NPC Natural Product Communications

2009
Vol. 4
No. 1
77 - 82

Flavone and Flavonol Glycosides from *Astragalus eremophilus* and *Astragalus vogelii*

Angela Perrone[a], Milena Masullo[a], Alberto Plaza[a,§], Arafa Hamed[b] and Sonia Piacente[a*]

[a]*Dipartimento di Scienze Farmaceutiche*, *Università degli Studi di Salerno*, *via Ponte don Melillo*, *84084 Fisciano (SA) Italy*

[§]*Laboratory of Bioorganic Chemistry*, *National Institute of Diabetes and Digestive and Kidney Diseases*, *National Institutes of Health*, *Bethesda, Maryland 20892-0820*, *USA* (actual address)

[b]*Faculty of Science*, *South Valley University*, *81528 Aswan*, *Egypt*

piacente@unisa.it

Received: August 1st, 2008; Accepted: November 20th, 2008

Two new rhamnocitrin glycosides (**1** and **2**) were isolated from the aerial parts of *Astragalus vogelii*, along with one known rhamnocitrin glycoside (**3**). Two known flavonol glycosides (**4** and **5**) and four known flavone derivatives (**6**-**9**) were isolated from the aerial parts of *Astragalus eremophilus*. Their structures were elucidated by extensive spectroscopic methods including 1D- (^{1}H, ^{13}C and TOCSY) and 2D-NMR (DQF-COSY, HSQC, HMBC) experiments, as well as ESIMS analysis.

Keywords: *Astragalus*, flavonol glycosides, flavone glycosides, rhamnocitrin.

Astragalus L., the largest genus in the family Leguminosae, comprises ca 2000 species, grouped into more than 100 subdivisions. *Astragalus* spp. are widely distributed throughout the temperate regions of the world, located principally in Europe, Asia and North America. About 37 species grow in North Africa from Mauritania, Ethiopia and Egypt to Saudi Arabia [1]. *Astragalus* roots are very old and well-known drugs in traditional medicine for the treatment of nephritis, diabetes, leukemia, uterine cancer and as an antiperspirant, diuretic and tonic [2]. *Astragalus* spp. have shown interesting pharmacological properties, including hepatoprotective, immuno-stimulant and antiviral activities [3,4]. Anti-inflammatory, analgesic, sedative and cardiovascular activities are also reported [3,5].

Polysaccharides, triterpenoid saponins and flavonoids are the main constituents that have been isolated from *Astragalus* spp. *Astragalus* polysaccharides are reported to have anticancer and immunostimulating effects [3,4], while cycloartane- and oleanene-type glycosides were found to exert leishmanicidal, antibacterial and antiviral activities [3,4,6-12].

Flavonoids of the *Astragalus* genus have been widely investigated and in particular flavonol glycosides are the most abundant class in *Astragalus* spp. Derivatives of kaempferol, quercetin, myricetin, isorhamnetin, rhamnetin and rhamnocitrin [13-22] have been isolated from several *Astragalus* spp., along with derivatives of apigenin [23]. These aglycons are generally glycosylated by one or more sugar units, which are frequently linked at the 3-OH position and less often at either 7-OH or 4'-OH [17,18,24-26].

As a part of our ongoing research of secondary metabolites from species belonging to the flora of Egypt, we have investigated two species of *Astragalus*, *A. vogelii* and *A. eremophilus* Boiss. for their flavonoidic profile. *A.vogelii* (Webb) Bornm. (syn = *A. prolixus* Sieb.) is an annual grey-canescent plant, known as Taweel or Qarn [27]. It is highly valued as fodder by the Bedouin, who claim it encourages fertility and milk production in their animals [28]. *A. eremophilus* Boiss. (syn = *A. falcinellus* Boiss.) is an annual plant known by the Arabic names Faga'aye and Omm el-qorein [27].

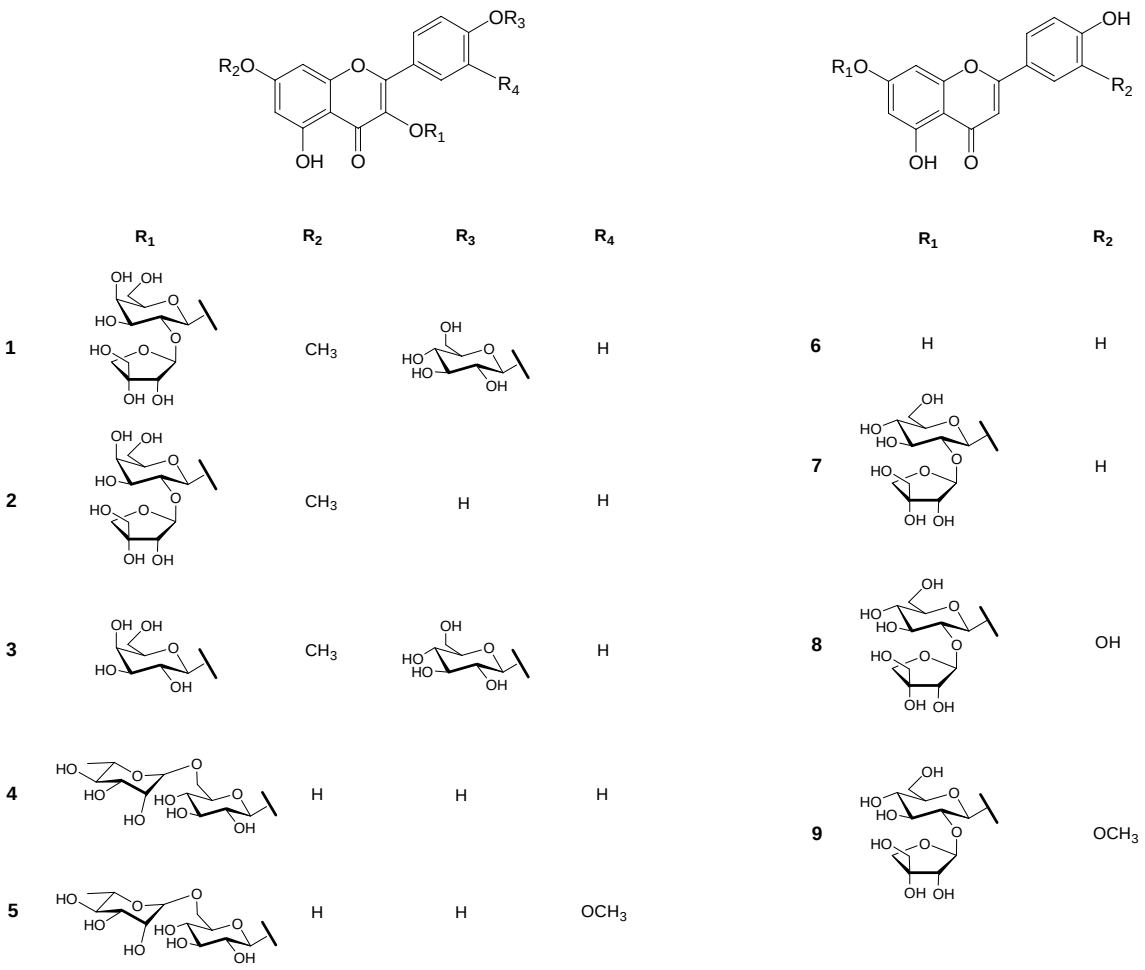

Figure 1: Compounds **1-9** isolated from the aerial parts of *Astragalus vogelii* and *A. eremophilus*..

This paper reports the isolation of two new flavonol glycosides (**1**, **2**), along with one known flavonol glycoside (**3**) from the aerial parts of *A. vogelii*, and two known flavonol glycosides (**4**, **5**), along with four known flavone derivatives from the aerial parts of *A. eremophilus* (**6-9**). Their structures were elucidated by extensive spectroscopic methods, including 1D- (^{1}H, ^{13}C and TOCSY) and 2D-NMR (DQF-COSY, HSQC, HMBC) experiments, as well as ESIMS analysis.

The EtOH extract of *A. vogelii* aerial parts was fractionated over Sephadex LH-20, obtaining the new compound **2**. The fractions containing flavonoid compounds were chromatographed by RP-HPLC to yield the new compound **1**, along with the known compound **3**. The EtOH extract of *A. eremophilus* aerial parts was fractionated over Sephadex LH-20. The fractions containing flavonoid compounds were chromatographed by RP-HPLC and further fractionated over Sephadex LH-20, followed by RP-HPLC to yield six known compounds, **4-9**.

The HRMALDITOF mass spectrum of **1** showed a major ion peak at *m/z* 779.2016 $[M+Na]^+$, ascribable to the molecular formula $C_{33}H_{40}O_{20}$ (calcd for $C_{33}H_{40}O_{20}Na$ 779.2011). The positive ESIMS spectrum of **1** gave the highest mass ion peak at *m/z* 779.1 $[M+Na]^+$ and a significant fragment in the MS/MS analysis at *m/z* 617.3 $[M+Na–162]^+$, due to the loss of an hexose unit.

The ^{1}H NMR spectrum of compound **1** showed signals for two *meta*-coupled aromatic protons at δ 6.35 (1H, d, J = 1.9 Hz, H-6) and δ 6.63 (1H, d, J = 1.9 Hz, H-8), two *ortho*-coupled aromatic protons at δ 7.24 (2H, d, J = 8.8 Hz, H-3', H-5') and δ 8.24 (2H, d, J = 8.8 Hz, H-2', H-4') and a signal corresponding to the methoxy group at δ 3.91 (3H, s). Comparison of these data with those reported in the literature allowed us the identification of the aglycon of **1** as 7-*O*-methyl-kaempferol, known as rhamnocitrin. To the authors' knowledge, rhamnocitrin was isolated for the first time as the 3-*O*-galactoside from *Anthyllis onobrychioides* (Leguminosae) [29].

Rhamnocitrin and its derivatives are often reported from the *Astragalus* genus, even though they are very unusual flavonols in the plant kingdom in comparison with other flavonols, such as kaempferol and quercetin [30].

The ^{1}H NMR spectrum displayed also signals corresponding to three anomeric protons at δ 5.57 (1H, d, *J* = 7.7 Hz), 5.46 (1H, d, *J* = 1.6 Hz) and 5.08 (1H, d, *J* = 7.5 Hz). The chemical shifts of all the individual protons of the four sugar units were ascertained from a combination of 1D-TOCSY and DQF-COSY spectral analysis, and the ^{13}C NMR chemical shifts of their attached carbons could be assigned unambiguously from the HSQC spectrum. These data showed the presence of one β-galactopyranosyl unit (δ 5.57), one β-apiofuranosyl unit (δ 5.46) and one β-glucopyranosyl unit (δ 5.08). The HMBC spectrum allowed us to establish the linkage sites of the sugar units: key correlation peaks between the proton signal at δ 5.46 (H-1_{api}) and the carbon resonance at δ 76.7 (C-2_{gal}), and the proton signal at δ 5.57 (H-1_{gal}) and the carbon resonance at δ 135.2 (C-3) were observed. The unusual 4'-*O*-glycosidation was confirmed by the diagnostic HMBC correlation between the proton signal at δ 5.08 (H-1_{glc}) and the carbon resonance at δ 160.7 (C-4'). Thus the structure of **1** was established as the new rhamnocitrin 3-*O*-[β-apiofuranosyl-(1→2)-β-galactopyranosyl]-4'-*O*-β-glucopyranoside.

The molecular formula of **2** was established to be $C_{27}H_{30}O_{15}$ by HRMALDITOFMS analysis (*m/z* 617.1489 $[M+Na]^+$, calcd for $C_{27}H_{30}O_{15}Na$, 617.1482). The positive ESIMS spectrum showed the major ion peak at *m/z* 617.2 $[M+Na]^+$. The MS/MS fragmentation showed peaks at *m/z* 485.0 $[M+Na–132]^+$, due to the loss of a pentose unit, *m/z* 323.0 $[M+Na–132–162]^+$, due to the loss of an hexose unit and at *m/z* 317.1 $[M+Na–300]^+$, ascribable to the loss of the aglycon moiety.

The ^{1}H NMR spectrum of compound **2** showed signals for two *meta*-coupled aromatic protons at δ 6.33 (1H, d, *J* = 1.9 Hz, H-6) and δ 6.61 (1H, d, *J* = 1.9 Hz, H-8), two *ortho*-coupled aromatic protons at δ 6.93 (2H, d, *J* = 8.8 Hz, H-3', H-5') and δ 8.16 (2H, d, *J* = 8.8 Hz, H-2', H-6'), and a signal corresponding to the methoxy group at δ 3.90 (3H, s). Additionally, for the sugar region of **2** in comparison with that of **1**, signals for only two anomeric protons at δ 5.54 (1H, d, *J* = 7.7 Hz, H-1_{gal}) and 5.48 (1H, d, *J* = 1.6 Hz, H-1_{api}) were observed in the ^{1}H NMR spectrum. These data, in combination with 1D-TOCSY, HSQC, HMBC, and DQF-COSY correlations, suggested that **2** differed from **1** only by the absence of the glucopyranosyl unit at C-4'. Therefore, compound **2** was identified as the new rhamnocitrin 3-*O*-β-apiofuranosyl-(1→2)-β-galactopyranoside.

Additionally, one known rhamnocitrin derivative, rhamnocitrin 3-*O*-β-galactopyranosyl-4'-*O*-β-glucopyranoside (**3**) was also isolated from the roots of *A. vogelii*, which is the first report of this compound from *Astragalus* genus.

Two known flavonol glycosides, kaempferol 3-*O*-α-rhamnopyranosyl-(1→6)-β-glucopyranoside (**4**) [31] and isorhamnetin 3-*O*-α-rhamnopyranosyl-(1→6)-β-glucopyranoside (**5**) [32], were isolated from the ethanol extract of the aerial parts of *A. eremophilus*, along with apigenin (**6**) [30], apiin (**7**) [33], and graveobiosides A (**8**) and B (**9**) [34]. Compounds **4**-**7** are often reported in *Astragalus* spp. while this is the first report of graveobiosides A and B from *Astragalus* genus.

Experimental

General procedures: Optical rotations were measured on a JASCO DIP 1000 polarimeter. UV measurements were obtained on a Beckman DU 670 spectrometer. IR spectra were obtained on a Bruker IFS-48 spectrometer. NMR experiments were performed on a Bruker DRX-600 spectrometer equipped with a Bruker 5 mm TCI CryoProbe at 300 K. All 2D-NMR spectra were acquired in CD_3OD (99.95%, Sigma Aldrich) and standard pulse sequences and phase cycling were used for DQF-COSY, HSQC, HMBC and ROESY spectra. Exact masses were measured by a Voyager DE mass spectrometer. Samples were analyzed by matrix-assisted laser desorption ionization time-of-flight (MALDITOF) mass spectrometry. A mixture of analyte solution and α-cyano-4-hydroxycinnamic acid (Sigma) was applied to the metallic sample plate and dried. Mass calibration was performed with the ions from ACTH (fragment 18-39) at 2465.1989 Da and angiotensin III at 931.5154 Da as internal standards. ESIMS analyses were performed using a ThermoFinnigan LCQ Deca XP Max ion-trap mass spectrometer equipped with Xcalibur software. Column chromatography was performed over Sephadex LH-20 (Pharmacia). Semi-preparative

RP-HPLC was performed on a Knauer Smartline Pump 1000 system equipped with a differential refractometer K-2301, a Waters XTerra Prep MSC_{18} column (300 x 7.8 mm i.d.), a Rheodyne injector and Clarity Lite software. TLC was performed on silica gel F254 (Merck) plates, and reagent grade chemicals (Carlo Erba) were used throughout.

Plant material: Fresh samples of *A. eremophilus* Boiss. aerial parts were collected at Hurghada (Red Sea, Egypt) in March 2004 and identified by Prof. M. G. Sheded. The voucher specimen (no. 11352) was deposited at the Botany Department Herbarium, Faculty of Science of Aswan, Egypt. Fresh samples of *A. vogelii* aerial parts were collected in March 2003 from the University Campus at Aswan, Egypt and identified by Prof. A. I. Hamed according to Täckholm [27]. The voucher specimens (no. 11323) are deposited in the Botany Department Herbarium, Aswan Faculty of Science, Egypt.

Extraction and isolation: The aerial parts (1100 g) of *A. vogelii* were powdered and exhaustively extracted with 80% EtOH (3 x 1.5 L) for 20 days by maceration at room temperature. The crude extract was concentrated under reduced pressure to a syrupy consistency (200 g). Part of the extract (2.5 g) was fractionated on Sephadex LH-20 (100 x 5 cm) using MeOH as the mobile phase. Ninety-five fractions (8 mL) were collected, obtaining compound **2** (8.1 mg) in fractions 51-52. Fractions 39-43 (47.7 mg) were chromatographed by RP-HPLC using MeOH-H_2O (9:11) as mobile phase (flow rate 2.5 mL/min) to yield compounds **1** (6.8 mg, t_R = 13.1 min) and **3** (4.0 mg, t_R = 16.2 min). The aerial parts (900 g) of *A. eremophilus* were extracted with 70% EtOH (3 x 1.5 L) for 20 days. The solvent was removed under reduced pressure to afford 57.9 g of crude extract. Part of the extract (2.5 x 3 g) was fractionated on Sephadex LH-20 (100 x 5 cm) using MeOH as the mobile phase. Sixty-nine fractions (8 mL) were obtained. Fractions 29-31 (29.6 mg) were chromatographed by RP-HPLC using MeOH-H_2O (9:11) as mobile phase (flow rate 2.0 mL/min) to yield compounds **5** (4.2 mg, t_R = 18.0 min) and **4** (3.5 mg, t_R = 20.0 min). The next fractions, containing flavonoid compounds (frs. 32-69, 1.79 g), were chromatographed on Sephadex LH-20 (100 x 5 cm) using MeOH:H_2O (3:2) as the mobile phase. Sixty-two fractions (8 mL) were obtained. Fractions 36-37 (25.8 mg) were chromatographed by RP-HPLC using MeOH-H_2O (57:43) as mobile phase (flow rate 2.0 mL/min) to yield compound **9** (3.8 mg, t_R = 8.8 min). Fractions 38-39 (10.3 mg) were chromatographed by RP-HPLC using MeOH-H_2O (3:2) as mobile phase (flow rate 2.0 mL/min) to yield compound **6** (2.6 mg, t_R = 11.1 min). Fractions 40-44 (11.0 mg) were chromatographed by RP-HPLC using MeOH-H_2O (1:1) as mobile phase (flow rate 2.0 mL/min) to yield compound **7** (2.3 mg, t_R = 8.7 min). Fractions 48-51 (10.0 mg) were chromatographed by RP-HPLC using MeOH-H_2O (9:11) as mobile phase (flow rate 2.0 mL/min) to yield compound **8** (2.5 mg, t_R = 9.0 min).

Rhamnocitrin 3-*O*-{Api(1→2)-Gal}-4'-O-Glc (**1**)

Amorphous yellow powder.
MP: 195-198°C.
$[\alpha]^{25}_D$: – 25.4 (*c* 0.2, MeOH).
IR (KBr) ν_{max}: 3415, 1655, 1250-1024 cm^{-1}.
UV (MeOH) λ_{max} (log ε): 262 (4.21), 290 (4.45), 358 (3.89) nm.
^{1}H NMR (600 MHz, CD_3OD): 6.35 (1H, d, *J* = 1.9 Hz, H-6), 6.63 (1H, d, *J* = 1.9 Hz, H-8), 8.24 (2H, d, *J* = 8.8 Hz, H-2', H-6'), 7.24 (2H, d, *J* = 8.8 Hz, H-3', H-5'), 3.91 (3H, s, OCH_3), sugars 5.57 (1H, d, *J* = 7.7 Hz, H-1_{gal}), 3.95 (1H, dd, *J* = 7.7, 9.7 Hz, H-2_{gal}), 3.70 (1H, dd, *J* = 9.7, 3.2 Hz, H-3_{gal}), 3.83 (1H, dd, *J* = 6.0, 3.2 Hz, H-4_{gal}), 3.49 (1H, m, H-5_{gal}), 3.63 (1H, dd, *J* = 6.0, 11.3 Hz, H-$6a_{gal}$), 3.58 (1H, dd, *J* = 6.0, 11.3 Hz, H-$6b_{gal}$), 5.46 (1H, d, *J* = 1.6 Hz, H-1_{api}), 4.07 (1H, d, *J* = 1.6 Hz, H-2_{api}), 4.05 (1H, d, *J* = 9.7 Hz, H-$4a_{api}$), 3.71 (1H, d, *J* = 9.7 Hz, H-$4b_{api}$), 3.74 (1H, d, *J* = 11.3 Hz, H-$5a_{api}$), 3.64 (1H, d, *J* = 11.3 Hz, H-$5b_{api}$), 5.08 (1H, d, *J* = 7.5 Hz, H-1_{glc}), 3.54 (1H, dd, *J* = 7.5, 9.0 Hz, H-2_{glc}), 3.54 (1H, dd, *J* = 9.0, 9.1 Hz, H-3_{glc}), 3.45 (1H, dd, *J* = 9.1, 9.1 Hz, H-4_{glc}), 3.54 (1H, m, H-5_{glc}), 3.96 (1H, dd, *J* = 3.5, 12.0 Hz, H-$6a_{glc}$), 3.76 (1H, dd, *J* = 5.0, 12.0 Hz, H-$6b_{glc}$).
^{13}C NMR (150 MHz, CD_3OD): 158.8 (C, C-2), 135.2 (C, C-3), 179.0 (C, C-4), 163.0 (C, C-5), 98.7 (CH, C-6), 167.2 (C, C-7), 92.6 (CH, C-8), 158.2 (C, C-9), 106.2 (C, C-10), 125.5 (C, C-1'), 131.9 (CH, C-2', C-6'), 116.9 (CH, C-3', C-5'), 160.7 (C, C-4'), 56.0 (CH_3, OCH_3), 100.8 (CH, C-1_{gal}), 76.7 (CH, C-2_{gal}), 75.3 (CH, C-3_{gal}), 70.2 (CH, C-4_{gal}), 76.8 (CH, C-5_{gal}), 61.7 (CH_2, C-6_{gal}), 110.5 (CH, C-1_{api}), 77.8 (CH, C-2_{api}), 80.4 (C, C-3_{api}), 75.2 (CH_2, C-4_{api}), 65.8 (CH_2, C-5_{api}), 101.4 (CH, C-1_{glc}), 74.6 (CH, C-2_{glc}), 77.9 (CH, C-3_{glc}), 71.1 (CH, C-4_{glc}), 77.9 (CH, C-5_{glc}), 62.1 (CH_2, C-6_{glc}).
ESIMS: *m/z* 779.1 $[M+Na]^+$.
ESIMS/MS: *m/z* 617.3 $[M+Na–162]^+$.
HRMALDITOFMS: *m/z* $[M+Na]^+$ calcd for $C_{33}H_{40}O_{20}Na$: 779.2011; found 779.2016.

Rhamnocitrin-3-*O*-{Api-(1→2)-Gal} (**2**)

Amorphous yellow powder.
MP: 180-185°C.
$[\alpha]^{25}_{D}$: – 47.3 (*c* 0.2, MeOH).
IR (KBr) ν_{max}: 3420, 1650, 1271-1050 cm^{-1}.
UV (MeOH) λ_{max} (log ε): 266 (4.32), 285 (4.58), 354 (3.94) nm.
^{1}H NMR (600 MHz, CD_3OD): 6.33 (1H, d, *J* = 1.9 Hz, H-6), 6.61 (1H, d, *J* = 1.9 Hz, H-8), 8.16 (2H, d, *J* = 8.8 Hz, H-2', H-6'), 6.93 (2H, d, *J* = 8.8 Hz, H-3', H-5'), 3.90 (3H, s, OCH_3), sugars 5.54 (1H, d, *J* = 7.7 Hz, H-1_{gal}), 3.97 (1H, dd, *J* = 7.7, 9.7 Hz, H-2_{gal}), 3.72 (1H, dd, *J* = 9.7, 3.2 Hz, H-3_{gal}), 3.85 (1H, dd, *J* = 6.0, 3.2 Hz, H-4_{gal}), 3.48 (1H, m, H-5_{gal}), 3.63 (1H, dd, *J* = 6.0, 11.3 Hz, H-$6a_{gal}$) 3.58 (1H, dd, *J* = 6.0, 11.3 Hz, H-$6b_{gal}$), 5.48 (1H, d, *J* = 1.6 Hz, H-1_{api}), 4.09 (1H, d, *J* = 1.6 Hz, H-2_{api}), 4.08 (1H, d, *J* = 9.7 Hz, H-$4a_{api}$), 3.73 (1H, d, *J* = 9.7 Hz, H-$4b_{api}$), 3.75 (1H, d, *J* = 11.3 Hz, H-$5a_{api}$), 3.66 (1H, d, *J* = 11.3 Hz, H-$5b_{api}$).
^{13}C NMR (150 MHz, CD_3OD): 158.9 (C, C-2), 135.3 (C, C-3), 179.0 (C, C-4), 163.6 (C, C-5), 98.5 (CH, C-6), 167.7 (C, C-7), 92.6 (CH, C-8), 158.7 (C, C-9), 106.8 (C, C-10), 122.8 (C, C-1'), 132.3 (CH, C-2', C-6'), 116.1 (CH, C-3', C-5'), 162.2 (C, C-4'), 56.2 (CH_3, OCH_3), 100.7 (CH, C-1_{gal}), 76.8 (CH, C-2_{gal}), 75.3 (CH, C-3_{gal}), 70.4 (CH, C-4_{gal}), 76.9 (CH, C-5_{gal}), 61.7 (CH_2, C-6_{gal}), 110.6 (CH, C-1_{api}), 77.9 (CH, C-2_{api}), 80.9 (C, C-3_{api}), 75.2 (CH_2, C-4_{api}), 66.0 (CH_2, C-5_{api}).
ESIMS: *m/z* 617.2 $[M+Na]^+$.
ESIMS/MS: *m/z* 485.0 $[M+Na–132]^+$, 323.0 $[M+Na–132–162]^+$, 317.1 $[M+Na–300]^+$.
HRMALDITOFMS: *m/z* $[M+Na]^+$ calcd for $C_{27}H_{30}O_{15}Na$: 617.1482; found 617.1489.

References

[1] Boulos L. (**1999**) *Flora of Egypt* (Azollaceae-Oxalidaceae) 1th ed., Alhadara Publishing, Cairo, Egypt, 419.

[2] Tang W. (**1992**) *Chinese Drugs of Plant Origin*, ed. Springer-Verlag, Berlin, 191.

[3] Rios LJ, Waterman PG. (**1997**) A review of the pharmacology and toxicology of *Astragalus*. *Phytotherapy Research*, *11*, 411-418.

[4] Verotta L, El-Sebakhy NA. (**2001**) *Studies in Natural Products Chemistry: Bioactive Natural Products*, Elsevier: Amsterdam, Vol. ***25*** (Part F).

[5] Isaev MI, Gorovits MB, Abubakirov NK. (**1989**) Progress in the chemistry of cycloartanes. *Chemistry of Natural Compounds*, ***25***, 131-147.

[6] Çaliş İ, Yuruker A, Tasdemir D, Wright AD, Sticher O, Luo Y-D, Pezzuto JM. (**1997**) Cycloartane triterpene glycosides from the roots of *Astragalus melanophrurius*. *Planta Medica*, ***63***, 183-186.

[7] Bedir E, Pugh M, Çaliş İ, Pasco DS, Khan IA. (**2000**) Immunostimulatory effects of cycloartane-type triterpene glycosides from *Astragalus species*. *Biological & Pharmaceutical Bulletin*, ***23***, 834-837.

[8] Yesilada E, Bedir E, Çaliş İ, Takaishi Y, Ohmoto Y. (**2005**) Effects of triterpene saponins from *Astragalus* species on *in vitro* cytokine release. *Journal of Ethnopharmacology*, ***96***, 71-77.

[9] Özipek M, Dönmez AA, Çaliş İ, Brun R, Rüedi P, Tasdemir D. (**2005**) Leishmanicidal cycloartane-type triterpene glycosides from *Astragalus oleifolius*. *Phytochemistry*, ***66***, 1168-1173.

[10] Verotta L, Guerrini M, El-Sebakhy NA, Asaad AM, Toaima SM, Abou-Sheer ME, Luo Y-D, Pezzuto JM. (**2001**) Cycloartane saponins from *Astragalus peregrinus* as modulators of lymphocyte proliferation. *Fitoterapia*, ***72***, 894-905.

[11] Verotta L, Guerrini M, El-Sebakhy NA, Asaad AM, Toaima SM, Abou-Sheer ME, Luo Y-D, Pezzuto JM. (**2002**) Cycloartane and oleanane saponins from Egyptian *Astragalus* spp. as modulators of lymphocyte proliferation. *Planta Medica*, ***68***, 986-994.

[12] Abdallah RM, Ghazy NM, El-Sebakhy NA, Pirillo A, Verotta L. (**1993**) Astragalosides from Egyptian *Astragalus spinosus* Vahl. *Pharmazie*, ***48***, 452-454.

[13] Alaniya MD, Komissarenko NF, Kemertelidze EP. (**1975**) Ascaside, a new flavonol glycoside of *Astragalus caucasicus*. *Khimiya Prirodnykh Soedinenii*, ***11***, 351-354.

[14] Nakabayashi T. (**1952**) Partition chromatography of tannins and pigments. XII. A flavonoid pigment astragalin (kaempferol-3-glucoside) in *Astragalus sinicus* flowers. *Nippon Nogei Kagaku Kaishi*, ***26***, 539-541.

[15] Norris FA, Stermitz FR. (**1970**) 4'-*O*-methylquercetin 3-glucoside from *Astragalus miser* var oblongifolius. *Phytochemistry*, ***9***, 229-230.

[16] Cui B, Kinjo J, Nakamura M, Nohara T. (**1991**) Constituents of leguminous plants. 25. A novel acylated flavonoid glycoside from *Astragalus complanatus*. *Tetrahedron Letters*, ***32***, 6135-6138.

[17] Cui B, Sakai Y, Takeshita T, Kinjo J, Nohara T. (**1992**) Constituents of leguminous plants. XXIV. Four new oleanene derivatives from the seeds of *Astragalus complanatus*. *Chemical & Pharmaceutical Bulletin*, ***40***, 136-138.

[18] Bedir E, Çalis İ, Piacente S, Pizza C, Khan IA. (**2000**) A new flavonol glycoside from the aerial parts of *Astragalus vulneraria*. *Chemical & Pharmaceutical Bulletin*, ***48***, 1994-1995.

[19] Semmar N, Jay M, Chemli R. (**2001**) Chemical diversification trends in *Astragalus caprinus* (Leguminosae), based on the flavonoid pathway. *Biochemical Systematics and Ecology*, ***29***, 727-738.

[20] Semmar N, Fenet B, Lacaille-Dubois M, Glucoff-Fiasson K, Chemli R, Jay M. (**2001**) Two new glycosides from *Astragalus caprinus*. *Journal of Natural Products*, ***64***, 656-568.

[21] Semmar N, Fenet B, Glucoff-Fiasson K, Comte G, Jay M. (**2002**) New flavonol tetraglycosides from *Astragalus caprinus*. *Chemical & Pharmaceutical Bulletin*, ***50***, 981-984.

[22] Semmar N, Fenet B, Glucoff-Fiasson K, Hasan A, Jay M. (**2002**) Four new flavonol glycosides from the leaves of *Astragalus caprinus*. *Journal of Natural Products*, ***65***, 576-579.

[23] Benbassat N, Nikolov S. (**1995**) Flavonoids from *Astragalus onobrychis. Planta Medica*, ***61***, 100.

[24] Cui B, Nakamura M, Kinjo J, Nohara T. (**1993**) Constituents of leguminous plants. XXXV. Chemical constituents of *Astragali semen*. *Chemical & Pharmaceutical Bulletin*, ***41***, 178-182.

[25] Yahara S, Kohjyouma M, Kohoda H. (**2000**) Flavonoid glycosides and saponins from *Astragalus shikokianus*. *Phytochemistry*, ***53***, 469-471.

[26] Guzhva NN, Sarsikov LC, Zaitsev VP. (**1992**) Polyphenol composition of the epigeal part of *Astragalus eximius*. *Chemistry of Natural Compounds*, ***28***, 120.

[27] Täckholm V. (**1974**) *Students' Flora of Egypt*, 2nd ed., Cairo University, Printed by Cooperative Printing Company, Beirut, 888.

[28] Badri M, Hamed AI. (**2000**) Nutrient value of some plants in an extremely arid environment (Wadi Allaqi Biosphere Reserve, Egypt). *Journal of Arid Environment*, ***44***, 347-356.

[29] Marco JA, Barberá O, Sanz JF, Sánchez-Parareda J. (**1986**) A new flavonol diglycoside from *Anthyllis onobrychioides*. *Journal of Natural Products*, ***49***, 151-152.

[30] Harborne JB, Mabry TJ. (**1982**) *The Flavonoids: Advances in Research*, Chapman & Hall, New York, 307.

[31] Sang S, Cheng X, Zhu N, Stark RE, Badmaev V, Ghai G, Rosen RT, Ho C-T. (**2001**) Flavonol glycosides and novel iridoid glycoside from the leaves of *Morinda citrifolia*. *Journal of Agricultural and Food Chemistry*, ***49,*** 4478-4481.

[32] Senatore F, D'Agostino M, Dini I. (**2000**) Flavonoid glycosides of *Barbarea vulgaris* L. (Brassicaceae). *Journal of Agricultural and Food Chemistry*, ***48***, 2659-2662.

[33] Subramanian SS, Nagarajan S. (**1970**) Flavonoids of three *Crotalaria* species. *Phytochemistry*, ***9***, 2581-2582.

[34] Farooq MO, Gupta SR, Kiamuddin M, Rahman W, Seshadri TR. (**1953**) Chemical examination of celery seeds. *Journal of Scientific & Industrial Research*, ***12B***, 400-407.