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Cycloartane-type glycosides from *Astragalus amblolepis*

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ABSTRACT

Five cycloartane-type triterpene glycosides were isolated from the methanol extract of the roots of *Astragalus amblolepis* Fischer along with one known saponin, 3-*O*-β-D-xylopyranosyl-16-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxy-cycloartane. Structures of the compounds were established as 3-*O*-β-D-xylopyranosyl-25-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxy-cycloartane, 3-*O*-[β-D-glucuronopyranosyl-(1 → 2)-β-D-xylopyranosyl]-25-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxy-cycloartane, 3-*O*-β-D-xylopyranosyl-24,25-di-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxy-cycloartane, 6-*O*-α-L-rhamnopyranosyl-16,24-di-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxy-cycloartane, 6-*O*-α-L-rhamnopyranosyl-16,25-di-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxy-cycloartane by using 1D and 2D-NMR techniques and mass spectrometry. To the best of our knowledge, the glucuronic acid moiety in cycloartanes is reported for the first time.

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1. Introduction

The genus *Astragalus* belonging to the Leguminosae family is widely distributed throughout the temperate regions of the world, located principally in Europe, Asia and North America. About 2000 species have been described, 372 of them in North America and 133 in Europe (Rios and Waterman, 1997; Davis, 1982). In the flora of Turkey, this genus is represented by ~380 species, which are listed under several sections (Davis, 1970).

The roots of *Astragalus* are used in traditional medicine as an antiperspirant, diuretic and tonic drug. It has also been used in the treatment of diabetes mellitus, nephiritis, leukemia and uterine cancer (Tang and Eisenbrand, 1992). 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.

The genus *Astragalus* appears highly uniform from chemical point of view, with two kinds of pharmacologically active principles and three different kinds of toxic compounds. In the former group, the polysaccharides and the saponins stand out, and in the second, the indolizidine alkaloids, the nitro compounds and 3-nitropropyl glucosides, and the selenium compounds. There are other interesting compounds, such as flavonoids in free and glycosidic forms, pterocarpans free and as glycosides and organic acid derivatives (Rios and Waterman, 1997).

Astragalus polysaccharides are known to have anticancer and immune enhancing properties in both *in vitro* and *in vivo* experiments (Simee and Verbiscar, 1995; Yang and Zhao, 1998; Liu et al., 1994).

Chemical studies on *Astragalus* saponins have indicated the presence of cycloartane-type triterpenoid glycosides which were found to exert biological activities, e.g. antiinflammatory, analgesic, diuretic, hypotensive and sedative effects (Isaev et al., 1989; Çalış et al., 1997).

Earlier investigations on Turkish *Astragalus* species resulted in the isolation of a series of cycloartane-type triterpenoidal saponins (Bedir et al., 1998, 1999, 2001a; Çalış et al., 1999, 2008; Tabanca et al., 2005). Continuing our studies on the constituents of *Astragalus* species, we investigated the roots of *Astragalus amblolepis* Fischer. This paper describes the isolation and structure elucidation of five new cycloartane-type glycosides.

2. Results and discussion

The HRMALDITOF mass spectrum of 3-*O*-β-D-xylopyranosyl-25-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxy-cycloartane (**1**) (*m/z* 809.4669 $[M+Na]^+$, calc. for $C_{41}H_{70}O_{14}Na$, 809.4663) supported a molecular formula of $C_{41}H_{70}O_{14}$. The ESI–MS mass spectrum showed the major ion peak at *m/z* 809.4 which was assigned to $[M+Na]^+$. The MS/MS of this ion showed peaks at *m/z* 629.3 $[M+Na-180]^+$, corresponding to the loss of an hexose

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Table 1
^{1}H NMR data (*J* in Hz) of the aglycon moieties of compounds **1–6** (600 MHz, δ ppm, in CD_3OD)[a].

	1	2	3	4	5	6
H_2-1	1.56, 1.26, *m*	1.56, 1.26, *m*	1.56, 1.26, *m*	1.56, 1.26, *m*	1.59, 1.29, *m*	1.59, 1.29, *m*
H_2-2	1.97, 1.69, *m*	1.97, 1.69, *m*	1.97, 1.69, *m*	1.97, 1.69, *m*	1.72, 1.65, *m*	1.72, 1.65, *m*
H-3	3.24, *dd* (11.3, 4.0)	3.24, *dd* (11.3, 4.0)	3.25, *dd* (11.3, 4.0)	3.24, *dd* (11.3, 4.0)	3.23, *dd* (11.3, 4.0)	3.23, *dd* (11.3, 4.0)
H-5	1.40, *d* (9.5)	1.40, *d* (9.5)	1.39, *d* (9.5)	1.40, *d* (9.5)	1.53, *d* (9.5)	1.53, *d* (9.5)
H-6	3.48, *ddd* (9.5, 9.5, 4.5)	3.48, *ddd* (9.5, 9.5,4.5)	3.46, *ddd* (9.5, 9.5, 4.5)	3.48, *ddd* (9.5, 9.5, 4.5)	3.42, *ddd* (9.5, 9.5, 4.5)	3.42, *ddd* (9.5, 9.5, 4.5)
H_2-7	1.50, 1.37, *m*	1.50, 1.37, *m*	1.48, 1.38, *m*	1.50, 1.37, *m*	1.85, 1.39, *m*	1.85, 1.39, *m*
H-8	1.81, *dd* (11.9, 4.2)	1.81, *dd* (11.9, 4.2)	1.82, *dd* (11.9, 4.2)	1.81, *dd* (11.9, 4.2)	1.79, *dd* (11.9, 4.2)	1.79, *dd* (11.9, 4.2)
H_2-11	2.00, 1.22, *m*	2.02, 1.22, *m*	2.00, 1.23, *m*	2.02, 1.22, *m*	2.00, 1.24, *m*	2.00, 1.24, *m*
H_2-12	1.73, 1.68, *m*	1.73, 1.68, *m*	1.71, 1.68, *m*	1.73, 1.68, *m*	1.71, 1.65, *m*	1.71, 1.65, *m*
H_2-15	2.05, *dd* (12.7, 8.0)	2.08, *dd* (12.7, 8.0)	2.05, *dd* (12.7, 8.0)	2.04, *dd* (12.7, 8.0)	2.07, *dd* (12.7, 8.0)	2.07, *dd* (12.7, 8.0)
	1.42, *dd* (12.7, 5.2)	2.06, *dd* (12.7, 5.2)	1.42, *dd* (12.7, 5.2)	1.43, *dd* (12.7, 5.2)	1.80, *dd* (12.7, 5.2)	1.80, *dd* (12.7, 5.2)
H-16	4.47, *ddd* (8.0, 8.0, 5.2)	4.27, *ddd* (8.0, 8.0, 5.2)	4.48, *ddd* (8.0, 8.0, 5.2)	4.46, *ddd* (8.0, 8.0, 5.2)	4.27, *ddd* (8.0, 8.0, 5.2)	4.27, *ddd* (8.0, 8.0, 5.2)
H-17	1.73, *dd* (9.9, 8.0)	1.84, *dd* (9.9, 8.0)	1.72, *dd* (9.9, 8.0)	1.75, *dd* (9.9, 8.0)	1.89, *dd* (9.9, 8.0)	1.89, *dd* (9.9, 8.0)
H_3-18	1.21, *s*	1.21, *s*	1.20, *s*	1.21, *s*	1.20, *s*	1.20, *s*
H_2-19	0.55, *d* (4.2)	0.55, *d* (4.2)	0.54, *d* (4.2)	0.55, *d* (4.2)	0.54, *d* (4.2)	0.54, *d* (4.2)
	0.41, *d* (4.2)	0.41, *d* (4.2)	0.40, *d* (4.2)	0.41, *d* (4.2)	0.40, *d* (4.2)	0.40, *d* (4.2)
H-20	1.90, *m*	2.10, *m*	1.90, *m*	1.84, *m*	2.09, *m*	1.92, *m*
H_3-21	0.97, *d* (6.5)	0.96, *d* (6.5)	0.97, *d* (6.5)	0.99, *d* (6.5)	0.96, *d* (6.5)	0.96, *d* (6.5)
H_2-22	1.81, 1.24, *m*	1.81, 1.24, *m*	1.81, 1.24, *m*	1.81, 1.24, *m*	1.69, 1.60, *m*	2.00, 0.89, *m*
H_2-23	1.59, 1.51, *m*	1.67, 1.40, *m*	1.59, 1.51, *m*	1.85, 1.79, *m*	1.60 (2H), *m*	1.83, 1.19, *m*
H-24	3.61, *dd* (2.4, 10.5)	3.41, *dd* (2.4, 10.5)	3.61, *dd* (2.4, 10.5)	3.77, *dd* (2.4, 10.5)	3.58, *dd* (2.4, 10.5)	3.41, *dd* (2.4, 10.5)
H_3-26	1.29, *s*	1.18, *s*	1.29, *s*	1.37, *s*	1.21, *s*	1.25, *s*
H_3-27	1.24, *s*	1.18, *s*	1.25, *s*	1.32, *s*	1.19, *s*	1.27, *s*
H_3-28	1.32, *s*	1.32, *s*	1.31, *s*	1.32, *s*	1.13, *s*	1.13, *s*
H_3-29	1.05, *s*	1.05, *s*	1.02, *s*	1.05, *s*	0.98, *s*	0.98, *s*
H_3-30	0.98, *s*	0.98, *s*	0.99, *s*	0.98, *s*	0.98, *s*	0.98, *s*

[a] Assignments confirmed by 1D-TOCSY, DQF-COSY, HSQC and HMBC experiments.

unit and at *m/z* 479.0 $[M+Na-180-150]^+$, due to the loss of a pentose unit. Taking into account the results of our comprehensive ^{1}H and ^{13}C NMR studies, the main features of a cyclopropane-type triterpene possessing an acyclic side chain were evident for compound **1**: characteristic signals due to cyclopropane–methylene protons as an AX system (δ 0.41, 0.55, J_{AX} = 4.2 Hz, H_2-19), six tertiary methyl groups (δ 1.05, 1.21, 0.98, 1.32, 1.24, 1.29; respectively, H_3-29, H_3-18, H_3-30, H_3-28, H_3-27, H_3-26) and a secondary methyl group (δ 0.97, *d*, *J* = 6.5, H_3-21) (Bedir et al., 2001b) (Table 1). Additionally, the resonances for two anomeric protons were observed at δ 4.30 (*d*, *J* = 7.5 Hz) and 4.55 (*d*, *J* = 7.5 Hz), indicative of the presence of two β-linked sugar units (Table 2). Thus, **1** was considered to be a cycloartane-type triterpene diglycoside.

The ^{13}C NMR spectrum contained 41 resonances (Table 3); 30 of them, attributed to the sapogenol moiety, were in good agreement with cyclocanthogenin (Isaev et al., 1989). Full assignment of the ^{1}H and ^{13}C signals of the aglycon part of **1**, accomplished by DQF-COSY and HSQC spectra, showed glycosylation shifts for C-3 (δ 89.6) and C-25 (δ 81.4). All connectivities within **1** were also confirmed by HMBC spectrum, which showed a long-range correlation between the anomeric proton signal at δ 4.30 (*d*, *J* = 7.5 Hz) and the carbon resonance at δ 89.6 (C-3), while the anomeric proton at δ 4.55 (*d*, *J* = 7.5 Hz) exhibited a long-range correlation with the carbon resonance at δ 81.4 (C-25), confirming the bidesmosidic character of **1**. Furthermore the following stereochemical data confirmed the presence of cyclocanthogenol as aglycon of compound **1**. The relative configurations of the oxygenated carbon atoms were determined from the magnitude of the vicinal proton–proton coupling constants: C-3-(β-OH) (δ 3.24, *dd*, *J* = 11.3, 4.0 Hz, H_{ax}-3), C-6-(α-OH) (δ 3.48, *ddd*, *J* = 9.5, 9.5, 4.5 Hz, H_{ax}-6), C-16-(β-OH) (δ 4.47,

Table 2
^{1}H NMR data (*J* in Hz) of the sugar moieties of compounds **1–6** (600 MHz, δ ppm, in CD_3OD)[a].

	1	2	3	4	5	6
H-1′	4.30 *d* (7.5)	4.31 *d* (7.5)	4.56 *d* (7.5)	4.30 *d* (7.5)	4.78 *d* (1.2)	4.78 *d* (1.2)
H-2′	3.22 *dd* (9.2, 7.5)	3.22 *dd* (9.2, 7.5)	3.65 *dd* (9.2, 7.5)	3.22 *dd* (9.2, 7.5)	3.86 *dd* (1.2, 3.2)	3.86 *dd* (1.2, 3.2)
H-3′	3.31 *dd* (9.2)	3.34, *dd* (9.2)	3.58, *dd* (9.2)	3.31, *dd* (9.2)	3.62 *dd* (3.2, 9.3)	3.62 *dd* (3.2, 9.3)
H-4′	3.49 *m*	3.49 *m*	3.54 *m*	3.49 *m*	3.42 *t* (9.3)	3.42 *t* (9.3)
H-5′	3.84 *dd* (11.7, 5.2)	3.85 *dd* (11.7, 5.2)	3.91 *dd* (11.7, 5.2)	3.84 *dd* (11.7, 5.2)	3.72 *m*	3.72 *m*
	3.20 *t* (11.7)	3.21 *t* (11.7)	3.24 *t* (11.7)	3.20 *t* (11.7)		
H-6′	–	–	–	–	1.29 *s*	1.29 *s*
H-1″	4.55 *d* (7.5)	4.24 *d* (7.5)	4.66 *d* (7.5)	4.45 *d* (7.5)	4.32 *d* (7.5)	4.30 *d* (7.5)
H-2″	3.20 *dd* (7.5, 9.0)	3.22 *dd* (7.5, 9.0)	3.35 *dd* (7.5, 9.0)	3.25 *dd* (7.5, 9.0)	3.18 *dd* (7.5, 9.0)	3.15 *dd* (7.5, 9.0)
H-3″	3.39 *dd* (9.0, 9.0)	3.38 *dd* (9.0, 9.0)	3.46 *dd* (9.0, 9.0)	3.42 *dd* (9.0, 9.0)	3.38 *dd* (9.0, 9.0)	3.39 *dd* (9.0, 9.0)
H-4″	3.30 *dd* (9.0, 9.0)	3.32 *dd* (9.0, 9.0)	3.49 *dd* (9.0, 9.0)	3.33 *dd* (9.0, 9.0)	3.39 *dd* (9.0, 9.0)	3.37 *dd* (9.0, 9.0)
H-5″	3.30 *m*	3.25 *m*	3.64 *d* 9.0)	3.33 *m*	3.27 *m*	3.24 *m*
H-6″	3.67 *dd* (4.5, 12.0)	3.71 *dd* (4.5, 12.0)	–	3.69 *dd* (4.5, 12.0)	3.69 *dd* (4.5, 12.0)	3.69 *dd* (4.5, 12.0)
	3.86 *dd* (3.5, 12.0)	3.85 *dd* (3.5, 12.0)		3.89 *dd* (3.5, 12.0)	3.89 *dd* (3.5, 12.0)	3.89 *dd* (3.5, 12.0)
H-1‴	–	–	4.55 *d* (7.5)	4.60 *d* (7.5)	4.45 *d* (7.5)	4.55 *d* (7.5)
H-2‴	–	–	3.20 *dd* (7.5, 9.0)	3.20 *dd* (7.5, 9.0)	3.25 *dd* (7.5, 9.0)	3.20 *dd* (7.5, 9.0)
H-3‴	–	–	3.39 *dd* (9.0, 9.0)	3.39 *dd* (9.0, 9.0)	3.42 *dd* (9.0, 9.0)	3.39 *dd* (9.0, 9.0)
H-4‴	–	–	3.30 *dd* (9.0, 9.0)	3.30 *dd* (9.0, 9.0)	3.33 *dd* (9.0, 9.0)	3.30 *dd* (9.0, 9.0)
H-5‴	–	–	3.30 *m*	3.30 *m*	3.33 *m*	3.30 *m*
H-6‴	–	–	3.67 *dd* (4.5, 12.0)	3.67 *dd* (4.5, 12.0)	3.69 *dd* (4.5, 12.0)	3.67 *dd* (4.5, 12.0)
			3.86 *dd* (3.5, 12.0)	3.86 *dd* (3.5, 12.0)	3.89 *dd* (3.5, 12.0)	3.86 *dd* (3.5, 12.0)

[a] Assignments confirmed by 1D-TOCSY, DQF-COSY, HSQC and HMBC experiments.

Table 3
^{13}C NMR data of compounds **1–6** (150 MHz, δ ppm, in CD_3OD)[a].

Position	1	2	3	4	5	6
1	33.1	33.1	33.1	33.1	33.2	33.2
2	30.3	30.3	30.3	30.3	30.9	30.9
3	89.6	89.6	89.8	89.6	79.0	79.0
4	42.7	42.7	43.1	42.7	42.5	42.5
5	54.5	54.7	54.6	54.5	53.1	53.1
6	69.4	69.4	69.4	69.4	81.4	81.4
7	38.7	38.7	38.7	38.7	35.6	35.6
8	48.7	48.7	48.7	48.7	48.0	48.0
9	21.6	21.6	22.3	21.6	21.7	21.7
10	29.8	29.8	30.0	29.8	29.8	29.8
11	26.7	26.7	26.6	26.7	26.5	26.5
12	33.5	33.5	34.0	33.5	33.3	33.3
13	46.1	46.1	46.8	46.1	46.2	46.2
14	47.5	47.5	47.6	47.5	48.0	48.0
15	48.6	48.4	48.4	49.0	49.0	49.0
16	73.2	83.4	73.0	72.7	83.7	83.7
17	57.9	58.1	58.2	57.6	57.1	57.1
18	18.9	18.9	19.0	18.9	18.9	18.9
19	31.5	31.5	31.5	31.5	31.3	31.3
20	29.8	29.8	29.8	31.6	31.4	31.4
21	17.9	17.9	18.7	18.0	17.9	17.9
22	33.6	33.7	33.6	34.1	34.3	34.3
23	28.4	28.6	28.4	28.4	29.4	29.4
24	76.7	78.6	76.8	85.2	90.4	79.1
25	81.4	73.6	81.3	81.0	73.7	81.6
26	22.9	24.9	23.0	25.3	24.7	20.9
27	22.6	24.9	22.6	22.7	26.5	23.2
28	28.3	28.3	28.7	28.3	28.9	28.9
29	16.3	16.3	16.5	16.3	16.2	16.2
30	20.2	20.1	20.2	20.1	20.1	20.1
1′	107.4	107.2	105.0	107.1	103.9	103.9
2′	75.1	75.0	81.4	75.1	72.5	72.5
3′	77.7	77.7	76.4	77.7	72.3	72.3
4′	70.8	70.8	71.0	70.8	73.6	73.6
5′	66.3	66.2	66.3	66.3	69.9	69.9
6′	–	–	–	–	17.7	17.7
1″	97.8	106.2	104.3	104.8	106.8	106.0
2″	75.0	75.0	75.5	75.4	75.5	75.5
3″	78.0	78.2	77.2	77.8	78.0	77.8
4″	71.2	71.2	73.6	71.3	71.2	71.2
5″	77.8	77.5	77.3	77.8	77.6	77.2
6″	62.2	62.4	177.0	62.4	62.4	62.4
1‴	–	–	97.7	98.4	104.8	98.4
2‴	–	–	75.0	75.0	75.4	75.0
3‴	–	–	78.0	78.0	77.8	78.0
4‴	–	–	71.2	71.2	71.3	71.2
5‴	–	–	77.8	77.8	77.8	77.8
6‴	–	–	62.2	62.2	62.4	62.2

[a] Assignments confirmed by DQF-COSY, HSQC, and HMBC experiments.

ddd, J = 8.0, 8.0, 5.2 Hz, H_{ax}-16). Moreover, ^{13}C-NMR data for C-24 was comparable to those reported for analogous compounds having a 24(*S*) configuration (Bedir et al., 2000; Fadeev et al., 1987; Hirotani et al., 1994).

As concerning the sugar moiety, examination of the remaining signals allowed us to identify one β-xylopyranosyl unit and one β-glucopyranosyl unit (Agrawal et al., 1985; Bedir et al., 2001b). The configuration of glucose and xylose units was established as D after hydrolysis of **1** with 1 N HCl, trimethylsilation and determination of retention time by GC.

Consequently, the structure of **1** was established as 3-*O*-β-D-xylopyranosyl-25-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxy-cycloartane (Fig. 1).

The HRMALDITOF mass spectrum of 3-*O*-β-D-xylopyranosyl,16-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxy-cycloartane (**2**) showed a major ion peak at *m/z* 809.4665 $[M+Na]^+$ ascribable to molecular formula $C_{41}H_{70}O_{14}$ (calc. for $C_{41}H_{70}O_{14}Na$, 809.4663). The NMR spectral data of **2** were consistent with **1** being a cycloartane-type triterpene diglycoside. Full assignments of ^{1}H and ^{13}C NMR data of the aglycon of **2** were deduced from its HSQC and HMBC spectra. All 1D and 2D NMR data of **2** were superimposable with those of the known compound 3-*O*-β-D-xylopyranosyl,16-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxy-cycloartane, isolated previously from *Tragacantha stipulosa* Boviss (Karimov et al., 1998) (Fig. 1, Tables 1–3).

The molecular formula of 3-*O*-[β-D-glucuronopyranosyl-(1 → 2)-β-D-xylopyranosyl]-25-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxy-cycloartane (**3**) and 3-*O*-β-D-xylopyranosyl-24,25-di-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxy-cycloartane (**4**) was unequivocally established to be $C_{47}H_{78}O_{20}$ and $C_{47}H_{80}O_{19}$ by HRMALDITOFMS analysis (*m/z* 985.4979 $[M+Na]^+$, calc. for $C_{47}H_{78}O_{20}Na$, 985.4984, and *m/z* 971.5199 $[M+Na]^+$, calc. for $C_{47}H_{80}O_{19}Na$, 971.5192), respectively.

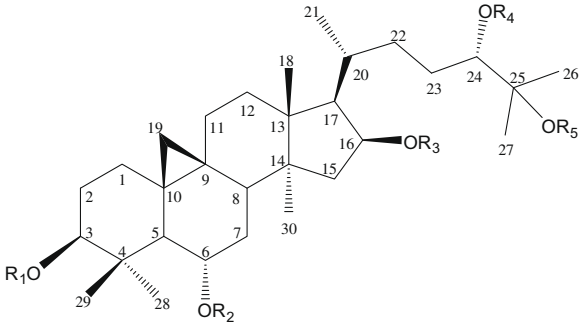
The NMR spectra of compounds **3** and **4** were also characteristic of cyclocanthogenol type glycosides (Tables 1–3). Furthermore, the ^{1}H NMR spectrum of **3** clearly showed three anomeric proton doublets at δ 4.66 (*J* = 7.5 Hz), 4.56 (*J* = 7.5 Hz) and 4.55 (*J* = 7.5 Hz) in the downfield region, indicative of β-linked sugar units. The ^{13}C NMR spectrum contained 47 resonances; 30 of them attributed to cyclocanthogenol moiety. Full assignment of the ^{1}H and ^{13}C signals of the aglycon part of **3** showed glycosylation shifts for C-3 (δ 89.8) and C-25 (δ 81.3). These results suggested that **3** was also a bidesmosidic saponin with sugar residues linked to C-3 and C-25 of cyclocanthogenol. A combination of 1D-TOCSY and HSQC experiments allowed us to unambiguously determine all sugar signals and to identify the sugar moieties as consisting of β-xylopyranosyl, β-glucopyranosyl and β-glucuronic acid units, respectively.

All connectivities, including the sites of attachment of sugar moieties on the aglycon of **3**, were determined by HMBC experiment (Fig. 2). In the HMBC spectrum, the anomeric proton signal at δ 4.56 (H-1′), assigned to the β-xylopyranose, showed long-range correlation with the carbon resonance at δ 89.8 (C-3). The second anomeric proton signal at δ 4.66 (H-1″), assigned to the β-glucuronic acid, showed long-range correlation with the carbon resonance at δ 81.4 (C-2′). The third anomeric proton signal at δ 4.55 (H-1‴), assigned to the β-glucopyranose, showed long-range correlation with the carbon resonance at δ 81.3 (C-25). The D configuration of glucose, xylose and glucuronic acid units was determined by acid hydrolysis followed by GC analysis.

Thus the structure of **3** was elucidated as 3-*O*-[β-D-glucuronopyranosyl-(1 → 2)-β-D-xylopyranosyl]-25-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxy-cycloartane. This compound (**3**) represents the first report of a cycloartane-type compound possessing glucuronic acid residue in plant kingdom (Fig. 1). Since most of the *Astragalus* members have not been studied phytochemically, it is too premature to draw a conclusion from chemotaxonomic point of view especially for *Astragalus* genus. Thus further studies are required to clarify chemotaxonomic significance of the presence of glucuronic acid moiety attached to cycloartanes in *Astragalus* species.

The ^{1}H NMR spectrum of **4** showed three anomeric proton resonances at δ 4.30 (*d, J* = 7.5 Hz), 4.45 (*d, J* = 7.5 Hz), and 4.60 (*d, J* = 7.5 Hz) correlated by HSQC to the resonances at δ 107.1, 104.8 and 98.4 (Tables 2 and 3). The three terminal sugar units were identified as a β-D-xylopyranose and two β-D-glucopyranoses, respectively, using a combination of 1D-TOCSY and HSQC spectra.

The ^{13}C-NMR resonances arising from the sapogenol moiety were similar to those of **3**, except for the signal assigned to C-24 (δ 85.2) exhibiting a significant glycosidation shift. These results suggested another tridesmosidic structure in which the three sugar units were attached to the hydroxyl groups at C-3, C-24 and C-25. The glycosidation sites were established by the HMBC experiment



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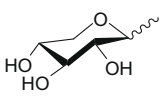
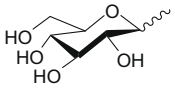
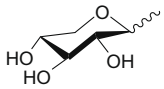
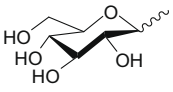
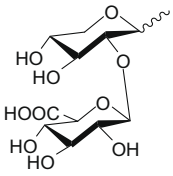
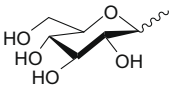
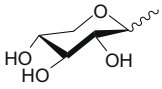
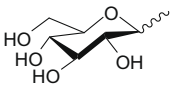
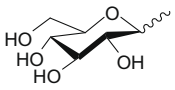
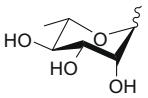
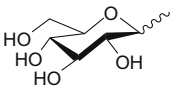
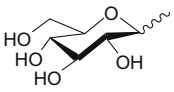
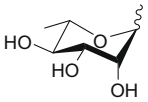
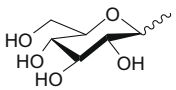
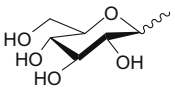
1		H	H	H	
2		H		H	H
3		H	H	H	
4		H	H		
5	H				H
6	H			H	

Fig. 1. Structures of **1**–**6**.

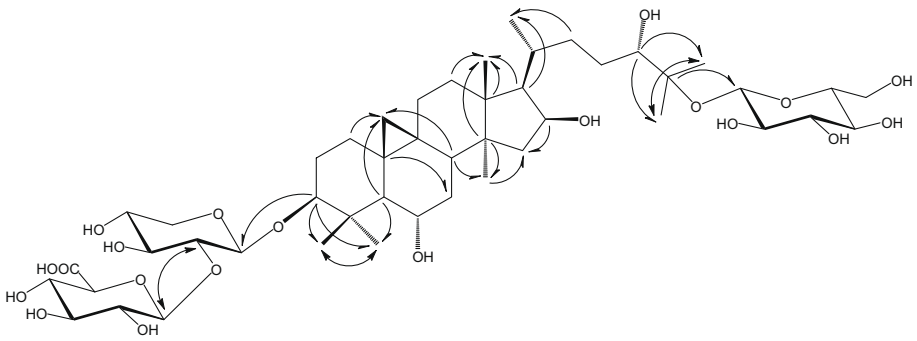


Fig. 2. Key HMBC of **3**.

which showed correlations between H-3 (δ 3.24) and C-1′ (δ 107.1) of the β-D-xylopyranosyl unit, H-24 (δ 3.77) and C-1″ (δ 104.8) of the first β-D-glucopyranosyl and between H-1‴ (δ 4.60) and C-25 (δ 81.0).

On the basis of these evidence, the structure of compound **4** was established as 3-*O*-β-D-xylopyranosyl-24,25-di-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxy-cycloartane (Fig. 1).

The HRMALDITOF mass spectrum of 6-*O*-α-L-rhamnopyranosyl-16,24-di-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxy-cycloartane (**5**) (*m/z* 985.5350 $[M+Na]^+$, calc. for $C_{48}H_{82}O_{19}Na$, 985.5348) supported a molecular formula of $C_{48}H_{82}O_{19}$. The ESI–MS mass spectrum showed the major ion peak at *m/z* 985.3 which was assigned to $[M+Na]^+$. The MS/MS of this ion showed peaks at *m/z* 805.3 $[M+Na-180]^+$, cor-

responding to the loss of an hexose unit and at *m/z* 641.4 $[M+Na-180-164]^+$, ascribable to the loss of a 6-deoxyhexose. The ^1H NMR spectrum of aglycon moiety of **5** displayed the typical signals of cycloartanes having acyclic side chain: six tertiary methyl groups, from signals at δ 0.98, 1.20, 0.98, 1.13, 1.21, 1.19, and a secondary methyl group from signal at δ 0.96 (*d*, $J = 6.5$ Hz, H_3-21), as well as the cyclopropane-methylene protons at δ 0.40, 0.54 ($J_{AX} = 4.2$ Hz, H_2-19) (Table 1). In addition, the resonances of three anomeric protons, indicative of the presence of one α- linked and two β-linked sugar moieties, were observed in the downfield region (δ 4.78, *d*, $J = 1.2$ Hz, H-1′; δ 4.32, *d*, $J = 7.5$ Hz, H-1″; δ 4.45, *d*, $J = 7.5$ Hz, H-1‴) (Table 2).

Combination of 1D-TOCSY and HSQC experiments allowed the sequential assignments of all proton resonances of each sugar residue, starting from anomeric protons. Furthermore, on the basis of chemical shifts, the multiplicity of the signal and the coupling constants, the three sugar residues were identified as α-rhamnopyranose and two β-glucopyranoses. The D configuration of glucose units and the L configuration of rhamnose unit were determined by acid hydrolysis followed by GC analysis.

The remaining ^1H and ^{13}C resonances of **5** arising from the sapogenol moiety were evident for the presence of cyclocanthogenol except for the signals ascribable to linkage sites exhibiting typical glycosidation shifts. Key correlation peaks observed in the HMBC spectrum of **5** between H-1′ of the rhamnosyl at δ 4.78 (*d*, $J = 1.2$, Hz) and C-6 (δ 81.4) of the aglycon, between H-1″ of the glucosyl at δ 4.32 (*d*, $J = 7.5$ Hz and C-16 (δ 83.7), and between H-1‴ of the second glucosyl unit at δ 4.45 (*d*, $J = 7.5$ Hz) and C-24 (δ 90.4), revealed the locations of glycosidic linkages to be C-6, C-16 and C-24 indicating the tridesmosidic nature of compound **5**.

The molecular formula of 6-*O*-α-L-rhamnopyranosyl-16,25-di-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxy-cycloartane (**6**) was determined to be $C_{48}H_{82}O_{19}$ by HRMALDITOFMS analysis (*m/z* 985.5341 $[M+Na]^+$, calc. for $C_{48}H_{82}O_{19}Na$, 985.5348). In the positive ESI–MS spectrum compound **6** showed a major ion peak at *m/z* 985.4 $[M+Na]^+$ and a significant fragment in MS/MS analysis at *m/z* 805.3 $[M+Na-180]^+$, ascribable to the loss of an hexose unit. The MS^3 fragmentation showed a peak at *m/z* 641.3 $[M+Na-180-164]^+$ corresponding to the loss of a 6-deoxyhexose unit. Full assignments of the proton and carbon signals of the aglycon and sugar parts of **6** in comparison with those of **5** showed their considerable structural similarity (Tables 1 and 2). The difference consisted only in the position of one of the glucose moieties. The sapogenol moiety showed an unambiguous glycosidation shift at C-25 (δ 81.4), while C-24 displayed no downfield shift due to the sugar attachment (δ 79.1). Furthermore, the HMBC experiment showed key correlations peaks between the anomeric proton of rhamnosyl unit (δ 4.78, *d*, $J = 1.2$ Hz, H-1′) and C-6 (δ 81.4), between the anomeric proton of the first glucose moiety (δ 4.27, *d*, $J = 7.5$ Hz, H-1″) and C-16 (δ 83.7), and H-1‴ (δ 4.55, *d*, $J = 7.5$ Hz) of the second glucose residue and C-25 (δ 81.6). Thus, compound **6** displayed the glycosidation at C-25 instead of the glycosidation at C-24 as for compound **5**.

Consequently, the structure of **5** and **6** was established as 6-*O*-α-L-rhamnopyranosyl-16,24-di-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxy-cycloartane and 6-*O*-α-L-rhamnopyranosyl-16,25-di-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxy-cycloartane, respectively (Fig. 1). Cycloartane glycosides with no sugar residue at C-3 position such as compounds **5** and **6** are rather unusual in nature. Moreover, as far as we have ascertained, a rhamnosyl unit at C-6 position is reported for the first time in cyclocanthogenol skeleton which is one of the most common aglycons in *Astragalus* genus together with cycloastragenol.

3. Experimental

3.1. 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%, Sigma–Aldrich) 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 analysed 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. ESI–MS 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 × 25 m). Column chromatography was carried out on Silica gel (JT Baker, 40 μm), Sephadex LH-20 (Amersham Biosciences, 17-0090-02) and RP (C-18, 40 μm) (Merck). TLC analyses were carried out on Silica gel 60 F_{254} (Merck) and RP-18 F_{254s} (Merck) plates. Compounds were detected by UV and 20% H_2SO_4/water spraying reagent followed by heating at 110 °C for 1–2 min.

3.2. Plant material

A. amblolepis was collected from Ersele Village, Pütürge-Malatya, East Anatolia, in October 2006 and identified by Serdar G. Senol (Deparment of Biology, Faculty of Sciences, Ege University, Izmir, Turkey). A voucher specimen was deposited in the Herbarium of Ege University, Izmir, Turkey (EGE 32574).

3.3. Extraction and isolation

Air-dried and powdered roots of *A. amblolepis* (600 g) were extracted with MeOH (3 × 3.5 l) at 60 °C. After filtration, the solvent was removed by rotary evaporation yielding 36 g of extract. The MeOH extract was dissolved in H_2O (400 ml), and successively partitioned with *n*-hexane (3 × 100 ml), CH_2Cl_2 (4 × 100 ml), and *n*-BuOH saturated with H_2O (6 × 100 ml). The *n*-BuOH extract (5 g) was subjected to vacuum liquid chromatography (VLC) using reversed-phase material (Lichroprep RP-18, 25-40 μm, 120 g) employing H_2O (850 ml), H_2O–MeOH (8:2, 200 ml; 6:4, 400 ml; 4:6, 1600 ml) and MeOH (800 ml) to give ten main fractions (1–10). Fractions 7, 8 and 9 eluted with H_2O:MeOH (4:6) were rich in saponins. Fraction 7 (615.2 mg) was subjected to high performance flash chromatography (HPFC, Biotage, Inc., A Dyax Corp. Company; Biotage SI 40 M column), and eluted with $CHCl_3$–MeOH mixtures [80% $CHCl_3$, 5 column volume (CV); 80–70% $CHCl_3$, 5 CV; 70% $CHCl_3$, 4 CV; 70–60% $CHCl_3$, 4 CV; 50% $CHCl_3$, 3 CV] and MeOH (4 CV) to yield 510 fractions. Fractions 374-488 and the first MeOH fraction (160.6 mg) were combined and applied to a reversed-phase column (Lichroprep RP-18, 25–40 μm, 50 g) using MeOH–H_2O (5:5, 400 ml; 6:4, 300 ml) to give **1** (4.1 mg). Fractions 287–310 (81.4 mg) were combined and fractionated over an open column chromatography using Si gel (50 g) as stationary phase. Elution was performed with $CHCl_3$–MeOH–H_2O mixtures (80:20:2, 300 ml; 70:30:3, 1700 ml; 61:32:7, 400 ml) to afford **2**

(19.8 mg). Fractions 8 and 9 (860.2 mg) from VLC were combined and subjected to Si gel (200 g) column chromatography. Elution was carried out with $CHCl_3$-MeOH–H_2O (85:15:0.5, 400 ml), $CHCl_3$-MeOH (80:20, 400 ml) and $CHCl_3$–MeOH–H_2O (80:20:1, 500 ml) yielding **3** (3.9 mg) and **4** (21.8 mg). Fractions 894–1110 (135 mg) were applied to column chromatography using reversed-phase material (Lichroprep RP-18, 25–40 μm, 40 g) employing MeOH:H_2O (6:4, 350 ml) to give **5** (5.2 mg) and **6** (13.7 mg).

3.4. Compound *1*

Amorphous powder; $[\alpha]_D^{25}$ +34.0 (*c* 0.1, MeOH); IR (KBr): ν_{max} = 3480 (>OH), 3025 (cyclopropane ring), 2870 (>CH), 1290–1030 (C–O–C) cm^{-1}; ESI–MS *m/z* 809.4 $[M+Na]^+$; MS/MS *m/z* 629.3 $[M+Na-180]^+$, *m/z* 479.0 $[M+Na-180-150]^+$; HRMALDI-TOFMS $[M+Na]^+$ *m/z* 809.4669 (calc. for $C_{41}H_{70}O_{14}Na$, 809.4663); ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data: see Tables 1–3.

3.5. Compound *2*

Amorphous powder; $[\alpha]_D^{25}$ 29.3 (*c* 0.1, MeOH); IR (KBr): ν_{max} = 3490 (>OH), 3033 (cyclopropane ring), 2889 (>CH), 1271–1025 (C–O–C) cm^{-1}; ESI–MS *m/z* 809.3 $[M+Na]^+$; MS/MS *m/z* 629.3 $[M+Na-180]^+$; HRMALDITOFMS $[M+Na]^+$ *m/z* 809.4665 (calc. for $C_{41}H_{70}O_{14}Na$, 809.4663); ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data: see Tables 1–3.

3.6. Compound *3*

Amorphous powder; $[\alpha]_D^{25}$ 15.8 (*c* 0.1, MeOH); IR (KBr): ν_{max} = 3477 (>OH), 3048 (cyclopropane ring), 2895 (>CH), 1285–1043 (C–O–C) cm^{-1}; ESI–MS *m/z* 985.3 $[M+Na]^+$; MS/MS *m/z* 805.3 $[M+Na-180]^+$, *m/z* 641 $[M+Na-180-164]^+$; HRMALDITOFMS $[M+Na]^+$ *m/z* 985.4979 (calc. for $C_{47}H_{78}O_{20}Na$, 985.4984); ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data: see Tables 1–3.

3.7. Compound *4*

Amorphous powder; $[\alpha]_D^{25}$ 22.7 (*c* 0.1 MeOH); IR (KBr): ν_{max} = 3483 (>OH), 3027 (cyclopropane ring), 2877 (>CH), 1279–1038 (C–O–C) cm^{-1}; ESI–MS *m/z* 971.4 $[M+Na]^+$; MS/MS *m/z* 791.4 $[M+Na-180]^+$, *m/z* 613.3 $[M+Na-180-180]^+$; HRMALDI-TOFMS $[M+Na]^+$ *m/z* 971.5199 (calc. for $C_{47}H_{80}O_{19}Na$, 971.5192); ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data: see Tables 1–3.

3.8. Compound *5*

Amorphous powder; $[\alpha]_D^{25}$ 17.9 (*c* 0.1, MeOH); IR (KBr): ν_{max} = 3472 (>OH), 3040 (cyclopropane ring), 2883 (>CH), 1283–1029 (C–O–C) cm^{-1}; ESI–MS *m/z* 985.3 $[M+Na]^+$; MS/MS *m/z* 805.3 $[M+Na-180]^+$, *m/z* 641.4 $[M+Na-180-164]^+$; HRMALDI-TOFMS $[M+Na]^+$ *m/z* 985.5350 (calc. for $C_{48}H_{82}O_{19}Na$, 985.5348); ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data: see Tables 1–3.

3.9. Compound *6*

Amorphous powder; $[\alpha]_D^{25}$ 24.4 (*c* 0.1, MeOH); IR (KBr): ν_{max} = 3486 (>OH), 3035 (cyclopropane ring), 2874 (>CH), 1292–1040 (C–O–C) cm^{-1}; ESI–MS *m/z* 985.4 $[M+Na]^+$; MS/MS *m/z* 805.3 $[M+Na-180]^+$, *m/z* 641.3 $[M+Na-180-164]^+$; HRMALDI-TOFMS $[M+Na]^+$ *m/z* 985.5341 (calc. for $C_{48}H_{82}O_{19}Na$, 985.5348); ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data: see Tables 1–3.

3.10. Acid hydrolysis

A solution (1 mg each) of compounds **1**, **3** and **5** in 1 N HCl (0.5 ml) was stirred at 80 °C for 4 h. After cooling, the solution was 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 °C 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, 1:1 v/v). The CH_2Cl_2 layer was analysed by GC using an L-Chirasil-Val column (0.32 mm × 25 m). Temperatures of the injector and detector were 200 °C for both. A temperature gradient system was used for the oven, starting at 100 °C for 1 min and increasing up to 180 °C at a rate of 5 °C/min. The peaks of the hydrolysate of **1** were detected at 10.96 and 12.01 (D-xylose) and 14.72 min (D-glucose). The peaks of D-glucuronic acid (15.81 min), D-glucose (14.70 min) and D-xylose (10.94 and 12.00 min) were detected in the hydrolysate of **3**. The peaks of the hydrolysate of **5** were detected at 14.71 min (D-glucose), 9.67 and 10.70 (L-rhamnose). Retention times for authentic samples after being treated in the same manner with 1-(trimethylsilyl)-imidazole in pyridine were detected at 15.81 (D-glucuronic acid), 14.71 min (D-glucose), 9.68 and 10.71 (L-rhamnose), 10.96 and 12.00 min (D-xylose).

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