

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

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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)-d-glucopyranosyl)-[β-d-glucopyranosyl(1→4)-l-rhamnopyranosyl (1→4)-d-glucopyranosyl]nuatigenin 26-O-β-d-glucopyranoside. Additionally, a known steroidal saponoside, aculeatiside A, 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 Phkidze, 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 furopspirostanol glycoside, multifidoside (2), from the aerial parts of V. fuhsii and V. multifida besides a known furopspirostanol 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-dihydroxy-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 isoscutellaren 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 Kızılcahamam-Isıkdağ 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 (Yıldızeli-Akdağ) 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 H₂O, followed by increasing concentrations of MeOH to yield four main fractions: A–D [A, 4.2 g (H₂O); B, 1.4 g (50% MeOH); C, 400 mg (75% MeOH); D, 1 g (MeOH)]. Fraction A₄ (1.4 g) was applied to MPLC using reversed phase material (LiChroprep C₁₈) with increasing amount of MeOH in H₂O. Fraction eluted with 75% MeOH was rechromatographed over Si gel using CH₂Cl₂-MeOH (9:1) solvent system to give 4 (20 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 Si gel by stepwise elution with CHCl₃-MeOH-H₂O (80:20:2) solvent system to give 1 (11 mg) and 2 (9 mg).

Aculeatiside A (1): IR νmax(KBr) cm⁻¹: 3420 (OH), 2929 (CH), 1456, 1039, 915, 860, 820. ¹H NMR (500 MHz, pyridine-d₅) (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 Hz, CH₃-21), 1.41 (3H, s, H-27). ¹³C NMR (125.6 MHz, pyridine-d₅), see Table II; HR-FAB-MS: (m/z) 1069.5 [M + Na]⁺.

Mulifidoside (2): [α]D = -78.0 (c 0.1, MeOH) IR νmax (KBr) cm⁻¹: 3418 (OH), 2927(CH), 1456, 1045, 910, 870, 820. ¹H NMR (300, 500 MHz, CD₃OD) (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). ¹³C NMR (125.6 MHz, CD₃OD), 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 C₅₁H₈₂O₂₂, requiring eleven degrees of unsaturation. The ¹H-NMR spectrum revealed the signals for six methyl groups at δH 0.81 (s, CH₃-18), 1.05 (s, CH₃-19), 1.08 (d, J = 7.0 Hz, CH₃-21), 1.41 (s, CH₃-27), 1.78 (d, J = 6.1 Hz, CH₃-6”), 1.64 (d, J = 6.1 Hz, CH₃-6”), an olefinic proton at δH 5.32 (brs, H-6), and four anomic protons at δH 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 (δH 4.95, 5.87*), J_AB = 10.0 Hz, H₂-26), predicting a tetracylicosidic steroidal structure. Thus, the signals at δH 1.64 and 1.78 arising from two secondary methyl protons were attributed to two rhamnose units. In the ¹³C-NMR spectrum, four anomic carbon signals were observed at δC 100.3, 105.5, 102.9 and 102.0, confirming the tetracylicosidic structure. All NMR assignments (Tables I, II) were based on COSY, HMOC 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 δC 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 decacylic. Hence, the aglycon was found to be hexacyclic. The ¹³C NMR data contained 27 carbon atoms for the steroidal aglycon. The quaternary carbon signals displayed at δC 120.3 and 83.9 indicated the dioxygenated C-22 carbon and C-25 of the aglycone, respectively, which are characteristic for the furospirostanol sapogenins (Agrawal et al., 1985). Thus, the signals at δC 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 H₂-26/C-25.
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 δC 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" (δH 5.87) and C-2' (δC 78.6); H-1" (δH 6.41) and C-4' (δC 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 H2-26/C-1" in the HMBC experiment. The signals attributed to compound 1 were in good agreement with those of aculeatiside A, a nuatigenin tetracygoside (Saijo et al., 1983). Therefore the structure of 1 was identified as aculeatiside A, 3-O-[α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl] nuatigenin 26-O-β-D-glucopyranoside.
Signals arising from an additional sugar unit. The δ five anomeric protons at δH NMR spectrum indicated the signals for six Glc-1 δH606 M. Ozipek et al. CH3-6 were attributed to two rhamnose units. The signals at δH and 13C 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 δH 0.81 (s, CH3-18), 1.05 (s, CH3-19), 0.99 (d, J = 7.0 Hz, CH3-21), 1.23 (s, CH3-27), 1.33 (d, J = 6.1 Hz, CH2-6i), 1.24 (d, J = 6.1 Hz, CH3-6i), an olefinic proton at δH 5.37 (d, J = 4.9), five anomic protons at δH 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 (δH 3.86 and 3.48, JAB = 10.0 Hz, H2-26), confirming the pentaglycosidic structure of compound 2. The signals at δH 1.24 and 1.33 arising from two secondary methyl protons were attributed to two rhamnose units. Five anomic carbon signals observed at δC 100.4, δC 105.5, δC 104.9, δC 102.2 and δC 102.6 in the 13C NMR spectrum verified the pentaglycosidic structure. The complete assignments (Tables I, II) of all proton and carbon resonances were based on the DQF-COSY, HSQSY, HMQC and HMBC experiments. The assignments attributed to the sugar moiety indicated two rhamnose and three glucose units. The 13C 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.

Table I. 1H NMR spectroscopic data for sugar moieties of aculeatiside A (1*) and multifidoside (2*) (Pyridine-d5*, CD3OD5).
nuatigenin, as compound 1. In the HBMC spectrum, the anomic proton of the glucose (H-1') (δ_H 4.49) exhibited a long-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 aglycone (δ_C 77.5) indicating the bisdesmosidic structure of 2. Additionally long-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);
H-1” of glucose (δH 4.58) and C-4” of rhamnose (δC 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)]β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranosyl] nuanigen 26-O-β-D-glucopyranoside.

For this novel structure, the trivial name multidoside is proposed by us.


