Secondary Metabolites from the Roots of *Astragalus trojanus*

Erdal Bedir,[†] Ihsan Çalis,*[,†] Rita Aquino,[‡] Sonia Piacente,[‡] and Cosimo Pizza[‡]

Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, TR-06100 Ankara, Turkey, and Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, 84084 Penta di Fisciano, Salerno, Italy

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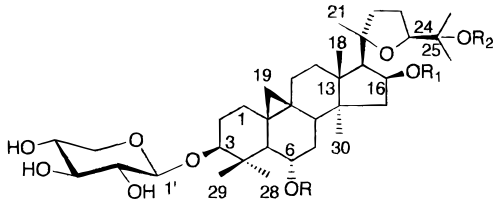
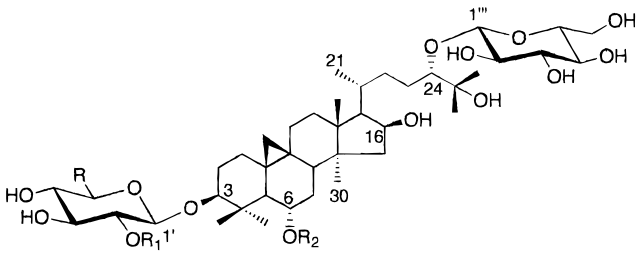
Six novel cycloartane-type glycosides were isolated from the roots of *Astragalus trojanus*. Two of these, compounds **1** and **2**, have (20*R*,24*S*)-epoxy-3*β*,6α,16*β*,25-tetrahydroxycycloartane as the aglycon, while compounds **3**−**6** possess 3*β*,6α,16*β*,(24*S*),25-pentahydroxycycloartane as the aglycon. The saccharide moieties linked to the C-3, C-6, and C-24 or C-25 positions of the aglycons in **1**−**6** contained either xylopyranose, glucopyranose, rhamnopyranose, or arabinopyranose units. Structure elucidation of compounds **1**−**6** was accomplished through the extensive use of 1D and 2D NMR techniques. In addition, a new oleanene glycoside (**7**) and a new tryptophan derivative (**8**) were also isolated and characterized.

In the flora of Turkey the genus *Astragalus* (Leguminosae) is represented by approximately 380 species.[1] *A. microcephalus* is used primarily in Turkey for the production of the economically important gum, tragacanth.[2] Our research program on the oligoglycosidic constituents of Turkish *Astragalus* species has led to the discovery of a number of cycloartane-type glycosides from several different *Astragalus* species. Eight cycloartane saponins were isolated from *A. melanophrurius*,[3] which showed a modest antibacterial activity and stimulated lymphocyte transfer in vitro, with further representatives isolated from *A. oleifolius*,[4] *A. microcephalus*,[5] and *A. brachypterus*.[6] In the present study, the constituents of the roots of *A. trojanus* have been examined, and here the isolation of eight new compounds is reported, with six (compounds **1**−**6**, named trojanosides A−F) belonging to the cycloartane glycoside class. In addition, an oleanene glycoside (**7**), and a tryptophan derivative (**8**), named, respectively, astrojanoside A and achillamide, were also isolated.



	R	R_1	R_2
1	1"-Glc	$COCH_3$	H
2	1"-Glc	H	1'''-Glc



	R	R_1	R_2
3	H	1"-Glc	H
4	CH_2OH	H	1"-Glc
5	H	1""-Rha	1"-Glc
6	H	1""-Ara	1"-Glc

Results and Discussion

Compounds **1** ($C_{43}H_{70}O_{15}$) and **2** ($C_{46}H_{76}O_{18}$) gave quasimolecular ion peaks in their negative ion FABMS at *m*/*z* 825 $[M - H]^-$ and *m*/*z* 915 $[M - H]^-$, respectively, and prominent peaks (see Experimental Section) due to the loss of a pentose (132 mass units) and a hexose (162 mass units) for **1** and two pentose units and a hexose for **2**. The NMR spectral data of saponins **1** and **2** (Table 1 and Experimental Section) revealed characteristic features of cycloartane glycosides.[3−6] The ^{1}H NMR spectra of **1** and **2** displayed diagnostic signals due to the cyclopropane-ring methylene protons as an AX system (δ 0.28 and 0.63, $J_{AX} = 4.5$ Hz, H-19a and H-19b in **1**) and seven tertiary methyl groups (at δ 1.03, 1.10, 1.16, 1.17, 1.30, and 1.35 × 2 in **1**) in the aglycon moiety. Additionally, resonances of anomeric protons were observed in the low-field region in the ^{1}H NMR spectrum at δ_H 4.34 (d, $J = 7.5$ Hz) and 4.31 (d, $J = 7.8$ Hz) for **1** and δ_H 4.32 (d, $J = 7.5$ Hz), 4.34 (d, $J = 7.5$ Hz), and 4.56 (d, $J = 7.5$ Hz) for **2**, indicative of the presence, respectively, of two and three *β*-linked sugar units. Full assignments of the proton and carbon signals of the aglycon part of **1** were secured by ^{1}H−^{1}H DQF COSY[7] and HSQC[8] spectra. It was apparent from the ^{1}H and ^{13}C NMR data of **2** that this compound was based on the same aglycon as **1**. The aglycon signals indicated cycloastragenol [(20*R*,24*S*)-epoxy-3*β*,6α,16*β*,25-tetrahydroxycycloartane] as this agly-

* To whom correspondence should be addressed. Tel.: 90 312-3103545/1089. Fax: 90 312-3114777. E-mail: acalis@dominet.in.com.tr.
[†] Hacettepe University.
[‡] Università degli Studi di Salerno.

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Table 1. ^{1}H and ^{13}C NMR Data of the Sugar Portion of Compounds **1**−**4**[a]

	1		**2**		**3**		**4**	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
	β-D-xyl (at C-3)		β-D-xyl (at C-3)		β-D-xyl (at C-3)		β-D-glc (at C-3)	
1′	4.31 d (7.8)	107.1	4.32 d (7.5)	105.2	4.41 d (7.5)	105.9	4.37 d (7.5)	104.8
2′	3.22 dd (7.8, 8.5)	75.1	3.19 dd (7.5, 9.0)	75.0	3.45 dd (7.5, 9.0)	78.5	3.21 dd (7.5, 9.0)	75.2
3′	3.32 t (8.5)	77.8	3.32 t (9.0)	77.6	3.45 t (9.0)	78.5	3.37 t (9.0)	78.2
4′	3.50 ddd (4.5, 8.5, 11)	70.9	3.50 ddd (4.5, 9.0, 11)	70.8	3.48 ddd (4.5, 8.5, 11)	71.2	3.31 t (9.0)	71.4
5′	3.21 t (11.0)	66.5	3.21 t (11.0)	66.1	3.21 t (11.0)	65.9	3.27 ddd (3.5, 4.5, 9.0)	77.5
	3.85 dd (4.5, 11.0)		3.86 dd (4.5, 11.0)		3.89 dd (4.5, 11.0)			
6′							3.71 dd (4.5, 12.0)	62.5
							3.87 dd (2.5, 12.0)	
	β-D-glc (at C-6)		β-D-xyl (at C-6)		α-L-rha (at C-2 xyl)		β-D-glc (at C-6)	
1″	4.34 d (7.5)	104.7	4.34 d (7.5)	105.2	5.37 d (1.5)	101.7	4.37 d (7.5)	105.8
2″	3.21 dd (7.5, 9.0)	75.1	3.19 dd (7.5, 9.0)	75.0	3.98 dd (1.5, 2.5)	71.8	3.21 dd (7.5, 9.0)	75.2
3″	3.33 t (9.0)	78.1	3.32 t (9.0)	77.6	3.78 dd (2.5, 9.0)	71.7	3.37 t (9.0)	78.2
4″	3.32 t (9.0)	70.4	3.50 ddd (4.5, 9.0, 11)	70.8	3.42 t (9.0)	73.6	3.31 t (9.0)	71.4
5″	3.25 ddd (3.5, 4.5, 9.0)	77.6	3.21 t (11.0)	66.1	4.01 m	69.7	3.27 ddd (3.5, 4.5, 9.0)	77.5
			3.86 dd (4.5, 11.0)		1.28 d (6.5)	18.0		
6″	3.67 dd (4.5, 12.0)	62.7					3.71 dd (4.5, 12.0)	62.2
	3.87 dd (3.5, 12.0)						3.87 dd (2.5, 12.0)	
			β-D-glc (at C-25)		β-D-glc (at C-24)		β-D-glc (at C-24)	
1‴			4.56 d (7.5)	98.4	4.44 d (7.5)	104.7	4.44 d (7.5)	104.8
2‴			3.18 dd (7.5, 9.0)	75.0	3.27 dd (7.5, 9.0)	75.2	3.28 dd (7.5, 9.0)	75.0
3‴			3.38 t (9.0)	77.9	3.40 t (9.0)	77.7	3.42 t (9.0)	77.9
4‴			3.37 t (9.0)	71.1	3.33 t (9.0)	71.2	3.31 t (9.0)	71.4
5‴			3.27 ddd (2.5, 4.5, 9.0)	77.5	3.33 ddd (3.0, 4.5, 9.0)	77.7	3.34 ddd (2.5, 4.5, 9.0)	77.8
6‴			3.68 dd (4.5, 11.0)	62.5	3.68 dd (4.5, 12.0)	62.2	3.70 dd (4.5, 12.0)	62.5
			3.89 dd (2.5, 11.0)		3.89 dd (3.0, 12.0)		3.90 dd (2.5, 12.0)	

[a] Assignments confirmed by 1D-TOCSY, 2D-HOHAHA, DQF−COSY, HSQC, and HMBC experiments.

con, which was glycosylated at C-3 (δ 89.9) and C-6 (δ 79.2) in **1**, and at C-3, C-6, and C-25 (δ 79.9) in **2**. The glycosylation shifts observed for these carbons suggested that **2** was a tridesmosidic saponin like astragaloside VII[9] and **1** was a bisdesmosidic saponin like cyclocephaloside II.[6] The aglycon signals of **1** were superimposable on those of cyclocephaloside II,[6] except for the presence of a $-COCH_3$ group ($COCH_3$ δ_C 20.6, $COCH_3$ δ_C 171.9, $COCH_3$ δ_H 2.06) and with the C-16 resonances (δ_C 77.6 and δ_H 5.48, ddd, J = 8.0, 8.0, 5.2 Hz) shifted downfield by 3.3 and 0.9 ppm, respectively, suggesting esterification at this position. The location of the acetoxy group at C-16 was confirmed unambiguously by the HMBC[10] (8 Hz) spectrum of **1**, which showed significant cross peaks, due to $^3J_{C-H}$ correlations between the H-16 (δ 5.48) and the CO (δ 171.9) signals.

The sugars were determined to be a xylopyranosyl and a glucopyranosyl in **1**, with two xylopyranosyls and a glucopyranosyl in **2**, by the use of monodimensional TOCSY,[11] and 2D DQF COSY and HSQC NMR experiments. Even at high field (600 MHz) the 1D sugar spectral region of **1** and **2** was complex, as most of the shifts overlapped and were found between δ 3.89 and 3.19. The isolated anomeric proton signals resonating at an uncrowded region of the spectrum (between δ 4.31 and 4.56) were used as the starting point for the 1D TOCSY experiments. Because of the selectivity of the multistep coherence transfer, the 1D TOCSY subspectra of the single monosaccharide unit could be extracted from the crowded overlapping region. Each subspectrum could be attributed to one set of coupled protons such as H−C (1) to H−C (5) (for xylose) or H−C (6) (for glucose) of an individual monosaccharide. The interpretation of the 1D TOCSY subspectra of the monosaccharide units was accomplished as summarized in Table 1, with the type of sugar and its configuration and conformation assigned from the 2D COSY spectrum. The HSQC spectrum established the absence of any glycosylation shift for all the carbon resonances and suggested all the sugars to be terminal units. Once the proton and carbon spectra had been completely assigned, an unambiguous determination of the sequence and linkage sites was obtained from the long-range C−H (HMBC) correlation. The sugar substituent at C-3 of compound **1** was identified from the following evidence: a 1D TOCSY subspectrum obtained by irradiation at δ 4.31 showed a set of coupled protons at δ 3.22, 3.32, and 3.50 (all CH), and 3.21 and 3.85 (CH_2) were assigned as H-1′ to H_2-5′ of a xylopyranose unit by the COSY spectrum. The cross peak of the ^3J long-range coupling between H-1′ (δ 4.31) and C-3 (δ 89.9) as well as H-1″ (δ 4.34) and C-6 (δ 79.2) of the aglycon provided definitive evidence for the position of these sugar moieties. Key correlation peaks in the HMBC spectrum of **2** were similarly observed between H-1′ (δ 4.32) of the xylose and C-3 of the aglycon, and between H-1″ of the second xylose unit at δ 4.34 and C-6, and between H-1‴ (δ_H 4.56) of the glucose and C-25 (δ_C 79.9), establishing the position of these sugars as shown. Thus **1** (trojanoside A) was assigned as 3-*O*-β-D-xylopyranosyl-6-*O*-β-D-glucopyranosyl-16-*O*-acetoxy-(20*R*,24*S*)-epoxy-3β,6α,25-trihydroxycycloartane, and **2** (trojanoside B) as 3-*O*-β-D-xylopyranosyl-6-*O*-β-D-xylopyranosyl-25-*O*-β-D-glucopyranosyl-(20*R*,24*S*)-epoxy-3β,6α,16β,25-tetrahydroxycycloartane.

The more polar compounds **3** ($C_{47}H_{80}O_{18}$), **4** ($C_{48}H_{82}O_{20}$), **5** ($C_{53}H_{90}O_{23}$), and **6** ($C_{52}H_{88}O_{23}$) showed $[M - H]^-$ peaks at m/z 931, 977, 1093, and 1079, respectively, in their FABMS. Their NMR spectral data were consistent with these compounds being cycloartane glycosides with cyclocanthogenin[6] as the aglycon. In compound **3**, signals typical of cyclopropane methylene protons at C-19 (δ 0.41 and 0.57, each d, J = 3.5 Hz), six tertiary (δ 0.98, 1.05, 1.19 × 2, 1.21, 1.32) and one secondary (δ 0.97, d, J = 6.0 Hz) methyl group(s), and a signal for a oxymethine group at C-16β (δ 4.43, ddd, J = 8.2, 8.0, 5.2 Hz) were apparent. Full assignments of the ^{1}H and ^{13}C NMR signals of the aglycon moiety were obtained from the ^{1}H−^{1}H DQF COSY and HSQC spectra. Glycosylation at C-3 and C-24 was indicated by the pronounced downfield shifts observed for C-3 (δ 89.5) and C-24 (δ 89.8) relative to the corresponding signals in cyclocanthogenin,[6] whereas the C-6 (δ_C 69.2, δ_H 3.47, ddd,

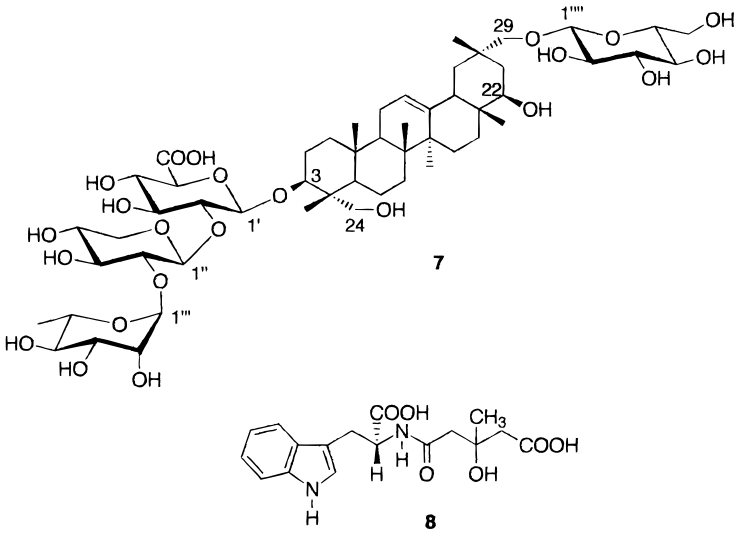
Table 2. ^{1}H and ^{13}C NMR Data of the Sugar Portion of Compounds **5**−**7**[a]

	5		**6**		**7**	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
	β-D-xyl (at C-3)		β-D-xyl (at C-3)		β-D-glcA (at C-3)	
1′	4.41 d (7.7)	105.9	4.50 d (7.7)	105.6	4.43 d (7.5)	105.2
2′	3.47 dd (7.7, 9.0)	78.7	3.45 dd (7.7, 9.0)	82.8	3.59 dd (7.5, 9.0)	78.6
3′	3.47 t (9.0)	78.1	3.55 t (9.0)	76.6	3.65 t (9.0)	76.8
4′	3.49 ddd (4.5, 9.0, 11)	71.1	3.54 ddd (4.5, 9.0, 11)	70.6	3.43 t (9.0)	74.0
5′	3.21 t (11.0)	66.1	3.22 t (11.0)	65.7	3.58 d (9.0)	77.0
	3.89 dd (4.5, 11.0)		3.89 dd (4.5, 11.0)			
6′						176.0
	β-D-glc (at C-6)		β-D-glc (at C-6)		β-D-xyl	
1″	4.35 d (7.8)	104.6	4.36 d (7.5)	104.7	4.81 d (7.4)	102.9
2″	3.22 dd (7.8, 9.0)	75.3	3.21 dd (7.5, 9.0)	75.3	3.41 dd (7.4, 8.6)	79.1
3″	3.36 t (9.0)	78.2	3.36 t (9.0)	78.2	3.58 t (8.6)	78.4
4″	3.33 t (9.0)	71.3	3.30 t (9.0)	71.4	3.54 m	71.0
5″	3.27 ddd (2.5, 4.5, 11)	77.5	3.28 ddd (2.5, 4.5, 11)	77.5	3.13 t (11.0)	66.5
					3.83 dd (4.5, 11.0)	
6″	3.68 dd (4.5, 11.0)	62.2	3.68 dd (4.5, 11.0)	62.2		
	3.89 dd (2.5, 11.0)		3.89 dd (2.5, 11.0)			
	β-D-glc (at C-24)		β-D-glc (at C-24)		α-L-rha (at C-2 xyl)	
1‴	4.44 d (7.5)	104.6	4.44 d (7.5)	104.7	5.22 d (1.5)	102.0
2‴	3.27 dd (7.5, 9.0)	75.2	3.27 dd (7.5, 9.0)	75.2	3.97 dd (1.5, 2.5)	72.2
3‴	3.40 t (9.0)	77.7	3.40 t (9.0)	77.6	3.78 dd (2.5, 9.0)	72.0
4‴	3.34 t (9.0)	71.3	3.34 t (9.0)	71.1	3.45 t (9.0)	74.0
5‴	3.33 ddd (2.5, 4.5, 11)	77.7	3.33 ddd (2.5, 4.5, 11)	77.7	4.16 m	69.0
6‴	3.68 dd (4.5, 11.0)	62.2	3.70 dd (4.5, 11.0)	62.5	1.29 d (6.5)	18.0
	3.89 dd (2.5, 11.0)		3.89 dd (2.5, 11.0)			
	α-L-rha (at C-2 xyl)		α-L-ara (at C-2 xyl)		β-D-glc (at C-29)	
1⁗	5.43 d (1.5)	101.6	4.51 d (5.8)	106.2	4.26 d (7.5)	104.8
2⁗	3.96 dd (1.5, 2.5)	71.9	3.68 dd (5.8, 8.2)	73.1	3.23 dd (7.5, 9.0)	75.1
3⁗	3.78 dd (2.5, 9.0)	71.7	3.59 dd (3.0, 8.2)	74.7	3.39 t (9.0)	78.1
4⁗	3.43 t (9.0)	73.5	3.82 m	69.1	3.31 t (9.0)	70.6
5⁗	4.06 m	69.5	3.54 dd (3.5, 12.0)	66.8	3.29 d (9.0)	77.9
6⁗	1.28 d (6.5)	18.0	3.93 dd (2.5, 12.0)		3.70 dd (4.5, 12.0)	62.6
					3.91 dd (2.5, 12.0)	

[a] Assignments confirmed by 1D-TOCSY, 2D-HOHAHA, DQF−COSY, HSQC and HMBC experiments.

J = 9.5, 9.5, 4.5) resonances indicated an unglycosylated C-6α hydroxyl group at this position. A combination of the 2D COSY, 1D TOCSY, and 2D HOHAHA spectra allowed the assignment of all the proton sugar resonances and permitted the identification of the sugar moieties as xylopyranosyl, rhamnopyranosyl, and glucopyranosyl (Table 1). The HSQC 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 glucopyranosyl and the rhamnopyranosyl units, suggesting these sugars to be terminal. A glycosylation shift was observed for C-2′ (δ 78.5) of the inner xylopyranosyl unit (Table 1). The results of the HMBC experiment suggested that compound **3** was a bisdesmosidic saponin in which the sugar residues were linked to C-3 and C-24 of cyclocanthogenin. Key correlation peaks observed in the HMBC spectrum of **3** between H-1′ of the xylosyl at δ 4.41 (d , J = 7.5 Hz) and C-3 (δ 89.5) of the aglycon and between H-1″ of the rhamnosyl at δ 5.37 (d, J = 1.5 Hz) and C-2′ (δ 78.5) allowed the disaccharide chain at C-3 to be determined as α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranoside. A cross peak between H-1‴ of the glucosyl at δ 4.44 (d, J = 7.5 Hz) and C-24 (δ 89.8) indicated that the terminal glucose was linked to C-24. Therefore, the structure of **3** (trojanoside C) was assigned as 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl]-24-*O*-β-D-glucopyranosyl-3β,6α,16β,(24*S*),25-pentahydroxycycloartane.

In the NMR spectra of compounds **4**−**6**, the ^{1}H and ^{13}C NMR resonances arising from the sapogenol moiety were very close to those of **3**, except for the signal ascribable to C-6 (δ_C 79.9; δ_H 3.58, ddd, J = 10.0, 10.0, 4.5 Hz), which provided evidence for significant glycosylation shifts. These results suggested a tridesmosidic structure for compounds **4**−**6** in which three sugar moieties were attached to the hydroxyl groups at C-3, C-6, and C-24. Also, in the case of compounds **4**−**6**, the proton coupling network within each sugar residue was traced out using a combination of 1D TOCSY and 2D COSY NMR methods and the ^{13}C NMR resonances were assigned by HSQC spectrum (Tables 1 and 2), which indicated the presence of three terminal β-D-glucopyranosyls in compound **4**. In turn, two terminal β-D-glucopyranosyl units, a terminal α-L-rhamnopyranosyl, and a 2-substituted (δ 78.7) β-D-xylopyranosyl unit are present in **5**; while two terminal β-D-glucopyranosyls, a terminal arabinopyranosyl, and a 2-substituted (δ 82.8) β-D-xylopyranosyl occurred in **6** (Tables 1 and 2). It was apparent that α-L-arabinose was in the pyranose form from the ^{13}C NMR data from the results of the ROESY experiments, as reported previously.[12] As before, direct evidence of the sugar sequence and the linkage sites were derived by HMBC experiments, which led to the structures shown. Compounds **4**−**6** have in common the presence of two glucosyl units linked to C-6 and C-24 of the aglycon but differ in the sugar moiety at C-3. Therefore, **4** (trojanoside D) was 3-*O*-β-D-glucopyranosyl-6-*O*-β-D-glucopyranosyl-24-*O*-β-D-glucopyranosyl-3β,6α,16β,(24*S*),25-pentahydroxycycloartane, **5** (trojanoside E) was 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl]-6-*O*-β-D-glucopyranosyl-24-*O*-β-D-glucopyranosyl-3β,6α,16β,(24*S*),25-pentahydroxycycloartane; and **6** (trojanoside F) was 3-*O*-[α-L-arabinopyranosyl-(1→2)-β-D-xylopyranosyl]-6-*O*-β-D-glucopyranosyl-24-*O*-β-D-glucopyranosyl-3β,6α,16β,(24*S*),25-pentahydroxycycloartane, respectively. To the our knowledge, tetraglycosidic-type cycloartanes such as trojanosides E and F have been isolated from an *Astragalus* species for the first time. This is only the second report of such metabolites as natural products.[13]



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8

Saponin **7** ($C_{53}H_{86}O_{23}$) showed a peak at *m/z* 1089 due to $[M - H]^-$ ion in the negative ion FABMS. Its NMR spectral data (see Experimental Section) indicated that this compound was a triterpene of the oleanene type[14,15] bearing four sugar units (Table 2). A combination of 1D and 2D NMR techniques led to the assignment of the ^1H and ^{13}C NMR signals due to the A, B, C, and D rings of a 3β,24-dihydroxyolean-12-ene sapogenol unit,[14] glycosylated at C-3. Ring E was shown to contain an oxymethine at C-22 (δ_C 77.0, δ_H 3.52 br m) and a glycosylated hydroxymethyl group at C-29 or C-30 (δ_C 80.1, δ_H 3.19 and 3.69, each d, J = 12.5 Hz). The location of the $-CH_2OR$ group at C-29α was derived from the resonance of Me-30 at δ 24.9 superimposable to that of abrisapogenol B.[14,15] In abrisapogenol E,[14,15] which possesses a $-CH_2OH$ group at C-30β, Me-29 was reported to resonate downfield (δ 28.3). 1D and 2D NMR data (Table 2) also indicated the presence of a terminal α-L-rhamnopyranosyl and a terminal β-D-glucopyranosyl as well as an inner 2-substituted β-D-glucuronopyranosyl (δ 78.6, C-2′) and a β-D-xylopyranosyl (δ 79.1, C-2″). The glucopyranosyl moiety at C-29 was evident from a HMBC correlation between the H-1‴″ (δ_H 4.26) and C-29 (δ_C 80.1) signals. The sequence of the trisaccharide chain at C-3 was derived by the observed HMBC correlations between H-1′ (δ 4.43) of the glucuronic acid residue and C-3 (δ 92.1) of the aglycon, as well as between H-1‴ (δ 5.22) of the terminal rhamnose residue and C-2″ (δ 79.1) of the inner xylopyranosyl. All the sugar signals assigned for the trisaccharide chain at C-3 were also in good agreement with those reported for wistariasaponins.[15] Thus, **7** (astrojanoside A) is 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl-(1→2)-β-D-glucuronopyranosyl]-29-*O*-β-D-glucopyranosyl-3β,22β,24,29-tetrahydroxy-olean-12-en.

The ^1H NMR spectrum of compound **8** ($C_{17}H_{20}O_6N_2$) showed the typical aromatic resonance pattern of a C-3-substituted indole derivative.[16] The ABCD-system of the benzene ring could be analyzed by a simple first order interpretation, and the signal for H-2 showed the typical small coupling constant of 1.5 Hz to N−H (Table 3). The ^{13}C NMR resonances in the aromatic region were compatible with a 3-substitued indole. The side chain attached at C-3 of the indole unit consisted of a free carboxylic group (δ 179.1), a methylene (δ 29.1), and a methine (δ 56.6) whose resonance indicated that it was an α-amino acid derivative. These groups appeared in the ^1H NMR spectrum as aliphatic proton signals, including a methine (δ 4.67, dd, J = 4.4, 7.9 Hz) and two neighboring geminal methylene protons (δ 3.15 and 3.41, each dd, J = 14.5, 7.9, 14.5, 4.4 Hz, respectively). Based on the results of COSY and HSQC spectra this moiety was identified as tryptophan.[16] The tryptophan moiety was connected to an acid via an amide bond (173.0, C-1′). Corresponding resonances for the acid component were two doublets for two $-CH_2$ groups (δ 2.38 and 2.42; δ 2.23 and 2.27) and a tertiary Me (δ 1.17, s) in the ^1H NMR spectrum. Detailed ^{13}C NMR spectral analysis led to the identification of dicrotalic acid (3-hydroxy-3-methyl-glutaric acid).[17] The FABMS $[M - H]^-$ ion at *m/z* 347 was fully compatible with the derived structure. The proposed connectivity between the tryptophan moiety and the dicrotalic acid residue was confirmed by the HMBC spectrum, which correlated the methine signal at δ 4.67 assigned for H-11 of tryptophan and the ^{13}C NMR signals at δ 173.0 (−CONH group of dicrotalic acid residue, C-1′) and at δ 179.1 (−COOH, C-12) and 29.1 ($-CH_2$, C-10), both of the tryptophan moiety. Thus, the structure of compound **8** was determined as *N*-[3-hydroxy-3-methyl-glutaroyl]-tryptophan, for which the trivial name achillamide is proposed.

Table 3. ^1H and ^{13}C NMR Data of Compound **8**[a]

	δ_H (J in Hz)	δ_C
tryptophan moiety		
2	7.19 d (1.5)	124.2
3		111.6
4		128.9
5	7.65 d (7.9)	119.6
6	7.01 t (7.9)	119.8
7	7.08 t (7.9)	122.1
8	7.33 d (7.9)	111.9
9		137.7
10	3.15 dd (7.9, 14.5)	29.1
	3.41 dd (4.4, 14.5)	
11	4.67 dd (4.4, 7.9)	56.6
12		179.1
dicrotalic acid		
1		173.0
2	2.38 d (14.5)	48.3
	2.42 d (14.5)	
3		71.3
4	2.23 d (14.9)	48.4
	2.27 d (14.9)	
5		180.0
6	1.17 s	27.7

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

Astrasieversianin I, astrasieversianin II, astragaloside I, astragaloside IV, astragaloside VII, and brachyoside C were also isolated from the roots of *A. trojanus* and

identified on the basis of their FABMS and NMR (^{1}H and ^{13}C) data, in comparison with literature values.[2,6,9,18,19]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin−Elmer 141 polarimeter using a sodium lamp operating at 589 nm as 0.1% w/v solutions in MeOH. UV measurements were performed on a Perkin−Elmer Lambda 7 spectrophotometer. IR spectra were measured on a Perkin−Elmer 2000 FT-IR spectrometer in KBr pellets. A Bruker DRX-600 spectrometer operating at 599.19 MHz for ^{1}H and 150.858 for ^{13}C using the UXNMR software package was used for NMR measurements in CD_3OD. The ^{1}H−^{1}H DQF COSY,[7] inverse detected ^{1}H−^{13}C HSQC[8] and HMBC,[10] and ROESY[20] 2D NMR experiments were run by employing the conventional pulse sequences. The 1D TOCSY NMR data[11] were acquired using waveform generator-based GAUSS-shaped pulses, with a mixing time ranging from 100 to 120 ms and a MLEV-17 spin-lock field of 10 kHz preceded by a 2.5 ms trim pulse. FABMS were recorded in a glycerol matrix in the negative ion mode on a VG ZAB instrument (Xe atoms of energy of 2−6 kV).

Plant Material. *Astragalus trojanus* Stev. (Leguminosae) was collected from Hacibozlar Village, Burhaniye-Balikesir, West Anatolia, Turkey, in August 1996. Voucher specimens (96−104) have been deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

Extraction and Isolation. The air-dried, powdered roots (250 g) of *A. trojanus* were extracted with 80% EtOH under reflux. The solvent was removed by rotary evaporation yielding 37 g of extract. The H_2O-soluble part of the EtOH extract was subjected to vacuum liquid chromatography using reversed-phase material (Sepralyte 40 μm, 100 g) as eluents, employing H_2O (400 mL), H_2O−MeOH (9:1, 200 mL; 8:2, 200 mL), and MeOH (800 mL). Fractions eluted with MeOH (18.35 g) were rich in saponins. An aliquot of this material (16 g) was subjected to open column chromatography (Si gel 60, 75 g) using $CHCl_3$ (200 mL), $CHCl_3$−MeOH (95:5, 200 mL; 90:10, 250 mL; 88:12, 100 mL; 86:14, 100 mL), and $CHCl_3$−MeOH−H_2O [84:16:0.5, 82:18:0.75, 80:20:1, 80:20:2, 78:22:2, 75:25:2.5, 72.5:27.5:2.5 (each 100 mL), 70:30:3 (200 mL); 67:33:4 (100 mL); 65:35:5 (100 mL); 61:32:7, 500 mL] and MeOH (300 mL), yielding 18 fractions (A−S) altogether.

Fractions B, C, and E (49 mg, 140 mg, and 73 mg, respectively) were further separately applied to Si gel (5 g) column chromatography. Elution was carried out with $CHCl_3$ and $CHCl_3$−MeOH (95:5), yielding astrasieversianin I (7 mg), astrasieversianin II (20 mg), and astragaloside I (12 mg). Fraction H (500 mg) was chromatographed on a Si gel column (40 g) eluted with EtOAc (200 mL), EtOAc−MeOH (97.5:2.5, 300 mL), and EtOAc−MeOH−H_2O (96:3:0.5, 100 mL; 96:4:1, 100 mL; 95:5:2, 100 mL; 94:6:2.5, 100 mL; 93:7:3, 700 mL) to yield three fractions (H1−H3). Fraction H3 (120 mg) was subjected to column chromatography using Si gel (6 g) as stationary phase, eluting with $CHCl_3$−MeOH (90:10, 50 mL; 85:15, 100 mL) to give compound **1** (18 mg). Fraction J (200 mg) was subjected to a Si gel column (25 g) using $CHCl_3$−MeOH−H_2O mixtures (80:20:2, 250 mL; 70:30:3, 200 mL) to yield astragaloside IV (50 mg). Fraction K (532 mg) was applied to column chromatograpy using Si gel (60 g) as stationary phase eluting with EtOAc−MeOH−H_2O (100:16.5:13.5, 100 mL, 95:16.5:13.5, 500 mL) to give compounds **2** (57 mg), **3** (15 mg), and astragaloside VII (25 mg). Fraction R (477 mg) was subjected to a Si gel column (30 g) using $CHCl_3$-MeOH−H_2O (70:30:3, 300 mL) to yield compound brachyoside C (25 mg). An aliquot of fraction S (1 g) was then subjected to MPLC on reversed-phase material (LiChrosorb C_{18}) using a stepwise H_2O−MeOH gradient (30% MeOH, 40%, 50%, 60%; each 200 mL; 70%, 400 mL), and the fractions were pooled into five main fractions (fractions S1−S5). Fractions S1 (80 mg), S2 (90 mg), and S5 (150 mg) were further separately applied to Si gel (10 g) column chromatography. Elutions were carried out with $CHCl_3$−MeOH−H_2O mixtures (70:30:3 and 61:32:7) yielding compounds **4** (5 mg), **5** (27 mg), **6** (9.5 mg), and **7** (9.5 mg). The remaining part of fraction S (1.8 g) was chromatographed on a Si gel column (40 g) eluted with $CHCl_3$−MeOH−H_2O (70:30:3, 68:32:4, 61:32:7) and MeOH to yield six fractions (fractions S6−S11). Fraction S10 (200 mg) was applied to vacuum−liquid chromatography using reversed-phase material (Sepralyte 40 μm). Elution was performed with H_2O−MeOH (8:2, 6:4) and MeOH to give three fractions (fractions S10a−S10c). Fractions S10a (65 mg) and S11 (125 mg) were dissolved in H_2O and partitioned with *n*-BuOH. The aqueous partition of fraction S10a yielded compound **8** (38 mg) and the *n*-BuOH partition of S-11 yielded compound **7** (15.5 mg).

Trojanoside A (**1**): $[\alpha]^{25}_D$ +20.1° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3420 (OH), 1735 (ester C=O), 1260 and 1049 cm^{-1} (C−O−C); ^{1}H NMR (600 MHz, CD_3OD) aglycon moiety δ 5.48 (1H, ddd, J = 8.0, 8.0, 5.2 Hz, H-16), 3.77 (1H, dd, J = 8.0, 5.0 Hz, H-24), 3.58 (1H, ddd, J = 10.0, 10.0, 4.5 Hz, H-6), 3.24 (1H, dd, J = 11.1, 4.5 Hz, H-3), 2.58 (1H, d, J = 8.0 Hz, H-17), 2.40 (1H, dd, J = 6.0, 12.0 Hz, H-22a), 2.33 (1H, m, H-15a), 2.06 (3H, s, C*H*$_3$CO), 1.98 (1H, m, H-2a), 1.96 (1H, m, H-8), 1.95 (1H, m, H-23a), 1.91 (1H, m, H-11a), 1.88 (1H, m, H-7a), 1.79 (1H, m, H-12a), 1.78 (1H, m, H-23b), 1.72 (1H, m, H-12b), 1.71 (1H, m, H-7b), 1.70 (1H, m, H-2b), 1.66 (1H, d, J = 10.0, H-5), 1.65 (1H, m, H-22b), 1.59 (1H, m, H-1a), 1.47 (1H, m, H-11b), 1.37 (1H, m, H-15b), 1.35 (6H, s, H$_3$-18, H$_3$-21), 1.31 (1H, m, H-1b), 1.30 (3H, s, H$_3$-28), 1.17 (3H, s, H$_3$-26), 1.16 (3H, s, H$_3$-27), 1.10 (3H, s, H$_3$-30), 1.03 (3H, s, H$_3$-29), 0.28 and 0.63 (each 1H, d, J_{AB} = 4.5 Hz, H$_2$-19); ^{13}C NMR (150 MHz, CD_3OD) aglycon moiety δ 171.9 (s, CH_3CO), 89.9 (d, C-3), 87.0 (s, C-20), 83.0 (d, C-24), 79.2 (d, C-6), 77.6 (d, C-16), 72.4 (s, C-25), 58.4 (d, C-17), 52.8 (d, C-5), 47.4 (s, C-14), 45.9 (s, C-13), 45.4 (d, C-8), 45.3 (t, C-15), 42.9 (s, C-4), 37.1 (t, C-22), 33.6 (t, C-7), 33.4 (t, C-12), 32.6 (t, C-1), 30.0 (t, C-2), 29.5 (s, C-10), 28.1 (q, C-28), 28.0 (t, C-19), 27.8 (q, C-21), 27.1 (t, C-23), 26.8 (t, C-11), 25.8 (q, C-27), 25.8 (q, C-26), 22.4 (s, C-9), 20.6 (q, *C*H$_3$CO), 20.3 (q, C-18), 20.1 (q, C-30), 16.3 (q, C-29); ^{1}H and ^{13}C NMR (600 MHz, CD_3OD) data of the sugar moiety, see Table 1; FABMS *m*/*z* 825 [M − H]$^-$, 693 [(M − H) − 132]$^-$, 663 [(M − H) −162]$^-$.

Trojanoside B (**2**): $[\alpha]^{25}_D$ +13.2° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3420 (OH), 1270 and 1040 cm^{-1} (C−O−C); ^{1}H NMR (600 MHz, CD_3OD) aglycon moiety δ 4.71 (1H, ddd, J = 8.0, 8.0, 5.2 Hz, H-16), 3.86 (1H, dd, J = 8.0, 5.0 Hz, H-24), 3.55 (1H, ddd, J = 10.0, 10.0, 4.5 Hz, H-6), 3.24 (1H, dd, J = 11.1, 4.5 Hz, H-3), 2.59 (1H, dd, J = 6.0, 12.0 Hz, H-22a), 2.41 (1H, d, J = 8.0 Hz, H-17), 2.17 (1H, m, H-23a), 2.04 (1H, m, H-23a), 2.03 (1H, m, H-15a), 1.97 (1H, m, H-2a), 1.94 (1H, m, H-8), 1.93 (1H, m, H-7a), 1.92 (1H, m, H-11a), 1.70 (1H, m, H-2b), 1.67 (1H, m, H-22b), 1.66 (1H, d, J = 10.0, H-5), 1.65 (1H, m, H-12a), 1.63 (1H, m, H-12b), 1.62 (1H, m, H-7b), 1.58 (1H, m, H-1a), 1.43 (1H, m, H-15b), 1.42 (1H, m, H-11b), 1.41 (3H, s, H$_3$-26), 1.31 (1H, m, H-1b), 1.29 (3H, s, H$_3$-28), 1.28 (3H, s, H$_3$-18), 1.26 (3H, s, H$_3$-27), 1.25 (3H, s, H$_3$-21), 1.05 (3H, s, H$_3$-30), 1.03 (3H, s, H$_3$-29), 0.28 and 0.61 (each 1H, d, J_{AB} = 4.5 Hz, H$_2$-19); ^{13}C NMR (150 MHz, CD_3OD) aglycon moiety δ 90.0 (d, C-3), 87.0 (s, C-20), 82.8 (d, C-24), 79.9 (s, C-25), 79.1 (d, C-6), 74.5 (d, C-16), 58.5 (d, C-17), 52.8 (d, C-5), 46.9 (s, C-14), 46.1 (s, C-13), 45.4 (d, C-8), 45.5 (t, C-15), 42.6 (s, C-4), 35.1 (t, C-22), 35.0 (t, C-7), 33.6 (t, C-12), 32.6 (t, C-1), 30.1 (t, C-2), 29.5 (s, C-10), 28.5 (t, C-19), 28.1 (q, C-28), 27.5 (q, C-21), 26.7 (t, C-11), 26.1 (t, C-23), 25.0 (q, C-27), 22.7 (q, C-26), 21.9 (s, C-9), 20.9 (q, C-18), 19.7 (q, C-30), 16.3 (q, C-29); ^{1}H and ^{13}C NMR (600 MHz, CD_3OD) data of the sugar moiety, see Table 1; FABMS *m*/*z* 915 [M − H]$^-$, 783 [(M − H) − 132]$^-$, 753 [(M − H) − 162]$^-$, 651 [(M − H) − (132 × 2)]$^-$, 489 [(M − H) − (132 × 2 +162]$^-$.

Trojanoside C (**3**): $[\alpha]^{25}_D$ −5.0° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3420 (OH), 2933 (CH), 1250 and 1024 cm^{-1} (C−O−C); ^{1}H NMR (600 MHz, CD_3OD) aglycon moiety δ 4.43 (1H, ddd, J = 8.2, 8.0, 5.2 Hz, H-16), 3.53 (1H, dd, J = 12.0, 4.5 Hz, H-24), 3.47 (1H, ddd, J = 9.5, 9.5, 4.5 Hz, H-6), 3.23 (1H, dd, J = 11.2, 4.5 Hz, H-3), 2.05 (1H, dd, J = 12.0, 8.0 Hz, H-15a), 2.03 (1H, m, H-11a), 1.97 (1H, m, H-2a), 1.90 (1H, m, H-22a), 1.88 (1H, m, H-20), 1.82 (1H, m, H-8), 1.72 (1H, m, H-12a), 1.71

(1H, m, H-2b), 1.66 (1H, m, H-12b), 1.64 (1H, m, H-23a), 1.62 (2H, m, H-17, H-23b), 1.49 (1H, m, H-7a), 1.45 (1H, dd, J = 12.0, 5.2 Hz, H-15b), 1.39 (1H, d, J = 9.5, H-5), 1.38 (1H, m, H-7b), 1.36 (1H, m, H-22b), 1.32 (3H, s, H_3-28), 1.25 (1H, m, H-11b), 1.21 (3H, s, H_3-26), 1.19 (3H, s, H_3-18, H_3-27), 1.05 (3H, s, H_3-29), 0.98 (3H, s, H_3-30), 0.97 (3H, d, J = 6.0 Hz, H_3-21), 0.41 and 0.57 (each 1H, d, J_{AB} = 3.5 Hz, H_2-19); ^{13}C NMR (150 MHz, CD_3OD) aglycon moiety δ 89.8 (d, C-24), 89.5 (d, C-3), 73.9 (s, C-25), 72.3 (d, C-16), 69.2 (d, C-6), 57.8 (d, C-17), 54.5 (d, C-5), 48.8 (t, C-15), 48.4 (d, C-8), 46.8 (s, C-14), 46.1 (s, C-13), 43.0 (s, C-4), 38.5 (t, C-7), 33.7 (t, C-12), 33.1 (t, C-1), 32.9 (t, C-22), 31.5 (t, C-19), 30.7 (d, C-20), 30.1 (t, C-2), 29.9 (s, C-10), 29.3 (t, C-23), 28.2 (q, C-28), 26.6 (t, C-11), 26.3 (q, C-27), 21.4 (s, C-9), 19.9 (q, C-30), 17.7 (q, C-21), 16.3 (q, C-29); ^{1}H and ^{13}C NMR (600 MHz, CD_3OD) data of the sugar moiety, see Table 1; FABMS m/z 931 $[M - H]^-$, 785 $[(M - H) - 146]^-$, 769 $[(M - H) - (162)]^-$, 491 $[(M - H) - (132 + 146 + 162]^-$.

Trojanoside D (**4**): $[\alpha]^{25}_D$ +22.5° (c 0.1, MeOH); IR (KBr) ν_{max} 3392 (OH), 2935 (CH), 1257 and 1044 cm^{-1} (C−O−C); ^{1}H and ^{13}C NMR data of the aglycon moiety were superimposable on those reported for brachyoside C;[6] ^{1}H and ^{13}C NMR (CD_3-OD) data of the sugar moiety, see Table 1; FABMS m/z 977 $[M - H]^-$, 815 $[(M - H) - 162]^-$, 653 $[(M - H) - (162 \times 2)]^-$, 491 $[(M - H) - (162 \times 3)]^-$.

Trojanoside E (**5**): $[\alpha]^{25}_D$ +2.6° (c 0.1, MeOH); IR (KBr) ν_{max} 3392 (OH), 2933 (CH), 1257, and 1044 cm^{-1} (C−O−C); ^{1}H and ^{13}C NMR data of the aglycon moiety were superimposable on those reported for brachyoside C;[6] ^{1}H and ^{13}C NMR (CD_3OD) of the sugar moiety, see Table 2; FABMS m/z 1093 $[M - H]^-$, 947 $[(M - H) - 146]^-$, 785 $[(M - H) - (146 + 162)]^-$, 653 $[(M - H) - (146 + 162 + 132)]^-$, 491 $[(M - H) - (162 \times 2 + 146 + 132)]^-$.

Trojanoside F (**6**): $[\alpha]^{25}_D$ +5.2° (c 0.1, MeOH); IR (KBr) ν_{max} 3420 (OH), 2924 (CH), 1270, and 1040 cm^{-1} (C−O−C); ^{1}H and ^{13}C NMR data of the aglycon moiety were superimposable on those reported for brachyoside C;[6] ^{1}H and ^{13}C NMR (CD_3OD) of the sugar moiety, see Table 2; FABMS m/z 1079 $[M - H]^-$, 947 $[(M - H) - 132]^-$, 785 $[(M - H) - (132 + 162)]^-$, 491 $[(M - H) - (162 \times 2 + 132 \times 2)]^-$.

Astrojanoside A (**7**): $[\alpha]^{25}_D$ +16.7° (c 0.1, MeOH); IR (KBr) ν_{max} 3393 (OH), 2926 (CH), 1749 (C=O), 1636 (C=C), 1271, and 1045 cm^{-1} (C−O−C); ^{1}H NMR (600 MHz, CD_3OD) aglycon moiety δ 5.30 (1H, t, J = 3.5 Hz, H-12), 4.12 (1H, d, J = 12.3, H-24a), 3.69 (1H, d, J = 12.5, H-29a), 3.52 (1H, br s, H-22), 3.36 (1H, dd, J = 11.1, 4.5 Hz, H-3), 3.26 (1H, d, J = 12.3, H-24b), 3.19 (1H, d, J = 12.5, H-29b), 2.20 (1H, m, H-2a), 2.13 (1H, dd, J = 2.5 and 11.5 Hz, H-8), 1.93 (1H, m, H-11a), 1.91 (1H, m, H-19a), 1.85 (1H, m, H-2b), 1.78 (1H, m, H-16a), 1.73 (1H, m, H-21a), 1.68 (1H, m, H-1a), 1.61 (2H, m, H-7a, H-9), 1.47 (1H, m, H-7b), 1.42 (1H, m, H-16b), 1.35 (1H, m, H-21b), 1.25 (3H, s, H_3-30), 1.20 (1H, m, H-15a), 1.15 (3H, s, H_3-27), 1.09 (1H, m, H-15b), 1.08 (3H, s, H_3-30), 1.06 (1H, m, H-1b), 1.01 (3H, s, H_3-26), 0.98 (1H, m, H-5), 0.94 (1H, m, H-19b), 0.91 (3H, s, H_3-25), 0.86 (3H, s, H_3-28); ^{13}C NMR (150 MHz, CD_3OD) aglycon moiety δ 144.8 (s, C-13), 124.0 (d, C-12), 92.1 (d, C-3), 80.1 (t, C-29), 77.0 (d, C-22), 63.8 (t, C-24), 57.5 (d, C-5), 48.9 (d, C-9), 46.1 (d, C-18), 45.5 (s, C-4), 43.5 (s, C-14), 42.1 (t, C-19), 40.8 (s, C-8), 39.9 (t, C-1), 38.7 (d, C-17), 37.6 (s, C-10), 37.1 (t, C-21), 36.0 (s, C-20), 34.4 (t, C-7), 30.1 (t, C-16), 26.9 (t, C-2), 26.6 (t, C-15), 25.1 (q, C-27), 24.9 (q, C-30), 24.5 (t, C-11), 22.8 (q, C-23), 20.1 (t, C-6), 20.0 (q, C-28), 17.5 (q, C-26), 16.2 (q, C-25); ^{1}H and ^{13}C NMR (CD_3OD) data of the sugar moiety, see Table 2; FABMS m/z 1089 $[M - H]^-$, 943 $[(M - H) - 146]^-$, 927 $[(M - H) - (162)]^-$.

Achillamide (**8**): $[\alpha]^{25}_D$ −29.0° (c 0.1, MeOH); UV (MeOH) λ_{max} 290, 281, 270, and 221 nm; IR (KBr) ν_{max} 3500 (NH), 3392 (OH), 1748, 1733, 1683 cm^{-1} (CO−NH, C=O); ^{1}H and ^{13}C NMR (600 MHz, CD_3OD), see Table 3; FABMS m/z 347 $[M - H]^-$.

Astrasieversianin I: ^{1}H and ^{13}C NMR (CD_3OD) data superimposable with those reported in the literature.[18]

Astrasieversianin II: ^{1}H and ^{13}C NMR (CD_3OD) data superimposable with those reported in the literature.[2,18]

Astragaloside I: ^{1}H and ^{13}C NMR (CD_3OD) data superimposable with those reported in the literature.[19]

Astragaloside IV: ^{1}H and ^{13}C NMR (CD_3OD) data superimposable with those reported in the literature.[19]

Astragaloside VII: ^{1}H and ^{13}C NMR (CD_3OD) data superimposable with those reported in the literature.[9]

Brachyoside C: ^{1}H and ^{13}C NMR (CD_3OD) data superimposable with those reported in the literature.[6]

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