A New Flavonol Glycoside from the Aerial Parts of *Astragalus vulneraria*

Erdal BEDIR,*[a,b] Ihsan ÇALIS,[a] Sonia PIACENTE,[d] Cosimo PIZZA,[d] and Ikhlas A. KHAN[b,c]

Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University,[a] TR-06100 Ankara, Turkey, National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences,[b] and Department of Pharmacognosy, School of Pharmacy,[c] The University of Mississippi, University, MS 38677, U.S.A., and Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno,[d] 84084 Fisciano, Salerno, Italy.

Received June 1, 2000; accepted August 9, 2000

A new flavonol glycoside, isorhamnetin 3-*O*-β-D-apiofuranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-galactopyranoside, and the known diglycoside, isorhamnetin 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside were isolated from the aerial parts of *Astragalus vulneraria*. Characterization of the two compounds was done by spectroscopic methods (1D and 2D NMR, and FAB-MS).

Key words *Astragalus vulneraria*; Leguminosae; flavonol glycoside; isorhamnetin

In the flora of Turkey the genus *Astragalus* (Leguminosae) is represented by approximately 380 species which are listed under several sections and are of economic importance for the production of gum.[1] Earlier investigations performed on *Astragalus* species resulted in the isolation of three major classes of compounds, polysaccharides, saponins, and isoflavonoids.[2—5] We previously reported the isolation and the structure determination of a series of cycloartane-type triterpenic saponins from *Astragalus* species.[6—12] Continuing our studies on the constituents of *Astragalus* species, we investigated the aerial parts of *A. vulneraria*. This paper describes the isolation and structure elucidation of a new flavonol glycoside, isorhamnetin 3-*O*-β-D-apiofuranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-galactopyranoside (**1**), in addition to the known glycoside, isorhamnetin 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside (**2**).

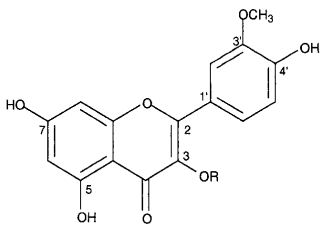
Compound (**1**) was isolated as a yellow amorphous powder. Its ^{1}H- and ^{13}C-NMR spectra showed the presence of aromatic systems and sugar moieties. The FAB mass spectrum of **1** in negative ion mode gave a quasi molecular ion peak $[M-H]^-$ at *m/z* 755. The fragments at *m/z* 623 $[(M-H)-132]^-$, 477 $[(M-H)-(132+146)]^-$ and 315 $[(M-H)-(132+146+162)]$ observed in FAB mass spectrum, together with the ^{13}C resonances suggested presence of one pentose unit, one deoxy hexose unit, and one hexose unit.

The ^{1}H-NMR resonances of two *m*-coupled doublets at δ 6.22 and 6.43 (J=1.5 Hz), which correlated with carbons at δ 99.8 (d), and 94.9 (d) in the HSQC spectrum, were characteristic of the two *meta*-related 6- and 8- protons with a 5,7-dihydroxy A-ring of a flavonoid.[13] A double doublet at δ 7.61 (J=7.5, 1.5 Hz), and two doublets at δ 6.93 (J=7.5 Hz), and 8.0 (J=1.5 Hz) were attributed to H-6′, H-5′, and H-2′, respectively, of the B ring. Additionally, the ^{1}H-NMR spectrum exhibited one singlet at δ 4.00 (3H) indicating the presence of a methoxy group, which showed a correlation with the carbon resonance at δ 148.4 (C-3′) in the HMBC spectrum. Based on these findings and by comparison of ^{1}H- and ^{13}C-NMR data with those of isorhamnetin 3-*O*-rutinoside,[13,14] the aglycone of **1** was identified as isorhamnetin.

The structure of the oligosaccharide unit was elucidated using 1D-TOCSY[15] and 2D NMR experiments. Selected 1D-TOCSY obtained by irradiating each anomeric proton resonance yielded subspectra of each sugar residue with high digital resolution. Each subspectrum contained the scalar-coupled protons within each sugar residue. In some cases, because of the small coupling constants, the distribution of magnetization around the spin system was impeded. Since in the TOCSY method the cross peaks represent both direct and relayed connectivities, we also recorded a DQF-COSY spectrum.[16] The results of 1D-TOCSY and DQF-COSY experiments allowed the sequential assignments of all proton resonances within each sugar residue, starting from the well isolated anomeric proton signals (Experimental). Thus, on the basis of the chemical shifts, multiplicity of the signals, absolute values of the coupling constants, and the three sugar residues were identified as α-rhamnopyranosyl, β-apiofuranosyl and β-galactopyranosyl.[17]

HSQC experiments,[18] which correlated all proton resonances with those of each corresponding carbon, permitted the assignments of the interglycosidic linkages by comparison of the observed carbon chemical shifts with those of the corresponding methylpyranosides, taking into account the known effects of glycosidation.[19] The absence of any ^{13}C-NMR glycosidation shift for the rhamnopyranosyl, and apiofuranosyl residues suggested these sugars to be terminal.

The position of each sugar residue was unambigously determined by the HMBC experiment[20] (Chart 2) which showed long-range correlations between C-3 (135.5, s) of the aglycon and H-1$_{gal}$ (δ 5.60, d, J=7.5 Hz), C-6$_{gal}$ (δ 67.3, t) and H-1$_{rha}$ (δ 4.55, d, J=1.5 Hz), C-2$_{gal}$ (δ 76.4, d) and H-1$_{api}$ (5.44, d, J=2.0 Hz). Resonances assigned to the sugar moieties of **1** were consistent with the presence of the same



1 R = —β-D-Gal (2→) β-D-Api
(6→) α-L-Rha

2 R = —β-D-Gal (2→) β-D-Api

Chart 1. Structures of **1** and **2**

* To whom correspondence should be addressed. e-mail: ebedir@olemiss.edu

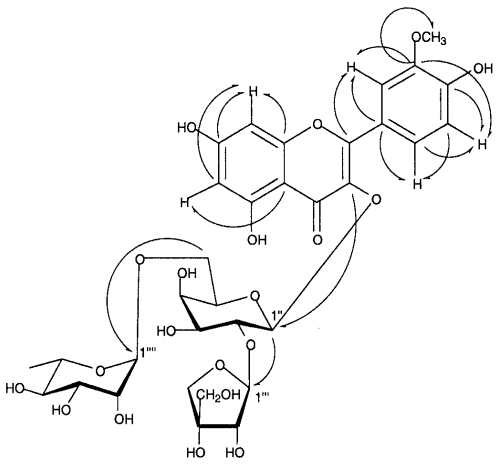


Chart 2. Key Heteronuclear Multiple-Bond Correlations (HMBC) of **1**

grouping to that found in kaempferol 3-*O*-β-D-apiofuranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-galactopyranoside,[21] confirming the proposed sugar sequence.

On the basis of these data, the structure of compound **1** was established as isorhamnetin 3-*O*-β-D-apiofuranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-galactopyranoside.

From the aerial parts of *A. vulneraria* isorhamnetin 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside (**2**) was also isolated and identified by comparison of its ^{1}H- and ^{13}C-NMR spectral data with literature values.[22]

Experimental

General Experimental A Bruker DRX-600 spectrometer operating at 599.19 MHz for ^{1}H and 150.86 for ^{13}C using the UXNMR software package was used for NMR measurements in CD_3OD solutions. 2D experiments: ^{1}H–^{1}H DQF-COSY[16] inverse detected ^{1}H–^{13}C HSQC[17] and HMBC[19] were obtained by employing the conventional pulse sequences as described previously. The selective excitation spectra, 1D TOCSY[15] were acquired using waveform generator-based GAUSS shaped pulses, mixing time ranging from 100 to 120 ms and a MLEV-17 spin-lock field of 10 kHz preceded by a 2.5 ms trim pulse. Optical rotations were measured on a Perkin-Elmer 141 polarimeter using a sodium lamp operating at 589 nm in 0.1% w/v solutions in MeOH. FAB-MS spectra were recorded in a glycerol matrix in the negative ion mode on a VG ZAB instrument (XE atoms of energy of 2—6 KV).

Plant Material *Astragalus vulneraria* DC. was collected from Polatli, Ankara, Central Anatolia, Turkey in June 1998. Voucher specimens have been deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Ankara.

Extraction and Isolation Air-dried aerial parts of *A. vulneraria* (380 g) were powdered and extracted with 80% EtOH (3.5 l×2) under reflux, and then filtered. The filtrate was concentrated to dryness *in vacuo* (27.7 g). The extract was subjected to VLC using normal-phase silica gel, employing pentane (200 ml, fr. 1), petroleum ether (400 ml, fr. 2), diethylether (500 ml, fr. 3), $CHCl_3$ (400 ml, fr. 4), $CHCl_3$–MeOH (90 : 10, 400 ml, fr. 5), and then $CHCl_3$–MeOH–H_2O mixtures (80 : 20 : 2, fr. 6; 70 : 30 : 3, fr. 7; 61 : 32 : 7, fr. 8, each 500 ml). fr. 7 (2.47 g) was subjected to column chromatography (silica gel, 60 g) eluted with $CHCl_3$–MeOH–H_2O mixtures (80 : 20 : 2, 100 ml; 70 : 30 : 3, 200 ml, 61 : 32 : 7, 400 ml) to give nine main fractions (Frs. A—I). Fraction B (55 mg) was chromatographed on a silica gel column (20 g), eluted with $CHCl_3$–MeOH–H_2O (70 : 30 : 3, 200 ml) to give compound **2** (26 mg). Fractionation of fr. 6 (300 mg) by open column chromatography (silica gel, 30 g) using EtOAc–MeOH–H_2O (90 : 17 : 13, 400 ml) gave compound **1** (16.5 mg).

Isorhamnetin-3-*O*-β-D-apiofuranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-galactopyranoside (**1**): $[\alpha]_D^{25}$ 38.4° (c=0.1, MeOH); ^{1}H-NMR (600 MHz, CD_3OD) aglycon moiety: δ: 8.00 (1H, d, J=1.5 Hz, H-2′), 7.61 (1H, dd, J=1.5, 7.5 Hz, H-6′), 6.43 (1H, d, J=1.5 Hz, H-8), 6.22 (1H, d, J=1.5 Hz, H-6), 4.00 (3H, s, $OC\underline{H}_3$); β-D-galactopyranose moiety: δ: 5.60 (1H, d, J=7.5 Hz, H-1″); 4.00 (1H, dd, J=7.5, 9.0 Hz, H-2″), 3.80 (1H, dd, J=4.0, 2.5 Hz, H-4″), 3.73 (1H, m, H-5″), 3.73 (1H, dd, J=12.0, 2.5 Hz, H-6a″), 3.67 (1H, dd, J=9.0, 4.0 Hz, H-3″), 3.47 (1H, dd, J=12.0, 4.5 Hz, H-6b″); β-D-apiofuranose moiety: δ: 5.44 (1H, d, J=2.0 Hz, H-1‴), 4.04 (1H, d, J=2.0 Hz, H-2‴), 4.04 (1H, d, J=10.0 Hz, H-4a‴), 3.73 (1H, br s, H-5a‴), 3.67 (1H, d, J=10.0 Hz, H-4b‴), 3.65 (1H, br s, H-5b‴); α-L-rhamnopyranose moiety: δ: 4.55 (1H, d, J=1.5 Hz, H-1⁗), 3.59 (1H, dd, J=1.5, 2.5 Hz, H-2⁗), 3.53 (1H, m, H-5⁗), 3.51 (1H, dd, J=8.4, 2.5 Hz, H-3⁗), 3.28 (t, J=8.4 Hz, H4⁗), 1.19 (d, J=6.5 Hz, H-6⁗); ^{13}C-NMR (150 MHz, CD_3OD) aglycon moiety: δ: 179.5 (s, C-4), 166.1 (s, C-7), 163.0 (s, C-5), 158.9 (s, C-2), 158.5 (s, C-9), 150.9 (s, C-4′), 148.4 (s, C-3′), 135.5 (s, C-3), 123.8 (d, C-6′), 123.0 (s, C-1′), 116.0 (d, C-5′), 114.6 (d, C-2′), 105.5 (s, C-10), 99.8 (d, C-6), 94.9 (d, C-8), 57.0 ($O\underline{C}H_3$); β-D-galactopyranose moiety: δ: 101.3 (d, C-1″), 76.4 (d, C-2″), 75.7 (d, C-5″), 75.5 (d, C-3″), 70.4 (d, C-4″), 67.3 (t, C-6″); β-D-apiofuranose moiety: δ: 110.7 (d, C-1‴), 79.5 (s, C-3‴), 78.0 (d, C-2‴), 75.7 (t, C-4‴), 66.5 (t, C-5‴); α-L-rhamnopyranose moiety: δ: 101.9 (d, C-1⁗), 73.5 (d, C-4⁗), 72.8 (d, C-3⁗), 72.1 (d, C-2⁗), 69.8 (d, C-5⁗), 18.1 (q, C-6⁗); FAB-MS *m/z* 755 $[M-H]^-$, 623 $[(M-H)-132]^-$, 477 $[(M-H)-(132+146)]^-$, 315 $[(M-H)-(132+146+162)]^-$.

Isorhamnetin-3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside (**2**): ^{1}H- and ^{13}C-NMR (600 MHz, CD_3OD) data were identical to those reported in the literature.[22]

References

1) Davis P. H., "Flora of Turkey and East Aegean Islands," University Press: Edinburgh 1970; Vol. 4, pp. 49—254.
2) Tang W., Eisenbrand G., "Chinese Drugs of Plant Origin," Springer-Verlag, Berlin, 1992.
3) Isaev M. I., Gorovits M. B., Abubakirov N. K., *Khim. Prir. Soedin.*, 156—175 (1988).
4) He Z. Q., Findlay J. A., *J. Nat. Prod.*, **54**, 810—815 (1991).
5) El-Sebakhy N., Asaad A. M., Abdallah R. M., Toaima S. M., Abdel-Kader S. M., Stermitz F. R., *Phytochemistry*, **36**, 1387—1389 (1994).
6) Calis I., Zor M., Saracoglu I., Isimer A., Rüegger H., *J. Nat. Prod.*, **59**, 1019—1023 (1996).
7) Bedir E., Calis I., Zerbe O., Sticher O., *J. Nat. Prod.*, **61**, 503—505 (1998).
8) Bedir E., Calis I., Aquino R., Piacente S., Pizza C., *J. Nat. Prod.*, **61**, 1469—1472 (1998).
9) Bedir E., Calis I., Aquino R., Piacente S., Pizza C., *J. Nat. Prod.*, **62**, 563—568 (1999).
10) Bedir E., Calis I., Aquino R., Piacente S., Pizza C., *Phytochemistry*, **51**, 1017—1020 (1999).
11) Calis I., Yusufoglu H., Zerbe O., Sticher O., *Phytochemistry*, **50**, 843—847 (1999).
12) Calis I., Yürüker A., Tasdemir D., Wright A. D., Sticher O., Luo Y. D., Pezzuto J. M., *Planta Med.*, **63**, 183—186 (1997).
13) Mabry T. J., Markham K. R., Thomas M. B., "The Systematic Identification of Flavonoids," Springer, Berlin, 1970, p. 261.
14) Harborne J. B., Mabry T. J., "The Flavonoids: Advances in Research," Chapman and Hall, New York, 1982, pp. 72—73.
15) Davis D. G., Bax A., *J. Am. Chem. Soc.*, **107**, 7198—7199 (1985).
16) Bodenhausen G., Freeman R., Morris G. A., Neidermeyer R., Turner J., *J. Magn. Reson.*, **25**, 559 (1977).
17) Agrawal P. K., Jain D. C., Gupta R. K., Thakur R. S., *Phytochemistry*, **24**, 2479—2496 (1985).
18) Bodenhausen G., Ruben D., *J. Chem. Phys. Lett.*, **69**, 185—186 (1980).
19) Breitmaier E., Voelter W., "Carbon-13 NMR Spectroscopy," VCH Verlagsgellsschaft Geulsh: Weinheim, Germany, 1987, pp. 380—393.
20) Martin G. E., Crouch R. C., *J. Nat. Prod.*, **54**, 1—70 (1991).
21) De Simone F., Dini A., Pizza C., Saturnino P., Schettino O., *Phytochemistry*, **29**, 3690—3692 (1990).
22) Burasheva G. Sh., Mukhamedyarova M. M., Chumbalov T. K., *Khim. Prir. Soedin.*, **11**, 426 (1975).