Triterpenoid Saponins from *Astragalus corniculatus*

Ilina Krasteva[a], Stefan Nikolov[a], Maki Kaloga[b], and Gisela Mayer[c]

[a] Department of Pharmacognosy, Faculty of Pharmacy, 2 Dunav St., 1000 Sofia, Bulgaria
[b] Institut für Pharmazie, Pharmazeutische Biologie, Königin Luise Straße 2+4, D-14195 Berlin, Germany
[c] Schering AG, Abt. IPCH Strukturanalyse, D-13353, Berlin, Germany

Reprint requests to Dr. I. Krasteva. Fax: (+359 2) 9879874. E-mail: ikrasteva@pharmfac.acad.bg

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Two new triterpenoid saponins were isolated from the ethanolic extract of the aerial parts of *Astragalus corniculatus* Bieb. The structures of the saponins were elucidated as 3β-O-[O-4-oxo-pentopyranosyl-(1 $\rightarrow$ 2)-β-D-glucopyranosyl]-21α-hydroxyolean-12-ene-28-oic acid (**1**) and 21α-hydroxyolean-12-ene-28-oic acid 3β-4-oxo-pentopyranoside (**2**) by chemical and spectral methods.

Key words: Astragalus corniculatus, Fabaceae, Triterpenes

Introduction

Astragalus corniculatus Bieb. (Fabaceae) is grown in North Bulgaria and distributed in Southeastern Romania, South Ukraine and Moldova. Many species of *Astragalus* L. have been recorded as yielding a wide range of saponins. Although tetracyclic triterpene saponins are widely distributed in genus *Astragalus*, pentacyclic triterpenes do not often occur [1 – 4]. In a previous work we reported on a protective effect of purified mixture of saponins from *A. corniculatus* Bieb. against myeloid Graffi tumors [5]. However, no work has been reported on the saponins of this species.

The present paper describes the isolation and identification of two new oleanane type saponins (**1** and **2**) from *A. corniculatus*.

Results and Discussion

Solvent partition and repeated chromatographic purification over silica gel and Sephadex LH-20 of the saponin mixture obtained from ethanolic extract of the aerial parts of *Astragalus corniculatus* afforded two new triterpenoid saponins, **1** and **2**. Sapogenin **1a** was obtained after acid hydrolysis of a saponin mixture, followed by column chromatography on Sephadex LH-20 and preparative TLC. Acid and enzymatic hydrolysis of **1** and **2** afforded the same genin **1a**, D-glucose and 4-oxo-pentose.

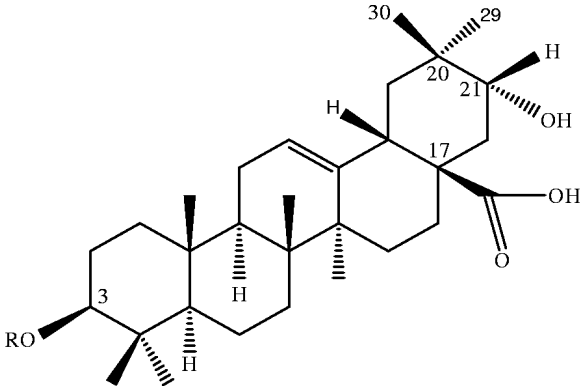
The negative ion FAB-MS spectrum of compound **1** showed the quasi-molecular ion [M-H]$^-$ peak at $m/z = 763$ together with sodium adducts at $m/z = 785$ $[(\mathrm{M}-\mathrm{H}+\mathrm{Na})-\mathrm{H}]^-$ and $m/z = 807$ $[(\mathrm{M}-2\mathrm{H}+2\mathrm{Na})-\mathrm{H}]^-$, and two other important ion peaks appeared at $m/z = 633$ [(M-H)-130]$^-$ and $m/z = 471$ [(M-H)-130-162]$^-$ indicating the respective elimination of 130 mass units and a glucosyl moiety. The positive ion FAB-MS of **1** exhibited a $[(\mathrm{M}-2\mathrm{H}+2\mathrm{Na})+\mathrm{H}]^+$ peak at $m/z = 809$. Its HR-ESI MS spectrum showed the [M-H]$^-$ ion peak at $m/z = 763.4328$, corresponding to the molecular formula $C_{41}H_{64}O_{13}$. The ^{1}H NMR spectrum of saponin **1** and the less polar compound **2** exhibited seven Me singlets and signals for an olefinic proton, and two oxymethine proton resonances at $\delta_\mathrm{H} = 3.21$ (overlap.) and 3.42 (br t-like, $J = 3.50$ Hz) for **1**, and $\delta = 3.20$ (dd, $J = 11.70$, 4.30 Hz, H-3α) and 3.42 (overlap.) for **2**, respectively. The ^{1}H and ^{13}C NMR data of **1** clearly revealed the presence of two sugar moieties. The anomeric proton signals appeared at $\delta_\mathrm{H} = 4.67$ (d, $J = 7.70$ Hz, H-1') and 4.45 (d, $J = 7.40$ Hz, H-1"), which showed 1,2 diaxial coupling. In the HMBC spectrum correlations were observed between these protons (H-1' and H-1") and C-3 and C-2', respectively. This identified the points of attachment of the sugar units as C-3 ($\delta_\mathrm{C} = 91.46$) and C-2' ($\delta = 81.33$).

The EIMS of sapogenin **1a** showed a fragment at $m/z = 454$ generated from the [M]$^+$ (absent in the spectrum). The presence of significant peaks at $m/z = 246$ [RDA product]$^+$ and $m/z = 201$ [246-COOH]$^+$ (base peak), suggested the presence of one hydroxyl group and a carboxyl group in the D/E rings. These fragments were recorded in the HR-EIMS at

Table 1. NMR spectral data of **1a** in CD_3OD (δ values).

Position	^{13}C	^{1}H	HMBC
1	39.85	1.64/1.10	
2	28.20	2.20, dt, 13.80, 3.00, H-2ax 1.79, m, H-2eq	
3	79.84	3.14, dd, 11.30, 4.80, H-3α	H_3-23, H_2-24
4	39.96	–	H_3-23, H_3-24
5	56.93	0.75, brd, 11.00, H-5	H_3-23, H_3-24, H_3-25
6	19.58	1.55/1.40	
7	34.39	1.34, m, H_2-7	H_3-26
8	40.20	–	H_3-26, H_3-27
9	49.32	1.58	H-12, H_3-25, H_3-26
10	38.25	–	
11	24.62	1.88, m, H_2-11	
12	122.49	5.23, t-like, 3.40, H-12	
13	146.63	–	H_2-11, H-18, H_3-27
14	40.34	–	H-18
15	29.60	–	H_3-27
16	24.97	1.62/1.58	
17	49.00	–	H-18, H-21
18	43.16	2.99, br dd, 13.70, 3.40	H-12, H-19ax
19	43.66	2.06, t-like, 13.70, H-19ax 0.97, m, H-19eq, overlap. with H_3-29	H-18, H-21, H_3-29, H_3-30
20	36.20	–	H-18, H-19ax, H-21, H_3-29, H_3-30
21	75.92	3.42, br t-like, 3.50, H-21β	H_3-29, H_3-30
22	41.25	1.90/1.70	
23	28.78	0.97, s, H_3-23	H-3α, H_3-24
24	16.33	0.77, s, H_3-24	H-3α, H_3-23
25	15.99	0.94, s, H_3-25	
26	18.26	0.88, s, H_3-26	
27	25.94	1.15, s, H_3-27	
28	180.38	–	H-22b
29	27.96	0.98, s, H_3-29	H-19ax, H-21, H_3-30
30	25.80	0.89, s, H_3-30	

$m/z = 454.34459$ (calcd. 454.34467 for $C_{30}H_{46}O_3$), $m/z = 246.16195$ (calcd. 246.16198 for $C_{16}H_{22}O_2$) and $m/z = 201.16422$ (calcd. 201.16433 for $C_{15}H_{21}$). In the desorption chemical ionisation (DCI) spectrum, **1a** gave a molecular ion peak at $m/z = 472$ [$C_{30}H_{48}O_4$]. The ^{1}H NMR spectrum of **1a** displayed signals for seven tertiary methyl groups at $\delta_H = 0.77$, 0.88, 0.89, 0.94, 0.97, 0.98, and 1.15 (each s), an olefinic proton at 5.23 (t-like, $J = 3.40$ Hz), and one-proton signal due to H-18 β, characteristic of olean-12-ene-28-oic acid type triterpenes [6] was observed at $\delta = 2.99$ (dd, $J = 13.70, 3.40$). This ^{1}H NMR spectrum indicated also the existence of two hydroxylated methine signals at $\delta_H = 3.14$ (dd, $J = 11.30, 4.80$ Hz, H-3α) and $\delta = 3.42$ (br t-like, $J = 3.50$ Hz). The position of the second OH group was determined to be C-21 by HMBC correlations of H-21 with C-17 ($\delta_C = 49.00$), C-19 ($\delta = 43.66$), C-20 ($\delta = 36.20$), C-29 ($\delta = 27.96$), and C-30 ($\delta = 25.80$) (Table 1).



1a: R = H;
1 : R = 4-oxo-pentopyranosyl-(1 → 2)-β-D-glcp;
2 : R = 4-oxo-pentopyranosyl.

Further the proton signal H-21 at $\delta = 3.42$ was observed as broad triple t-like signal ($J = 3.50$ Hz). This confirmed that the hydroxyl group on ring E of **1a** must be α-located [7]. The NOE associations in the NOESY spectrum between H-21 / H_3-29 and H_3-30 supported the α-configuration of the 21-hydroxy group.

The structure of **1a** was therefore determined as 3β,21α-dihydroxyolean-12-ene-28-oic acid and **1** as 3β-O-[O-4-oxo-pentopyranosyl-(1 → 2)-β-D-glucopyranosyl]-21α-hydroxyolean-12-ene-28-oic acid.

Compound **2** showed the quasi-molecular ion $[M-H]^-$ at $m/z = 601$ and a prominent fragment ion peak at $m/z = 471$ $[(M-H)-130]^-$ in the negative ion FAB-MS spectrum. The positive FAB-MS revealed an $[(M-2H+2Na)+H]^+$ ion at $m/z = 647$. Its HR-ESI MS spectrum gave the $[M-H]^-$ ion peak at $m/z = 601.3798$, indicating the molecular formula $C_{35}H_{54}O_8$. The ^{13}C NMR spectrum of **2** was similar to that of **1** but featured one less glucosyl moiety, and displayed a signal characteristic of an anomeric carbon at $\delta_C = 106.72$ (C-1'). The glycosidic connectivity in **2** was further confirmed by correlation between anomeric proton ($\delta = 4.34$, d, $J = 7.70$ Hz) and C-3 ($\delta = 90.96$) of the aglycone. On the other hand, the large coupling constant ($J = 7.70$ Hz) observed for this anomeric proton indicated it to be axially oriented.

Thus, we conclude that saponin **2** is 21α-hydroxyolean-12-ene-28-oic acid 3β-4-oxo-pentopyranoside.

Structure determination of these compounds was established on the basis of spectral data. The ^{13}C and ^{1}H NMR spectra of **1**, **1a** and **2** have been assigned using ^{13}C DEPT, ^{1}H, ^{1}H COSY, HETCOR, HMBC and NOESY techniques.

Experimental Section

General

Melting points are uncorrected. Optical rotations were measured with a Perkin-Elmer 343 polarimeter. IR spectra were obtained with a Shimadzu FTIR-8101 M spectrometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100.6 MHz) spectra were recorded on Bruker DPX – 400 and Bruker AMX – 400 instruments using TMS as internal standard. All spectra were recorded in CD_3OD. HMBC experiments were optimised for $^{2-3}J_{H/C} = 8$ Hz. EIMS, HR-EIMS, FAB-MS and DCI/NH_3 spectra were carried out Varian MAT CH_7A, Finnigan MAT 711, Finnigan MAT CH_5DF and Finnigan TSQ 700 spectrometers, respectively. HR-ESI MS spectra were recorded on a Q-Tof Dremier mass spectrometer (Firma Waters). Thin-layer chromatographic study (TLC) was carried out on Kieselgel 60 F_{254} (0.24 mm thick, Merck) plates, using the solvent systems 1-BuOH-AcOH-H_2O (4 : 1 : 1) and $CHCl_3$-MeOH (25 : 1). The spots were visualized by spraying with anisaldehyde/conc. H_2SO_4 (for saponins and sapogenins) and thymol/conc. H_2SO_4 solution (for sugars), followed by heating at 110 °C. Column chromatography (CC) was carried out with Sephadex LH-20 and silica gel 60 (70 – 230 mesh, Merck). Prep. TLC was performed on silica gel plates (Kieselgel 60, 0.5 mm thick, Merck).

Plant material, extraction and isolation

Astragalus corniculatus Bieb. herbs were collected in July 1999 in Northern Bulgaria. The plant was identified by Dr. D. Pavlova from the Department of Botany, Faculty of Biology, Sofia University, where voucher specimen has been deposited (SO95265). The air-dried plant material (1 kg) was powdered and extracted exhaustively with 50% EtOH. The extract was filtrated, concentrated under reduced pressure and successively treated with $CHCl_3$ and EtOAc respectively. The residue was dissolved in MeOH. After filtration and addition of Me_2CO a precipitate (45 g) was obtained. 20 g of the precipitate were chromatographed on a silica gel column eluting with $CHCl_3$-MeOH-H_2O (98 : 72 : 9) to give five crude fractions (A – E). Fr. C was repeatedly chromatographed on Sephadex LH-20 eluting with MeOH and on silica gel ($CHCl_3$-MeOH-H_2O 98 : 72 : 9) to yield a fraction, containing compounds **1** and **2**. This fraction (182 mg) was purified by prep. TLC on silica gel G developed in 1-BuOH-AcOH-H_2O (4 : 1 : 1) to give **1** (23 mg) and **2** (20 mg). Another 20 g of the precipitate were refluxed with 8% H_2SO_4 for 6 h and separated by centrifugation and filtration. The obtained solution was neutralized and evaporated to give 6 g of a crude sapogenin mixture, which was separated into 10 fractions (each 150 ml) by CC on Sephadex LH-20 eluted with methanol to give three main fractions (I – III). Fr. II was rechromatographed on Sephadex LH-20 with MeOH and appropriate fractions were combined (IIa – IIc). Fraction IIb (129 mg) was subjected to prep. TLC with $CHCl_3$-MeOH (25 : 1) to afford a sapogenin **1a** (15 mg).

Isolated sapogenin and saponins

3β, 21α*-dihydroxyolean-12-ene-28-oic acid* (**1a**): Amorphous powder, m. p. 240 – 243 °C; $[\alpha]_D^{20} = +15.3°$ (MeOH, *c* 0.30). – ^{1}H and ^{13}C NMR: (Table 1). – MS (EI, 70 eV): *m*/*z* (rel. int.) 454 [M-H_2O]$^+$, 246 [RDA product], 201 (base peak); DCI/NH_3: *m*/*z* = 472 [M]$^+$; HR-EIMS *m*/*z* = 454.34459 (calcd. 454.34467 for $C_{30}H_{46}O_3$).

3β*-O-[O-4-oxo-pentopyranosyl-*(1 → 2)*-*β*-D-glucopyranosyl]-*21α*-hydroxyolean-12-ene-28-oic acid* (**1**): Amorphous powder, m. p. 191 – 192 °C; $[\alpha]_D^{20} = +8°$ (MeOH, *c* 0.35). – IR (KBr) ν_{max} 3421 (OH), 2929, 1725 (C=O), 1618, 1075, 1044 cm^{-1}. – ^{1}H NMR (400 MHz, CD_3OD): $\delta = 0.85$, 0.87, 0.90, 0.95, 0.98, 1.07, 1.15 (3H each, tertiary methyls), 0.77 (br d, *J* = 11.00 Hz), 3.21 (overlap., H-3α), 3.42 (br t-like, *J* = 3.50 Hz, H-21β), 4.67 (d, *J* = 7.70 Hz, H-1'), 3.86 (br d, *J* = 11.00 Hz, H-6'b), 3.61 (dd, *J* = 11.00, 6.00 Hz, H-6'a), 4.45 (d, *J* = 7.40 Hz, H-1"), 3.23 – 3.60 (8H, glycosyl protone). – ^{13}C NMR (100.6 MHz, CD_3OD): δ = 39.91 (C-1), 27.10 (C-2), 91.46 (C-3), 40.45 (C-4), 57.14 (C-5), 19.45 (C-6), 34.38 (C-7), 40.43 (C-8), 48.11 (C-9), 37.87 (C-10), 24.59 (C-11), 122.60 (C-12), 146.40 (C-13), 41.72 (C-14), 28.61 (C-15), 25.75 (C-16), 48.78 (C-17), 43.35 (C-18), 43.55 (C-19), 37.09 (C-20), 76.32 (C-21), 41.19 (C-22), 28.50 (C-23), 16.96 (C-24), 16.07 (C-25), 18.19 (C-26), 25.90 (C-27), (C-28 not observed), 27.92 (C-29), 25.76 (C-30), 104.67 (C-1'), 81.33 (C-2'), 77.84 (C-3'), 71.89 (C-4'), 78.25 (C-5'), 63.06 (C-6'), 105.38 (C-1"), 75.80 (C-2"), 78.25 (C-3"), 205.32 (C-4"), 73.54 (C-5"). – FAB-MS (negative): *m*/*z* = 763 [M-H]$^-$, 633 [(M-H)–130]$^-$, 471 [(M-H)–130-162]$^-$; FAB-MS (positive): *m*/*z* = 809 [(M-2H + 2Na) + H]$^+$; *m*/*z* = 764 [M]$^+$; HR-ESI MS (negative ion mode) *m*/*z* = 763.4328 [M-H]$^-$ (calcd. for $C_{41}H_{64}O_{13}$, 763.4323).

21α*-hydroxy olean-12-ene-28-oic acid* 3β*-4-oxo-pentopyranoside (**2**):* Amorphous powder, m. p. 232 – 235 °C. – $[\alpha]_D^{20} = +3.2°$ (MeOH, *c* 0.25). – ^{1}H NMR (400 MHz, CD_3OD): $\delta = 0.85$, 0.91, 0.92, 0.95, 0.98, 1.05, 1.16 (3H each, tertiary methyls), 0.80 (br d, *J* = 11.20 Hz), 3.20 (dd, *J* = 11.70, 4.30 Hz, H-3α), 3.42 (overlap. H-21β), 5.24 (m, H-12), 4.34 (d, *J* = 7.70 Hz, H-1'), 3.24 (dd, *J* = 8.80, 7.70 Hz, H-2'), 3.39 (d, *J* = 8.80 Hz, H-3'), 3.41 (d, *J* = 9.40 Hz, H-5'a), 3.56 (d, *J* = 9.40 Hz, H-5'b). – ^{13}C NMR (100.6 MHz, CD_3OD): δ = 39.93 (C-1), 27.85 (C-2), 90.96 (C-3), 40.49 (C-4), 57.18 (C-5), 19.42 (C-6), 34.38 (C-7), 40.23 (C-8), 48.37 (C-9), 37.98 (C-10), 24.62 (C-11), 122.70 (C-12), 145.68 (C-13), 41.25 (C-14), 29.63 (C-15), 25.00 (C-16), 48.60 (C-17), 43.34 (C-18), 43.04 (C-19), 37.14 (C-20), 75.73 (C-21), 41.17 (C-22), 28.61 (C-23), 17.08

(C-24), 16.11 (C-25), 18.20 (C-26), 25.87 (C-27), 180.46 (C-28), 27.98 (C-29), 25.82 (C-30), 106.70 (C-1'), 75.51 (C-2'), 78.08 (C-3'), 208.31 (C-4'), 73.72 (C-5'). – FAB-MS (negative): $m/z = 601$ [M-H]$^-$, 471 [(M-H)–130]$^-$, 623 [(M-H+Na)–H]$^-$, 645 [(M-2H+2Na)+H]$^-$, FAB-MS (positive): $m/z = 647$ [(M-2H+2Na)+H]$^+$; $m/z = 602$ [M]$^+$; HR-ESI MS (negative ion mode) $m/z = 601.3798$ [M-H]$^-$ (calcd. for $C_{35}H_{53}O_8$, 601.3795).

Acid hydrolysis: Each glycoside (5 mg) was refluxed with 7% methanolic HCl (5 ml) for 3 h. The MeOH was evaporated, the mixture was diluted with H_2O, and the hydrolysate was partitioned between EtOAc and H_2O. The aglycone-containing organic phase was concentrated, and analysed by TLC. The aglycone of **1** and **2** found to be identical with sapogenin **1a**. The aqueous layer was neutralized with Ag_2CO_3, filtered, concentrated, and tested for carbohydrates by co-TLC with authentic samples using EtOAc-MeOH-HAc-H_2O (12 : 3 : 3 : 2) as eluent. In addition the filtrate from **2** was evaporated to afford a residue, which was chromatographed on a silica gel column (20 g) with $CHCl_3$-MeOH (1 : 0, 2 l; 95 : 5, 5 l) as eluent to give 4-oxo-pentose (*ca*. 1.2 mg). Positive FAB-MS *m/z*: 131 [(M+H)-H_2O]$^+$, 172 [M+H+Na]$^+$.

Enzymatic hydrolysis: The glycosides (**1** and **2** respectively, 2 mg) were dissolved in 5 ml of H_2O at 40 °C. After cooling to r. t. CH_3COOH (10%) was added to pH 5.5. To this mixture 2 mg of Luizym®, an enzyme preparation from *Aspergillus oryzae* (Luitpold Pharma, D-81379 München) was appended. After 5 d at r. t. in the dark the sediment was removed by filtration, the liquid was concentrated to 50 ml under reduced pressure, and subsequently extracted three times with 50 ml of $CHCl_3$.

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