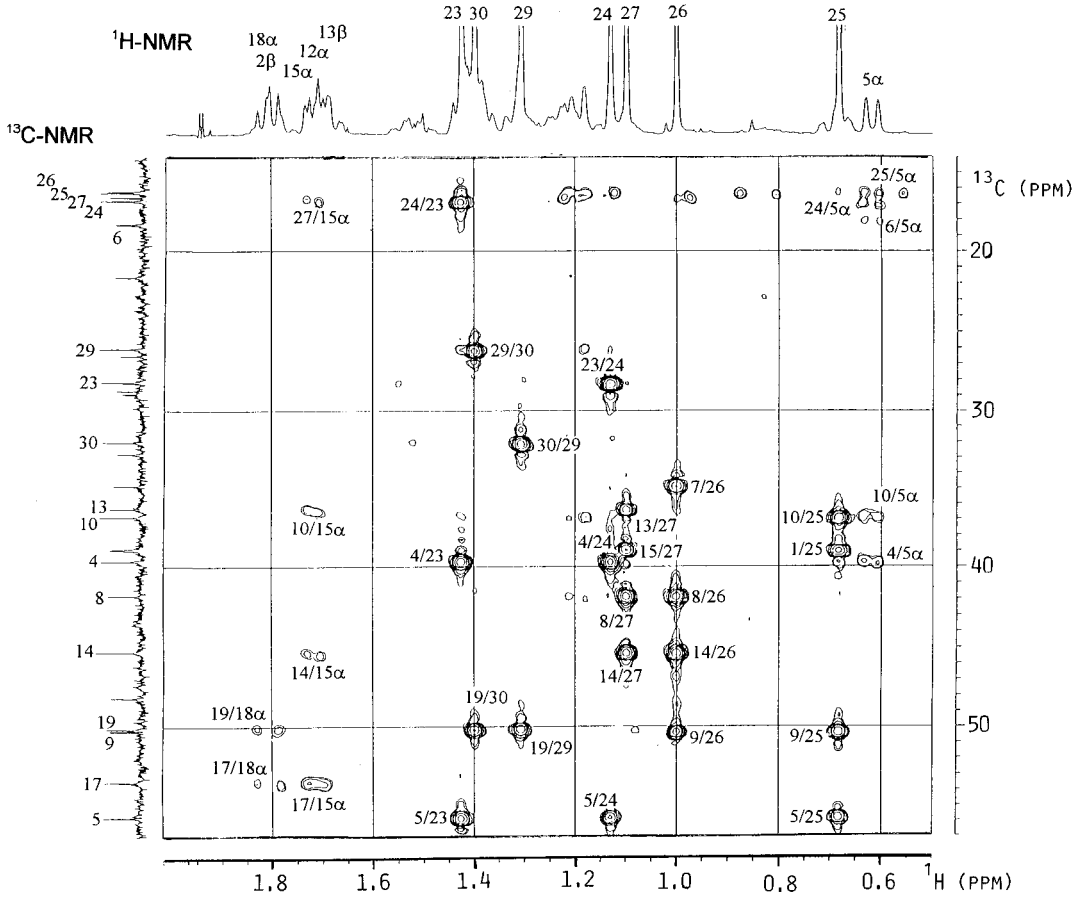
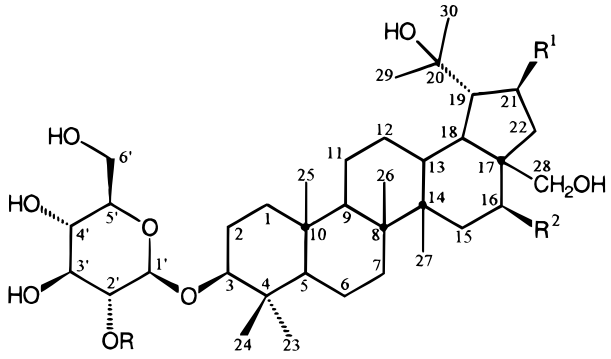


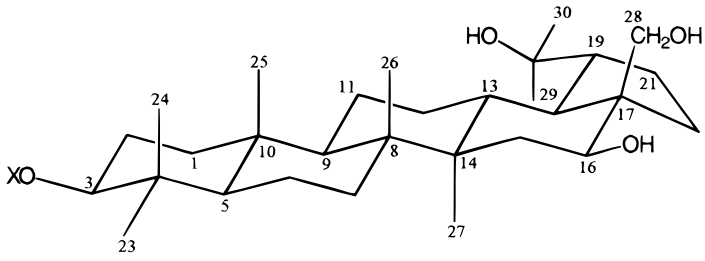
Figure 1. Section of the HMBC spectrum of **1**. In the identification of the cross peaks the first number indicates the carbon and the second the hydrogen atom.

(a)



	R	R^1	R^2
1	SO_2OH	H	OH
2	H	OH	H
3	SO_2OH	OH	H

(b)



X = glucosyl sulfate

Scheme 1. (a) Structures of snatzkein E (**1**), A (**2**) and B (**3**); (b) stereoprojection of the aglycone of **1**; whether R^3 is H or CH_2CH_2OH is still under investigation.

surement proved to be the method of choice allowing the consecutive assignment of all seven methyl groups, and in this way the two- and three-bond correlations of the protons of these methyl moieties made the assignment of most of the skeletal signals feasible. The mutual HMBC peaks of two methyl groups show their geminal position. The high chemical shift (δC-20 = 72.4) of the intermediate quaternary carbon atom and the cross peak between HO ($\delta = 4.94$) and one of the methyls prove the existence of an exocyclic carbinol group. This group is attached to C-19, as proved by HMBC correlations H-30/C-19, H-29/C-19 and H-18/C-20 (Table 2). The H-22β signal appears without any overlapping and this correlation to C-18 and C-19 proves unam-

Table 3 Comparison of the ^1H and ^{13}C NMR data for the glycosidic moiety of 1 and 2

		1			**2**		
Atom		^1H	J(Hz)	^{13}C	^1H	J(Hz)	^{13}C
1′		4.97	7.8	104.3	4.90	7.8	106.8
2′		5.07	8.5	81.0	4.01	8.1, 8.6	75.7
3′		4.44	8.9	78.4	4.22	8.7, 8.8	78.7
4′		4.12	8.9	71.9	4.18	9.1, 8.9	71.8
5′		3.92		77.8	3.98		78.2
6′	a	4.25	11.8, 5.7	62.9	4.37	10.5, 5.4	63.0
	b	4.49	11.8, 2.2		4.56	11.7, 2.6	

biguously the existence of a five-membered ring in the molecule. From the high chemical shifts of the H-16 and C-16 signals, it is evident that in **1** the OH group is attached to C-16, and not to C-21 as in Snatzkein A and B (**2** and **3**). The J(H-16,H-15β) = 12.2 Hz coupling and, further, the ROESY cross peaks H-16/H-18α and H-16/H-27α proved the β-position of the hydroxy group. The assignment of the quaternary C-8 and C-14 signals correlates well with their chemical shifts measured for **2** and **3**. The stereochemistry of the ring system (Scheme 1), the positions of all atoms and substitutions could be obtained beyond doubt from ^{1}H,^{1}H coupling constants, as far as identifiable, and ROESY cross peaks (Tables 1 and 2). In all instances pertinent information was redundant.

The same all-*trans*-fused pentacyclic ring system was recently reported as an aglycone by Tsichritzis and Jakupovic,[6] but without the β-OH group at C-16. Snatzkein A (**2**) and B (**3**) possess the same skeleton, but with a β-OH group in position 21 (Scheme 1).[1a]

The ^{1}H and ^{13}C NMR data for the glucosidic moieties of **1** and **2** are compared in Table 3. From the ^{1}H and ^{13}C chemical shifts and the ^{1}H,^{1}H coupling constants, it is straightforward to conclude that the sugar moiety is β-glucose. It is attached to C-3 as proved by the C-3 chemical shift, HMBC and ROESY cross peaks (Table 2). To our surprise, we found H-2′ (δ = 5.07) and C-2′ chemical shifts (δ = 80.1) which show, by comparison with the data for **2** that the glucose in **1** carries a substituent at C-2′. However, we did not find any further signals for such a substituent which, therefore, cannot contain hydrogen and carbon atoms. The positive FAB mass spectrum showed a peak at m/z 763 corresponding to [glucoside + SO_3 + 2 Na − H]$^+$ and, in addition, a peak at m/z 265 relating to a 2′-SO_2ONa-glucosyl cation. Hence the new substituent is a sulfate group which was further confirmed by negative FAB peaks at m/z 80 [SO_3]$^-$ and 97 [SO_3OH]$^-$. This prompted us to re-investigate **3**, which was considered earlier[1a] to be a glucosyl ether by negative FAB-MS. It gave the same peaks at m/z 80 and 97 as **1**. Therefore, we have to conclude the Snatzkein B (**3**)[1a] also contains a sulfate group. Apparently the CH_2CH_2OH signals which we observed in the spectra of **3** have to be attributed to a 2:1 host–guest complex of **3** and diglycol.

Conformational analysis

The five-membered ring in the aglycone is fairly rigid owing to the *trans*-ring junction and substitution. The out-of-plane atom of the envelope is C-17. This is the only possible five-membered ring conformation where J(H-21α,H-22β) ≈ 0 Hz, as observed. Further confirmation comes from the two coupling constants J(H-18α,H-19β) ≈ J(H-21β,H-22α) ≈ 10.5 Hz.

Similarly to **2** and **3**, we find in **1** a chemical shift difference for C-29 and C-30 of *ca.* 6 ppm (δ = 32.0 and 26.1), indicating again that one of these two methyl groups experiences one more γ-*gauche* interaction than the other. The ROESY cross peak between H-30 and H-21α in the spectra of **2** and **3** was decisive for determining the stereochemical assignment of these two diastereotopic geminal methyl groups. In **1**, however, the H-21α signal is obscured by severe overlap, and a straightforward identification of a corresponding ROESY cross peak (both H-21 and H-30) is not possible. Previously, we have reported for **2** and **3** that the rotation about the C-19—C-20 bond is restricted, and in the preferred conformation the hydroxy group is directed towards C-12 (see Fig. 2 in Ref. 1a). Owing to the ambiguity mentioned above, we cannot exclude another conformation of **1** with one methyl group oriented towards C-12. Since, however, this situation would create enormous steric congestion, we prefer to assume a conformation similar to that in **2** and **3**. This would lead to the same stereochemical assignment, namely pro-*R* for C-29 and pro-*S* for C-30.

The chemical shifts of the methylene protons of the 28-CH_2OH group are significantly different ($\Delta\delta$ = 0.81). The ROESY spectrum indicates that the proton with the larger chemical shift (H-28b) is closer to H-15β and H-26 whereas H-28a is closer to H-22β. This leads to the conclusion that the OH group is preferentially oriented outwards, a conformation possibly stabilized by a hydrogen bridge between the two OH groups at C-16 and C-28. If this is true, a stereochemical differentiation is possible: H-28a is pro-*S* and H-28b is pro-*R*, i.e. the sequence of the chemical shifts of these two protons is opposite to that in **2** and **3** owing to the neighbourhood of the 16β-OH in **1**.

The orientation of the aglycone and the glucose with respect to each other is apparently very similar in all three compounds **1**–**3** (see Fig. 3 in Ref. 1a). The weak ROESY cross peak observed for H-1′ and H-2α is not contradictory to this; molecular models suggest a distance of 3.5–4 Å.

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