Macrophyllosaponin E: A Novel Compound from the Roots of *Astragalus oleifolius*

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Macrophyllosaponin E, a novel cycloartane-type triterpene, has been isolated from the roots of *Astragalus oleifolius*. The structure elucidation of the compound was achieved by a combination of one- and two-dimensional NMR techniques [^{1}H–^{1}H-correlation spectroscopy (COSY), ^{1}H–^{13}C-heteronuclear multiple guantum correlation spectroscopy (HMQC), and ^{1}H–^{13}C-heteronuclear multiple-bond correlation spectroscopy (HMBC)] and high resolution electrospray ionization Fourier transformation mass spectrometry (HR-ESI-FT-MS).

Key words *Astragalus oleifolius*; cycloartane-type triterpene; macrophyllosaponin E, Leguminosae

Astragalus L., the largest genus in the family Leguminosae, is represented by 380 species in the flora of Turkey.[1)] The roots of *Astragalus* species represent a very old and well-known drug in traditional medicine for its usage as an antiperspirant, diuretic and tonic drug. It has also been used in the treatment of diabetes mellitus, nephritis, leukemia and uterine cancer.[2)] In the district of Anatolia, located in South Eastern Turkey, an aqueous extract of the roots of *Astragalus* is traditionally used against leukemia and for its wound-healing properties. Known biologically active constituents of *Astragalus* roots represent two major classes of compounds, polysaccharides and saponins.[2)] *Astragalus* polysaccharides are already known to have anticancer and immune enhancing properties in both *in vitro* and *in vivo* experiments.[3—5)] Chemical studies on *Astragalus* saponins have reported the presence of cycloartane-type triterpenoid glycosides which were found to exert biological activities (*e.g.* anti-inflammatory, analgesic, diuretic, hypotensive and sedative effects).[6)]

Our earlier investigations performed on *Astragalus* species resulted in the isolation of a series of cycloartane-type triterpenic saponins,[7—13)] as well as the compounds which were evaluated in lymphocyte stimulation tests showed immunomodulatory activity. The activity is relatively potent and thereby deserves further attention.[8)]

We previously reported the isolation and the structure determination of macrophyllosaponins A—D from *Astragalus oleifolius*.[7)] In our continuing search, we have isolated a novel cycloartane-type triterpene glycoside, named as macrophyllosaponin E (**1**). This paper deals with the isolation and the structural elucidation of **1**.

The high resolution electrospray ionization Fourier transformation mass spectrometry (HR-ESI-FT-MS) spectra of **1** exhibited ion peak for $[M+Na]^+$ at *m/z* 839.4721, which is compatible with the molecular formulae $C_{42}H_{72}O_{15}$.

Taking into account the results of our comprehensive ^{1}H- and ^{13}C-NMR studies and previous knowledge derived from metabolites isolated from the genus *Astragalus*,[7—13)] the main features of a cycloartane-type triterpene were evident: characteristic signals due to cyclopropane-methylene protons as an AX system (δ 0.44, 0.79, J_{AX}=4.5 Hz, H_2-19), six tertiary methyl groups (δ 0.89, 1.05, 1.07, 1.09, 1.2, 1.27; respectively, H_3-29, H_3-18, H_3-30, H_3-28, H_3-27, H_3-26) and a secondary methyl group (0.95 d, J=6.3; H_3-21). Additionally, the resonances for two anomeric protons were observed at δ 4.54 (d, J=7.8 Hz) and 4.36 (d, J=7.8 Hz), indicative of the presence of two β-linked sugar units. Thus, **1** was considered to be a cycloartane-type triterpene diglycoside. This observation was supported by the ^{13}C-NMR spectral data of **1** (Table 1). The ^{1}H- and ^{13}C-NMR data (Table 1) supported the assignment of the sugar moieties in **1** as two β-D-glucopyranose.[14)] The remaining carbon and proton resonances were consistent with $C_{30}H_{52}O_5$ for the aglycon moiety. This implied five saturated ring systems because there were no olefinic protons.

Among the sugar signals, the remaining signals showed correlations with the resonances at highfield, indicating the presence of four protons geminal to oxygenated carbons of the sapogenol moiety (δ 3.47, dd, J=5.8, 11.6 Hz, H-24; δ 3.54, dd, J=3.6, 2.7 Hz, H-1; δ 3.57 ddd, 10.5, 8.3, 5.0 Hz, H-7; δ 3.73, dd, J=4.3, 12.0 Hz, H-3). The resonances for the oxygenated carbons also indicated the presence of four oxymethine carbons (δ 70.2, d, C-7; δ 73.1, d, C-1; δ 77.0, d, C-24; δ 84.1, d, C-3) and an oxygenated quaternary carbon (δ 80.5, s, C-25).

To complete the assignment of the chemical shifts of the triterpene skeleton and its substitution pattern had to be determined for which task the ^{1}H–^{1}H-correlation spectroscopy (COSY) spectrum proved to be most useful. Detailed examination of this spectrum indicated the presence of seven spin systems (Chart 2). In order to establish the interfragment relationship, a heteronuclear multiple-bond correlation experiment (HMBC) was performed (Chart 2). Thus, the anomeric proton at δ 4.36 (d, J=7.8 Hz) showed a long range correlation with the carbon resonance at δ 84.10 (d, C-3) while the anomeric proton at δ 4.54 (d, J=7.8 Hz) exhibited a long range correlation with the carbon resonance at δ 80.50 (s, C-25), indicating the bidesmosidic structure of **1**.

^{13}C-NMR data for C-24 is comparable to those reported for analagous compounds having a 24(*S*) configuration.[15,16)] It has to be mentioned that the ^{13}C-NMR data can be regarded as characteristic parameters in the determination of absolute configurations of C-24. In the case of 24(*R*) configuration $\delta_{C\text{-}24}$ gives resonance at 80.0—80.5 ppm,[17,18)] while for the 24(*S*) configuration $\delta_{C\text{-}24}$ gives resonance at 77.0—77.2

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Table 1. ^{1}H- and ^{13}C-Assignments of **1**, (in CD_3OD; at 500 and 125 MHz, Respectively)

C/H	δ (ppm), J (in Hz)	δ (ppm)	C/H	δ (ppm), J (in Hz)	δ (ppm)
1	3.54 dd (2.7, 3.6)	73.1 d	23	1.45 m	28.0 t
2	2.17 m, 1.86 ddd (11.8, 2.7, 1.6)	36.4 t	24	3.47 dd (5.8, 11.6)	77.0 d
3	3.73 dd (12.0, 4.3)	84.1 d	25	—	80.5 s
4	—	40.7 s	26	1.27 s	22.0 q
5	2.16 t (12.0, 4.8)	39.3 d	27	1.23 s	21.6 q
6	1.05 m, 1.75 m	31.0 t	28	1.09 s	24.8 q
7	3.57 ddd (10.5, 8.3, 5.0)	70.2 d	29	0.89 s	13.5 q
8	1.56 m	55.1 d	30	1.07 s	18.2 q
9	—	21.0 s			
10	—	30.4 s	1′	4.36 d (7.8)	105.7 d
11	2.27 m, 1.36 m	26.0 t	2′	3.22[a)]	74.3 d
12	1.70 m	33.0 t	3′	3.37[a)]	77.2[b)] d
13	—	45.9 s	4′	3.30[a)]	70.8 d
14	—	48.9 s	5′	3.28[a)]	76.8[c)] d
15	1.58 m	37.5 t	6′	3.84 dd (10.3, 2.0)	61.8 t
16	1.36 m, 1.96 m	28.6 t		3.65[a)]	
17	1.58 m	52.2 d	1″	4.54 d (7.8)	97.0 d
18	1.05 s	17.4 q	2″	3.20[a)]	74.7 d
19	0.44 d (4.5), 0.79 d (4.5)	28.4 t	3″	3.37[a)]	77.3[b)] d
20	1.45 m	36.2 d	4″	3.30[a,b)]	70.6 d
21	0.95 d (6.3)	17.9 q	5″	3.28[a)]	77.0[c)] d
22	1.56 m, 1.35 m	33.7 t	6″	3.91 dd (10.1, 2.0)	62.1 t
				3.65[a)]	

Assignments confirmed by COSY, HMQC and HMBC experiments. *a*) Signal pattern was unclear due to overlapping. *b*, *c*) Assignments may be interchangeable.

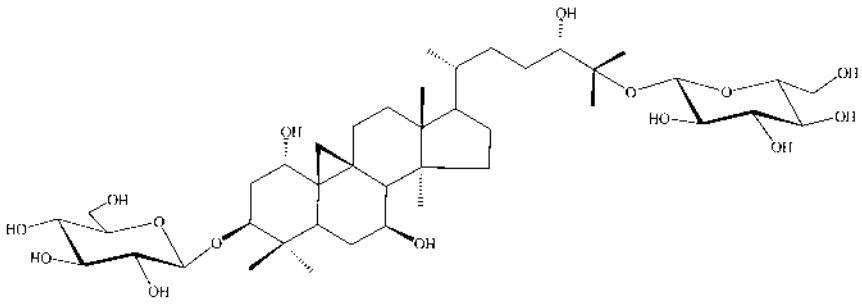

Chart 1. Structure of **1**

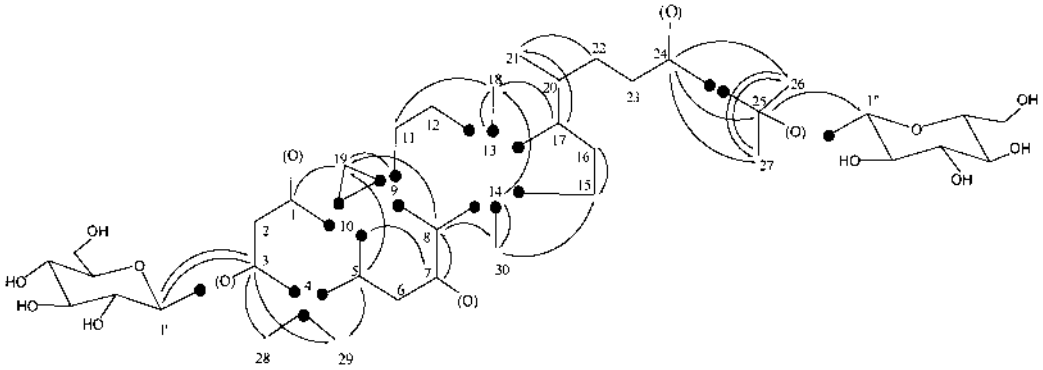

Chart 2. Partial Structures Deduced from 2D-NMR Measurements (HMQC, COSY) and Key HMBC of **1**

ppm.[15,16] Additionally, the relative configurations of the oxygenated carbon atoms were determined from the magnitude of the vicinal proton–proton coupling constants to be: C-1 (α-OH; δ 3.54 dd, J=2.7, 3.6 Hz, H_{eq}-1), C-3 (β-OH; δ 3.73 dd, J=12.0, 4.3 Hz, H_{ax}-3), C-7 (β-OH; δ 3.57 ddd, J=10.5, 8.3, 5.0 Hz, H_{ax}-7), and C-24 (δ 3.47 dd, J=11.6, 5.8 Hz, H-24).

Consequently, the structure of **1** was established as 3,25-di-*O*-β-D-glucopyranosyl-1α,3β,7β,24(*S*),25-pentahydroxycycloartane.

Experimental

General The 1D- and 2D-NMR spectra were obtained on a Bruker® Avance DRX 500 FT spectrometer operating at 500 and 125 MHz, respectively. The chemical shift values are reported as parts per million (ppm) units relative to tetramethylsilane (TMS) for ^{1}H- and ^{13}C-; and the coupling constants are in Hz (in parentheses). For the ^{13}C-NMR spectra, multiplicities were determined by a distortionless enhancement by polarization transfer (DEPT) experiment. HR-ESI-FT-MS were obtained using a Bruker BioApex

FT-MS in ESI mode.

Chromatographic Conditions TLC: precoated Si 250F plates (Baker); developing system: $CHCl_3$–MeOH–H_2O (70 : 30 : 3); visualization: 30% H_2SO_4. Column chromatography: silica gel 230—400 mesh (Merck).

Plant Material *Astragalus oleifolius* DC. was collected from Ahlatlibel, Ankara, Central Anatolia, Turkey in May 1994. Voucher specimens (94-004) have been deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

Extraction and Isolation Air-dried and powdered roots of *A. oleifolius* (250 g) were kept in EtOH–H_2O (4 : 1; 2 l) overnight, then refluxed for 2 h and filtered. The filtrate was concentrated to dryness *in vacuo* (65.0 g, yield 26%). An aliquot of the extract (30.0 g) was partitioned in H_2O and subjected to vacuum liquid chromatography (VLC) using reversed phase material (Sepralyte 40 μm; 250 g), employing H_2O, H_2O–MeOH (90 : 10→10 : 90) and MeOH as the eluents. Fractions rich in saponins were combined (7.0 g). This fraction further subjected to column chromatography (silica gel, 150 g) eluted with CH_2Cl_2–MeOH (85 : 15) and CH_2Cl_2–MeOH–H_2O mixtures (80 : 20 : 1; 80 : 20 : 2; 70 : 30 : 3) to give six main fractions (frs. A—F). Fr. E (166 mg) was subjected to VLC using reversed phase material as stationary phase (Sepralyte 40 μm; 15 g). Elution with increasing amount of MeOH in H_2O (40 : 60→70 : 30) yielded compound **1** (11.5 mg).

Compound **1** 3,25-di-*O*-β-D-glucopyranosyl-1α,3β,7β,24(*S*),25-pentahydroxy-cycloartane: ^{1}H- and ^{13}C-NMR: see Table 1. HR-ESI-FT-MS: $[M+Na]^+$ at *m/z* 839.4721.

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