Triterpene Glycosides from the Roots of Astragalus flavescens

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · JANUARY 2008

Impact Factor: 3.8 · DOI: 10.1021/np0703500 · Source: PubMed

Triterpene Glycosides from the Roots of *Astragalus flavescens*

Sibel Avunduk,[†,‡] Anne-Claire Mitaine-Offer,[§] Özgen Alankuş-Çalişkan,[†] Tomofumi Miyamoto,[∥] Serdar Gökhan Şenol,[⊥] and Marie-Aleth Lacaille-Dubois*[,§]

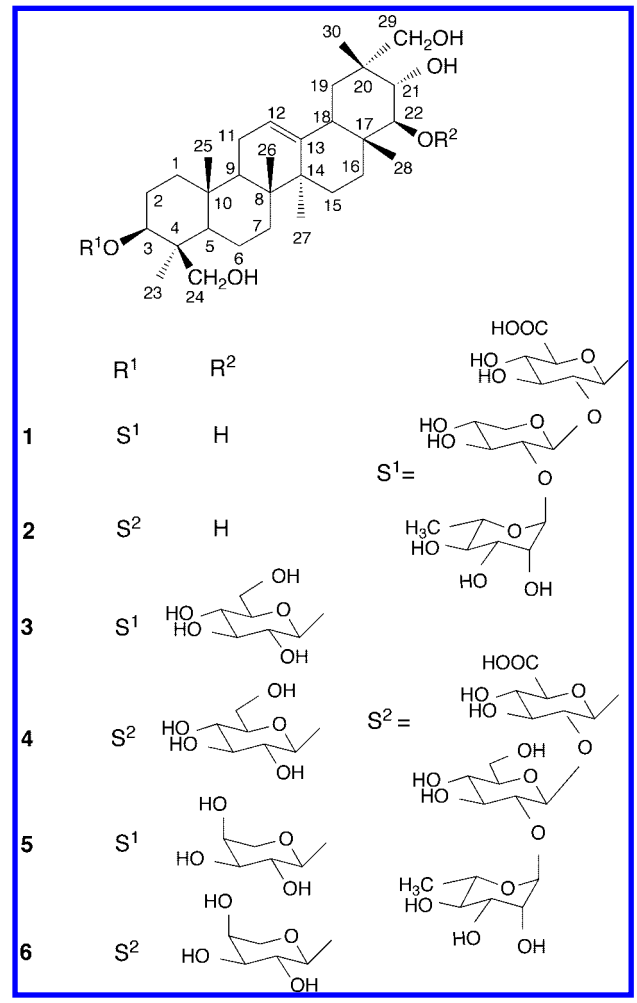
Department of Chemistry, Faculty of Sciences, Ege University 2, Bornova, Izmir, 35100 Turkey, Medical Laboratory Programme, Vocational School of Health Care, Mugla University, Marmaris, Mugla, 48187 Turkey, Laboratoire de Pharmacognosie, UMIB, UPRES-EA 3660, Faculté de Pharmacie, Université de Bourgogne, 7, Bd. Jeanne D'Arc, BP 87900, 21079 Dijon Cedex, France, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, 812-8582, Japan, and Department of Botany, Faculty of Sciences, Ege University 2, Bornova, Izmir, 35100 Turkey

Received July 18, 2007

Six new triterpene saponins, 3-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucuronopyranosyl-21-epi-kudzusapogenol A (**1**), 3-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucuronopyranosyl-21-epi-kudzusapogenol A (**2**), 3-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucuronopyranosyl-22-*O*-*β*-D-glucopyranosyl-21-epi-kudzusapogenol A (**3**), 3-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucuronopyranosyl-22-*O*-*β*-D-glucopyranosyl-21-epi-kudzusapogenol A (**4**), 3-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucuronopyranosyl-22-*O*-α-L-arabinopyranosyl-21-epi-kudzusapogenol A (**5**), and 3-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucuronopyranosyl-22-*O*-α-L-arabinopyranosyl-21-epi-kudzusapogenol A (**6**), were isolated from the roots of *Astragalus flavescens*, together with the known trajanoside B, azukisaponin V, and astragalosides IV, VII, and VIII. Their structures were established mainly by 2D NMR techniques and mass spectrometry.

The genus *Astragalus* is the largest one in the Fabaceae family and is represented by 380 species in the flora of Turkey.[1] Several *Astragalus* species are used in traditional medicine as an antiperspirant, diuretic, and tonic drug and for the treatment of diabetes mellitus, nephritis, and leukemia.[2] In China, many pharmaceutical preparations containing *Astragalus* extract or isolated compounds have been used: Water decoctions of the roots of *A. membranaceus* and *A. sinicus* were incorporated into pharmaceutical preparations for treatment of toothache or for oral hygiene.[3] Chemical studies on *Astragalus* saponins have provided oleanane-type and cycloartane-type triterpenoid glycosides that exhibited cytotoxic, immunostimulatory, hepatoprotective, or antiviral activities.[3] In this paper we describe six new triterpene saponins (**1**−**6**) and the known compounds trajanoside B,[4] azukisaponin V,[5] and astragalosides IV,[6] VII,[6] and VIII,[7] isolated from the roots of *Astragalus flavescens* Boiss. Their structures were elucidated mainly by NMR analyses, including 1D and 2D NMR (^{1}H−^{1}H COSY, TOCSY, NOESY, HSQC, HMBC), and mass spectrometry.

The aglycon of the six new saponins (**1**–**6**) was recognized to be an oleanane-type triterpene by ^{1}H NMR and ^{13}C NMR analysis (Table 1). The ^{1}H NMR spectrum of **1** showed signals for six angular methyl groups as singlets, one olefinic proton at δ_H 5.22 (br t, J = 3.0 Hz) (H-12), three oxygen-bearing methine protons at δ_H 3.32 (H-3), 4.36 (br s) (H-21), and 3.78 (br s) (H-22), and two primary OH functions at δ_H 3.34 (d, J = 10.9 Hz), 4.20 (H_2-24), and 3.50 (d, J = 10.5 Hz), 3.96 (H_2-29). In the HMBC spectrum, cross-peaks between δ_H 1.35 (s) (H_3-23) and δ_C 90.6 (C-3) and δ_C 62.4 (C-24) placed a secondary OH at C-3 and primary OH at C-24. The downfield shift to δ_C 90.6 (C-3) suggested a glycosidic link at C-3. In the NOESY spectrum, correlations between δ_H 3.32 (H-3) and δ_H 0.74 (H-5) and δ_H 1.35 (s) (H_3-23) confirmed the α-axial orientation of H-3. Cross-peaks between the protons of the methyl angular groups at δ_H 0.59 (s) (H_3-25) and δ_H 0.83 (H_3-26) and protons of the primary OH function at δ_H 3.34 (d, J = 10.9 Hz) and 4.20 (H_2-24) indicated a β-axial orientation of CH_2OH-24. Moreover, correlations between H-18 at δ_H 2.58 (dd, J = 13.1, 2.9 Hz) and the protons of the methyl angular groups at δ_H 1.18 (s) (H_3-28) revealed a β-axial orientation of these protons and proved



* To whom correspondence should be addressed. Tel: 0033-3-80393229. Fax: 0033-3-80393300. E-mail: malacd@u-bourgogne.fr.
† Ege University.
‡ Mugla University
§ Université de Bourgogne.
∥ Kyushu University.
⊥ Ege University.

10.1021/np0703500 CCC: $40.75
Published on Web 12/15/2007

Table 1. ^{13}C NMR and ^{1}H NMR Data of the Aglycons of Compounds **1**–**6** in Pyridine-d_5 (δ ppm, J in parentheses in Hz)[a]

		1		**2**		**3**		**4**		**5**		**6**	
		δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	CH_2	38.6	0.75, 1.30	38.6	0.75, 1.30	38.4	0.74, 1.29	38.4	0.74, 1.29	38.4	0.77, 1.30	38.4	0.77, 1.30
2	CH_2	26.3	0.90, 1.19	26.3	0.90, 1.19	26.0	nd	26.0	nd	26.0	nd	26.0	nd
3	CH	90.6	3.32	91.2	3.30	90.8	3.30	91.2	3.28	90.9	3.33	91.2	3.33
4	C	43.7		43.3		44.1		43.9		44.0		43.8	
5	CH	55.9	0.74	55.8	0.74	56.1	0.74	56.0	0.74	55.7	0.74	55.6	0.74
6	CH_2	18.2	1.12, 1.42	18.2	1.12, 1.42	18.0	nd	18.0	nd	18.2	nd	18.2	nd
7	CH_2	32.5	1.16, 1.37	32.5	1.16, 1.37	32.2	1.15, 1.36	32.2	1.15, 1.36	32.2	1.13, 1.38	32.2	1.13, 1.38
8	C	39.7		39.7		39.9		39.9		40.0		40.0	
9	CH	47.2	1.46	47.2	1.46	47.2	1.45	47.2	1.45	47.2	1.44	47.2	1.44
10	C	36.0		35.9		36.1		36.0		36.0		35.9	
11	CH_2	23.7	1.64, 1.70	23.7	1.64, 1.70	23.9	1.67, 1.70	23.9	1.67, 1.70	23.9	1.64, 1.66	23.9	1.64, 1.66
12	CH	122.1	5.22 br t (3.0)	122.2	5.22 br t (3.0)	122.0	5.18 br t (3.0)	122.0	5.18 br t (3.0)	122.0	5.22 br t (3.0)	122.0	5.22 br t (3.0)
13	C	144.2		144.2		144.0		144.0		144.0		144.0	
14	C	41.6		41.6		41.5		41.5		41.6		41.6	
15	CH_2	26.3	1.73, 2.46	26.3	1.73, 2.46	26.0	nd	26.0	nd	26.1	nd	26.1	nd
16	CH_2	26.8	0.94, 1.98	26.8	0.94, 1.98	26.9	1.00, 1.97	26.9	1.00, 1.97	27.2	0.98, 1.98	27.2	0.98, 1.98
17	C	37.0		37.0		38.5		38.5		38.2		38.2	
18	CH	42.8	2.58 dd (13.1, 2.9)	42.8	2.58 dd (13.1, 2.9)	43.3	2.43 dd (13.1, 3.0)	43.3	2.43 dd (13.1, 3.0)	43.2	2.43 dd (14.3, 2.9)	43.2	2.43 dd (14.3, 2.9)
19	CH_2	40.6	1.23, 2.45 t (13.1)	40.6	1.23, 2.45 t (13.1)	38.6	1.29, 2.54 t (13.1)	38.6	1.29, 2.54 t (13.1)	38.6	2.53 t (14.3), nd	38.6	2.53 t (14.3), nd
20	C	40.6		40.6		41.0		41.0		41.0		41.0	
21	CH	69.6	4.36 br s	69.6	4.36 br s	70.2	4.43 br s	70.2	4.43 br s	70.0	4.44 br s	70.0	4.44 br s
22	CH	79.2	3.78 br s	79.2	3.78 br s	93.0	3.78 br s	93.0	3.78 br s	93.0	3.75 br s	93.0	3.75 br s
23	CH_3	22.3	1.35 s	22.3	1.30 s	22.5	1.34 s	22.5	1.30 s	22.9	1.33 s	22.9	1.30 s
24	CH_2	62.4	3.34 d (10.9), 4.20	62.6	3.22 d (10.9), 4.18	62.8	3.34 d (10.6), 4.20	63.0	3.21 d (10.5), 4.18	63.0	3.34, 4.20	63.2	3.22 d (10.5), 4.17
25	CH_3	15.1	0.59 s	15.2	0.56 s	15.1	0.58 s	15.2	0.55 s	15.2	0.57 s	15.3	0.54 s
26	CH_3	16.5	0.83 s	16.5	0.83 s	16.5	0.80 s	16.5	0.80 s	16.7	0.81 s	16.7	0.81 s
27	CH_3	26.3	1.23 s	26.3	1.23 s	26.8	1.24 s	26.8	1.24 s	26.9	1.22 s	26.9	1.22 s
28	CH_3	21.8	1.18 s	21.8	1.18 s	22.9	1.26 s	22.9	1.26 s	23.0	1.24 s	23.0	1.24 s
29	CH_2	70.6	3.50 d (10.5), 3.96	70.6	3.50 d (10.5), 3.96	70.0	3.43 d (10.5), 3.98	70.0	3.43 d (10.5), 3.98	69.9	3.41 d (10.7), 3.96	69.9	3.41 d (10.7), 3.96
30	CH_3	17.0	1.38 s	17.0	1.38 s	17.1	1.35 s	17.1	1.35 s	17.1	1.28 s	17.1	1.28 s

[a] Multiplicities were assigned from DEPT spectra. Overlapped proton signals are reported without designated multiplicity. Nd: Not determined.

Table 2. ^{13}C NMR and ^{1}H NMR Data of the Sugar Moieties of Compounds **1–6** in Pyridine-d_5 (δ ppm, J in parentheses in Hz)[a]

	1		**2**		**3**		**4**		**5**		**6**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
3-*O*-GlcA-1	104.6	4.78 d (7.6)	104.5	4.80 d (7.6)	104.6	4.77 d (7.4)	104.6	4.80 d (7.4)	104.5	4.80 d (7.4)	104.5	4.81 d (7.4)
2	77.8	4.18	78.0	4.25	78.2	4.17	78.2	4.24	78.0	4.17	78.0	4.24
3	75.9	4.23	75.9	4.26	75.9	4.23	75.9	4.23	75.9	4.23	75.9	4.23
4	73.5	4.16	73.5	4.16	73.7	4.13	73.7	4.13	73.7	4.14	73.7	4.14
5	78.0	4.41 d (9.1)	78.1	4.48 d (9.0)	78.0	4.40	78.1	4.46	78.0	4.40	78.1	4.47 d (9.3)
6	nd		nd		nd		nd		nd		nd	
Xyl-1	102.0	5.40 d (7.6)			102.0	5.40 d (7.1)			102.0	5.40 d (7.1)		
2	77.8	4.17			78.2	4.18			78.0	4.15		
3	78.8	3.98 t (10.7)			79.0	3.97			78.9	3.98		
4	69.6	4.26			69.9	4.29			69.5	4.26		
5	66.1	3.38 t (12.1), 4.30			66.5	3.37 t (12.0), 4.30			66.2	3.38 t (12.3), 4.30		
Glc-1			101.5	5.57 d (7.6)			101.4	5.58 d (7.4)			101.4	5.58 d (7.5)
2			77.8	4.16			78.2	4.15			78.0	4.13
3			78.2	4.09 t (9.0)			78.5	4.08			78.2	4.09
4			70.6	4.30			70.6	4.30			70.2	4.30
5			77.4	3.55			77.6	3.54			77.5	3.55
6			61.4	4.17, 4.27			61.5	4.18, 4.26			61.1	4.17, 4.26
Rha-1	101.5	6.11 br s	101.3	6.15 br s	101.7	6.11 br s	101.4	6.15 br s	101.6	6.10 br s	101.4	6.14 br s
2	71.7	4.65 br s	71.7	4.65 br s	72.0	4.65 br s	72.0	4.65 br s	71.9	4.65 br s	71.9	4.65 br s
3	71.7	4.60 dd (9.2, 2.9)	71.7	4.60 dd (9.2, 2.9)	72.1	4.59 dd (9.3, 2.9)	72.1	4.59 dd (9.3, 2.9)	71.9	4.60 dd (9.3 2.5)	71.9	4.60 dd (9.3 2.5)
4	73.6	4.26	73.6	4.26	73.8	4.26	73.8	4.26	73.8	4.26	73.8	4.26
5	68.9	4.91 dq (10.5, 6.2)	68.9	4.91 dq (10.5, 6.2)	68.9	4.90 dq (10.0, 6.6)	68.9	4.90 dq (10.0, 6.6)	68.9	4.91 dq (10.0, 6.2)	68.9	4.91 dq (10.0, 6,2)
6	18.3	1.70 d (5.5)	18.3	1.70 d (5.5)	18.5	1.70 d (5.7)	18.5	1.70 d (5.7)	18.3	1.70 d (5.5)	18.3	1.70 d (5.5)
22-*O*-Glc-1					107.5	4.90 d (6.7)	107.5	4.90 d (6.7)				
2					75.9	4.00	75.9	4.00				
3					78.2	4.15	78.2	4.15				
4					71.0	4.10	71.0	4.10				
5					77.7	3.80	77.7	3.80				
6					62.2	4.25, 4.39	62.2	4.25, 4.39				
Ara-1									108.1	4.77 d (6.9)	108.1	4.77 d (6.9)
2									73.2	4.40	73.2	4.40
3									74.6	4.06	74.6	4.06
4									69.2	4.20	69.2	4.20
5									67.1	3.64 d (11.6), 4.22	67.1	3.64 d (11.6), 4.22

[a] Multiplicities were assigned from DEPT spectra. Overlapped proton signals are reported without designated multiplicity. Nd: Not determined.

the *cis* D/E ring junction. In the HMBC spectrum, a correlation between δ_H 1.18 (s) (H_3-28) and a carbinol carbon at δ_C 79.2 revealed the location of C-22. In the COSY spectrum, the cross-peak between H-22 at δ_H 3.78 (br s) and H-21 at δ_H 4.36 (br s) proved the position of the latter. Their multiplicity as broad singlets suggested equatorial/equatorial coupling, with β-equatorial orientation of H-21 and α-equatorial orientation of H-22. The NOESY correlations between protons of an angular methyl group at δ_H 1.38 (s) and H-18 at δ_H 2.58 (dd, J = 13.1, 2.9 Hz) and H-21 at δ_H 4.36 (br s) revealed its β-axial orientation and was attributed to CH_3-30. Therefore, the protons of the primary OH function at δ_H 3.50 (d, J = 10.5 Hz), 3.96, which gave HMBC correlations with C-21 at δ_C 69.6, was finally attributed to CH_2OH-29 with α-equatorial orientation. The aglycon is thus the C-21 epimer of kudzusapogenol A[8] and was named 21-epi-kudzusapogenol A. For the pharmacological importance of this type of aglycone, the antiherpes virus type 1 activity of kudzusapogenol A[9] and its preventive effect on in vitro immunological liver injury of rat primary hepatocyte cultures[10] were evaluated. The structure–activity relationship studies showed that the C-29 hydroxy group eliminates anti-HSV-1 activity and reduces the hepatoprotective activity, while the hydroxy group at C-21 enhances the hepatoprotective activity.[9,10]

The monosaccharides obtained by acid hydrolysis of the MeOH extract were identified as D-glucuronic acid, D-glucose, D-xylose, L-arabinose, and L-rhamnose by TLC and by measurement of their optical rotation after purification. In the ^{1}H NMR spectra of the compounds, the relatively large $^3J_{H\text{-}1,H\text{-}2}$ values of the GlcA, Glc, Xyl, and Ara (between 6.7 and 7.6 Hz) moieties indicated a β anomeric proton for GlcA, Glc, and Xyl and an α anomeric proton for Ara. The broad singlet of the anomeric proton of the Rha unit indicated an α-orientation.[11]

Compound **1** exhibited a pseudomolecular ion peak (HRESIMS, positive-ion mode) at *m/z* 967.4884 $[M + Na]^+$ (calcd 967.4879), consistent with a molecular formula of $C_{47}H_{76}O_{19}Na$. Its FABMS (negative-ion mode) showed a quasimolecular ion peak at *m/z* 943 $[M - H]^-$, indicating a molecular weight of 944. The ^{1}H NMR spectrum of **1** displayed signals for three anomeric protons at δ_H 6.11 (br s), 5.40 (d, J = 7.6 Hz), and, 4.78 (d, J = 7.6 Hz), which gave correlations, in the HSQC spectrum, with anomeric carbon signals at δ_C 101.5, 102.0, and 104.6, respectively. The ring protons of the three sugars were assigned starting from the readily identifiable anomeric protons by means of COSY, TOCSY, HSQC, and HMBC spectroscopic experiments (Table 2). Units of one β-D-glucuronopyranosyl (GlcA), one β-D-xylopyranosyl (Xyl), and one α-L-rhamnopyranosyl (Rha) were identified. Correlations observed in the HMBC spectrum between an anomeric signal at δ_H 4.78 (d, J = 7.6 Hz) (GlcA-1) and δ_C 90.6 (C-3), and in the NOESY spectrum between δ_H 4.78 (GlcA-1) and δ_H 3.32 (H-3), confirmed the substitution at C-3 of the aglycon by a GlcA. A correlation in the HMBC spectrum between δ_H 5.40 (d, J = 7.6 Hz) (Xyl-1) and δ_C 77.8 (GlcA-2), and the reverse correlation between δ_H 4.18 (GlcA-2) and δ_C 102.0 (Xyl-1), revealed the (1→2) linkage between Xyl and GlcA. Moreover, correlation between δ_H 6.11 (br s) (Rha-1) and δ_C 77.8 (Xyl-2), and the reverse correlation between δ_H 4.17 (Xyl-2) and δ_C 101.5 (Rha-1), revealed the (1→2) linkage between these two sugars. On the basis of the above results, the structure of **1** was elucidated as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl-(1→2)-β-D-glucuronopyranosyl-21-epi-kudzusapogenol A.

Compound **2** exhibited a pseudomolecular ion peak (HRESIMS) at *m/z* 997.4980 $[M + Na]^+$ (calcd 997.4984), consistent with a molecular formula of $C_{48}H_{78}O_{20}Na$. Its FABMS (negative-ion mode) displayed a quasimolecular ion peak at *m/z* 973 $[M - H]^-$, indicating a molecular weight of 974, differing from **1** by 30 amu. The ^{1}H and ^{13}C NMR signals of **2** assigned from the 2D NMR spectra were almost superimposable on those of **1** except for the characteristic signals of a glucopyranosyl moiety instead of a xylopyranosyl moiety (Table 2). In the HMBC spectrum, the (1→2) linkages between Rha and Glc, and between Glc and GlcA, were proved by the cross-peaks between δ_H 4.16 (Glc-2) and δ_C 101.3 (Rha-1), and between δ_H 4.25 (GlcA-2) and δ_C 101.5 (Glc-1). The structure of **2** was thus established as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl-21-epi-kudzusapogenol A.

Compound **3** exhibited a pseudomolecular ion peak at *m/z* 1129.5402 $[M + Na]^+$ (calcd 1129.07), consistent with a molecular formula of $C_{53}H_{86}O_{24}Na$. Its FABMS showed a quasimolecular ion peak at *m/z* 1105 $[M - H]^-$, indicating a molecular weight of 1106, differing from **1** by 162 amu. A significant fragment ion peak was observed at *m/z* 959 $[(M - H) - 146]^-$, which revealed the elimination of one 6-desoxyhexosyl moiety. The ^{1}H and ^{13}C NMR signals of **3** assigned from 2D NMR spectra were almost superimposable on those of **1** (Table 1) except for those at C-22 of the aglycon and those of an additional glucopyranosyl moiety. The downfield shift to δ_C 93.0 (glycosylation shift = 13.8 ppm) in comparison with compound **1** suggested a glycosidic linkage at C-22. A correlation in the HMBC spectrum between an anomeric signal at δ_H 4.90 (d, J = 6.7 Hz) (Glc-1) and δ_C 93.0 proved attachment of a glucopyranosyl unit at C-22. These observations were used to assign the structure of **3** as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl-(1→2)-β-D-glucuronopyranosyl-22-*O*-β-D-glucopyranosyl-21-epi-kudzusapogenol A.

Compound **4** had a pseudomolecular ion peak at *m/z* 1159.5508 $[M + Na]^+$ (calcd 1159.12), consistent with a molecular formula of $C_{54}H_{88}O_{25}Na$. Its FABMS displayed a quasimolecular ion peak at *m/z* 1135 $[M - H]^-$, indicating a molecular weight of 1136, differing from **2** by 162 amu. A significant fragment ion peak was observed at *m/z* 989 $[(M - H) - 146]^-$, which revealed the elimination of one 6-desoxyhexosyl moiety. Once again, comparing with the NMR data of **2**, changes were observed at the C-22 position that indicated glycosylation by a glucopyranosyl unit as described in compound **3** (Table 1). The structure of **4** was thus elucidated as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl-22-*O*-β-D-glucopyranosyl-21-epi-kudzusapogenol A.

Compound **5** exhibited a pseudomolecular ion peak at *m/z* 1099.5295 $[M + Na]^+$ (calcd 1099.5301), consistent with a molecular formula of $C_{52}H_{84}O_{23}Na$. Its FABMS showed a quasimolecular ion peak at *m/z* 1075 $[M - H]^-$, indicating a molecular weight of 1076, differing from **3** by 30 amu. In the ^{13}C NMR spectrum, the downfield shift of C-22 (δ_C 93.0) of the aglycon suggested a glycosidic linkage. Moreover, a cross-peak in the HMBC spectrum between an anomeric signal of an arabinopyranosyl moiety at δ_H 4.77 (d, J = 6.9 Hz) (Ara-1) and δ_C 93.0 proved a substitution at C-22 by this sugar. Thus, the structure of **5** was assigned as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl-(1→2)-β-D-glucuronopyranosyl-22-*O*-α-L-arabinopyranosyl-21-epi-kudzusapogenol A.

Compound **6** exhibited a pseudomolecular ion peak at *m/z* 1129.5409 $[M + Na]^+$ (calcd 1129.5407), consistent with a molecular formula of $C_{53}H_{86}O_{24}Na$. Its FABMS displayed a quasimolecular ion peak at *m/z* 1105 $[M - H]^-$, indicating a molecular weight of 1106, differing from **5** by 30 amu. A significant fragment ion peak was observed at *m/z* 959 $[(M - H) - 146]^-$, which revealed the elimination of one 6-desoxyhexosyl moiety. Compared to the NMR data of **5**, changes were observed only at the oligosaccharidic chain linked to C-3 of the aglycon, which showed the same sequence, 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl, as described in compounds **2** and **4** (Table 2). The structure of compound **6** was thus elucidated as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl-22-*O*-α-L-arabinopyranosyl-21-epi-kudzusapogenol A.

The known compounds were identified as trajanoside B,[4] azukisaponin V,[5] and astragalosides IV,[6] VII,[6] and VIII,[7] by comparison of their spectroscopic data with literature values.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a AA-OR automatic polarimeter. For 1D and 2D NMR spectra (^{1}H−^{1}H COSY, TOCSY, NOESY, HSQC, and HMBC) see ref 12. HRESIMS (positive-ion mode) was carried out on a Q-TOF 1-micromass spectrometer; FABMS (negative-ion mode, glycerol matrix), on a JEOL SX 102 mass spectrometer. TLC and HPTLC precoated silica gel plates $60F_{254}$ (Merck) and the solvent system $CHCl_3$–MeOH–H_2O (13:7:2, lower phase) were used. The spray reagent for saponins was Komarowsky reagent (2% 4-hydroxybenzaldehyde in MeOH–50% H_2SO_4, 5:1). Isolations were carried out using an open column liquid chromatographic (CC) system [silica gel 60 (Merck, 63–200 μm), Sephadex LH-20 (Pharmacia)], a vacuum liquid chromatographic (VLC) system [reversed-phase RP-18 (25–40 μm)], and a medium-pressure liquid chromatographic (MPLC) system [Gilson pump M 303, Büchi glass column (460 × 15 mm and 250 × 15 mm), Büchi precolumn (110 × 15 mm), silica gel 60 (Merck, 15–40 μm), RP-18 (25–40 μm)].

Plant Material. *Astragalus flavescens* Boiss. roots were collected from Ödemis Province in July 2003 and identified by one of the authors (S.G.S.). A voucher specimen (EGE. 19536) was deposited at the Ege University Botanical Garden & Herbarium Research and the Application Center, Izmir, Turkey.

Extraction and Isolation. The dried, powdered roots of *A. flavescens* (1.4 kg) were extracted with MeOH (3 × 5 L) at room temperature, then concentrated to dryness and washed with *n*-hexane (2 × 200 mL). The remaining extract was obtained as a gummy mixture (60.8 g), of which 25 g was subjected to VLC on RP-18 (25–40 μm) (385 g, 7 × 17 cm) with H_2O containing increasing amounts of MeOH, to give nine fractions. The fraction eluted with MeOH–H_2O (3:7) (240 mg) was submitted to successive VLC on RP-18 (20 g, 2 × 7 cm, H_2O–MeOH mixtures), yielding compounds **1** (8 mg) and **2** (7 mg), and MPLC on silica gel ($CHCl_3$–MeOH–H_2O, 13:7:2, lower phase, 8:5:1) to give astragaloside VIII and azukisaponin V. The fraction eluted with MeOH–H_2O (1:1) was separated into four fractions, A−D. B (500 mg) was subjected to successive CC on silica gel (50 g, 4 × 20 cm) eluted with $CHCl_3$–MeOH–H_2O (70:30:3, 750 mL; 61:32:7, 750 mL; 32:25:5, 800 mL) and MPLC on RP-18 using MeOH–H_2O gradients, yielding compounds **3** (4 mg) and **4** (6 mg). Fraction D was subjected to successive VLC on RP-18 (H_2O–MeOH mixtures) and CC on Sephadex LH-20 (MeOH), yielding compounds **5** (8 mg) and **6** (7 mg). The fraction eluted with MeOH–H_2O (2:8) (240 mg) was submitted to successive VLC on RP 18 (20 g, 2 × 7 cm, H_2O–MeOH mixtures) and CC on silica gel (50 g, 2 × 7 cm) using $CHCl_3$–MeOH–H_2O (40:10:1, 200 mL; 70:30:3, 200 mL; 61:32:7, 400 mL) to afford trajanoside B (17.6 mg), astragaloside IV (35.7 mg), and astragaloside VII (15 mg).

Acid Hydrolysis. A 200 mg sample of the MeOH extract was refluxed with 2 N CF_3COOH for 2 h. After extraction with $CHCl_3$, the aqueous layer was repeatedly evaporated to dryness with MeOH until neutral. Five sugars were identified as glucuronic acid, glucose, xylose, rhamnose, and arabinose, by comparison with authentic samples on TLC in $CHCl_3$–MeOH–H_2O (8:5:1). After preparative TLC of the sugar mixture in this solvent, the optical rotation of each purified sugar was measured.

Compound 1: white, amorphous powder; $[\alpha]^{25}_D$ –8.0 (*c* 0.05, MeOH); ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; negative FABMS *m/z* 943 $[M - H]^-$; positive HRESIMS *m/z* 967.4884 $[M + Na]^+$ (calcd 967.4879).

Compound 2: white, amorphous powder; $[\alpha]^{25}_D$ –7.5 (*c* 0.05, MeOH); ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; negative FABMS *m/z* 973; positive HRESIMS *m/z* 997.4980 $[M + Na]^+$ (calcd 997.4984).

Compound 3: white, amorphous powder; $[\alpha]^{25}_D$ –6.2 (*c* 0.05, MeOH); ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; negative FABMS *m/z* 1105 $[(M - H)]^-$, 959 $[(M - H) - 146]^-$; positive HRESIMS *m/z* 1129.5402 $[M + Na]^+$ (calcd 1129.5407).

Compound 4: white, amorphous powder; $[\alpha]^{25}_D$ –4.5 (*c* 0.05, MeOH); ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; negative FABMS *m/z* 1135 $[(M - H)]^-$, 989 $[(M - H) - 146]^-$; positive HRESIMS *m/z* 1159.5508 $[M + Na]^+$ (calcd 1159.5512).

Compound 5: white, amorphous powder; $[\alpha]^{25}_D$ +3.1 (*c* 0.05, MeOH); ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; negative FABMS *m/z* 1075 $[(M - H)]^-$; positive HRESIMS *m/z* 1099.5295 $[M + Na]^+$ (calcd 1099.5301).

Compound 6: white, amorphous powder; $[\alpha]^{25}_D$ +8.5 (*c* 0.05, MeOH); ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; negative FABMS *m/z* 1105 $[(M - H)]^-$, 959 $[(M - H) - 146]^-$; positive HRESIMS *m/z* 1129.5409 $[M + Na]^+$ (calcd 1129.5407).

Acknowledgment. This work was supported by Ege University Research Foundation under contract number 2004 Fen-064. S.A. is also grateful to The Scientific and Technical Research Council of Turkey (TUBÝTAK) for National Scholarship Programme for PhD Students.

References and Notes

(1) Davis, P. H. *Flora of Turkey and East Aegean Islands*; Edinburgh University Press: Edinburgh, 1970; *Vol. 4*, pp 49–254.
(2) Tang, W.; Eisenbrand, G. *Chinese Drugs of Plant Origin*; Springer-Verlag: Berlin, 1992.
(3) Verrota, L., El-Sebakhy, N. A. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, 2001; Vol. 25, pp 179−234.
(4) Bedir, E.; Calis, I.; Aquino, R. *J. Nat. Prod.* **1999**, *62*, 563–568.
(5) Kitagawa, I.; Wang, H. K.; Saito, M.; Yoshikawa, M. *Chem. Pharm. Bull.* **1983**, *31*, 683–688.
(6) Kitagawa, I.; Wang, H. K.; Yoshikawa, M. *Chem. Pharm. Bull.* **1983**, *31*, 716–722.
(7) Konoshima, T.; Kozuka, M.; Haruna, M.; Ito, K.; Kimura, T.; Tokuda, H. *Chem. Pharm. Bull.* **1989**, *37*, 2731–2735.
(8) Kinjo, J.; Miyamoto, I.; Murakami, K.; Kida, K.; Tomimatsu, T.; Yamasaki, M.; Nohara, T. *Chem. Pharm. Bull.* **1985**, *33*, 1293–1296.
(9) Ikeda, T.; Yokomizo, K.; Okawa, M.; Tsuchihashi, R.; Kinjo, J.; Nohara, T.; Uyeda, M. *Biol. Pharm. Bull.* **2005**, *28*, 1779–1781.
(10) Arao, T.; Udayama, M.; Kinjo, J.; Nohara, T. *Planta Med.* **1998**, *64*, 413–416.
(11) Mimaki, Y.; Yokosuka, A.; Hamanaka, M.; Sakuma, C.; Yamori, T.; Sashida, Y. *J. Nat. Prod.* **2004**, *67*, 1511–1516.
(12) Mitaine-Offer, A.-C.; Miyamoto, T.; Semmar, N.; Jay, M.; Lacaille-Dubois, M.-A. *Magn. Reson. Chem.* **2006**, *44*, 713–716.

NP0703500