Trojanosides I—K: New Cycloartane-Type Glycosides from the Aerial Parts of *Astragalus trojanus*

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Three new cycloartane-type triterpene glycosides have been isolated from the aerial parts of *Astragalus trojanus*. The structures were established mainly by a combination of one- and two-dimensional NMR techniques [^{1}H–^{1}H-correlation spectroscopy (COSY), ^{1}H–^{13}C-heteronuclear multiple quantum correlation spectroscopy (HMQC), and ^{1}H–^{13}C-heteronuclear multiple-bond correlation spectroscopy (HMBC)] and high resolution electrospray ionization mass spectrometry (HR-ESI-MS) as 3-*O*-β-(2′,3′-di-*O*-acetyl)-D-xylopyranosyl-6-*O*-β-D-glucopyranosyl-16-*O*-acetoxy-20(*R*),24(*S*)-epoxycycloartane-3β,6α,16β,25-tetrol, 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-(3′,4′-di-*O*-acetyl)-D-xylopyranosyl]-6-*O*-β-D-xylopyranosyl-20(*R*),24(*S*)-epoxycycloartane-3β,6α,16β,25-tetrol, 3-*O*-β-D-xylopyranosyl-6,16-di-*O*-β-D-glucopyranosyl-20(*R*),24(*S*)-epoxycycloartane-3β,6α,16β,25-tetrol.

Key words *Astragalus trojanus*; cycloartane-type triterpene; trojanoside; 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, tonic and diuretic. 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 chemical compounds, polysaccharides and saponins.[2] *Astragalus* polysaccharides are 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 of *Astragalus species* resulted in the isolation of a series of cycloartane-type triterpenic saponins.[7—13] We previously reported the isolation and the structure determination of trojanosides A—H from *Astragalus trojanus*.[10,11] In our continuing search, we have isolated three new cycloartane-type triterpene glycosides, named as trojanosides I—K (**1**, **2**, **5**), from the aerial parts of *Astragalus trojanus*. Here we report the isolation and the structural elucidation of **1**, **2** and **5**.

The IR spectrum of compound **1** indicated the presence of hydroxyl (3419 cm^{-1}) and ester carbonyl (1726 cm^{-1}) functionalities. The high resolution electrospray ionization mass spectrometry (HR-ESI-MS) spectrum of **1** exhibited an ion peak for $[M+Na]^+$ at *m/z* 933.3207, which is compatible with the molecular formulae $C_{47}H_{74}O_{17}$.

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 0.23, 0.64, J_{AX}=4.4 Hz, H_2-19), seven tertiary methyl groups (δ 1.04, 1.23, 1.35, 1.40, 1.42, 1.43, 1.70; respectively, H_3-30, H_3-29, H_3-27, H_3-21, H_3-18, H_3-26, H_3-28). Additionally, the resonances for three acetyl methyls at δ 2.00, 2.07 and 2.09 and two anomeric protons at δ 4.80 (d, J=7.7 Hz, H-1′) and 4.85 (d, J=7.6 Hz), indicative of the presence of two β-linked sugar units, were observed. Thus, **1** was considered to be a cycloartane-type triterpene diglycoside. Inspection of the ^{13}C-NMR of **1** (Table 1) showed 47 signals, 6 of which were in good accordance with the presence of three acetyl groups (δ 21.3, 21.3, 21.7, 170.4, 170.8, 171.0), 30 of which were attributed to a triterpenic aglycone, and the remaining 11 resonances indicated the presence of a pentosyl and a hexosyl moieties.

Full assignments of the proton and carbon signals of the aglycon part of **1** were secured from its double quantum filtered correlation spectroscopy (DQF-COSY) and heteronuclear multiple quantum correlation spectroscopy (HMQC) spectra. The resonances assigned to the sapogenol moiety were in good agreement with trojanoside A,[10] possessing cycloastragenol [20(*R*),24(*S*)-epoxycycloartane-3β,6α,16β,25-tetrol] as aglycon, which was glycosylated at C-3 [δ 89.6 (**1**); δ 89.9 (trojanoside A)] and C-6 [δ 79.1 (**1**); δ 79.2 (trojanoside A)], and acetylated at C-16(O) [δ 76.6, C-16, δ 5.66, ddd, J=5.2, 7.8, 7.9 Hz, H-16 (**1**); δ 77.6, C-16, δ 5.48 ddd, J=5.2, 8.0, 8.0 Hz, H-16 (trojanoside A)]. In order to substantiate our findings, deacetyl derivative of **1** was obtained by an alkaline deacetylation. Alkaline deacetylation of **1** followed by TLC analysis showed the expected glycoside astragaloside IV.[14]

After subtraction of the aglycon and acetyl group signals from the total HMQC spectrum, evaluation of the remaining signals permitted identification of the sugar moieties as β-xylopyranosyl, and β-glucopyranosyl.[15] We favour D-configuration of monosaccharide residues, consistent with all other naturally occurring cycloartane-type glycosides present in the genus *Astragalus*.

Complete assignments of each sugar proton system were achieved by considering DQF-COSY, while the carbons were assigned from HMQC and heteronuclear multiple-bond correlation spectroscopy (HMBC) spectra. From the cross peaks in the DQF-COSY spectrum of **1**, it was easy to differentiate

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Table 1. ^{1}H-NMR Data of **1**—**5** (500 MHz, δ ppm, in C_5D_5N)

	1	**2**	**3**	**4**	**5**
H-3	3.39 dd (4.5, 11.1)	3.24 dd (11.6, 4.4)	3.34 dd (4.3, 11.6)	3.33 dd (3.5, 11.1)	3.49 dd (11.5, 4.2)
H-6	3.94 m	3.75 m	3.75 m	3.72 m	3.66 m
H-16	5.66 ddd (5.2, 7.8, 7.9)	5.03 dd (7.1, 7.3)	5.05 dd (5.1, 7.5)	5.03 dd (5.1, 7.5)	4.47 dd (7.5, 5.1)
H-17	2.62 d (8.0)	2.56 d (7.7)	2.58 d (7.7)	2.58 d (7.5)	2.45 d (7.7)
H_3-18	1.42 s	1.38 s	1.37 s	1.35 s	1.58 s
H_2-19	0.23 d, 0.64 d (4.4)	0.15 d, 0.60 d (4.0)	0.09 d, 0.59 d (4.0)	0.03 d, 0.59 d (3.4)	0.16 d, 0.52 d (3.5)
H_3-21	1.40 s	1.30 s	1.29 s	1.35 s	1.72 s
H-24	3.96[b]	3.87 dd (5.3, 12.2)	3.87[b]	3.85 dd (3.4, 13.5)	4.04 dd (3.1, 2.7)
H_3-26	1.43 s	1.56 s	1.56 s	1.56 s	1.46 s
H_3-27	1.35 s	1.28 s	1.29 s	1.28 s	1.37 s
H_3-28	1.70 s	1.65 s	1.72 s	1.73 s	2.03 s
H_3-29	1.23 s	1.27 s	1.35 s	1.35 s	1.35 s
H_3-30	1.04 s	1.11 s	1.15 s	1.16 s	0.92 s
H-1′	4.80 d (7.7)	4.92 d (4.7)	4.75 d (6.9)	4.73 d (7.6)	4.83 d (7.4)
H-2′	5.37 dd (9.4, 7.9)	4.15[b]	4.13[b]	4.19[b]	4.05[b]
H-3′	5.56 dd (9.4, 9.1)	5.51 t (6.6)	5.63 t (8.7)	4.10[b]	4.20[b]
H-4′	4.25[b]	5.13 dd (6.5, 4.4)	4.15[b]	4.15[b]	4.17[b]
H-5′	3.66 t (10.7), 4.28[b]	3.66[b], 4.32 dd (12.1, 4.1)	3.65[b], 4.28[b]	3.63[b], 4.29[b]	3.64[b], 4.35[b]
H-1″	4.85 d (7.6)	5.64 br s	5.73 br s	5.72 br s	4.85 d (7.7)
H-2″	4.08 dd (9.2, 8.9)	4.44[b]	4.58[b]	4.79[b]	4.03[b]
H-3″	4.23[b]	4.44[b]	4.45[b]	4.64[b]	4.20[b]
H-4″	4.21[b]	4.23 m	4.25[b]	4.34[b]	4.15[b]
H-5″	3.94[b]	4.44[b]	4.58[b]	4.84[b]	3.94[b]
H-6″	4.27[b], 4.39 dd (11.7, 2.9)	1.63 d (6.1)	1.67 d (6.1)	1.68 d (6.0)	4.35[b], 4.57[b]
H-1‴	—	4.79 d (7.2)	4.79 d (7.2)	4.75 d (7.4)	4.75 d (7.7)
H-2‴	—	3.95 dd (8.1, 7.7)	3.97[b]	3.94[b]	4.00[b]
H-3‴	—	4.08 t (8.5)	4.15[b]	4.07[b]	4.20[b]
H-4‴	—	4.14[b]	4.15[b]	4.15[b]	4.15[b]
H-5‴	—	3.64[b], 4.28 dd (11.3, 5.0)	3.65[b], 4.28[b]	3.63[b], 4.29[b]	3.94[b]
H-6‴	—	—	—	—	4.35[b], 4.57[b]

a) Assignments confirmed by COSY, TOCSY, HMQC and HMBC experiments. *b*) ^{1}H-NMR chemical shifts assigned on the basis of a DQF-COSY experiment. Multiplicity of the signals are unclear due to overlapping. $COC\underline{H}_3$: 2.00, 2.07, 2.09 for **1**, 1.91, 2.07 for **2**, 2.07 for **3**.

H-2_{xyl} (δ 5.37 dd, J=7.9, 9.4 Hz) from the anomeric proton signal at δ 4.80 (d, J=7.7 Hz, H-1_{xyl}). H-2_{xyl} showed correlation with H-3_{xyl} (δ 5.56, dd, J=9.1, 9.4 Hz), while the latter proton showed cross peaks with H-4_{xyl} (δ 4.25) which, in turn, coupled with H_2-5_{xyl} (δ 3.66, t, J=10.7 Hz; δ 4.28). Thus, the location of the acetoxy groups were ascertained from downfield acetylation shifts observed for H-2_{xyl} and H-3_{xyl} (*ca.* 1.4 ppm). This assumption was supported by long-range correlations (HMBC) between H-2_{xyl} and CO at δ 170.4, and H-3_{xyl} and CO at 171.0 ppm. Once the proton and the carbon spectra had been completely assigned, an unambiguous determination of the linkage sites was obtained from the long-range C–H (HMBC) correlation. Thus, the anomeric proton of the β-D-xylopyranose moiety (δ 4.80, d, J=7.7 Hz, H-1_{xyl}) displayed a long-range correlation to C-3 (δ 89.96), while the anomeric proton of β-D-glucopyranose moiety (δ 4.85, d, J=7.6 Hz) showed HMBC connectivity to C-6 (δ 79.1). Moreover, the location of the acetoxy groups at C-16 was confirmed unambiguously by the HMBC spectrum of **1**, which showed significant cross peaks due to $^3J_{C-H}$ correlations between H-16 (δ 5.66, ddd, J=5.2, 7.8, 7.9 Hz) and CO at δ 170.8.

On the basis of this evidence, the structure of **1** was established as 3-*O*-β-(2′,3′-di-*O*-acetyl)-D-xylopyranosyl-6-*O*-β-D-glucopyranosyl-16-*O*-acetoxy-20(*R*),24(*S*)-epoxycycloartane-3β,6α,16β,25-tetrol, for which the trivial name trojanoside I is proposed.

The molecular formula of **2** was determined as $C_{50}H_{80}O_{19}$ by HR-ESI-MS which exhibited an ion peak at *m/z* 1007.3524 $[M+Na]^+$. The ^{1}H-NMR spectrum of **2** suggested the presence of seven tertiary and two acetyl methyl groups, from signals at (δ 1.11, 1.27, 1.28, 1.30, 1.38 1.56, 1.65, 1.91, 2.07, each s), as well as the characteristic signals of cyclopropane-methylene protons at δ 0.15, 0.60 (J_{AX}=4.0 Hz, H_2-19). Additionally, the resonances of three anomeric protons, indicative of the presence of three sugar moieties, were observed in the downfield region at δ 4.92 (d, J=4.7 Hz, H-1′), and 5.64 (br s, H-1″), and δ 4.79 (d, J=7.2 Hz, H-1‴).

Combination of DQF-COSY, and total correlation spectroscopy (TOCSY) experiments allowed the sequential assignments of all proton resonances within each sugar residue, starting from the readily identifiable anomeric protons. Thus, on the basis of chemical shifts, the multiplicity of the signals, and the coupling constants, the three sugar residues were identified as α-rhamnopyranose and two β-xylopyranose.[15] The common L-configuration for α-rhamnopyranose and D-configuration for β-xylopyranose were assumed, according to those most often encountered among the plant glycosides in each case. The HMQC experiments correlated each ^{1}H-NMR sugar signal to the corresponding carbon resonance and showed the absence of any glycosylation shift for the ^{13}C-NMR resonances of the rhamnopyranosyl residue, suggesting this sugar to be terminal. Additionally, in the ^{1}H-NMR spectrum, the H-3′ and H-4′ protons of the xylose residue were observed at δ 5.51 (t, J=6.6 Hz) and δ 5.13 (dd, J=4.4, 6.5 Hz), respectively, showing the downfield shift due to acetylation.

After subtraction of the sugar and acetyl group signals, the

Table 2. ^{13}C Assignments of **1**—**5** (125 MHz, δ ppm, in C_5D_5N)

Position	1	2	3	4	5
1	32.3 t	32.7 t	32.7 t	32.6 t	32.4 t
2	30.2 t	30.4 t	30.8 t	30.2 t	30.3 t
3	89.6 d	89.2 d	88.8 d	87.6 d	88.8 d
4	42.7 s	43.2 s	43.3 s	42.7 s	42.8 s
5	52.6 d	52.8 d	52.8 d	52.1 d	52.7 d
6	79.1 d	78.5 d	78.1 d	77.2 d	80.1 d
7	34.0 t	34.3 t	34.4 t	33.7 t	35.1 t
8	45.4 d	44.6 d	44.0 d	42.7 d	46.1 d
9	22.1 s	22.1 s	22.1 s	21.4 s	20.3 s
10	29.1 s	29.0 s	28.8 s	28.0 s	29.4 s
11	26.3 t	27.2 t	27.1 t	26.5 t	26.7 t
12	33.3 t	34.1 t	33.6 t	33.7 t	33.2 t
13	46.8 s	46.1 s	46.1 s	45.4 s	46.9 s
14	47.1 s	47.0 s	47.0 s	46.3 s	46.9 s
15	45.7 t	46.6 t	46.4 t	45.4 t	47.4 t
16	76.6 d	74.3 d	74.3 d	73.6 d	83.7 d
17	58.0 d	59.0 d	58.9 d	58.1 d	59.9 d
18	20.8 q	21.2 q	20.9 q	20.0 q	21.3 q
19	28.2 t	27.3 t	26.3 t	24.8 t	29.6 t
20	86.2 s	88.2 s	88.2 s	87.5 s	87.2 s
21	27.2 q	29.5 q	28.4 q	28.8 q	26.4 q
22	37.3 t	35.8 t	35.8 t	35.1 t	38.8 t
23	27.1 t	27.2 t	27.3 t	26.7 t	25.9 t
24	83.3 d	82.5 d	82.5 d	81.8 d	84.5 d
25	71.3 s	72.1 s	72.1 s	71.5 s	72.0 s
26	28.5 q	29.0 q	29.5 q	28.3 q	27.6 q
27	27.2 q	28.0 q	28.0 q	27.4 q	26.5 q
28	28.5 q	28.7 q	29.0 q	27.5 q	28.9 q
29	16.9 q	17.3 q	17.5 q	17.0 q	16.8 q
30	20.3 q	20.5 q	20.0 q	19.6 q	20.3 q
1′	104.5 d	104.5 d	106.6 d	105.8 d	107.8 d
2′	73.6 d	75.2 d	77.4 d	78.0 d	75.8 d
3′	77.3 d	72.6 d	79.0 d	79.6 d	78.7 d
4′	69.3 d	70.3 d	69.9 d	71.2d	71.4 d
5′	67.2 t	61.7 t	67.7 t	67.0 t	67.2 t
1″	105.4 d	102.9 d	103.1 d	102.0 d	105.7 d
2″	76.1 d	71.5 d	72.8 d	72.6 d	75.7 d
3″	79.7 d	73.3 d	73.3 d	72.6 d	79.4 d
4″	72.3 d	74.6 d	74.6 d	74.3 d	72.2 d
5″	78.8 d	72.9 d	71.4 d	69.8 d	78.1 d
6″	63.5 t	19.5 t	19.5 t	18.9 t	63.0 t
1‴	—	106.6 d	106.0 d	106.0 d	106.6 d
2‴	—	76.2 d	76.3 d	75.6 d	75.7 d
3‴	—	79.3 d	79.2 d	78.4 d	79.0 d
4‴	—	71.9 d	72.0 d	71.6 d	72.0 d
5‴	—	67.8 t	67.1 t	67.0 t	78.5 d
6‴	—	—	—	—	63.4 t

$\underline{C}OCH_3$: 170.4, 170.8, 171.0 for **1**, 171.0, 171.1 for **2**, 171.5 for **3**, $CO\underline{C}H_3$: 21.2, 21.3, 21.7 for **1**, 21.2, 21.7 for **2**, 22.0 for **3**.

remaining ^1H and ^{13}C resonances of **2** arising from the sapogenol moiety were very close to those of **1** except for the signal ascribable to H-16 (δ 5.03, dd, J=7.1, 7.3 Hz) exhibiting no acylation shift. Thus, the presence of a cycloastragenol moiety in compound **2**, a common feature of cycloartane-type triterpenoids isolated from *Astragalus* species, was evident. Compound **2** affords, upon deacetylation, compound **4** whose structure is identical to that of astrasieversianin XV.[16] Key correlation peaks observed in the HMBC spectrum of **2** between H-1′ of the xylosyl at δ 4.92 (d, J=4.7 Hz) and C-3 (δ 89.2) of the aglycon, between H-1″ of the rhamnosyl at δ 5.64 (br s) and C-2′ (δ 75.2) allowed the disaccharide chain at C-3 to be determined as α-L-rhamnopyranosyl-(1→2)-β-(3′,4′-di-*O*-acetyl)-D-xylopyranoside. The cross peak of the ^3J long-range coupling between H-1‴ (δ 4.79, d, J=7.2 Hz) and C-6 (δ 78.5) provided evidence for the position of the second xylose moiety.

Based on these results, **2** was assigned as 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-(3′,4′-di-*O*-acetyl)-D-xylopyranosyl]-6-*O*-β-D-xylopyranosyl-20(*R*),24(*S*)-epoxycycloartane-3β,6α,16β,25-tetrol, and it was named trojanoside J.

HR-ESI-MS of **3** showed an ion peak for $[M+Na]^+$ at *m/z* 965.5188, in agreement with the molecular formula $C_{48}H_{78}O_{18}$. Detailed examination of 1D- and 2D-NMR spectra of **3** and comparison with those of **2** showed their considerable structural similarity. The differences consisted only in the signals of the sugar chain which was attached at C-3 of the sapogenol moiety. The difference appeared to be due to the absence of an acetyl group at the xylose residue, as determined by ^1H- and ^{13}C-NMR and HR-ESI-MS. Detailed examination of DQF-COSY, HMQC and HMBC spectra of **3** allowed unambiguous assignment of sugar chain at C-3 of aglycon, identified as α-L-rhamnopyranosyl-(1→2)-β-(3′-*O*-acetyl)-D-xylopyranosyl.

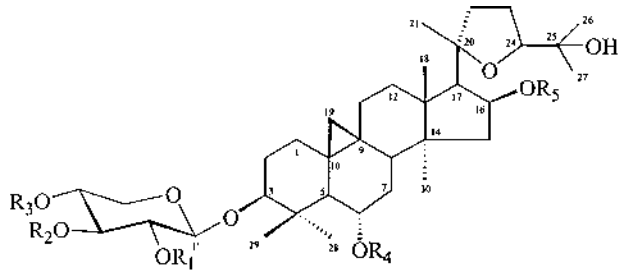
On the basis of these evidence, the structure of **3**, was established as 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-(3′-*O*-acetyl)-D-xylopyranosyl]-6-*O*-β-D-xylopyranosyl-20(*R*),24(*S*)-epoxycycloartane-3β,6α,16β,25-tetrol (astrasieversianin IX), isolated previously from *Astragalus sieversianus*.[16]

The molecular formula of **5** was determined as $C_{47}H_{78}O_{19}$ by HR-ESI-MS which exhibited an ion peak at *m/z* 969.4943 $[M+Na]^+$. The IR spectrum of **5** indicated the presence of hydroxyl (3389 cm^{-1}) functionalities. The NMR spectral data of **1** (Tables 1, 2) were consistent with **5** being a cycloartane-type glycoside. Full assignments of the proton and carbon signals of the aglycon part of **5** were secured from its DQF-COSY and HMQC spectra. The resonances assigned to the sapogenol moiety were in good agreement with cycloastragenol which was glycosylated at C-3, C-6 and C-16. The glycosylation shifts observed for these carbons (δ 88.8, C-3; δ 80.1, C-6; δ 83.7, C-16) suggested that compound **5** was a rarely encountered tridesmosidic saponin. The sugars were determined to be two glucopyranosyl and a xylopyranosyl in **5**, by the use of 1D- and 2D-NMR experiments. The HMQC spectrum established the absence of any glycosylation shift for all the carbon resonances of sugar moieties and suggested all the sugars to be terminal units, confirming the tridesmosidic structure of **5**.

The position of each sugar residue was unambigously determined by the HMBC experiment (Chart 1) which showed long-range correlations between C-3 (δ 88.8) and H-1′$_{xyl}$ (δ 4.75), C-6 (δ 80.1) and H-1″$_{glu}$ (δ 4.85), C-16 (δ 83.7) and H-1‴$_{glu}$ (δ 4.83).

Based on these results, the structure of **5** was determined as 3-*O*-β-D-xylopyranosyl-6,16-di-*O*-β-D-glucopyranosyl-20(*R*),24(*S*)-epoxycycloartane-3β,6α,16β,25-tetrol, and it was named trojanoside K.

Astrasieversianin I, astrasieversianin II, astrasieversianin XV (**4**), astragaloside I, astragaloside II, astragaloside IV, astragaloside VII, and trojanoside H were also isolated from the aerial parts of *A. trojanus*, identified on the basis of their HR-ESI-MS, and NMR (^1H- and ^{13}C-) data, in comparison with literature values.[10,14,16,17]



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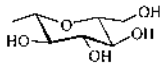
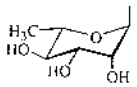
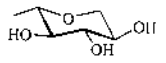
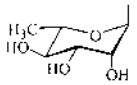
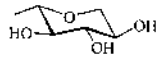
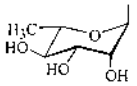
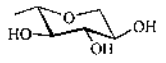
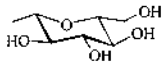
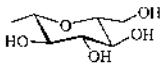
1	Ac	Ac	H		Ac
2		Ac	Ac		H
3		Ac	H		H
4		II	H		H
5	H	H	H		

Chart 1. Structure of **1**—**5**

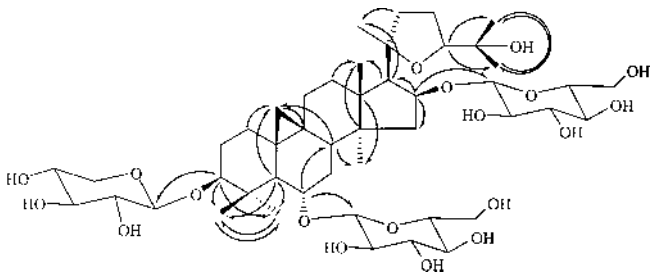


Chart 2. Key HMBC of **5**

Experimental

General Experimental 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-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 (80 : 20 : 2, 70 : 30 : 3, 61 : 32 : 7); visualization: Van.H_2SO_4. Column chromatography: silica gel 230—400 mesh (Merck), RP (C-18, 40 mμ) (Merck).

Plant Material *Astragalus trojanus* Stev. (Leguminosae) was collected from Hacibozlar Village, Burhaniye-Balikesir, West Anatolia, in July 1999. The plant specimen was determined by Professor Dr. Zeki Aytac (Department of Botany, Gazi University, Etiler, Ankara, Turkey).

Extraction and Isolation The air-dried, powdered aerial parts (4.5 kg) of *A. trojanus* were extracted with 99% EtOH under reflux. The solvent was removed by rotary evaporation yielding 200 g extract. One hundred and eighty grams of the EtOH extract was subjected to vacuum liquid chromatograpy (VLC) using silica gel (1.3 kg) as the stationary phase eluting with $CHCl_3$–MeOH (90 : 10, 85 : 15) and $CHCl_3$–MeOH–H_2O (80 : 20 : 1, 70 : 30 : 3) to give 19 fractions (fractions A—T). Fractions E (1.4 g), F (1.9 g), H (1.6 g) and I (2.76 g) were combined and subjected to VLC using reversed-phase material (Sepralyte 40 μm, 500 g). Elution with MeCN–H_2O mixtures (25→60%) yielded 11 fractions (fractions EI 1—11). Fraction EI-6 (758 mg) was subjected to a silica gel column (100 g) using $CHCl_3$, $CHCl_3$–MeOH (95 : 5, 90 : 10) and $CHCl_3$–MeOH–H_2O (85 : 15 : 0.5) to give trojanoside I (**1**; 8 mg) and fraction EI-6b (450 mg). Fraction EI-6b was subjected to a flash column chromatography on silica gel (50 g) using EtOAc–MeOH–H_2O (250 : 12.5 : 1.25, 227.5 : 15 : 2.5) yielding fraction EI-6b-1 (290 mg). Fraction EI-9 (430 mg) and fraction EI-6b-1 (290 mg) were combined and chromatographed on silica gel (120 g) using EtOAc–MeOH–H_2O (100 : 15 : 2.5) to give 6 fractions (fractions EI-9a—f). Fraction EI-9f (75.2 mg) was subjected to silica gel column (25 g) using EtOAc–MeOH–H_2O (230 : 15 : 5) to give trojanoside J (**2**; 29.2 mg). Fraction S (9.3 g) and fraction T (7.1 g) were combined and resolved by VLC on reversed-phase material (Sepralyte 40 μm, 250 g), employing MeOH–H_2O (10→70%) to give 4 fractions (fractions ST 1—4). Fraction ST-2 (4.0 g) was subjected to a silica gel column (350.0 g) using $CHCl_3$–MeOH–H_2O (80 : 20 : 2) to give astrasieversianin IX (**3**; 11.0 mg). Fraction R (4.04 g) was further purified by VLC on reversed-phase material (Sepralyte 40 μm, 250 g), eluted with MeOH–H_2O (10→90%) to give fraction R-1 (1.4 g). Fraction R-1 was subjected to silica gel column (140 g) using $CHCl_3$–MeOH–H_2O (80 : 20 : 1, 80 : 20 : 2) to yield astrasieversiannin XV (**4**; 526.0 mg). Fraction ST-1 (3.5 g) was chromatographed on reversed-phase material (Sepralyte 40 μm, 250 g), employing MeOH–H_2O (40→70%) to give trojanoside K (**5**, 70.0 mg).

Trojanoside I (**1**): 3-*O*-β-(3′,4′-Di-*O*-acetyl)-D-xylopyranosyl-6-*O*-β-D-glucopyranosyl-16-*O*-acetoxy-20(*R*),24(*S*)-epoxy-3β,6α,25-trihydroxycycloartane: White powder, IR (KBr) ν_{max} 3419, 2936, 1726, 1461, 1379, 1262, 1078, 1041 cm^{-1}. ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. HR-ESI-MS: $[M+Na]^+$ at *m*/*z* 933.3207.

Trojanoside J (**2**): 3-*O*-[α-L-Rhamnopyranosyl-(1→2)-β-(3′,4′-di-*O*-acetyl)-D-xylopyranosyl]-6-*O*-β-D-xylopyranosyl-20(*R*),24(*S*)-epoxy-3β,6α,16β,25-tetrahydroxycycloartane: White powder, IR (KBr) ν_{max} 3408, 2928, 1745, 1371, 1244, 1042 cm^{-1}. ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. HR-ESI-MS: $[M+Na]^+$ at *m*/*z* 1007.3524.

Astrasieversianin IX (**3**): 3-*O*-[α-L-Rhamnopyranosyl-(1→2)-β-(3′-*O*-

acetyl)-D-xylopyranosyl]-6-*O*-β-D-xylopyranosyl-20(*R*),24(*S*)-epoxy-3β,6α,16β,25-tetrahydroxycycloartane: White powder, IR (KBr) ν_{max} 3395, 2934, 1726, 1461, 1373, 1262, 1057 cm^{-1}. ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. HR-ESI-MS: $[M+Na]^+$ at *m*/*z* 965.5188.

Astrasieversianin XV (**4**): 3-*O*-[α-L-Rhamnopyranosyl-(1→2)-β-D-xylopyranosyl]-6-*O*-β-D-xylopyranosyl-20(*R*),24(*S*)-epoxy-3β,6α,16β,25-tetrahydroxycycloartane: White powder, IR (KBr) ν_{max} 3369, 2933, 1450, 1367, 1043 cm^{-1}. ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. HR-ESI-MS: $[M+Na]^+$ at *m*/*z* 923.5083.

Trojanoside K (**5**): 3-*O*-β-D-Xylopyranosyl-6,16-di-*O*-β-D-glucopyranosyl-20(*R*),24(*S*)-epoxy-3β,6α,16β,25-tetrahydroxycycloartane: White powder, IR (KBr) ν_{max} 3389, 2933, 1648, 1455, 1367, 1261, 1075, 1044 cm^{-1}. ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. HR-ESI-MS: $[M+Na]^+$ at *m*/*z* 969.4943.

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