Nuatigenin-Type Steroidal Saponins from *Veronica fuhsii* and *V. multifida*

Meltem Ozipek,[a] Iclal Saracoglu,[a] Yukio Ogihara[b] and İhsan Çaliş[a,*]

[a] Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, TR-06100 Ankara, Turkey. Fax: +90-312-3114777. E-mail: icalis@hacettepe.edu.tr

[b] Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan

[*] Author for correspondence and reprint requests

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A new nuatigenin-type steroidal saponin, multifidoside (**2**), was isolated from the aerial parts of *Veronica fuhsii* and *V. multifida* and its structure was identified as 3-*O*-{[α-L-rhamnopyranosyl-(1→2_{glu})]-[β-D-glucopyranosyl-(1→4_{rha})-α-L-rhamnopyranosyl (1→4_{glu})]-β-D-glucopyranosyl}nuatigenin 26-*O*-β-D-glucopyranoside. Additionally, a known steroidal saponoside, aculeatiside A (**1**), from *V. fuhsii*, a phenylethanoid glycoside, verpectoside A (**3**), and a flavon glycoside, isoscutellarein 7-*O*-(2″-*O*-6″-*O*-acetyl-β-D-allopyranosyl-β-D-glucopyranoside) (**4**) from *V. multifida* were isolated.

Introduction

Veronica species (Scrophulariaceae) contain mainly iridoid glycosides and some phenylethanoid glycosides and flavonoid compounds (Lahloub, 1989; Lahloub *et al.*, 1993; Taskova *et al.*, 1998; Taskova *et al.*, 1999; Chari *et al.*, 1981; Aoshima *et al.*, 1994). However saponins of *Veronica* species were rarely reported (Tamas, *et al.*, 1984; Bogacheva *et al.*, 1980; Gvazava and Pkhidze, 1988). Our previous studies have shown the presence of iridoid glycosides in *Veronica multifida* L. (Ozipek *et al.*, 2000) and *V. fuhsii* FREYN *et* SINT (Ozipek *et al.*, 1998) as well as phenylethanoid glycosides, plantamajoside and fuhsioside, and a flavone glucoside, luteolin 7-*O*-glucoside, in the latter plant (Ozipek *et al.*, 1999). We now report a new furospirostanol glycoside, multifidoside (**2**), from the aerial parts of *V. fuhsii* and *V. multifida* besides a known furospirostanol glycoside, aculeatiside A, from *V. fuhsii* (**1**). In addition, a phenylethanoid glycoside (**3**) and a flavone glycoside (**4**), isolated from *V. multifida*, are also reported. Compound (**3**) was identified as verpectoside A (3,4-dihidroxy-β-phenylethoxy-*O*-[α-L-arabinopyranosyl-(1→2)]-[α-L-rhamnopyranosyl-(1→3)]-4-*O*-feruloyl-β-D-glucopyranoside (Saracoglu *et al.*, 2002) and (**4**) as isoscutellarein 7-*O*-(2″-*O*-6″-*O*-acetyl-β-D-allopyranosyl-β-D-glucopyranoside) (Lenherr *et al.*, 1984) from their UV, IR and NMR spectroscopic data by comparison with reported data.

Material and Methods

General experimental procedures

Optical rotation was measured on a Autopol IV Rudolph Research Analytical polarimeter using a sodium lamp operating at 589 nm. IR spectra were measured on a Perkin-Elmer 2000 FT-IR spectrometer in KBr pellets. NMR spectra were recorded on a Joel JNM-A500, Bruker AMX 300 and DRX 500 spectrometers. HR-FAB-MS spectra were obtained on an Ion Spec Ultima FTMS spectrometer.

Plant material

Veronica fuhsii (Scrophulariaceae) was collected from Kizilcahamam-Isikdagi in May 1988. The voucher specimen (HUEF-88148) has been deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey. *Veronica multifida* was collected from Sivas (Yildizeli-Akdag) in June 1998. The voucher specimen (HUEF-98048) has been deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

Extraction and isolation

Air-dried aerial parts (220 g) of *V. fuhsii* were extracted with MeOH (2 × 1 l). The methanolic extract was evaporated in vacuo. The residue (48 g) was dissolved in water and then extracted with petroleum ether and the petroleum ether phase discarded. The aqueous phase was concentrated and chromatographed over polyamide column eluting with H_2O, followed by increasing concentrations of MeOH to yield four main fractions: A-D [A_1-A_5, 9.2 g (H_2O); B, 675 mg (50% MeOH); C, 400 mg (75% MeOH); D, 1 g (MeOH)]. Fraction A_4 (1.4 g) was applied to MPLC using reversed phase material (LiChroprep C_{18}) with increasing amount of MeOH in H_2O. Fraction eluted with 75% MeOH was rechromatographed over Si gel by stepwise elution with a $CHCl_3$-MeOH-H_2O (80:20:2→70:30:3) solvent system to give **1** (11 mg) and **2** (9 mg).

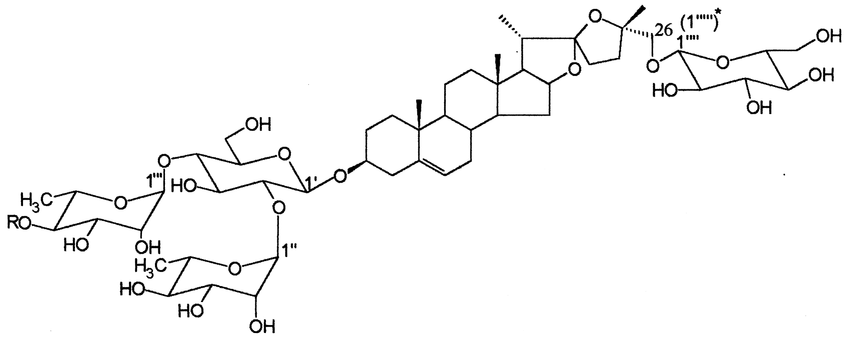
Air-dried aerial parts of *V. multifida* (100 g) were extracted twice with methanol (each 1 l). The methanolic extract was evaporated in vacuo. The residue (20 g) was dissolved in water and then extracted with petroleum ether and the petroleum ether phase discarded. The remaining aqueous phase was concentrated (12 g) and chromatographed over a polyamide column eluting with H_2O, followed by increasing concentrations of MeOH to yield four main fractions: A–D [A, 4.2 g (H_2O); B_1–B_3, 1.4 g (50% MeOH); C_1–C_3, 1.9 g (75% MeOH); D, 1.3 g (MeOH)]. B_2 (250 mg) was chromatographed over Si gel by stepwise elution with a CH_2Cl_2-MeOH (9:1→6:4) solvent system and then rechromatographed over Sephadex LH-20 with MeOH to give **2** (4 mg) and **3** (1.5 mg). C_2 (225 mg) was chromatographed over Si gel using CH_2Cl_2-MeOH-H_2O (80:20:2→60:40:4) solvent system and then rechromatographed over Sephadex LH-20 with MeOH to give **4** (20 mg).

Aculeatiside A (**1**): IR υ_{max}(KBr) cm^{-1}: 3420 (OH), 2929 (CH), 1456, 1039, 915, 860, 820. ^1H NMR (500 MHz, pyridine-d_5) (sugar moiety see Table I); δ 4.23 (H-3, overlapped), 5.32 (1H, *br s* , H-6), 4.72 (1H, *t*, *J* = 7.3 Hz, H-16), 1.74 (H-17, overlapped), 4.20 (1H, *d*, *J* = 10.0, H-26a), 3.91 (1H, *d*, *J* = 10.0, H-26b), 0.81 (3H, *s*, H-18), 1.05 (3H, *s*, H-19), 1.08 (3H, *d*, *J* = 7.0, H-21), 1.41 (3H, *s*, H-27). ^{13}C NMR (125.6 MHz, pyridine-d_5), see Table II; HR-FAB-MS: (*m/z*) 1069.5 $[M + Na]^+$

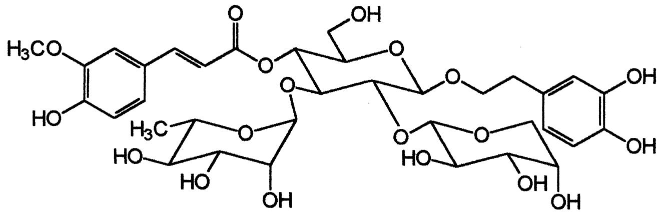
Mulifidoside (**2**): $[\alpha]^D{}_{20}$-78.0 (*c* 0.1, MeOH) IR υ_{max} (KBr) cm^{-1}: 3418 (OH), 2927(CH), 1456, 1045, 910, 870, 820. ^1H NMR (300, 500 MHz, CD_3OD) (sugar moiety, see Table I); δ 3.60 (1H, *t*, *J* = 9.2, H-3), 5.37 (1H, *d*, *J* = 4.9, H-6), 4.45 (1H, *t*, *J* = 7.3 Hz, H-16), 1.76 (1H, *t*, *J* = 7.0, H-17), 3.86 (1H, *d*, *J* = 10.0, H-26a), 3.48 (1H, *d*, *J* = 10.0, H-26b), 0.81 (3H, *s*, H-18), 1.05 (3H, *s*, H-19), 0.99 (3H, *d*, *J* = 7.0, H-21), 1.23 (3H, *s*, H-27). ^{13}C NMR (125.6 MHz, CD_3OD), see Table II; HR-FAB-MS: (*m/z*) 1231.5 $[M + Na]^+$.

Results and Discussion

Compound **1** was obtained as a white amorphous powder. The high resolution (HR)-FAB-MS of compound **1** exhibited a pseudomolecular ion peak at *m/z* 1069.5 $[M + Na]^+$ which is compatible with the molecular formula $C_{51}H_{82}O_{22}$, requiring eleven degrees of unsaturation. The ^1H-NMR spectrum revealed the signals for six methyl groups at δ_H 0.81 (s, CH_3-18), 1.05 (s, CH_3-19), 1.08 (*d*, *J* = 7.0 Hz, CH_3-21), 1.41 (s, CH_3-27), 1.78 (*d*, *J* = 6.1 Hz, CH_3-6″), 1.64 (*d*, *J* = 6.1 Hz, CH_3-6‴), an olefinic proton at δ_H 5.32 (brs, H-6), and four anomeric protons at δ_H 4.95 (*d*, *J* = 7.9 Hz), 4.98*, 5.87 (brs) and 6.41 (brs), and a pair of hydroxymethylene protons as an AB system (δ_H 4.20 and 3.91, J_{AB} = 10.0 Hz, H_2-26), predicting a tetraglycosidic steroidal structure. Thus, the signals at δ_H 1.64 and 1.78 arising from two secondary methyl protons were attributed to two rhamnose units. In the ^{13}C-NMR spectrum, four anomeric carbon signals were observed at δ_C 100.3, 105.5, 102.9 and 102.0, confirming the tetraglycosidic structure. All NMR assignments (Tables I, II) were based on COSY, HMQC and HMBC experiments. The chemical shifts and coupling constants of the signals assigned to the sugar moiety indicated the presence of two glucose and two rhamnose units. The signals observed at δ_C 140.7 and 121.9 were assigned to the olefinic carbon atoms. Since this last functional group accounted for one double bond equivalent, **1** was considered as decacyclic. Hence, the aglycon was found to be hexacyclic. The ^{13}C NMR data contained 27 carbon atoms for the steroidal aglycon. The quaternary carbon signals displayed at δ_C 120.3 and 83.9 indicated the dioxygenated C-22 carbon and C-25 of the agylcone, respectively, which are characteristic for the furospirostanol sapogenins (Agrawal *et al.*, 1985). Thus, the signals at δ_C 24.4 and 77.5 were assigned to the methyl (C-27) and hydroxymethyl (C-26) groups, respectively. This assumption was also confirmed by the long-range correlations between H_2-26/C-25



1 R=H
2* R= Glucose



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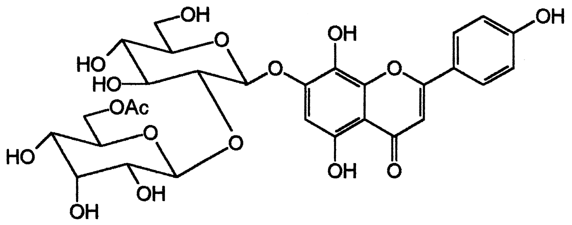

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Fig. 1. Chemical structures of the isolated compounds **2**.

and H-27/C-25. The remaining carbon and proton signals attributed to the aglycone were in good accordance to those of nuatigenin reported (Saijo *et al.*, 1983; Mimaki and Sashida, 1990). The downfield shifts at δ_C 78.1 (C-3) and 77.5 (C-26) showed that the glycosyl residues linked to the hydroxyls at C-3 and C-26 which indicated the bisdesmosidic structure of the compound **1**. The HMBC correlations observed between H-1″ (δ_H 5.87) and C-2′ (δ_C 78.6); H-1‴ (δ_H 6.41) and C-4′ (δ_C 77.8); revealed the sites of the linkages of sugar moiety attached to C-3 of the sapogenol. Finally, the remaining glucose unit was found to be linked to the hydroxymethyene group of the sapogenin moiety, which was confirmed by the long-range correlations between H-1″″/C-26 *visa verse* H_2-26/C-1″″ in the HMBC experiment. The signals attributed to compound **1** were in good agreement with those of aculeatiside A, a nuatigenin tetraglycoside (Saijo *et al.*, 1983). Therefore the structure of **1** was identified as aculeatiside A, 3-*O*-[α-L-rhamnopyranosyl-(1→2_{glu})-α-L-rhamnopyranosyl-(1→4_{glu})-β-D-glucopyranosyl] nuatigenin 26-*O*-β-D-glucopyranoside.

Table I. ^1H NMR spectroscopic data for sugar moieties of aculeatiside A (**1***) and multifidoside (**2**$^\#$) (Pyridine-d_5*, CD_3OD#).

$\mathbf{1}^*$		$\mathbf{2}^\#$	
H	δ_H ppm, J [Hz]	H	δ_H ppm, J [Hz]
Glc-1′	4.95 δ (7.9)	Glc-1′	4.49 δ (7.9)
2′	4.23*	2′	3.38*
3′	4.23*	3′	3.57*
4′	4.40*	4′	3.54 t (7.5)
5′	3.65 m	5′	3.32*
6′	4.23* 4.11*	6′	3.79 dd (12.0/2.0) 3.65 dd (12.0/6.0)
Rha-1″	5.87 brs	Rha-1″	5.19 δ (1.5)
2″	4.84 dd (1.7/3.4)	2″	3.92 dd (3.4/1.7)
3″	4.64 dd (3.4/9.4)	3″	3.67*
4″	4.37*	4″	3.38*
5″	4.98*	5″	4.12 dd (10.0/6.1)
6″	1.78 δ (6.1)	6″	1.33 δ (6.1)
Rha-1‴	6.41 brs	Rha-1‴	4.85 brs
2‴	4.70 dd (1.7/3.4)	2‴	3.85 dd (3.4/1.7)
3‴	4.56*	3‴	3.67*
4‴	4.34*	4‴	3.65*
5‴	4.95*	5‴	4.02 dq (10.0/6.1)
6‴	1.64 δ (6.1)	6‴	1.24 δ (6.1)
		Glc-1⁗	4.58 δ (7.9)
		2⁗	3.19 dd (7.9/9.0)
		3⁗	3.37*
		4⁗	3.28*
		5⁗	3.26*
		6⁗	3.87 dd (12.0/2.0) 3.70 dd (12.0/6.0)
Glc-1⁗	4.98*	Glc-1′′′′′	4.30 δ (7.9)
2⁗	4.05 dd (7.9/9.0)	2′′′′′	3.22 dd (7.9/9.0)
3⁗	4.28*	3′′′′′	3.37*
4⁗	4.28*	4′′′′′	3.28*
5⁗	3.96 m	5′′′′′	3.26*
6⁗	4.56 dd (12.0/2.0) 4.43 dd (16.0/6.0)	6′′′′′	3.87 dd (12.0/2.0) 3.70 dd (12.0/6.0)

* Signal pattern unclear due to overlapping.

Compound **2** was also obtained as a white amorphous powder. The HR-FAB-MS of compound **2** exhibited a pseudomolecular ion peak at *m/z* 1231.5 $[M + Na]^+$ which is compatible with the molecular formula $C_{57}H_{92}O_{27}$. The signals observed in ^1H and ^{13}C NMR spectra were very similar to those of compound **1**. The only difference is the signals arising from an additional sugar unit. The ^1H NMR spectrum indicated the signals for six methyl groups at δ_H 0.81 (s, CH_3-18), 1.05 (s, CH_3-19), 0.99 (*d*, $J = 7.0$ Hz, CH_3-21), 1.23 (s, CH_3-27), 1.33 (*d*, $J = 6.1$ Hz, CH_3-6″), 1.24 (*d*, $J = 6.1$ Hz, CH_3-6‴), an olefinic proton at δ_H 5.37 (*d*, $J = 4.9$), five anomeric protons at δ_H 4.49 (*d*, $J = 7.9$ Hz), 4.58 (*d*, $J = 7.9$ Hz), 4.30 (*d*, $J = 7.9$ Hz), 5.19 (*d*, $J = 1.5$ Hz), 4.85 (brs), and a pair of hydroxymethylene protons as an AB system (δ_H 3.86 and 3.48, $J_{AB} = 10.0$ Hz, H_2-26), confirming the pentaglycosidic structure of compound **2**. The signals at δ_H 1.24 and 1.33 arising from two secondary methyl protons were attributed to two rhamnose units. Five anomeric carbon signals observed at δ_C100.4, δ_C 105.5, δ_C 104.9, δ_C 102.2 and δ_C 102.6 in the ^{13}C NMR spectrum verified the pentaglycosidic structure. The complete assignments (Tables I, II) of all proton and carbon resonances were based on the DQF-COSY, HSQS, HMQC and HMBC experiments. The assignments attributed to the sugar moiety indicated two rhamnose and three glucose units. The ^{13}C NMR spectrum showed 27 carbon atoms for the steroidal aglycone moiety. The chemical shifts and coupling constants of the signals assigned to the aglycone moiety indicated the presence of the same steroidal aglycone,

C	DEPT	**1*** δ_C	**2**# δ_C	C	DEPT	**1*** δ_C	C	**2**# δ_C
1	CH_2	37.5	38.5	Glc-1′	CH	100.3	Glc-1′	100.4
2	CH_2	30.2	30.7	2′	CH	78.6	2′	79.3
3	CH	78.1	79.2	3′	CH	78.0	3′	78.0
4	CH_2	39.0	39.4	4′	CH	77.8	4′	79.6
5	C	140.7	141.8	5′	CH	77.0	5′	76.6
6	CH	121.9	122.5	6′	CH_2	61.3	6′	61.9
7	CH_2	32.2	32.8	Rha-1″	CH	102.9	Rha-1″	102.2
8	CH	31.6	32.8	2″	CH	72.5	2″	72.1
9	CH	50.3	51.7	3″	CH	72.8	3″	72.3
10	C	37.1	38.0	4″	CH	74.1	4″	73.9
11	CH_2	21.1	21.9	5″	CH	69.5	5″	69.7
12	CH_2	39.8	40.9	6″	CH_3	18.7	6″	17.9
13	C	40.1	41.5	Rha-1′′′	CH	102.0	Rha-1′′′	102.6
14	CH	56.5	57.7	2′′′	CH	72.6	2′′′	72.2
15	CH_2	32.3	33.1	3′′′	CH	72.7	3′′′	72.3
16	CH	81.0	82.1	4′′′	CH	73.9	4′′′	83.1
17	CH	62.5	63.2	5′′′	CH	70.4	5′′′	69.2
18	CH_3	16.2	16.5	6′′′	CH_3	18.5	6′′′	17.9
19	CH_3	19.4	19.8		CH		Glc-1′′′′	105.5
20	CH	38.6	39.3		CH		2′′′′	76.1
21	CH_3	15.2	15.0		CH		3′′′′	78.2
22	C	120.3	121.7		CH		4′′′′	71.6
23	CH_2	33.1	33.5		CH		5′′′′	78.0
24	CH_2	34.0	33.7		CH_2		6′′′′	62.7
25	C	83.9	85.2	Glc-1′′′′	CH	105.5	Glc-1′′′′′	104.9
26	CH_2	77.5	77.5	2′′′′	CH	75.4	2′′′′′	75.2
27	CH_3	24.4	24.2	3′′′′	CH	78.5	3′′′′′	77.7
				4′′′′	CH	71.6	4′′′′′	71.4
				5′′′′	CH	78.6	5′′′′′	78.1
				6′′′′	CH_2	62.7	6′′′′′	62.7

Table II. ^{13}C NMR spectroscopic data for aculeatiside A (**1***) and multifidoside (**2**#) (Pyridine-d_5*, CD_3OD#).

nuatigenin, as compound **1**. In the HBMC spectrum, the anomeric proton of the glucose (H-1′) (δ_H 4.49) exhibited a longe-range coupling between C-3 of the aglycone (δ_C 79.2) whereas H-1′′′′′ of glucose (δ_H 4.30) showed a correlation with C-26 of the agylcone (δ_C 77.5) indicating the bisdesmosidic structure of **2**. Additionally longe-range correlations observed between H-1″ of rhamnose (δ_H 5.19) and C-2′ of glucose (δ_C 79.3); H-1‴ of rhamnose (δ_H 4.85) and C-4′ of glucose (δ_C 79.6);

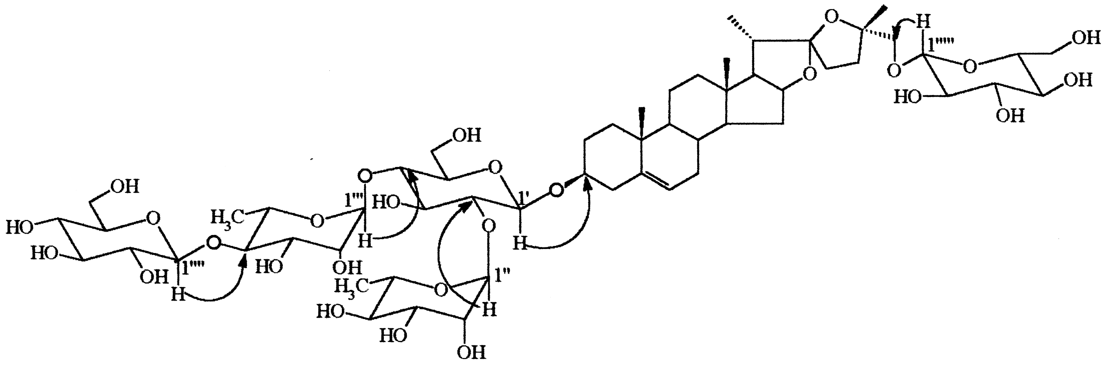

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Fig. 2. Selected HMBC correlations for multifidoside (**2**).

H-1'''' of glucose (δ_H 4.58) and C-4''' of rhamnose (δ_C 83.1), revealing the sites of the interglycosidic linkages in the sugar moiety attached to C-3 of the sapogenol.

Consequently, the structure of compound **2** was established as 3-*O*-{[α-L-rhamnopyranosyl-(1→2_{glu})]-[β-D-glucopyranosyl-(1→4_{rha})-α-L-rhamnopyranosyl (1→4_{glu})]-β-D-glucopyranosyl} nuatigenin 26-*O*-β-D-glucopyranoside.

For this novel structure, the trival name multifidoside is proposed by us.

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