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Cycloartane Glycosides from *Astragalus erinaceus*

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Abstract: One new cycloartane-type saponin, 3-*O*-[β-D-xylopyranosyl-(1→2)-β-D-xylopyranosyl]-6-*O*-β-D-glucuronopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxyxyxloartane (**1**) was isolated from the MeOH extract of whole plant parts of *Astragalus erinaceus* along with 5 known saponins (**2**-**6**), cyclodissectoside, cycloastragenol, 6-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxycycloartane, oleifolioside B and 3,6-di-*O*-β-D-xylopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxycycloartane, respectively. Their structures were established by the extensive use of 1D- and 2D-NMR experiments along with ESIMS and HRMS analysis. The glucuronic acid moiety in cycloartanes is a very unusual finding.

Keywords: *Astragalus erinaceus;* Leguminosae; cycloartane; glucuronic acid; saponin.

1. Introduction

The legume genus *Astragalus* L., with an estimated 2500 species of herbaceous perennial and annual species in the subfamily Papilionoideae of the Fabaceae [1]. In Turkey there are 445 species, of which 224 are endemic [2,3]. The dried roots of some *Astragalus* species (Radix Astragali) are well known in traditional medicine as remedy for animal bites and poisons, eye diseases, wounds and burns, nephritis, diabetes mellitus, hypertension, cirrhosis, throat diseases, leukaemia and uterine cancer. They are also famed for their antimicrobial, antiperspirant, anti-inflammatory, diuretic and tonic effects [4-6]. Earlier investigations on Turkish *Astragalus* species resulted in the isolation of a series of oleanane- and cycloartane-type triterpenoidal saponins [7-15]. Previous studies have shown that cycloartane- and oleanane-type glycosides isolated from *Astragalus* species show interesting biological properties, including immunostimulating [11,16,17], anti-protozoal [18], antiviral [19], cytotoxic [20], cardiotonic [21], wound healing [22] and adjuvant activities [23]. As a part of our ongoing research of new bioactive compounds from Turkish *Astragalus* species, we carried out a study on *Astragalus erinaceus* Fisch. et Mey. ex Fischer (Leguminosae).

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This paper reports the isolation of one new cycloartane-type triterpene glycoside (**1**) from the methanol extract of the whole plant of *A. erinaceus* along with five known cycloartane-type glycosides (**2**-**6**). Their structures were elucidated by extensive spectroscopic methods including 1D- (^{1}H, ^{13}C and TOCSY) and 2D-NMR (DQF-COSY, HSQC, HMBC, and ROESY) experiments as well as ESIMS and HRMS analysis.

2. Materials and Methods

2.1. General Procedures

Optical rotations were measured on a JASCO DIP 1000 polarimeter. IR measurements were obtained on a Bruker IFS-48 spectrometer. NMR experiments were performed on a Bruker DRX-600 spectrometer (Bruker BioSpinGmBH, Rheinstetten, Germany) equipped with a Bruker 5 mm TCI CryoProbeat 300 K. All 2D-NMR spectra were acquired in CD_3OD (99.95%, SigmaAldrich) and standard pulse sequences and phase cycling were used for DQF-COSY, HSQC, and HMBC spectra. The NMR data were processed using UXNMR software. 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 standard. ESIMS analyses were performed using a ThermoFinnigan LCQ Deca XP Max iontrap mass spectrometer equipped with Xcalibur software. GC analysis was performed on a Termo Finnigan Trace GC apparatus using a l-Chirasil-Val column (0.32 mm x 25 m).

2.2 Plant Material

Astragalus erinaceus Fisher & C.A.Mey. was collected from Gürpınar Village, north of Koçgüden village, from altitude of 2820 m, Van, Turkey in September 2010, and was identified by Dr. Fevzi Özgökçe (Department of Biology, Faculty of Science & Art, Yüzüncü Yıl University, Van, Turkey). Voucher specimen has been deposited in the Herbarium of Yüzüncü Yıl University, Van, Turkey (VANF 13824).

2.3 Extraction and Isolation

Air-dried and grinded plant material (400 g) were extracted with MeOH (2x4 L) at 60 °C. After filtration, the solvent was removed by rotary evaporation yielding (40.87g) of MeOH extract. The MeOH extract was dissolved in H_2O (200 mL), and successively partitioned with *n*-hexane (2x200 mL), CH_2Cl_2 (3x200 mL), and *n*-BuOH saturated with H_2O (3x150 mL). The n-BuOH extract (16.61g) was subjected to vacuum liquid chromatography (VLC) using reversed-phase material (RP-18) employing H_2O (600 mL), H_2O-MeOH (8:2, 1650 mL; 6:4 , 2550 mL; 4:6, 1800 mL; 3:7, 3450 mL; 2:8, 2700 mL) and MeOH (1800 mL) to give 6 main fractions. Fraction 4 (2.51g) was applied to an open column chromatography using silica gel (370 g) as stationary phase. Elution performed with $CHCl_3$-MeOH-H_2O (80:20:2) to give compound **5** (43.9 mg). Subfraction 4.1 (417 mg) was subjected to silica gel column chromatoghraphy (114 g) with the solvent system $CHCl_3$-MeOH-H_2O (75:25:2.5) to give compound **6** (130.5mg). The main fraction 3 (1.99g) from RP-VLC column was subjected to silica gel column chromatography (258g) with the solvent system $CHCl_3$-MeOH-H_2O (80:20:2) to give compound **4** (20mg), compound **2** (8.8 mg) and compound **1** (19.0mg). The main fraction 6

(543mg) from RP-VLC column was applied to silica gel CC (100 g). Elution was performed with $CHCl_3$-MeOH (9:1) to yield compound **3** (15.9mg)

2.4 Acid Hydrolysis and GC Analysis

A solution of compound 1 (1 mg) in 1 N HCl was heated at 80 ^oC for 4 h. The mixture was cooled at 0 ^oC and then concentrated by blowing with N_2. The residue was dissolved in 1-(trimethylsilyl)-imidazole and pyridine (0.1 mL), and the solution was stirred at 60 oC for 5 min. After drying the solution with a stream of N_2, the residue was partitioned between H_2O and CH_2Cl_2 (1 mL each), with the organic layer was analysed by GC using an L-Chirasil-Val column (0.32 mm x 25 m). Temperatures of the injector and detector were 200 ^oC for both. A temperature gradient system was used for the oven, starting at 100 ^oC for 1 min and increasing up to 180 ^oC at a rate of 5 ^oC /min. The peaks from the hydrolysate of 1 were detected at 10.96 and 12.02 (D-xylose) and 15.83 min (D-glucuronic acid). Retention times for authentic samples in the same experimental conditions were detected at 10.96 and 12.02 (D-xylose)), and 5.90 min (L-rhamnose), 15.81min (D-glucuronic acid), respectively.

3. Results and Discussion

Compound **1** was obtained as white powder with $[\alpha]_D$ + 30.8 (c= 0.1, MeOH), and its molecular formula was determined to be $C_{46}H_{77}O_{19}$ by HRESIMS data (955,4882 $[M + Na]^+$, calcd 955,4879). The ESIMS spectrum showed a major ion peak at *m/z* 955 which was assigned to $[M+Na]^+$. The MS/MS of this ion showed a peak at *m/z* 779 $[M+Na-176]^+$, corresponding to the loss of a glucuronopyranosyl unit. In the MS3 spectrum peaks at *m/z* 629 $[M+Na-176–150]^+$, corresponding to the loss of a pentose unit, and 479 $[M+Na-176–150–150]^+$, due to the loss of a pentose unit, were observed.

The ^1H NMR spectrum of **1** showed signals due to a cyclopropane methylene at δ 0.59 and 0.24 (each 1H, d, J = 4.2 Hz), six tertiary methyl groups at δ 1.30 (3H, s), 1.18 (6H, s), 1.17 (3H, s) and 1.00 (6H, s), a secondary methyl group at δ 0.97 (d, J = 6.5 Hz), and four methine proton signals at δ 4.44 (ddd, J = 8.0, 8.0, 5.2 Hz), 3.53 (ddd, J = 9.5, 9.5, 4.5), 3.40 (dd, J = 10.5, 2.4 Hz) and 3.22 (dd, J = 11.2, 4.0 Hz), which were indicative of secondary alcoholic functions. The NMR data of the aglycon moiety of **1** were in good agreement with those reported for cyclocanthogenin with glycosidation shifts for C-3 (δ 89.7) and C-6 (δ 79.1) [24].

For the sugar region in the ^1H NMR spectrum, three anomeric protons at δ 4.58 (d, J = 7.5 Hz), 4.57 (d, J = 7.5 Hz) and 4.33 (d, J = 7.2 Hz) were observed. Complete assignments of the ^1H and ^{13}C NMR signals of the sugar portion were accomplished by 1D-TOCSY, HSQC, HMBC and DQF-COSY experiments which led to the identification of two β-xylopyranosyl units (δ 4.58, δ 4.57) and one β-glucuronoyranosyl unit (δ 4.33). The determination of the sequence and linkage sites was obtained from the HMBC correlations which showed key correlation peaks between the proton signals at δ 4.58 (H-1_{xyl}) and the carbon resonance at δ 82.5 (C-2_{xyl}), δ 4.57 (H-1_{xyl}) and δ 89.7 (C-3) and the proton signal at δ 4.33 (H-1_{glcA}) and the carbon resonance at δ 79.1 (C-6). The D configuration of glucuronic acid and xylose units were established after hydrolysis of **1** followed by GC analysis [25, 26]. Thus, the compound **1** was identified as 3-*O*-[β-D-xylopyranosyl-(1→2)-β-D-xylopyranosyl]-6-*O*-β-D-glucuronopyranosyl-3β,6α,16β,24(*S*),25- pentahydroxyxyxloartane.

Additionally, five known cycloartane-type triterpene glycosides, cyclodissectoside (**2**) [27], cycloastragenol (**3**) [28], 6-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxycycloartane (**4**) [12], oleifolioside B (**5**) [18] and 3,6-di-*O*-β-D-xylopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxycycloartane (**6**) [12] were isolated.

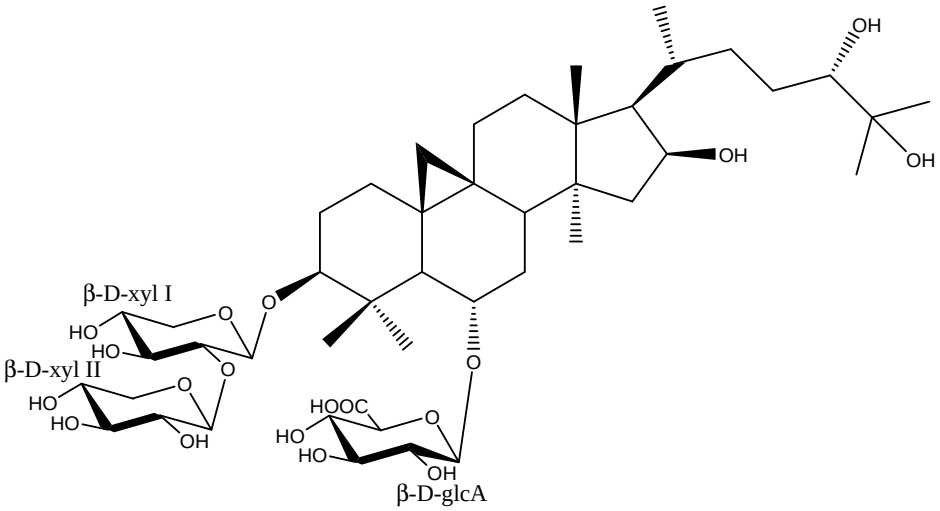

Figure 1 Structure of compound **1**.

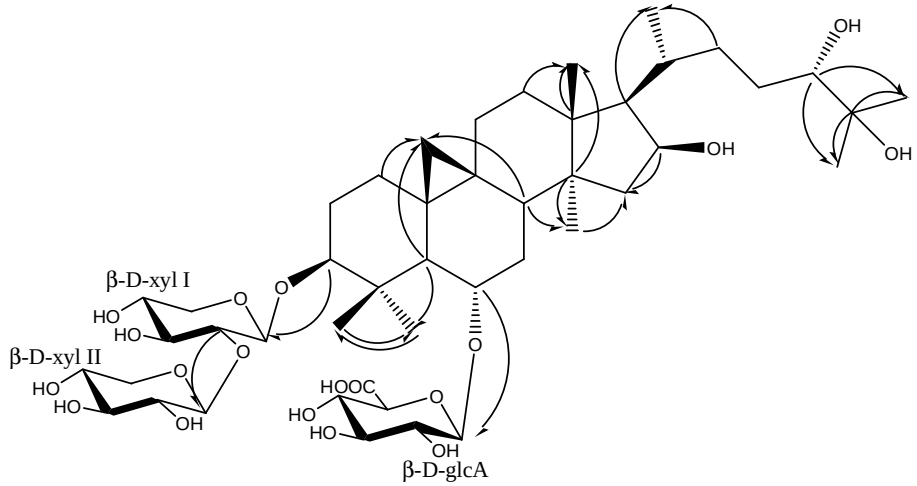

Figure 2 Key HMBC of compound **1**.

Table 1. ^{13}C and ^{1}H NMR data (*J* in Hz) of the aglycon and sugar moieties of compound **1** (600Mz, δ ppm, in CD_3OD).

Position	δ_C	δ_H (*J* in Hz)	Position	δ_C	δ_H (*J* in Hz)
1	32.5	1.29,1.58, m			β-D-Xyl I (at C-3)
2	30.2	1.96,1.70, m	1	105.0	4.57, d (7.5)
3	89.7	3.22, dd (11.2, 4.0)	2	82.5	3.59, dd (7.5, 9.2)
4	42.8	-	3	77.0	3.60, t (9.2)
5	52.8	1.63,d (9.5)	4	70.8	3.53, m
6	79.1	3.53, ddd (9.5, 9.5, 4.5)	5	65.6	3.88, dd (5.2, 11.7) 3.23, t (11.7)
7	34.1	1.90,1.65, m			β-D-Xyl II (at C-2_{xyl})
8	46.2	1.89, dd (12.0, 4.2)	1	105.0	4.58, d (7.5)
9	22.0	-	2	76.7	3.36, dd (7.4, 9.0)
10	29.5	-	3	77.0	3.38, t (9.0)
11	26.8	1.84, 1.42, m	4	70.7	3.53, m
12	33.8	1.81, m	5	65.6	3.88, dd (5.2, 11.7) 3.23, t (11.7)
13	46.2	-			β-D-GlcA (at C-6)
14	46.6	-	1	104.9	4.33, d (7.2)
15	47.6	1.41, dd (12.7, 5.2) 2.07, dd (12.7, 8.0)	2	76.7	3.28, dd (7.5, 9.0)
16	72.8	4.44, ddd (8.0, 8.0, 5.2)	3	77.2	3.46, dd (9.0, 9.0)
17	57.7	1.72, dd (9.9, 8.0)	4	73.2	3.46, dd (9.0, 9.0)
18	18.4	1.17, s	5	76.7	3.59, d (9.0)
19	17.7	0.24, d (4.2) 0.59, d (4.2)	6	176.2	-
20	29.7	1.90, m			
21	18.6	0.97, d (6.5)			
22	33.8	1.24, 1.81, m			
23	28.7	1.65,1.45, m			
24	78.0	3.40, dd (10.5, 2.4)			
25	73.5	-			
26	25.2	1.18, s			
27	25.2	1.18, s			
28	28.5	1.30, s			
29	16.5	1.00, s			
30	20.3	1.00, s			

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP.

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