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Cycloartane glycosides from *Astragalus aureus*

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ABSTRACT

Eight cycloartane-type triterpene glycosides (**1**–**8**) were isolated from *Astragalus aureus* Willd along with ten known cycloartane-type glycosides (**9**–**18**). Their structures were established by the extensive use of 1D and 2D-NMR experiments along with ESIMS and HRMS analyses. Compounds **1**–**5** are cyclocanthogenin glycosides, whereas compounds **6**–**8** are based on cyclocephalogenin as aglycon, more unusual in the plant kingdom, so far reported only from *Astragalus* spp. Moreover, for the first time monoglycosides of cyclocanthogenin (**5**) and cyclocephalogenin (**7**, **8**) are reported. All of the compounds tested for their cytotoxic activities against a number of cancer cell lines. Among the compounds, only **8** exhibited activity versus human breast cancer (MCF7) at 45 μM concentration.

1. Introduction

The genus *Astragalus* belonging to the Leguminosae family is widely distributed throughout the temperate regions of the world, located principally in Europe, Asia and North America. About 2000 species have been described, 380 of which are represented in the flora of Turkey (Ríos and Waterman, 1997; Davis 1970). The roots of various *Astragalus* species represent very old and well-known drugs in traditional medicine for the treatment of nephritis, diabetes, uterine cancer and as antiperspirant, diuretic, and tonic (Tang and Eisenbrand, 1992). In Turkish folk medicine, the aqueous extracts of some *Astragalus* species (declared by the healer) are used to treat leukemia as well as for wound healing (Çalış et al., 1997; Bedir et al., 2000a).

Besides polysaccharides, saponins are the major class of biologically active compounds from *Astragalus* species thoroughly investigated (Tang and Eisenbrand, 1992). Chemical studies on *Astragalus* saponins have resulted in isolation of cycloartane- and oleanane-type glycosides (Ríos and Waterman, 1997; Verotta and El-Sebakhy, 2001). A series of cycloartane-type triterpenoidal saponins were isolated from our earlier investigations on Turkish *Astragalus* species (Bedir et al., 1998a,b, 1999; Polat et al., 2009, 2010; Horo et al., 2010).

Cycloartane- and oleanane-type glycosides from *Astragalus* species have shown interesting biological properties, including immunostimulating (Çalış et al., 1997; Bedir et al., 2000a; Yesilada et al., 2005), anti-protozoal (Özipek et al., 2005), antiviral (Gariboldi et al., 1995), and cytotoxic activities (Tian et al., 2005). On the basis of interesting activities reported for many *Astragalus* species and for cycloartane glycosides, and as a part of our ongoing research of new bioactive compounds from Turkish *Astragalus* species, we carried out a study on *Astragalus aureus* Willd (Leguminosae). This paper reports the isolation of eight new cycloartane-type triterpene glycosides (**1**–**8**) from the methanol extract of *A. aureus* along with ten known cycloartane-type glycosides (**9**–**18**). 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 and HRMS analysis. Additionally, all of the compounds were screened for their antiproliferative activities versus a number of cancer cell lines.

2. Results and discussion

The HRMALDITOF mass spectrum of **1** (*m/z* 1057.5563 $[M+Na]^+$, calcd for $C_{51}H_{86}O_{21}Na$, 1057.5559) supported a molecular formula of $C_{51}H_{86}O_{21}$. The ESIMS spectrum showed a major ion peak at *m/z* 1057.65 which was assigned to $[M+Na]^+$. The MS/MS of this ion

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showed a peak at *m/z* 907.53 $[M+Na-150]^+$, corresponding to the loss of a pentose unit. In the MS^3 spectrum peaks at *m/z* 761.46 $[M+Na-150-146]^+$, corresponding to the loss of a deoxy–hexose unit, and 629.42 $[M+Na-150-146-132]^+$, due to the loss of a pentose unit, were observed. A detailed comparison of the NMR data (^1H, ^{13}C, HSQC, HMBC, COSY) of compounds **1**–**5** (Fig. 1) showed that the aglycon moiety was identical in all five compounds. In particular, the ^1H NMR spectrum of **1** showed signals due to a cyclopropane methylene at δ 0.59 and 0.25 (each 1H, d, *J* = 4.2 Hz), six tertiary methyl groups at δ 1.28 (3H, s), 1.20 (3H, s), 1.18 (3H, s), 1.17 (3H, s), 1.03 (3H, s) and 1.01 (3H, s), a secondary methyl group at δ 0.98 (d, *J* = 6.5 Hz), and four methine proton signals at δ 4.48 (ddd, *J* = 8.0, 8.0, 5.2 Hz), 3.54 (ddd, *J* = 9.5, 9.5, 4.5 Hz), 3.41 (dd, *J* = 10.5, 2.4 Hz) and 3.23 (dd, *J* = 11.3, 4.0 Hz), which were indicative of secondary alcoholic functions. The NMR data of the aglycon moiety of **1** were in good agreement with those reported for cyclocanthogenin with glycosidation shifts for C-3 (δ 89.6) and C-6 (δ 79.0) (Isaev et al., 1992). The ^{13}C-NMR chemical shift for C-24

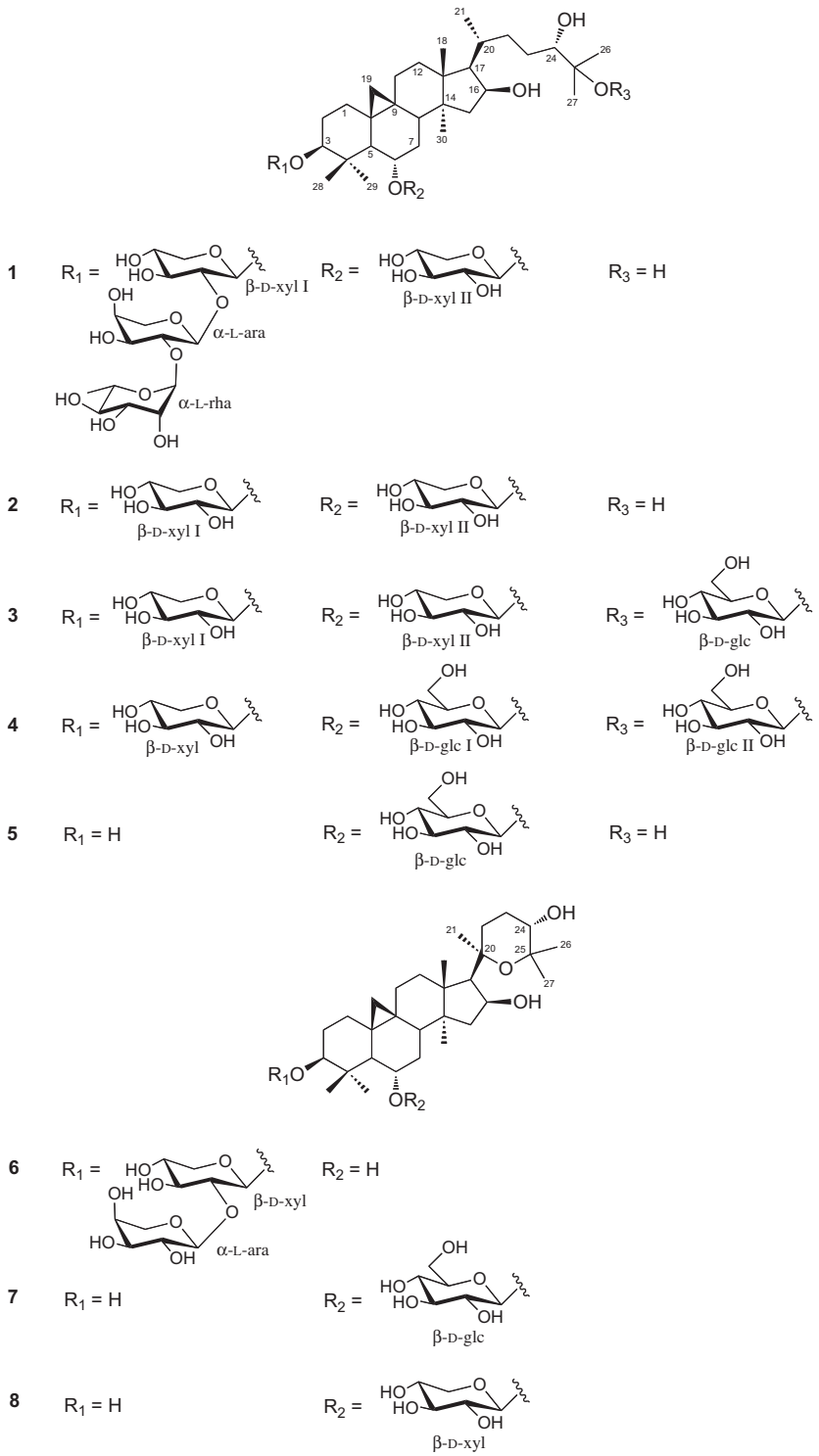

Fig. 1. Structures of compounds **1**–**8**.

can be regarded as a characteristic parameter in the determination of the configurations of C-24. In the case of the 24*R* configuration, the chemical shift for C-24 gives resonance at 79.9–80.6 ppm (Isaev et al., 1983, 1982; Agzamova and Isaev; 1998), while C-24 gives resonance at 77.0–78.2 ppm for the 24*S* configuration (Isaev et al., 1992; Bedir et al., 2000b; Hirotani et al., 1994a,b; Fadeev et al., 1988). The ^{13}C NMR data of compound **1** are comparable to those reported for analogous compounds having a 24*S* configuration. The relative configurations of the oxygenated carbons were determined by the multiplicity of the vicinal proton–proton coupling constants to be β-OH for C-3 (δ 3.23, dd, *J* = 11.3, 4.0 Hz. H_{ax}-3), α-OH for C-6 (δ 3.54, ddd, *J* = 9.5, 9.5, 4.5 Hz, H_{ax}-6) and β-OH for C-16 (δ 4.48, ddd, *J* = 8.0, 8.0, 5.2 Hz, H_{ax}-16). Additionally, the relative configuration of C-20 was derived by the ROESY spectrum that showed key correlations between Me-30α (δ 1.01), H-16α (δ 4.48) and H-17α (δ 1.74) signals, and between H-17α and Me-21α (δ 0.98) signals.

For compound **1**, a secondary methyl group at δ 1.31 (d, *J* = 6.5 Hz) along with four anomeric protons at δ 5.06 (d, *J* = 1.2 Hz), 4.98 (d, *J* = 3.7 Hz), 4.49 (d, *J* = 7.5 Hz) and 4.33 (d, *J* = 7.5 Hz) were additionally observed. The chemical shifts of all the individual protons of the four sugar units were ascertained from a combination of 1D-TOCSY and DQF-COSY spectral analyses, and the ^{13}C chemical shifts of their relative attached carbons were assigned unambiguously from the HSQC spectrum (Table 1). These data showed the presence of one α-rhamnopyranosyl unit (δ 5.06), two β-xylopyranosyl units (δ 4.49 and 4.33) and one α-arabinopyranosyl unit (δ 4.98). The α configuration of the rhamnose unit was deduced from the H-1/C-1 *J* value = 169 Hz, measured from the residual direct correlation observed in the HMBC spectrum, in agreement with that reported for the α anomer of rhamnopyranose (Kasai et al., 1979). For the L-arabinopyranosyl unit the value of $J_{H1\text{-}H2}$ coupling constant has been reported not to be diagnostic on its own, owing to the high conformational mobility of arabinopyranosides ($^4C_1 \rightarrow {}^1C_4$) (Breitmaier and Voelter, 1987). Evidence supporting an α-L-arabinopyranoside configuration in rapid conformational exchange was obtained from ROESY experiments. NOe's were observed from H_{ara}-1 to H_{ara}-2 and H_{ara}-1 to H_{ara}-3 as expected for $^1C_4$ and $^4C_1$ conformations respectively. The nOe H_{ara}-1-H_{ara}-3 would not be expected for both $^1C_4$-$^4C_1$-β-L-arabinopyranosides. An nOe was also observed between H_{ara}-1 and H_{ara}-5 as expected for an α-L-arabinopyranoside in a $^4C_1$ conformation.

Glycosidation shifts were observed for C-$2_{xyl\ I}$ (δ 79.9) and C-2_{ara} (δ 75.9). An unambiguous determination of the sequence and linkage sites was obtained from the HMBC spectrum, which showed key correlation peaks between the proton signal at δ 4.49 (H-$1_{xyl\ I}$) and the carbon resonance at δ 89.6 (C-3), δ 4.98 (H-1_{ara}) and δ 79.9 (C-2_{xyl}), δ 5.06 (H-1_{rha}) and δ 75.9 (C-2_{ara}), and the proton signal at δ 4.33 (H-$1_{xyl\ II}$) and the carbon resonance at δ 79.0 (C-6). The D configuration of xylose unit and the L configuration of arabinose and rhamnose units were established after hydrolysis of **1** with 1 N HCl, trimethylsilation and determination of the retention times by GC (De Marino et al., 2003).

On the basis of all these evidence, the structure of the new compound **1** was established as 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→2)-β-D-xylopyranosyl]-6-*O*-β-D-xylopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxycycloartane.

The molecular formula of compound **2** was established as $C_{40}H_{68}O_{13}$ by HRMALDITOFMS analysis (*m/z* 779.4561 $[M + Na]^+$, calcd for $C_{40}H_{68}O_{13}Na$, 779.4558). The ^{1}H NMR spectrum of **2**

Table 1
^{1}H NMR data (*J* in Hz) of the sugar portions of compounds **1**–**5** (600 MHz, δ ppm, in CD_3OD).

	1		**2**		**3**		**4**		**5**[a]	
	δ_C	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	δ_H (*J* in Hz)
	β-D-Xyl I (at C-3)		β-D-Xyl I (at C-3)		β-D-Xyl I (at C-3)		β-D-Xyl I (at C-3)		β-D-Glc (at C-6)	
1	105.9	4.49, d (7.5)	106.9	4.31, d (7.5)	107.0	4.31, d (7.5)	106.8	4.31, d (7.5)	104.6	4.36, d (7.5)
2	79.9	3.42, dd (9.2, 7.5)	75.1	3.22, dd (9.2, 7.5)	75.1	3.22, dd (9.2, 7.5)	75.0	3.22, dd (9.2, 7.5)	75.4	3.21, dd (7.5, 9.0)
3	77.0	3.54, t (9.2)	77.7	3.33, t (9.2)	77.6	3.32, t (9.2)	78.0	3.34, t (9.2)	78.2	3.36, t (9.0)
4	71.0	3.54, m	71.0	3.50, m	70.9	3.49, m	71.0	3.50, m	71.3	3.31, t (9.0)
5	66.2	3.90, dd (11.7, 5.2)	66.5	3.86, dd (11.7, 5.2)	66.3	3.86, dd (11.7, 5.2)	66.3	3.85, dd (11.7, 5.2)	77.4	3.28, ddd (3.5, 4.5, 9.0)
		3.23, t (11.7)		3.20, t (11.7)		3.20, t (11.7)		3.21, t (11.7)		
6									62.7	3.88, dd (3.5, 12.0)
										3.69, dd (4.5, 12.0)
	α-L-Ara (at C-2_{xyl})		β-D-Xyl II (at C-6)		β-D-Xyl II (at C-6)		β-D-Glc I (at C-6)			
1	101.8	4.98, d (3.7)	105.1	4.32, d (7.5)	105.1	4.33, d (7.5)	104.6	4.36, d (7.5)		
2	75.9	3.90, dd (8.5, 3.7)	75.1	3.22, dd (9.2, 7.5)	75.1	3.20, dd (9.2, 7.5)	75.0	3.20, dd (7.5, 9.0)		
3	72.1	3.84, dd (8.5, 3.0)	77.7	3.33, t (9.2)	77.6	3.32, t (9.2)	78.2	3.36, t (9.0)		
4	67.9	3.84, m	71.0	3.50, m	70.9	3.49, m	71.3	3.30, t (9.0)		
5	63.2	3.97, dd (11.9, 2.0)	66.5	3.86, dd (11.7, 5.2)	66.3	3.86, dd (11.7, 5.2)	77.2	3.31, ddd (3.5, 4.5, 9.0)		
		3.48, dd (11.9, 3.0)		3.20, t (11.7)		3.20, t (11.7)				
6							62.7	3.86, dd (3.5, 12.0)		
								3.68, dd (4.5, 12.0)		
	α-L-Rha (C-2_{ara})				β-D-Glc (at C-25)		β-D-Glc II (at C-25)			
1	101.8	5.06, d (1.2)			97.6	4.55, d (7.5)	97.5	4.55, d (7.5)		
2	72.1	3.90, dd (1.2, 3.2)			75.0	3.19, dd (7.5, 9.0)	75.0	3.19, dd (7.5, 9.0)		
3	72.1	3.72, dd (3.2, 9.3)			77.9	3.40, t (9.0)	77.9	3.41, t (9.0)		
4	73.8	3.43, t (9.3)			71.3	3.31, t (9.0)	71.3	3.33, t (9.0)		
5	70.1	3.89, m			77.5	3.31, ddd (3.5, 4.5, 9.0)	77.4	3.28, ddd (3.5, 4.5, 9.0)		
6	17.9	1.31, d (6.5)			62.4	3.86, dd (3.5, 12.0)	62.7	3.86, dd (3.5, 12.0)		
		3.68, dd (4.5, 12.0)				3.68, dd (4.5, 12.0)		3.68, dd (4.5, 12.0)		
	β-D-Xyl II (at C-6)									
1	105.2	4.33, d (7.5)								
2	75.4	3.22, dd (9.2, 7.5)								
3	77.7	3.34, t (9.2)								
4	71.0	3.50, m								
5	66.3	3.86, dd (11.7, 5.2)								
		3.20, t (11.7)								

[a] The chemical shift values of the sugar portion of **7** were superimposable with those reported for **5**.

showed signals for two anomeric protons at δ 4.31 (d, *J* = 7.5 Hz) and 4.32 (d, *J* = 7.5 Hz). The HSQC, HMBC, DQF-COSY and 1D-TOCSY data led to identify these sugar units as two β-xylopyranosyl units. The HMBC correlations between the proton signal at δ 4.31 ($H\text{-}1_{xyl\ I}$) and the carbon resonance at δ 89.6 (C-3) and the proton signal at δ 4.32 ($H\text{-}1_{xyl\ II}$) and the carbon resonance at δ 79.0 (C-6) allowed us to determine the linkage site of the sugar units. Thus, the new compound **2** was elucidated as 3,6-di-*O*-β-D-xylopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxycycloartane.

The HRMALDITOF mass spectrum of **3** (*m/z* 941.5090 $[M + Na]^+$, calcd for $C_{46}H_{78}O_{18}Na$, 941.5086) supported a molecular formula of $C_{46}H_{78}O_{18}$. The ^1H NMR spectrum of **3** displayed, in addition to signals of the aglycon moiety, signals for three anomeric protons at δ 4.55 (d, *J* = 7.5 Hz), 4.33 (d, *J* = 7.5 Hz) and 4.31 (d, *J* = 7.5 Hz). On the basis of HSQC, HMBC, DQF-COSY and 1D-TOCSY correlations two β-xylopyranosyl units (δ 4.33 and 4.31) and one β-glucopyranosyl (δ 4.55) were identified. Glycosidation shifts for the aglycon moiety were observed for C-3 (δ 89.6), C-6 (δ 79.0) and C-25 (δ 81.2). Key correlation peaks in the HMBC spectrum were observed between the proton signal at δ 4.31 ($H\text{-}1_{xyl\ I}$) and the carbon resonance at δ 89.6 (C-3), δ 4.33 ($H\text{-}1_{xyl\ II}$) and δ 79.0 (C-6), and between the proton signal at δ 4.55 ($H\text{-}1_{glc}$) and the carbon resonance at δ 81.2 (C-25). The configuration of glucose and xylose was determined as D via hydrolysis followed by GC analysis. Therefore, the structure of **3** was established as 3,6-di-*O*-β-D-xylopyranosyl-25-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxycycloartane accommodating a very rare chemical feature in nature, a tridesmosidic arrangement.

The molecular formula of **4** was established as $C_{47}H_{80}O_{19}$ by HRMALDITOFMS analysis (*m/z* 971.5197 $[M + Na]^+$, calcd for $C_{47}H_{80}O_{19}Na$, 971.5192). The comparison of the NMR data of compound **4** with those of compound **3** allowed us to determine that the two compounds differed only by the presence of a β-glucopyranosyl unit at C-6 in **4** instead of the β-xylopyranosyl unit in **3**. Thus, compound **4** was elucidated as 3-*O*-β-D-xylopyranosyl-6,25-di-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxycycloartane revealing another tridesmosidic glycoside.

The HRMALDITOF mass spectrum of **5** (*m/z* 677.4246 $[M + Na]^+$, calcd for $C_{36}H_{62}O_{10}Na$, 677.4241) supported a molecular formula of $C_{36}H_{62}O_{10}$. The ^1H NMR spectrum of **5** displayed, in the sugar region, only one anomeric proton signal at δ 4.36 (d, *J* = 7.5 Hz). On the basis of 1D-TOCSY, HSQC and DQF-COSY data this sugar unit was identified as β-glucopyranosyl unit. The HMBC spectrum showed the correlation between the proton signal at δ 4.36 ($H\text{-}1_{glc}$) and the carbon resonance at δ 80.1 (C-6), allowing us to elucidate compound **5** as 6-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxycycloartane.

The molecular formula of **6** was established as $C_{40}H_{66}O_{13}$ by HRMALDITOFMS analysis (*m/z* 777.4405 $[M + Na]^+$, calcd for $C_{40}H_{66}O_{13}Na$, 777.4401). The ESIMS mass spectrum showed the major ion peak at *m/z* 777.74 which was assigned to $[M + Na]^+$. In the MS/MS spectrum peaks at *m/z* 645.36 $[M + Na\text{-}132]^+$, corresponding to the loss of a pentose unit, 495.41 $[M + Na\text{-}150]^+$, corresponding to the loss of a further pentose unit, were observed. It was apparent from their ^1H and ^{13}C NMR data that **6**–**8** possessed the same aglycon moiety. In particular, the ^1H NMR spectrum of **6** showed signals due to a cyclopropane methylene at δ 0.57 and 0.43 (each 1H, d, *J* = 4.2 Hz), seven tertiary methyl groups at δ 1.54 (3H, s), 1.49 (3H, s), 1.32 (6H, s), 1.22 (3H, s), 1.05 (3H, s) and 0.97 (3H, s) and four methine proton signals at δ 4.64 (ddd, *J* = 8.0, 8.0, 5.2 Hz), 3.47 (ddd, *J* = 9.5, 9.5, 4.5 Hz), 3.51 (br s) and 3.22 (dd, *J* = 11.3, 4.0 Hz). The NMR data of compound **6** were in agreement with those reported for 3β,6α,16β,24α-tetrahydroxy-20(*R*),25-epoxycycloartane named cyclocephalogenin, previously reported from *Astragalus* spp. (Bedir et al., 1998b). The relative configurations of the oxygenated carbons C-3, C-6, C-16 and C-20 were determined as reported for compound **1**. The α orientation of the –OH group at C-24 was determined by the ROE correlation between Me-27 (δ 1.32) and H-24β (δ 3.51) signals.

For the sugar region in the ^1H NMR spectrum, two anomeric protons at δ 4.49 (d, *J* = 3.7 Hz) and 4.48 (d, *J* = 7.5 Hz) were observed. Complete assignments of the ^1H and ^{13}C NMR signals of the sugar portion were accomplished by 1D-TOCSY, HSQC and DQF-COSY experiments which led to the identification of one β-xylopyranosyl unit (δ 4.48) and one α-arabinopyranosyl unit (δ 4.49). The determination of the sequence and linkage sites was obtained from the HMBC correlations which showed key correlation peaks between the proton signals at δ 4.48 ($H\text{-}1_{xyl}$) and the carbon resonance at δ 89.3 (C-3), and the proton signal at δ 4.49 ($H\text{-}1_{ara}$) and the carbon resonance at δ 82.9 ($C\text{-}2_{xyl}$). Thus, compound **6** was elucidated as 3-*O*-[α-L-arabinopyranosyl-(1→2)-β-D-xylopyranosyl]-3β,6α,16β,24α-tetrahydroxy-20(*R*),25-epoxycycloartane.

The molecular formulas of compounds **7** and **8** were established as $C_{36}H_{60}O_{10}$ and $C_{35}H_{58}O_9$ by HRMALDITOFMS analysis (*m/z* 675.4089 $[M + Na]^+$, calcd for $C_{36}H_{60}O_{10}Na$, 675.4084, and *m/z* 645.3979 $[M + Na]^+$, calcd for $C_{35}H_{58}O_9Na$, 645.3983), respectively. The comparison of the NMR data of compounds **7** and **8** showed that the two compounds differed only for the sugar unit attached at C-6. The combination of 1D-TOCSY, HSQC and DQF-COSY data led to determine the sugar units as β-glucopyranosyl (δ 4.36, d, *J* = 7.5 Hz) for **7** and β-xylopyranosyl (δ 4.33, d, *J* = 7.5 Hz) for **8**.

Therefore, the structures of compounds **7** and **8** were established as 6-*O*-β-D-glucopyranosyl-3β,6α,16β,24α-tetrahydroxy-20(*R*),25-epoxycycloartane and 6-*O*-β-D-xylopyranosyl-3β,6α,16β,24α-tetrahydroxy-20(*R*),25-epoxycycloartane, respectively.

Compounds **1**–**5** were based on cyclocanthogenin, one of the most common aglycons in *Astragalus* genus, whereas compounds **6**–**8** were cyclocephalogenin glycosides, more unusual in the plant kingdom, so far reported only from *Astragalus* spp. (Bedir et al., 1998a; Sukhina et al., 2007; Agzamova and Isaev, 1999; Semmar et al., 2001). Particularly, monoglycosides of cyclocanthogenin (**5**) and cyclocephalogenin (**7**, **8**) are reported for the first time.

Additionally, ten known cycloartane-type glycosides, 3-*O*-[α-L-rhamnopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→2)-*O*-β-D-xylopyranosyl]-6-*O*-β-D-glucopyranosyl-3β,6α,16β,24(*S*),25-pentahydroxycycloartane (**9**) (Horo et al., 2010), oleifolioside B (**10**) (Özipek et al., 2005), cyclocanthoside G (**11**) (Isaev et al., 1992), cyclocanthoside E (**12**) (Isaev et al., 1992), 3-*O*-[α-L-rhamnopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→2)-*O*-β-D-xylopyranosyl]-6-*O*-β-D-glucopyranosyl-3β,6α,16β,24α-tetrahydroxy-20(*R*),25-epoxycycloartane (**13**) (Horo et al., 2010), 3-*O*-[α-L-arabinopyranosyl-(1→2)-*O*-β-D-xylopyranosyl]-6-*O*-β-D-glucopyranosyl-3β,6α,16β,24α-tetrahydroxy-20(*R*),25-epoxycycloartane (**14**) (Horo et al., 2010), cyclocanthoside F (**15**), (Agzamova and Isaev, 1999), cyclocephaloside I (**16**) (Bedir et al., 1998a), cyclotrisectoside (**17**) (Sukhina et al., 2007) and macrophyllosaponin B (**18**) (Çalış et al., 1996) were isolated.

The antiproliferative activity of cycloartane glycosides has been reported against several cancer lines including solid tumor (HepG2), blood tumor (HL-60) and drug resistant tumor (R-HepG2) (Kikuchi et al., 2007). In particular 16β,23:23,26:24,25-triepoxy-, 16β,23:16α,24-diepoxy- and 16β,23;22β,25-diepoxycycloartane derivatives have been reported for their cytotoxic activities (Nian et al., 2010; Watanabe et al., 2002). Moreover, cancer chemopreventive effects of natural and semisynthetic cycloartane-type and related triterpenoids have been reported (Tian et al., 2005). Leishmanicidal (Özipek et al., 2005) and immunomodulatory (Çalis et al., 1997) activities have been reported for cyclocanthogenin and cycloastragenol derivatives.

On the basis of the biological activities reported for cycloartane glycosides, the antiproliferative activity of compounds **1**–**18** was tested in several cancer cell lines including HL-60 (human promy-

elocytic leukemia), MCF-7 (human breast cancer), HT-29 (human colon carcinoma), A549 (human lung adenocarcinoma), PC3 (human prostate cancer). Compounds **1**–**18** were tested in a range of concentrations between 1 and 50 μM, but only few compounds showed a weak activity. Compound **17** exhibited an IC_{50} value of 30 μM against MCF7 cells, while compound **8** exhibited an IC_{50} value of 45 μM against HL-60 cells. Compounds **12** and **14** showed an IC_{50} value of 50 μM against A549 and PC3 cells. All the other compounds showed no activity (data not shown). Etoposide used as positive control showed IC_{50} values ranging from 0.5 μM (HL-60 cells) to 16 μM (A549 cells).

3. Experimental

3.1. General

Optical rotations were measured on a JASCO DIP 1000 polarimeter. IR measurements were obtained on a Bruker IFS-48 spectrometer. NMR experiments were performed on a Bruker DRX-600 spectrometer (Bruker BioSpinGmBH, Rheinstetten, Germany) equipped with a Bruker 5 mm TCI CryoProbeat 300 K. All 2D-NMR spectra were acquired in CD_3OD (99.95%, SigmaAldrich) and standard pulse sequences and phase cycling were used for DQF-COSY, HSQC, and HMBC spectra. The NMR data were processed using UXNMR software. Exact masses were measured by a Voyager DE mass spectrometer. Samples were analyzed by matrix-assisted laser desorption ionization time-of-flight (MALDITOF) mass spectrometry. A mixture of analyte solution and a-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 iontrap mass spectrometer equipped with Xcalibur software. GC analysis was performed on a Termo Finnigan Trace GC apparatus using a l-Chirasil-Val column (0.32 mm × 25 m).

3.2. Plant material

A. aureus Willd (Whole plant; Section: Adiaspastus) was collected from Karlıova, Bingöl, 40 km from Çat to Karlıova, from 2180 m altitude, East Anatolia, Turkey in July 2009, and identified by Fevzi Özgökçe (Department of Biology, Faculty of Science and Art, Yüzüncü Yıl University, Van, Turkey). Voucher specimen has been deposited in the Herbarium of Yüzüncü Yıl University, Van, Turkey (VANF 13646).

3.3. Extraction and isolation

Air-dried and powdered plant material of *A. aureus* (Whole plant, 700 g) was extracted with MeOH (2 × 3.5 l) at 60 °C. After filtration, the solvent was removed by rotary evaporation yielding 72 g of extract. The MeOH extract was dissolved in H_2O (350 ml), and successively partitioned with hexane (2 × 200 ml), EtOAc (2 × 200 ml) and *n*-BuOH saturated with H_2O (3 × 200 ml). The *n*-BuOH extract (27 g) was subjected to vacuum liquid chromatography (VLC) using reversed- phase material (Lichroprep RP-18, 25–40 μm, 150 g) employing H_2O (1000 ml), H_2O–MeOH (8:2, 1200 ml; 6:4, 2400 ml; 4:6, 3200 ml; 2:8, 1600 ml) and MeOH (800 ml) to give seventeen main fractions (1–17). Fraction 7 (3.2 g) was submitted to silica gel (330 g) column chromatography with the solvent system $CHCl_3$–MeOH–H_2O (90:10:1, 2000 ml; 80:20:2, 2500 ml; 70:30:3, 1500 ml) yielding **7** (93.3 mg), **18** (32.3 mg), **2** (25.6 mg), **16** (127.5 mg) and 25 subfractions. Subfraction 5 (150 mg) was subjected to silica gel (30 g) column chromatography. Elution was carried out with $CHCl_3$–MeOH–H_2O (90:10:1, 500 ml) to give **8** (10.4 mg). Subfraction 8 (70 mg) was fractionated over an open column chromatography using silica gel (30 g). Elution was performed with $CHCl_3$–MeOH–H_2O mixtures (90:10:1, 500 ml) to afford **6** (8.4 mg). Subfraction 9 (100 mg) was submitted to silica gel (30 g) column chromatography with the solvent system $CHCl_3$–MeOH–H_2O (90:10:1, 750 ml) to give **5** (5 mg). Subfraction 19 (100 mg) was subjected to silica gel (30 g) column chromatography with the solvent system $CHCl_3$–MeOH–H_2O (90:10:1, 750 ml) to yield **14** (27 mg). Subfraction 17 (100 mg) was applied to a reversed phase column (Lichroprep RP-18, 25–40 μm, 30 g) using MeOH–H_2O (6:4, 500 ml) to give **12** (7.3 mg). Subfraction 21 (118 mg) was chromatographed on a reversed phase material (Lichroprep RP-18, 25–40 μm, 30 g), employing MeOH–H_2O (6:4, 500 ml) yielding **15** (49 mg) and **10** (8 mg). Subfraction 22 (140 mg) was purified on a reversed phase column (Lichroprep RP-18, 25–40 μm, 30 g) and eluted with MeOH–H_2O (6:4, 500 ml) to give **17** (9.6 mg) and **3** (24 mg). Subfraction 23 (128 mg) was subjected to a reversed phase column (Lichroprep RP-18, 25–40 μm, 30 g) employing MeOH–H_2O (6:4, 750 ml) to afford **1** (28 mg). Subfraction 24 (165 mg) was applied to a reversed phase column (Lichroprep RP-18, 25–40 μm, 30 g) using MeOH–H_2O (6:4, 750 ml) to give **13** (17.8 mg) and **11** (10 mg). Subfraction 25 (116 mg) was chromatographed on a reversed phase material (Lichroprep RP-18, 25–40 μm, 30 g), employing MeOH–H_2O (6:4, 600 ml) to afford **4** (16 mg) and **9** (16 mg).

3.4. 3-O-[α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 2)-β-D-xylopyranosyl]-6-O-β-D-xylopyranosyl-3β,6α,16β,24(S),25-pentahydroxycycloartane (*1*)

Amorphous white solid; $C_{51}H_{86}O_{21}$; $[\alpha]_D^{25}$ +37.2° (*c* 0.1 MeOH); IR ν_{max}^{KBr} cm^{-1}: 3474 (>OH), 3035 (cyclopropane ring), 2953 (>CH), 1750 (C=O), 1255 and 1068 (C–O–C); ^{13}C NMR (CD_3OD, 150 MHz) aglycon moiety: δ 32.7 (C-1), 30.2 (C-2), 89.6 (C-3), 42.5 (C-4), 52.9 (C-5), 79.0 (C-6), 34.3 (C-7), 45.6 (C-8), 21.9 (C-9), 29.1 (C-10), 26.8 (C-11), 33.8 (C-12), 46.6 (C-13), 47.0 (C-14), 47.7 (C-15), 73.0 (C-16), 57.6 (C-17), 18.0 (C-18), 27.7 (C-19), 29.8 (C-20), 18.5 (C-21), 34.1 (C-22), 28.3 (C-23), 78.1 (C-24), 73.5 (C-25), 25.2 (C-26), 25.2 (C-27), 28.0 (C-28), 16.6 (C-29), 19.9 (C-30); ^1H NMR (CD_3OD, 600 MHz) aglycon moiety: δ 4.48 (1H, ddd, *J* = 8.0, 8.0, 5.2 Hz, H-16), 3.54 (1H, ddd, *J* = 9.5, 9.5, 4.5 Hz, H-6), 3.41 (1H, dd, *J* = 10.5, 2.4 Hz, H-24), 3.23 (1H, dd, *J* = 11.3, 4.0 Hz, H-3), 1.74 (1H, dd, *J* = 9.9, 8.0 Hz, H-17), 1.28 (3H, s, Me-28), 1.20 (3H, s, Me-27), 1.18 (3H, s, Me-26), 1.17 (3H, s, Me-18), 1.03 (3H, s, Me-29), 1.01 (3H, s, Me-30), 0.98 (3H, d, J = 6.5 Hz, Me-21), 0.59 and 0.25 (each 1H, d, *J* = 4.2 Hz, H_2-19); for the ^1H (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the sugar portion see Table 1; ESI–MS *m/z* 1057.65 $[M + Na]^+$; MS/MS *m/z* 907.53 $[M + Na\text{-}150]^+$, MS^3 *m/z* 761.46 $[M + Na\text{-}150\text{–}146]^+$, 629.40 $[M + Na\text{-}150\text{–}146\text{–}132]^+$; HRMALDITOFMS $[M + Na]^+$ *m/z* 1057.5563 (calcd for $C_{51}H_{86}O_{21}Na$, 1057.5559).

3.5. 3,6-Di-O-β-D-xylopyranosyl-3β,6α,16β,24(S),25-pentahydroxycycloartane (*2*)

Amorphous white solid; $C_{40}H_{68}O_{13}$; $[\alpha]_D^{25}$ +29.2° (*c* 0.1 MeOH); IR ν_{max}^{KBr} cm^{-1}: 3487 (>OH), 3043 (cyclopropane ring), 2950 (>CH), 1269 and 1059 (C–O–C); ^1H (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the aglycon moiety were superimposable with those reported for **1**; for the ^1H (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the sugar portion see Table 1; ESI–MS *m/z* 779.54 $[M + Na]^+$, MS/MS *m/z* 629.38 [M + Na-

150]$^+$, 479.38 [M + Na-150–150]$^+$; HRMALDITOFMS [M + Na]$^+$ *m/z* 779.4561 (calcd for $C_{40}H_{68}O_{13}Na$, 779.4558).

3.6. 3,6-Di-O-β-D-xylopyranosyl-25-O-β-D-glucopyranosyl-3β,6α,16β,24(S),25-pentahydroxycycloartane (**3**)

Amorphous white solid; $C_{46}H_{78}O_{18}$; $[\alpha]_D^{25}$ +35.6° (*c* 0.1 MeOH); IR ν_{max}^{KBr} cm^{-1}: 3482 (>OH), 3040 (cyclopropane ring), 2945 (>CH), 1259 and 1051 (C–O–C); ^{13}C NMR (CD_3OD, 150 MHz) aglycon moiety: δ 32.6 (C-1), 30.1 (C-2), 89.6 (C-3), 42.7 (C-4), 52.7 (C-5), 79.0 (C-6), 34.0 (C-7), 45.7 (C-8), 22.0 (C-9), 29.0 (C-10), 26.6 (C-11), 33.7 (C-12), 46.3 (C-13), 47.2 (C-14), 47.4 (C-15), 72.9 (C-16), 57.6 (C-17), 17.9 (C-18), 27.4 (C-19), 30.0 (C-20), 18.3 (C-21), 34.0 (C-22), 28.4 (C-23), 76.5 (C-24), 81.2 (C-25), 22.4 (C-26), 23.0 (C-27), 28.0 (C-28), 16.3 (C-29), 19.6 (C-30); ^{1}H NMR (CD_3OD, 600 MHz) aglycon moiety: δ 4.48 (1H, ddd, *J* = 8.0, 8.0, 5.2 Hz, H-16), 3.54 (1H, ddd, *J* = 9.5, 9.5, 4.5 Hz, H-6), 3.61 (1H, dd, *J* = 10.5, 2.4 Hz, H-24), 3.23 (1H, dd, *J* = 11.3, 4.0 Hz, H-3), 1.73 (1H, dd, *J* = 9.9, 8.0 Hz, H-17), 1.27 (3H, s, Me-28), 1.29 (3H, s, Me-27), 1.25 (3H, s, Me-26), 1.17 (3H, s, Me-18), 1.03 (3H, s, Me-29), 1.01 (3H, s, Me-30), 0.97 (3H, d, J = 6.5 Hz, Me-21), 0.59 and 0.24 (each 1H, d, *J* = 4.2 Hz, H_2-19); for the ^{1}H (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the sugar portion see Table 1; ESI–MS *m/z* 941.59 [M + Na]$^+$, MS/MS *m/z* 791.43 [M + Na-150]$^+$, MS^3 *m/z* 611.37 [M + Na-150–180]$^+$, MS^4 *m/z* 479.31 [M + Na-150–180–132]$^+$; HRMALDITOFMS [M + Na]$^+$ *m/z* 941.5090 (calcd for $C_{46}H_{78}O_{18}Na$, 941.5086).

3.7. 3-O-β-D-xylopyranosyl-6,25-di-O-β-D-glucopyranosyl-3β,6α,16β,24(S),25-pentahydroxycycloartane (**4**)

Amorphous white solid; $C_{47}H_{80}O_{19}$; $[\alpha]_D^{25}$ +32.5° (*c* 0.1 MeOH); IR ν_{max}^{KBr} cm^{-1}: 3477 (>OH), 3038 (cyclopropane ring), 2934 (>CH), 1263 and 1054 (C–O–C); ^{1}H (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the aglycon moiety were superimposable with those reported for **3**; for the ^{1}H (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the sugar portion see Table 1; ESI–MS *m/z* 971.56 [M + Na]$^+$, MS/MS *m/z* 791.46 [M + Na-180]$^+$, MS^3 *m/z* 611.40 [M + Na-180–180]$^+$, MS^4 *m/z* 479.32 [M + Na-180–180–132]$^+$; HRMALDITOFMS [M + Na]$^+$ *m/z* 971.5197 (calcd for $C_{47}H_{80}O_{19}Na$, 971.5192).

3.8. 6-O-β-D-glucopyranosyl-3β,6α,16β,24(S),25-pentahydroxycycloartane (**5**)

Amorphous white solid; $C_{36}H_{62}O_{10}$; $[\alpha]_D^{25}$ + 27.8° (*c* 0.1 MeOH); IR ν_{max}^{KBr} cm^{-1}: 3485 (>OH), 3047 (cyclopropane ring), 2930 (>CH), 1254 and 1061 (C–O–C); ^{13}C NMR (CD_3OD, 150 MHz) aglycon moiety: δ 32.7 (C-1), 30.7 (C-2), 79.3 (C-3), 42.6 (C-4), 52.9 (C-5), 80.1 (C-6), 34.8 (C-7), 46.6 (C-8), 22.3 (C-9), 29.7 (C-10), 26.6 (C-11), 33.8 (C-12), 46.6 (C-13), 47.9 (C-14), 47.9 (C-15), 73.0 (C-16), 57.7 (C-17), 18.5 (C-18), 29.0 (C-19), 29.6 (C-20), 18.3 (C-21), 33.7 (C-22), 28.2 (C-23), 78.2 (C-24), 73.8 (C-25), 25.2 (C-26), 25.5 (C-27), 28.4 (C-28), 15.7 (C-29), 19.9 (C-30); ^{1}H NMR (CD_3OD, 600 MHz) aglycon moiety: δ 4.47 (1H, ddd, *J* = 8.0, 8.0, 5.2 Hz, H-16), 3.58 (1H, ddd, *J* = 9.5, 9.5, 4.5 Hz, H-6), 3.41 (1H, dd, *J* = 10.5, 2.4 Hz, H-24), 3.25 (1H, dd, *J* = 11.3, 4.0 Hz, H-3), 1.74 (1H, dd, *J* = 9.9, 8.0 Hz, H-17), 1.25 (3H, s, Me-28), 1.19 (6H, s, Me-26, Me-27), 1.18 (3H, s, Me-18), 0.98 (3H, s, Me-29), 1.01 (3H, s, Me-30), 0.98 (3H, d, J = 6.5 Hz, Me-21), 0.61 and 0.30 (each 1H, d, *J* = 4.2 Hz, H_2-19); for the ^{1}H (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the sugar portion see Table 1; ESI–MS *m/z* 677.56 [M + Na]$^+$, MS/MS *m/z* 497.37 [M + Na-180]$^+$; HRMALDITOFMS [M + Na]$^+$ *m/z* 677.4246 (calcd for $C_{36}H_{62}O_{10}Na$, 677.4241).

3.9. 3-O-[α-L-arabinopyranosyl-(1→2)-β-D-xylopyranosyl]-3β,6α,16β,24α-tetrahydroxy-20(R),25-epoxycycloartane (**6**)

Amorphous white solid; $C_{40}H_{66}O_{13}$; $[\alpha]_D^{25}$ +10.5° (*c* 0.1 MeOH); IR ν_{max}^{KBr} cm^{-1}: 3488 (>OH), 3053 (cyclopropane ring), 2939 (>CH), 1274 and 1050 (C–O–C); ^{13}C NMR (CD_3OD, 150 MHz) aglycon moiety: δ 33.2 (C-1), 30.2 (C-2), 89.3 (C-3), 43.1 (C-4), 54.3 (C-5), 69.3 (C-6), 38.7 (C-7), 48.4 (C-8), 21.7 (C-9), 30.4 (C-10), 26.8 (C-11), 34.3 (C-12), 46.4 (C-13), 47.5 (C-14), 48.2 (C-15), 74.8 (C-16), 61.2 (C-17), 21.0 (C-18), 31.8 (C-19), 80.3 (C-20), 28.0 (C-21), 26.6 (C-22), 23.7 (C-23), 69.3 (C-24), 76.5 (C-25), 28.2 (C-26, C-27, C-28), 16.2 (C-29), 20.4 (C-30); ^{1}H NMR (CD_3OD, 600 MHz) aglycon moiety: δ 4.64 (1H, ddd, *J* = 8.0, 8.0, 5.2 Hz, H-16), 3.47 (1H, ddd, *J* = 9.5, 9.5, 4.5 Hz, H-6), 3.51 (1H, br s, H-24), 3.22 (1H, dd, *J* = 11.3, 4.0 Hz, H-3), 2.01 (1H, dd, *J* = 9.9, 8.0 Hz, H-17), 1.54 (3H, s, Me-21), 1.49 (3H, s, Me-18), 1.32 (6H, s, Me-28, Me-27), 1.22 (3H, s, Me-26), 1.05 (3H, s, Me-29), 0.97 (3H, s, Me-30), 0.57 and 0.43 (each 1H, d, *J* = 4.2 Hz, H_2-19); for the ^{1}H (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the sugar portion see Table 2; ESI–MS *m/z* 777.74 [M + Na]$^+$, MS/MS *m/z* 645.36 [M + Na-132]$^+$, 495.41 [M + Na-132–150]$^+$; HRMALDITOFMS [M + Na]$^+$ *m/z* 777.4405 (calcd for $C_{40}H_{66}O_{13}Na$, 777.4401).

3.10. 6-O-β-D-glucopyranosyl-3β,6α,16β,24α-tetrahydroxy-20(R),25-epoxycycloartane (**7**)

Amorphous white solid; $C_{36}H_{60}O_{10}$; $[\alpha]_D^{25}$ +15.2° (*c* 0.1 MeOH); IR ν_{max}^{KBr} cm^{-1}: 3479 (>OH), 3048 (cyclopropane ring), 2947 (>CH), 1257 and 1065 (C–O–C); ^{13}C NMR (CD_3OD, 150 MHz) aglycon moiety: δ 33.2 (C-1), 31.0 (C-2), 79.1 (C-3), 42.2 (C-4), 52.7 (C-5), 80.2 (C-6), 35.1 (C-7), 46.8 (C-8), 21.8 (C-9), 29.9 (C-10), 26.3 (C-11), 34.3 (C-12), 46.5 (C-13), 47.7 (C-14), 47.7 (C-15), 74.9 (C-16), 60.8 (C-17), 21.0 (C-18), 30.1 (C-19), 80.2 (C-20), 28.3 (C-21), 26.5 (C-22), 23.7 (C-23), 69.4 (C-24), 76.1 (C-25), 28.2 (C-26), 28.3 (C-27, C-28), 15.4 (C-29), 19.9 (C-30); ^{1}H NMR (CD_3OD, 600 MHz) aglycon moiety: δ 4.64 (1H, ddd, *J* = 8.0, 8.0, 5.2 Hz, H-16), 3.58 (1H, ddd, *J* = 9.5, 9.5, 4.5 Hz, H-6), 3.51 (1H, br s, H-24), 3.24 (1H, dd, *J* = 11.3, 4.0 Hz, H-3), 2.02 (1H, dd, *J* = 9.9, 8.0 Hz, H-17), 1.54 (3H, s, Me-21), 1.47 (3H, s, Me-18), 1.32 (3H, s, Me-27), 1.27 (3H, s, Me-28), 1.22 (3H, s, Me-26), 0.98 (3H, s, Me-29), 0.99 (3H, s, Me-30), 0.62 and 0.35 (each 1H, d, *J* = 4.2 Hz, H_2-19); for the ^{1}H (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the sugar portion see Table 1; ESI–MS *m/z* 675.47 [M + Na]$^+$, MS/MS *m/z* 495.29 [M + Na-180]$^+$; HRMALDITOFMS [M + Na]$^+$ *m/z* 675.4089 (calcd for $C_{36}H_{60}O_{10}Na$, 675.4084).

Table 2
^{1}H NMR data (*J* in Hz) of the sugar portions of compounds **6** and **8** (600 MHz, δ ppm, in CD_3OD).

	6		**8**	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
	β-D-Xyl (at C-3)		β-D-Xyl (at C-6)	
1	105.2	4.48, d (7.5)	104.9	4.33, d (7.5)
2	82.9	3.46, dd (9.2, 7.5)	74.9	3.20, dd (9.2, 7.5)
3	76.3	3.55, t (9.2)	77.7	3.32, t (9.2)
4	70.5	3.54, m	70.9	3.49, m
5	65.7	3.88, dd (11.7, 5.2) 3.22, t (11.7)	66.2	3.86, dd (11.7, 5.2) 3.20, t (11.7)
	α-L-Ara (at C-2$_{xyl}$)			
1	105.9	4.49, d (3.7)		
2	73.0	3.68, dd (8.5, 3.7)		
3	73.7	3.59, dd (8.5, 3.0)		
4	69.1	3.89, m		
5	66.8	3.91, dd (11.9, 2.0) 3.55, dd (11.9, 3.0)		

3.11. 6-O-β-D-xylopyranosyl-3β,6α,16β,24α-tetrahydroxy-20(R),25-epoxycycloartane (**8**)

Amorphous white solid; $C_{35}H_{58}O_9$; $[\alpha]_D^{25}$ +8.2° (*c* 0.1 MeOH); IR ν_{max}^{KBr} cm^{-1}: 3471 (>OH), 3050 (cyclopropane ring), 2943 (>CH), 1268 and 1057 (C–O–C); ^1H (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the aglycon moiety were superimposable with those reported for **7**; for the ^1H (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the sugar portion see Table 2; ESI–MS *m/z* 645.50 $[M + Na]^+$, MS/MS *m/z* 495.30 $[M + Na-150]^+$; HRMALDITOFMS $[M + Na]^+$ *m/z* 645.3983 (calcd for $C_{35}H_{58}O_9Na$, 645.3979).

3.12. Acid hydrolysis

The configuration of sugar units were established after hydrolysis of **1** and **3** with 1 N HCl, trimethylsilation, and determination of the retention times by GC operating in the experimental conditions previously reported by De Marino et al., 2003.

The peaks of the hydrolysate of **1** were detected at 8.94 and 9.81 min (L-arabinose), 9.70 and 10.72 (L-rhamnose) and 10.99 and 12.02 (D-xylose). The peaks of D-xylose (10.98 and 12.01 min) and D-glucose (14.73 min) were detected in the hydrolysate of **3**. Retention times for authentic samples after being treated in the same manner with 1-(trimethylsilyl)-imidazole in pyridine were detected at 8.92 and 9.80 (L-arabinose), 9.67 and 10.70 (L-rhamnose), 10.98 and 12.00 min (D-xylose), and 14.71 min (D-glucose).

3.13. Antiproliferative activity

HL-60 (human promyelocytic leukemia), MCF-7 (human breast cancer), HT-29 (human colon carcinoma), A549 (human lung adenocarcinoma), PC3 (human prostate cancer) cells obtained from the European Collection of Cell Cultures were cultured in DMEM medium supplemented with 2 mM L-glutamine, 10% heat-inactivated fetal bovine serum (FBS), 1% penicillin/streptomycin at 37 °C in an atmosphere of 95% O_2 and 5% CO_2. The cells were used up to a maximum of 10 passages.

Human cancer cells (3×10^3) were plated in 96-well culture plates in 90 μL of culture medium and incubated at 37 °C in humidified 5% CO_2. The next day, 10 μL aliquots of serial dilutions of each test compound (1–50 μM) and etoposide, used as positive control, were added to the cells and incubated for 48 h. Cell viability was assessed through the MTT assay. Briefly, 25 μL of MTT (5 mg/mL) were added and the cells were incubated for an additional 3 h. Thereafter, cells were lysed and the dark blue crystals solubilized with 100 μL of a solution containing 50% *N*,*N*-dimethylformamide, 20% SDS (Sodium Dodecyl Sulfate) with an adjusted pH of 4.5. The optical density (OD) of each well was measured with a microplate spectrophotometer (Titertek Multiskan MCC/340) equipped with a 620 nm filter. Cells viability in response to treatment was calculated as percentage of control cells treated with solvent DMSO at the final concentration 0.1%:% viable cells = (100 × OD treated cells)/OD control cells.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2011.02.006.

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