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Cycloartane glycosides from *Astragalus campylosema* Boiss. ssp. *campylosema*

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ABSTRACT

Four cycloartane glycosides, 3-*O*-[α-L-arabinopyranosyl-(1 → 2)-β-D-xylopyranosyl]-3β,6α,16β,23α,25-pentahydroxy-20(*R*),24(*S*)-epoxycycloartane (**1**), 3-*O*-[α-L-arabinopyranosyl-(1 → 2)-β-D-xylopyranosyl]-16-*O*-hydroxyacetoxy-23-*O*-acetoxy-3β,6α,25-trihydroxy-20(*R*),24(*S*)-epoxycycloartane (**2**), 3-*O*[α-L-arabinopyranosyl-(1 → 2)-β-D-xylopyranosyl]-3β,6α,23α,25-tetrahydroxy-20(*R*),24(*R*)-16β,24;20,24-diepoxycycloartane (**3**), 3-*O*-[α-L-arabinopyranosyl-(1 → 2)-β-D-xylopyranosyl]-25-*O*-β-D-glucopyranosyl-3β,6α,16β,25-tetrahydroxy-20(*R*),24(*S*)-epoxycycloartane (**4**), along with three known cycloartane glycosides were isolated from the MeOH extract of the roots of *Astragalus campylosema* ssp. *campylosema*. Their structures were established by the extensive use of 1D- and 2D-NMR experiments along with ESIMS and HRMS analysis. The occurrence of the hydroxyl function at position 23 (**1**–**2**) and of the ketalic function at C-24 (**3**) are very unusual findings in the cycloartane class.

1. Introduction

The genus *Astragalus* L. is one of the largest and most widely distributed genera belonging to the family Leguminosae, comprising 380 species distributed mainly in the flora of Turkey (Davis, 1970). *Astragalus* species, growing wild in Turkey, are economically important for the production of gum tragacanth, a very well-known foodstuff and pharmaceutical emulsifier (Çalış and Sticher, 1996). In Turkish folk medicine, the aqueous extracts of some *Astragalus* species (undeclared by the healer) are used to treat leukaemia as well as for wound healing (Çalış et al., 1997; Bedir et al., 2000). Roots of these plants are also used as antiperspirant, diuretic and tonic and for the treatment of nephritis, diabetes, leukemia, uterine cancer (Çalış et al., 1996).

Polysaccharides and saponins are the major classes of chemical compounds isolated from *Astragalus* species. The former compounds are reported to possess cytotoxic and immunostimulating effects (Ríos and Waterman, 1997; Bedir et al., 2000; Li, 2000) but the saponins are the class of constituents more investigated. In the course of studies on Turkish *Astragalus* species several cycloartane- and oleanane-type triterpene glycosides were isolated and their structures were elucidated (Bedir et al., 1998a,b, 1999a,b, 2000; Çalış et al., 1999, 1997, 1996; Özipek et al., 2005). Cycloartane- and oleanane-type glycosides from *Astragalus* species show interesting biological properties, including immunostimulating (Yesilada et al., 2005; Çalış et al., 1997; Bedir et al., 2000), anti-protozoal (Özipek et al., 2005), antiviral (Gariboldi et al., 1995) and cytotoxic activities (Radwan et al., 2004).

As a part of our ongoing research of new bioactive compounds from Turkish *Astragalus* species, we have investigated the roots of *Astragalus campylosema* Boiss. (Leguminosae). This paper reports the isolation of four new cycloartane glycosides (**1**–**4**) along with three known compounds (**5**–**7**). Their structures were elucidated by extensive spectroscopic methods including 1D- (^{1}H, ^{13}C and TOCSY) and 2D-NMR (DQF-COSY, HSQC, HMBC, and ROESY) experiments as well as ESIMS analysis.

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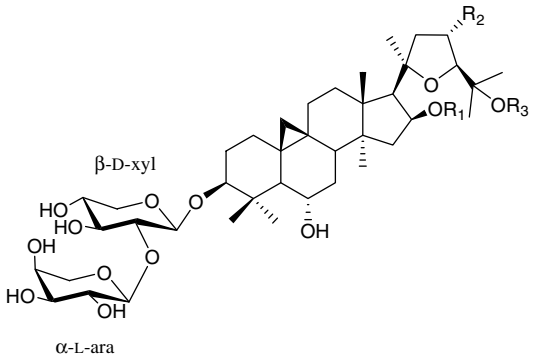
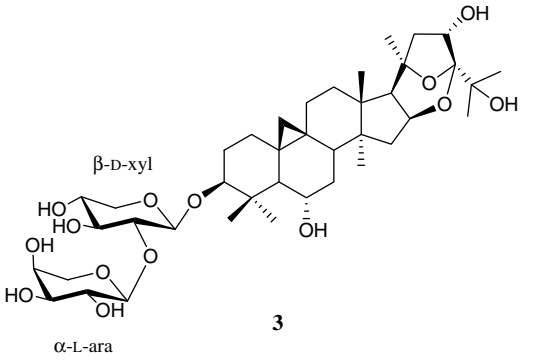


1 $R_1 = R_3 = H$ $R_2 = OH$

2 $R_1 = COCH_2OH$ $R_2 = OCOCH_3$ $R_3 = H$

4 $R_1 = R_2 = H$ $R_3 = \beta$-D-glc



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2. Results and discussion

Compounds **1–3**. A detailed comparison of the sugar region NMR data (^{1}H, ^{13}C, 1D-TOCSY, HSQC, HMBC, DQF-COSY) and ESIMS data of compounds **1–3** showed that the disaccharide chain was identical in the three compounds. In particular for the sugar portion, compound **1** showed in the ^{1}H NMR spectrum signals corresponding to two anomeric protons at δ 4.48 (1H, *d*, *J* = 7.5 Hz) and 4.50 (1H, *d*, *J* = 6.8 Hz) (Table 1). Complete assignments of the ^{1}H and ^{13}C NMR signals of the sugar portion were accomplished by 1D-TOCSY, HSQC, HMBC and DQF-COSY experiments which led to the identification of one β-xylopyranosyl unit (δ 4.48) and one α-arabinopyranosyl unit (δ 4.50). The determination of the sequence and linkage sites was obtained from the HMBC correlations between the proton signals at δ 4.48 (H-1_{xyl}) and the carbon resonance at δ 89.5 (C-3), and the proton signal at δ 4.50 (H-1_{ara}) and the carbon resonance at δ 83.2 (C-2_{xyl}). Thus, the sugar sequence of compounds **1–3** was established as 3-*O*-α-L-arabinopyranosyl-(1 → 2)-β-D-xylopyranoside.

The HRMALDITOF mass spectrum of **1** (*m*/*z* 793.4355 [M+Na]$^+$, calcd for $C_{40}H_{66}O_{14}Na$, 793.4350) supported a molecular formula of $C_{40}H_{66}O_{14}$. The ESIMS mass spectrum showed the sodiated ion peak at *m*/*z* 793 which was assigned to [M+Na]$^+$. The MS/MS of this ion showed peaks at *m*/*z* 661 [M+Na−132]$^+$, corresponding to the loss of an arabinopyranosyl unit, at *m*/*z* 511 [M+Na−132−150]$^+$, ascribable to the loss of a xylopyranosyl unit and at *m*/*z* 305 [M+Na−488]$^+$, due to the loss of the aglycon moiety.

The ^{1}H NMR spectrum showed for the aglycon moiety signals due to a cyclopropane methylene at δ 0.58 and 0.42 (each 1H, *d*, *J* = 4.2 Hz), seven tertiary methyl groups at δ 1.42, 1.33, 1.30, 1.28, 1.27, 1.05 and 1.02, and five methine proton signals at δ 4.68 (*ddd*, *J* = 8.0, 8.0, 5.2 Hz), 4.47 (*ddd*, *J* = 9.3, 6.3, 3.4 Hz), 3.59 (*d*, *J* = 6.3 Hz), 3.48 (*ddd*, *J* = 9.5, 9.5, 4.5 Hz) and 3.24 (*dd*, *J* = 11.3, 4.0 Hz) which were indicative of secondary alcoholic functions (Table 3). On the basis of DQF-COSY, HSQC and HMBC spectra and by comparison of these data with those of cyclostragenol (Kitagawa et al., 1983), it was observed that the aglycon of compound **1** differs from cycloastragenol only by the presence of an additional secondary alcoholic function at C-23 (δ_H 4.47, δ_C 74.2). The α-orientation of hydroxyl group on C-23 was derived by the ROESY spectrum, which showed key correlation peaks between H-23 (δ 4.47) and H-22β (δ 3.09) signals and between Me-21 (δ 1.42) and H-22α (δ 4.47), H-24 (δ 4.47) and H-17 (δ 2.34) signals. Thus, the new aglycon of **1** was identified as 3β,6α,16β,23α,25-pentahydroxy-20(*R*),24(*S*)-epoxycycloartane and compound **1** as the new 3-*O*-[α-L-arabinopyranosyl-(1 → 2)-β-D-xylopyranosyl]-3β,6α,16β,23α,25-pentahydroxy-20(*R*),24(*S*)-epoxycycloartane.

Table 1
^{13}C and ^{1}H NMR data (*J* in Hz) of the sugar portions of compounds **1–4** (600 MHz, CD_3OD)

	1–3		**4**	
	β-D-Xyl		β-D-Xyl	
1	105.8	4.48 *d* (7.5)	105.6	4.48 *d* (7.5)
2	83.2	3.46 *dd* (9.2, 7.5)	83.1	3.46 *dd* (9.2, 7.5)
3	76.7	3.55 *t* (9.2)	76.7	3.55 *t* (9.2)
4	70.8	3.54 *m*	70.7	3.54 *m*
5	65.8	3.89 *dd* (11.7, 5.2)	65.7	3.88 *dd* (11.7, 5.2)
		3.22 *t* (11.7)		3.22 *t* (11.7)
	α-L-Ara		α-L-Ara	
1	106.4	4.50 *d* (6.8)	106.2	4.50 *d* (6.8)
2	73.4	3.68 *dd* (8.5, 6.8)	73.3	3.68 *dd* (8.5, 6.8)
3	73.9	3.59 *dd* (8.5, 3.0)	73.8	3.59 *dd* (8.5, 3.0)
4	69.3	3.83 *m*	69.3	3.83 *m*
5	67.0	3.92 *dd* (11.9, 2.0)	66.9	3.92 *dd* (11.9, 2.0)
		3.55 *dd* (11.9, 3.0)		3.56 *dd* (11.9, 3.0)
			β-D-Glc	
1			98.2	4.55 *d* (7.5)
2			74.8	3.17 *dd* (9.0, 7.5)
3			77.9	3.36 *t* (9.0)
4			71.1	3.35 *t* (9.0)
5			77.3	3.27 *ddd* (9.0, 4.5, 2.5)
6			62.4	3.84 *dd* (11.0, 2.5)
				3.68 *dd* (11.0, 4.5)

The molecular formula of compound **2** was established as $C_{44}H_{70}O_{17}$ by HRMALDITOF mass spectrum (*m*/*z* 893.4511 [M+Na]$^+$, calcd for $C_{44}H_{70}O_{17}Na$, 893.4519). The positive ESIMS spectrum showed the sodiated ion peak at *m*/*z* 893 [M+Na]$^+$. Its MS/MS fragmentation showed peaks at *m*/*z* 817 [M+Na−76]$^+$, due to the loss of an hydroxyacetate molecule. The MS^3 fragmentation showed peaks at *m*/*z* 757 [M+Na−76−60]$^+$, corresponding to the loss of an acetate molecule, at *m*/*z* 685 [M+Na−76−132]$^+$, ascribable to the loss of an arabinopyranosyl unit and at *m*/*z* 305 [M+Na−76−512]$^+$, due to the loss of the aglycon moiety.

The ^{1}H NMR spectrum for the aglycon portion of **2** in comparison to that **1** showed in addition the presence of a signal at δ 2.08 and two signals at δ 4.24 (1H, *d*, *J* = 16.8 Hz) and 4.11 (1H, *d*, *J* = 16.8 Hz). Moreover a detailed analysis of the ^{13}C NMR data and the HSQC and HMBC correlations suggested that aglycon of **2** differed from **1** only by the presence of a –$COCH_3$ and a –$COCH_2OH$ group (Tables 2 and 3). The HMBC correlations between the proton signal at δ 5.48 (H-16) and the carbon resonance at δ 173.8 (*C*O-CH_2OH) and the proton signal at δ 5.18 (H-23) and the carbon resonance at δ 172.1 (*C*OCH_3) confirmed the location of the –$COCH_2OH$ group at C-16 and the –$COCH_3$ group at C-23, respectively. Thus, the structure of new compound **2** was established as 3-*O*-[α-L-arabinopyranosyl-(1 → 2)-β-D-xylopyranosyl]-16-*O*-hydroxyacetoxy-23-*O*-acetoxy-3β,6α,16β,23α,25-pentahydroxy-20(*R*),24(*S*)-epoxycycloartane.

Table 2
^{13}C NMR data of the aglycon moieties of compounds **1**–**4** (600 MHz, CD_3OD)

Position	1	2	3	4
1	33.1	33.2	33.1	32.9
2	30.5	30.3	30.4	30.2
3	89.5	89.4	89.3	89.4
4	42.9	43.0	43.0	43.2
5	54.6	54.3	54.6	54.6
6	69.2	69.1	69.3	69.1
7	38.8	38.5	39.1	38.8
8	48.5	48.3	48.0	48.5
9	21.5	21.2	21.3	21.5
10	30.2	30.1	30.1	30.2
11	26.6	26.7	27.2	26.6
12	33.7	33.3	34.2	33.6
13	45.3	46.3	45.0	45.8
14	46.4	46.7	45.8	46.9
15	46.5	46.8	43.7	46.3
16	74.3	77.6	75.5	74.3
17	59.4	58.5	61.7	58.9
18	21.9	21.1	23.5	22.0
19	31.9	31.7	33.1	31.8
20	87.9	86.0	84.6	88.4
21	29.7	28.3	30.1	28.2
22	46.6	44.8	43.6	35.3
23	74.2	76.0	77.6	26.1
24	90.4	85.6	108.6	82.9
25	71.5	71.0	75.0	79.8
26	26.1	26.0	24.3	23.0
27	27.7	27.0	25.7	25.2
28	28.4	28.3	28.5	28.4
29	15.9	16.0	16.0	16.1
30	20.0	20.5	20.2	20.2
$-OCOCH_3$	–	172.1		–
$-OCOCH_3$	–	20.7		–
$-OCOCH_2OH$	–	173.8		–
$-OCOCH_2OH$	–	61.3		–

The HRMALDITOF mass spectrum of **3** (*m/z* 791.4199 $[M+Na]^+$, calcd for $C_{40}H_{64}O_{14}Na$, 791.4194) supported a molecular formula of $C_{40}H_{64}O_{14}$. The ESIMS mass spectrum showed the sodiated ion peak at *m/z* 791 which was assigned to $[M+Na]^+$. The MS/MS of this ion showed peaks at *m/z* 659 $[M+Na-132]^+$, corresponding to the loss of an arabinopyranosyl unit, at *m/z* 509 $[M+Na-132-150]^+$, ascribable to the loss of a xylopyranosyl unit and at *m/z* 305 $[M+Na-486]^+$, due to the loss of the aglycon moiety.

As concerning the aglycon moiety, the ^1H NMR spectrum showed signals due to a cyclopropane methylene at δ 0.61 and 0.42 (each 1H, *d*, *J* = 4.2 Hz), six tertiary methyl groups at δ 1.52, 1.36, 1.34, 1.31, 1.30, 1.04 and 0.99, and four methine proton signals at δ 4.35 (*ddd*, *J* = 8.0, 8.0, 5.2 Hz), 4.17 (*d*, *J* = 6.3 Hz), 3.46 (*ddd*, *J* = 9.5, 9.5, 4.5 Hz) and 3.23 (*dd*, *J* = 11.3, 4.0 Hz) (Table 3). Comparison of ^1H and ^{13}C NMR data of the aglycon region of **3** with those of compound **1** revealed that the aglycon of compound **3** differs from that of **1** by the absence of the signals of C-24 (δ_H 3.59, *d*, *J* = 6.3 Hz, δ_C = 90.4) and by the presence of a ketalic function at δ 108.6 (Tables 2 and 3). HMBC correlations between the proton signals at δ 1.31 (Me-26), 1.36 (Me-27), 4.17 (H-23) and δ 1.44 (H-22) and the carbon resonance at δ 108.6 suggested for the aglycon of compound **3** the structure 3β,6α,23α,25-tetrahydroxy-16β,24;20,24-diepoxycycloartane. An examination of molecular models indicates that the heterocycles can be fused as the 20*R*, 24*R*- and 20*S*, 24*S*-configurations. Therefore, establishing the stereochemistry of one of these asymmetric centers defines the configuration of the other chiral atom (Agzamova and Isaev, 1995). Thus, the new compound **3** was identified as 3-*O*-[α-L-arabinopyranosyl-(1 → 2)-β-D-xylopyranosyl]-3β,6α,23α,25-tetrahydroxy-20(*R*),24(*R*)-16β,24;20,24-diepoxycycloartane. Compounds of this class are very unusual in the plant kingdom: 16β,24;20,24-diepoxycycloartane-type derivatives have been isolated only from *Astragalus alopecurus* (Agzamova and Isaev, 1995), *Souliea vaginata* and *Beesia calthaefolia* (Sakurai et al., 1990).

Compound **4** showed in the positive ESIMS a sodiated ion peak at *m/z* 939 $[M+Na]^+$ and a significant fragment in MS/MS analysis at *m/z* 759 $[M+Na-180]^+$, ascribable to the loss of an hexose unit. The MS^3 fragmentation showed peaks at *m/z* 627 $[M+Na-180-132]^+$ and *m/z* 477 $[M+Na-180-132-150]^+$, corresponding to the stepwise loss of two pentose units, and at *m/z* 305 $[M+Na-180-454]^+$, due to the loss of the aglycon moiety. Its molecular formula was established unequivocally as $C_{46}H_{76}O_{18}$ by HRMALDITOF mass spectrum (*m/z* 939.4934 $[M+Na]^+$, calcd for $C_{46}H_{76}O_{18}Na$, 939.4929). The NMR data of the sugar region of **4** in comparison with those of compounds **1**–**3** showed the presence of an additional anomeric signal at δ 4.55 (*d*, *J* = 7.5 Hz). On the basis of 1D-TOCSY, HSQC, HMBC, DQF-COSY correlations this sugar unit was identified as β-glucopyranose (Table 1). The ^1H and ^{13}C NMR data of **4** indicated cycloastragenol (Kitagawa et al., 1983) as aglycon with glycosidation shifts for C-3 (δ 89.4) and C-25 (δ 79.8) (Table 2 and 3). Key correlation peaks in the HMBC spectrum were observed between $H\text{-}1_{xyl}$ (δ 4.48) and C-3 (δ 89.4), $H\text{-}1_{ara}$ (δ 4.50) and $C\text{-}2_{xyl}$ (δ 83.1) and between $H\text{-}1_{glc}$ (δ 4.55) and C-25 (δ 79.8). Therefore, the new structure of **4** was assigned as 3-*O*-[α-L-arabinopyranosyl-(1 → 2)-β-D-xylopyranosyl]-25-*O*-β-D-glucopyranosyl-3β,6α,16β,25-tetrahydroxy-20(*R*),24(*S*)-epoxycycloartane.

Additionally, three known cycloartane glycosides namely 3-*O*-[α-L-arabinopyranosyl-(1 → 2)-β-D-xylopyranosyl]-3β,6α,16β,25-tetrahydroxy-20(*R*),24(*S*)-epoxycycloartane (**5**) (Isaev et al., 1983a), askendoside C (**6**) (Isaev et al., 1983b) and askendoside G (**7**) (Isaev, 1996) were isolated.

Thus the occurrence of compounds **1**–**2** characterized by hydroxylation of ring E and by the esterification of the alcoholic function at C-23 with hydroxyacetic acid, respectively, along with compound **3** showing as particular feature the ketalic function at C-24 is an unusual finding which makes unique the cycloartane profile of *A. campylosema*.

3. Experimental

3.1. General

Optical rotations were measured on a Rudolph Research Analytical Autopol IV 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 CryoProbe at 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, HMBC and ROESY spectra. The NMR data were processed using UXNMR software. Exact masses were measured by a Voyager DE mass spectrometer. Samples were analyzed 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. ESIMS analyses were performed using a ThermoFinnigan LCQ Deca XP Max ion-trap mass spectrometer equipped with Xcalibur software. GC analysis was performed on a ThermoFinnigan Trace GC apparatus using a l-Chirasil-Val column (0.32 mm × 25 m).

3.2. Plant material

Astragalus campylosema Boiss. subsp. *campylosema* was collected from Tutak, 1 km from Çobanobası village to Tutak, Ağrı,

Table 3
^{1}H NMR data (*J* in Hz) of the aglycon moieties of compounds **1**–**4** (600 MHz, CD_3OD)

Position	1	2	3	4
1	1.58, 1.25, *m*	1.57, 1.25, *m*	1.57, 1.24, *m*	1.58, 1.25, *m*
2	1.95, 1.71, *m*	1.95, 1.71, *m*	1.95, 1.70, *m*	1.95, 1.71, *m*
3	3.24, *dd* (11.3, 4.0)	3.23, *dd* (11.3, 4.0)	3.23, *dd* (11.3, 4.0)	3.23, *dd* (11.3, 4.0)
4	–	–	–	–
5	1.39, *d* (9.5)	1.38, *d* (9.5)	1.37, *d* (9.5)	1.39, *d* (9.5)
6	3.48, *ddd* (9.5, 9.5, 4.5)	3.47, *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)
7	1.49, 1.38, *m*	1.45, 1.40, *m*	1.46, 1.38, *m*	1.49, 1.38, *m*
8	1.83, *dd* (11.9, 4.2)	1.84, *dd* (11.9, 4.2)	1.77, *dd* (11.9, 4.2)	1.83, *dd* (11.9, 4.2)
9	–	–	–	–
10	–	–	–	–
11	2.06, 1.25, *m*	2.06, 1.28, *m*	2.14, 1.22, *m*	2.06, 1.25, *m*
12	1.73, 1.69, *m*	1.81, 1.76, *m*	1.70 (2H), *m*	1.71, 1.71, *m*
13	–	–	–	–
14	–	–	–	–
15	1.98, *dd* (12.7, 8.0) 1.42, *dd* (12.7, 5.2)	2.28, *dd* (12.7, 8.0) 1.39, *dd* (12.7, 5.2)	1.86, *dd* (12.7, 8.0) 1.44,[a]	1.98, *dd* (12.7, 8.0) 1.45, *dd* (12.7, 5.2)
16	4.68, *ddd* (8.0, 8.0, 5.2)	5.48, *ddd* (8.0, 8.0, 5.2)	4.35, *ddd* (8.0, 8.0, 5.2)	4.69, *ddd* (8.0, 8.0, 5.2)
17	2.34, *d* (8.0)	2.59, *d* (8.0)	2.58, *d* (8.0)	2.39, *d* (8.0)
18	1.27, *s*	1.34, *s*	1.30, *s*	1.31, *s*
19	0.58, *d* (4.2) 0.42, *d* (4.2)	0.59, *d* (4.2) 0.42, *d* (4.2)	0.61, *d* (4.2) 0.42, *d* (4.2)	0.58, *d* (4.2) 0.41, *d* (4.2)
20	–	–	–	–
21	1.42, *s*	1.46, *s*	1.52, *s*	1.26, *s*
22	3.09, *dd* (13.7, 9.3) 1.69,[a]	3.07, *dd* (13.7, 9.3) 1.61, *dd* (13.7, 3.4)	3.03, *dd* (13.7, 6.3) 1.44,[a]	2.58, *m* 1.69, *m*
23	4.47, *ddd* (9.3, 6.3, 3.4)	5.18, *ddd* (9.3, 6.3, 3.4)	4.17, *d* (6.3)	2.20, *m* 2.02, *m*
24	3.59, *d* (6.3)	3.78, *d* (6.3)	–	3.86, *dd* (8.2, 6.0)
25	–	–	–	–
26	1.28, *s*	1.16, *s*	1.31, *s*	1.26, *s*
27	1.30, *s*	1.18, *s*	1.36, *s*	1.41, *s*
28	1.33, *s*	1.32, *s*	1.34, *s*	1.33, *s*
29	1.05, *s*	1.04, *s*	1.04, *s*	1.04, *s*
30	1.02, *s*	1.07, *s*	0.99, *s*	1.03, *s*
—OCOCH_3		2.08, *s*		
—COCH_2OH		4.24, *d* (16.8) 4.11, *d* (16.8)		

[a] Overlapped with other signals.

East Anatolia, Turkey in June 23, 2005. Samples of plant material were identified by one of the co-author, A.A. Dönmez. Voucher specimen (AAD 12252) has been deposited at the HUB.

3.3. Extraction and isolation

The air-dried powdered roots (120 g) were macerated with MeOH (1000 ml) at room temperature 24 h. After filtration of the MeOH through a funnel, extracted plant material was washed by MeOH (500 ml). The combined MeOH used in the maceration and washing was removed by rotary evaporation yielding 8.7 g of crude extract (yield 7.25%). The crude extract was dissolved in H_2O (30 ml) and applied to VLC using reversed phase material (LiChroprep C-18, 26 × 260 mm), eluting with H_2O (A; 200 ml), 20% and 50% MeOH (B and C; each 100 ml) and MeOH (D; 200 ml). Fraction D eluted with MeOH was rich in cycloartane glycosides (1778 mg). An aliquot of fraction D (1250 mg) was further applied to MPLC on reversed phase silica gel (LiChrosorb C-18, 26 × 260 mm) using stepwise MeOH–H_2O gradient (50–100% MeOH, 35 ml/fraction) to give 50 fractions. Fractions 3–15 (611 mg) were poor in cyloartane glycosides. Fractions 16–18 were rich in compound **1** (80 mg) which was further purified on a silica gel column (50 g) eluting with CH_2Cl_2–MeOH–H_2O mixtures (80:20:1 and 80:20:2, 250 ml and 550 ml, respectively) to yield pure **1** (13 mg). Fractions 19–21 (47 mg) consisted of crude compound **3**. Final purification was performed on a silica gel column (30 g) using CH_2Cl_2–MeOH–H_2O mixture (80:20:1) to give pure **3** (5 mg). Fractions 22–23 (108 mg) was chromatographed on a silica gel column (55 g) eluting with CH_2Cl_2–MeOH–H_2O mixtures (from 80:20:1 to 50:50:5) to give compounds **2** (9 mg) and **4** (6 mg), respectively. Fractions 24–26 (208 mg) were also applied to a silica gel column (50 g) using CH_2Cl_2–MeOH–H_2O mixtures (80:20:2, 70:30:3 and 60:40:4) as eluent to give compounds **6** (28 mg) and **7** (53 mg), respectively. Fractions 29–30 (55 mg) and 31–33 (45 mg) were rich in compound **5** of which later were further subjected to a normal phase silica gel column chromatography using $CHCl_2$–MeOH–H_2O mixture (80:20:2) to yield the compound **5** (19 mg).

3.4. 3-O-[α-L-Arabinopyranosyl-(1 → 2)-β-D-xylopyranosyl]-3β,6α,16β,23α,25-pentahydroxy-20(R),24(S)-epoxycycloartane (*1*)

Amorphous white solid; $C_{40}H_{66}O_{14}$; $[\alpha]_D^{35}$ + 40° (MeOH; *c* 0.1); IR ν_{max}^{KBr} cm^{-1}: 3472 (>OH), 3035 (cyclopropane ring), 2932 (>CH), 1273 and 1040 (C–O–C); for ^{13}C and ^{1}H NMR data of the sugar portion, see Table 1; for ^{13}C and ^{1}H NMR data of the aglycon moiety, see Tables 2 and 3, respectively; ESI-MS *m*/*z* 793 [M+Na]$^+$; MS/MS *m*/*z* 661 [M+Na−132]$^+$, *m*/*z* 511 [M+Na−132−150]$^+$, *m*/*z* 305 [M+Na−488]$^+$; HRMALDITOFMS [M+Na]$^+$ *m*/*z* 793.4355 (calcd. for $C_{40}H_{66}O_{14}Na$, 793.4350).

3.5. 3-O-[α-L-Arabinopyranosyl-(1 → 2)-β-D-xylopyranosyl]-16-O-hydroxyacetoxy-23-O-acetoxy-3β,6α,16β,23α,25-pentahydroxy-20(R),24(S)-epoxycycloartane (*2*)

Amorphous white solid; $C_{44}H_{70}O_{17}$; $[\alpha]_D^{35}$ + 67° (MeOH; *c* 0.1); IR ν_{max}^{KBr} cm^{-1}: 3480 (>OH), 3044 (cyclopropane ring), 2928 (>CH),

1734 (C=O), 1277–1035 (C—O—C); for ^{13}C and ^{1}H NMR data of the sugar portion, see Table 1; for ^{13}C and ^{1}H NMR data of the aglycon moiety, see Table 2 and 3, respectively; ESI-MS *m/z* 893 $[M+Na]^+$; MS/MS *m/z* 817 $[M+Na-76]^+$, *m/z* 757 $[M+Na-76-60]^+$, *m/z* 685 $[M+Na-76-132]^+$, *m/z* 305 $[M+Na-76-512]^+$; HRMALDITOFMS $[M+Na]^+$ *m/z* 893.4519 (calcd. for $C_{44}H_{70}O_{17}Na$, 893.4511).

3.6. 3-O-[α-L-Arabinopyranosyl-(1 → 2)-β-D-xylopyranosyl]-3β,6α,23α,25-tetrahydroxy-20(R),24(R)-16β,24; 20,24-diepoxycycloartane (**3**)

Amorphous white solid; $C_{40}H_{64}O_{14}$; $[\alpha]_D^{24}$ $[\alpha]_D^{35}$ + 20° (MeOH; *c* 0.1); IR ν_{max}^{KBr} cm^{-1}: 3470 (>OH), 3040 (cyclopropane ring), 2930 (>CH), 1280-1045 (C—O—C); for ^{13}C and ^{1}H NMR data of the sugar portion, see Table 1; for ^{13}C and ^{1}H NMR data of the aglycon moiety, see Tables 2 and 3, respectively; ESI-MS *m/z* 791 $[M+Na]^+$; MS/MS *m/z* 659 $[M+Na-132]^+$, *m/z* 509 $[M+Na-132-150]^+$, *m/z* 305 $[M+Na-486]^+$; HRMALDITOFMS $[M+Na]^+$ *m/z* 791.4199 (calcd. for $C_{40}H_{64}O_{14}Na$, 791.4194).

3.7. 3-O-[α-L-Arabinopyranosyl-(1 → 2)-β-D-xylopyranosyl]-25-O-β-D-glucopyranosyl-3β,6α,16β,25-tetrahydroxy-20(R),24(S)-epoxycycloartane (**4**)

Amorphous white solid; $C_{46}H_{76}O_{18}$; $[\alpha]_D^{35}$ + 24° (MeOH; *c* 0.1); IR ν_{max}^{KBr} cm^{-1}: 3475 (>OH), 3047 (cyclopropane ring), 2925 (>CH), 1270–1043 (C—O—C); for ^{13}C and ^{1}H NMR data of the sugar portion, see Table 1; for ^{13}C and ^{1}H NMR data of the aglycon moiety, see Table 2 and 3, respectively; ESI-MS *m/z* 939 $[M+Na]^+$; MS/MS *m/z* 759 $[M+Na-180]^+$, *m/z* 627 $[M+Na-180-132]^+$, *m/z* 477 $[M+Na-180-132-150]^+$, *m/z* 305 $[M+Na-180-454]^+$; HRMALDI-TOFMS $[M+Na]^+$ *m/z* 939.4934 (calcd. for $C_{46}H_{76}O_{18}Na$, 939.4929).

3.8. Acid hydrolysis

A solution (1 mg each) of compounds **1** and **4** 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 analyzed 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** was detected at 8.90 and 9.79 min (L-arabinose) and 10.97 and 12.00 (D-xylose). The peaks of the hydrolysate of **4** were detected at 8.91 and 9.81 min (L-arabinose), 14.74 min (D-glucose), 10.96 and 12.02 (D-xylose). Retention times for authentic samples after being treated in the same manner with 1-(trimethylsilyl)-imidazole in pyridine were detected at 8.80 and 9.72 min (D-arabinose), 14.71 min (D-glucose), 10.98 and 12.00 min (D-xylose), 8.92 and 9.80 min (L-arabinose), 14.64 min (L-glucose) and 11.03 and 12.06 min (L-xylose).

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