Triterpene Glycosides from *Astragalus angustifolius*

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Key words
- *Astragalus angustifolius*
- Leguminosae
- cycloartane saponins
- oleanane saponins

Abstract

Six new cycloartane-type (**1**–**6**) and four new oleanane-type (**7**–**10**) triterpene glycosides were isolated from *Astragalus angustifolius* Lam., together with five known triterpene glycosides. Their structures were established by the extensive use of 1D and 2D-NMR experiments along with ESIMS and HRMS analysis. Compounds **1**–**3** are glycosides of cycloastragenol, while compounds **4**–**6** show the C-24 epimer of cycloastragenol as aglycone, encountered for the first time in nature. All compounds were evaluated for their antiproliferative activity in Hela, H-446, HT-29, and U937 cell lines. Only compound **8** displayed a weak activity with IC_{50} values of 36 and 50 µM against Hela and HT-29 cell lines, respectively.

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Introduction

Astragalus L. (Leguminosae) is the genus comprising the highest number of species among the spermatophytes. The exact number of species in this genus has not been established yet, but it is estimated to be around 3000 [1]. In Turkey it is represented by 445 species, of which 224 are endemic [2]. Earlier investigations on Turkish *Astragalus* species resulted in the isolation of a series of oleanane- and cycloartane-type saponins [3–11]. Previous studies have shown interesting biological activities, including immunostimulating [7, 12], anti-protozoal [13], antiviral [14], cytotoxic [15], cardiotonic [16], wound healing [17], and adjuvant properties [18] for cycloartane- and oleanane-type glycosides isolated from *Astragalus* species. In particular, cycloartane glycosides showed antiproliferative activity against several cancer lines including solid tumor (HepG2), blood tumor (HL-60), and drug resistant tumor (R-HepG2) [19].

Continuing our studies on the constituents of *Astragalus* species, the investigation of the whole parts of *Astragalus angustifolius* Lam., used as an anti-inflammatory remedy in Turkish folk medicine [2], was carried out. Here we report the isolation and structure elucidation of six new cycloartane-type triterpene glycosides (**1**–**6**; **Fig. 1**) and four new oleanane-type triterpene glycosides (**7**–**10**; **Fig. 2**) from the methanol extract of *A. angustifolius*, along with three known cycloartane glycosides (**11**–**13**) and two known oleanane glycosides (**14, 15**).

The antiproliferative activity of compounds **1**–**15** was tested in different cancer cell lines including Hela, H-446, HT-29, and U937. Only compound **8** displayed a weak activity with an IC_{50} of 36 and 50 µM against Hela and HT-29 cell lines, respectively.

Materials and Methods

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) 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-

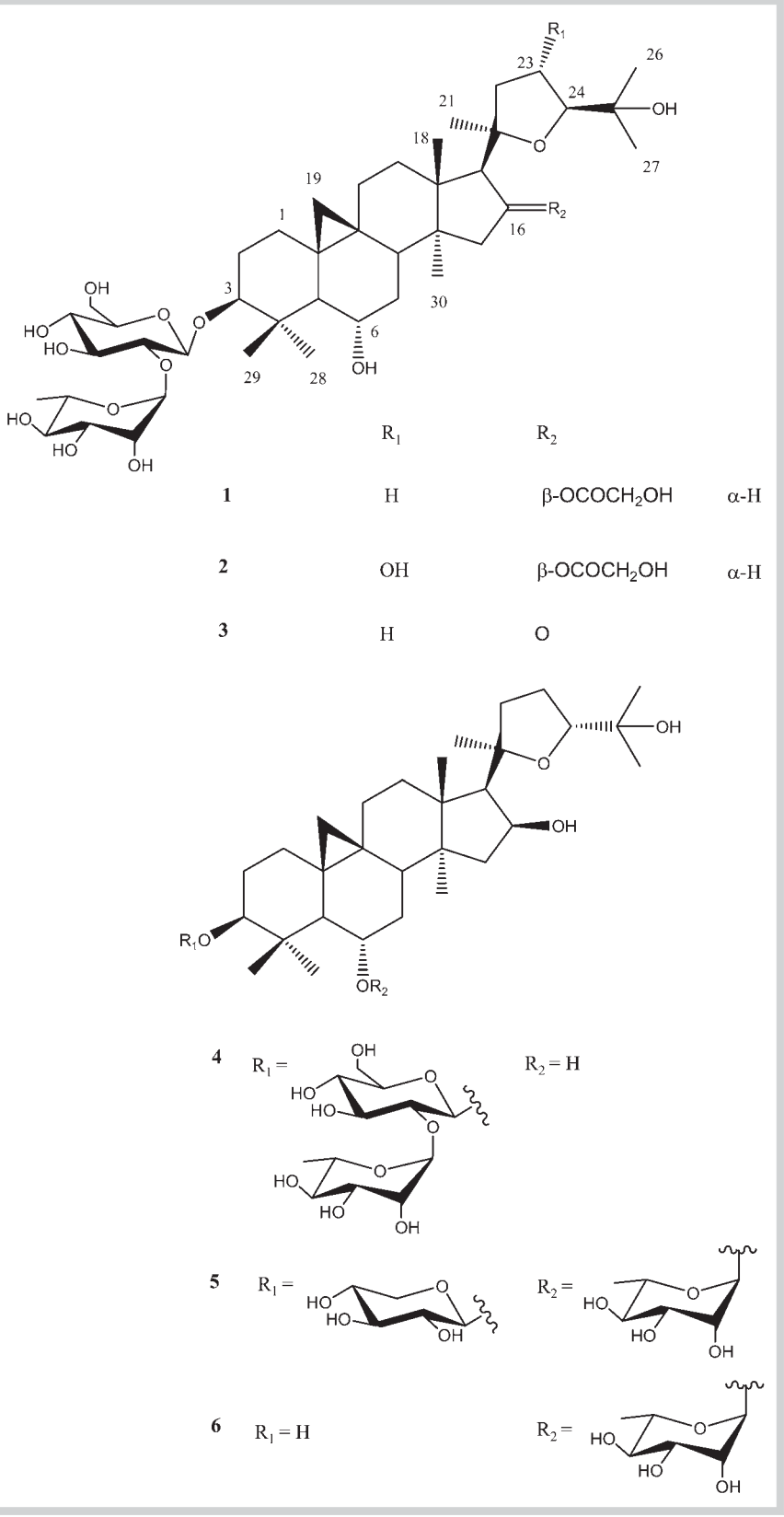
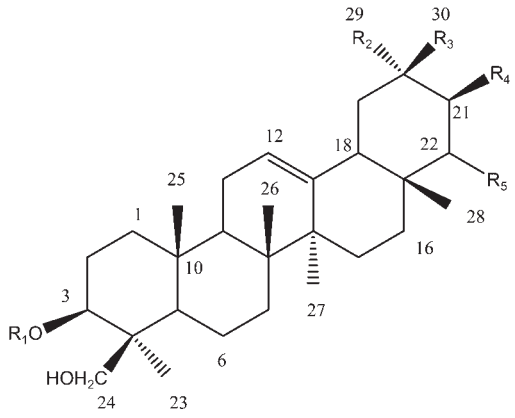


Fig. 1 Chemical structures of compounds **1–6**.

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 α-cyano-4-hydroxycinnamic acid at 190.0504 Da as the 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



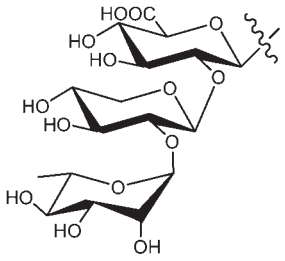
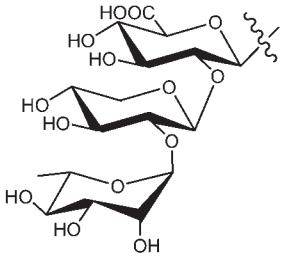
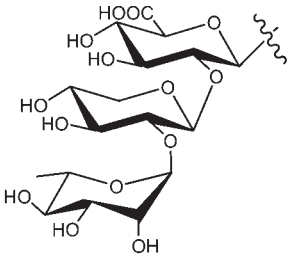
	R_1	R_2	R_3	R_4	R_5
7		CH_2OH	CH_3	OH	α-OH
8		CH_3	COOH	H	β-OH
9		CH_3	CH_3	H	β-O-β-D-Glc
10	H	CH_2O-β-D-Glc	CH_3	H	β-OH

Fig. 2 Chemical structures of compounds **7**–**10**.

Finnigan Trace GC apparatus using a l-Chirasil-Val column (0.32 mm × 25 m).

Plant material

Astragalus angustifolius Lam. (whole plant) was collected at Çaltili Village, Çingi hill, from an altitude of 1200 m, Hafik-Sivas, Turkey in July 2009. Samples of plant material were identified by Serdar G. Senol (Deparment of Biology, Faculty of Sciences, Ege University, Izmir, Turkey). A voucher specimen has been deposited in the Herbarium of Ege University, Izmir, Turkey (EGE 40790).

Extraction and isolation

Air-dried and powdered plant material of *Astragalus angustifolius* (whole plant, 367 g) was extracted with MeOH (3.5 L × 2) at 60 °C. The methanolic solution was then evaporated to dryness under reduced pressure and gave 28 g of a dark residue. This residue

Table 1 ^{13}C and ^{1}H NMR data (*J* in Hz) of the sugar portions of compounds **1, 5**, and **6** (600 Mz, δ ppm, in CD_3OD).

	1[a]		**5**		**6**	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
	β-D-Glc (at C-3)		β-D-Xyl (at C-3)		α-L-Rha (at C-6)	
1	105.7	4.44, d (7.5)	107.8	4.28, d (7.5)	104.2	4.80, d (1.2)
2	79.7	3.45, dd (7.5, 9.0)	75.5	3.21, dd (7.5, 9.2)	72.6	3.86, dd (1.2, 3.2)
3	79.7	3.48, t (9.0)	77.7	3.31, t (9.2)	70.1	3.71, dd (3.2, 9.7)
4	72.2	3.31, t (9.0)	71.4	3.49, m	73.8	3.42, t (9.7)
5	77.7	3.26, ddd (3.5, 4.5, 9.0)	66.7	3.84, dd (5.2, 11.7) 3.19, t (11.7)	72.3	3.61, m
6	62.6	3.88, dd (3.5, 12) 3.69, dd (4.5, 12)			17.5	1.26, d (6.5)
	α-L-Rha (at C-2_{glc})		α-L-Rha (at C-6)			
1	102.0	5.42, d (1.2)	104.2	4.78, d (1.2)		
2	72.0	3.99, dd (1.2, 3.2)	72.5	3.86, dd (1.2, 3.2)		
3	72.0	3.79, dd (3.2, 9.7)	70.0	3.71, dd (3.2, 9.7)		
4	74.2	3.42, t (9.7)	73.7	3.41, t (9.7)		
5	70.0	4.01, m	72.3	3.61, m		
6	17.6	1.23, d (6.5)	17.6	1.27, d (6.5)		

[a] The chemical shift values of the sugar portion of **2–4** were superimposable with those reported for **1**

was suspended in H_2O (350 mL), and successively partitioned with *n*-hexane (200 mL × 2), EtOAc (200 mL × 2), and *n*-BuOH saturated with H_2O (200 mL × 3). The *n*-BuOH extract (7.7 g) was subjected to vacuum liquid chromatography (VLC) using reversed-phase material (Lichroprep RP-18, 25–40 µm, 150 g, 50 cm × 5 cm) employing H_2O (1000 mL), H_2O–MeOH (8 : 2, 1200 mL; 6 : 4, 2400 mL; 4 : 6, 3000 mL; 2 : 8, 1600 mL) and MeOH (600 mL) to give ten main fractions (A–J). Fraction A (780 mg) was submitted to silica gel (100 g) column chromatography with the solvent system $CHCl_3$-MeOH-H_2O (80 : 20 : 2, 1500 mL; 70 : 30 : 3, 2000 mL, 150 cm × 1.5 cm) to give 7 subfractions. Subfraction 1 (120 mg) was chromatographed on a reversed-phase material (Lichroprep RP-18, 25–40 µm, 25 g, 50 cm × 3 cm), employing MeOH-H_2O (8 : 2, 500 mL) yielding **8** (15 mg) and **13** (9 mg). Subfraction 2 (58 mg) was purified on a reversed-phase column (Lichroprep RP-18, 25–40 µm, 25 g, 50 cm × 3 cm) and eluted with MeOH-H_2O (6 : 4, 500 mL) to give **7** (1.1 mg). Fraction B (300 mg) was subjected to silica gel (42 g) column chromatography with the solvent system EtOAc-MeOH-H_2O (100 : 17.5 : 13.5, 800 mL, 60 cm × 1.5 cm) to yield **14** (10 mg) and **9** (10 mg). Fraction D (240 mg) was submitted to silica gel (42 g, 60 cm × 1.5 cm) column chromatography, eluted with $CHCl_3$-MeOH-H_2O (80 : 20 : 2, 1500 mL; 70 : 30 : 3, 2000 mL) to give **6** (2 mg), **11** (1 mg), **10** (1.2 mg), **5** (23 mg), and **12** (9 mg). Fraction E (60 mg) was subjected to silica gel (38 g) column chromatography with the solvent system $CHCl_3$-MeOH-H_2O (90 : 10 : 1, 600 mL; 80 : 20 : 2, 1500 mL, 100 cm × 1.5 cm) to yield **3** (9 mg) and **1** (7 mg). Fraction D (160 mg) was submitted to silica gel (38 g) column chromatography with the solvent system $CHCl_3$-MeOH-H_2O (90 : 10 : 1, 100 mL; 80 : 20 : 2, 1500 mL; 70 : 30 : 3, 2000 mL, 60 cm × 1.5 cm) to give **4** (4 mg), **2** (12 mg), and **15** (10 mg).

Compound ***1***: Amorphous white solid; $[\alpha]_D^{25}$ + 19.01 (*c* 0.1 MeOH); IR (KBr) ν_{max} 3470, 3040, 2950, 1260, and 1058 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the sugar portion, see **Table 1**; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the aglycone moiety, see **Table 2**; ESI-MS (pos. ione mode) *m/z* 879 $[M+Na]^+$; MS/MS *m/z* 803 $[M+Na-76]^+$, *m/z* 657 $[M+Na-76-146]^+$, 477 $[M+Na-76-146-180]^+$; HRMALDITOFMS *m/z* 879.4722 $[M+Na]^+$ (calcd. for $C_{44}H_{72}O_{16}Na$, 879.4718).

Compound ***2***: Amorphous white solid; $[\alpha]_D^{25}$ + 41.4 (*c* 0.1 MeOH); IR (KBr) ν_{max} 3480, 3035, 2945, 1260, and 1055 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the sugar portion are superimposable with those reported for **1**; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the aglycone moiety, see **Table 2**; ESI MS (pos. ione mode) *m/z* 895 $[M+Na]^+$, MS/MS *m/z* 819 $[M+Na-76]^+$, *m/z* 673 $[M+Na-76-146]^+$, *m/z* 493 $[M+Na-76-146-180]^+$; HRMALDITOFMS *m/z* 895.4669 $[M+Na]^+$ (calcd. for $C_{44}H_{72}O_{17}Na$, 895.4667).

Compound ***3***: Amorphous white solid; $C_{42}H_{68}O_{14}$; $[\alpha]_D^{25}$ − 23.1 (*c* 0.1 MeOH); IR (KBr) ν_{max} 3475, 3030, 2948, 1740, 1258, and 1050 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the sugar portion are superimposable with those reported for **1**; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the aglycone moiety, see **Table 2**; ESI-MS (pos. ione mode) *m/z* 819 $[M+Na]^+$, MS/MS *m/z* 673 $[M+Na-146]^+$, *m/z* 493 $[M+Na-146-180]$; HRMALDITOFMS *m/z* 819.4512 $[M+Na]^+$ (calcd. for $C_{42}H_{68}O_{14}Na$, 819.4507).

Compound ***4***: Amorphous white solid; $[\alpha]_D^{25}$ − 5.21 (*c* 0.1 MeOH); IR (KBr) ν_{max} 3460, 3038, 2935, 1250, and 1050 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the sugar portion are superimposable with those reported for **1**; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the aglycone moiety, see **Table 2**; ESI-MS (pos. ione mode) *m/z* 821 $[M+Na]^+$, MS/MS *m/z* 675 $[M+Na-146]^+$, *m/z* 495 $[M+Na-146-180]^+$; HRMALDITOFMS *m/z* 821.4666 $[M+Na]^+$ (calcd. for $C_{42}H_{70}O_{14}Na$, 821.4663).

Compound ***5***: Amorphous white solid; $[\alpha]_D^{25}$ + 15.1 (*c* 0.1 MeOH); IR (KBr) ν_{max} 3460, 3035, 2940, 1240, and 1050 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the sugar portion, see **Table 1**; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the aglycone moiety, see **Table 2**; ESI-MS (pos. ione mode) *m/z* 791 $[M+Na]^+$, MS/MS *m/z* 645 $[M+Na-146]^+$, *m/z* 495 $[M+Na-146-150]^+$; HRMALDITOFMS *m/z* 791.4561 $[M+Na]^+$ (calcd. for $C_{41}H_{68}O_{13}Na$, 791.4558).

Compound ***6***: Amorphous white solid; $[\alpha]_D^{25}$ − 8.09 (*c* 0.1 MeOH); IR (KBr) ν_{max} 3450, 3042, 2938, 1250, and 1052 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the sugar portion, see **Table 1**; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the aglycone moiety, see **Table**

Table 2 ^{13}C and ^{1}H NMR data (*J* in Hz) of the aglycone moieties of compounds **1–6** (600 Mz, δ ppm, in CD_3OD).

	1		**2**		**3**		**4**		**5**		**6**	
	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)	δ_C	δ_H (*J* in Hz)
1	32.9	1.60, 1.24, m	33.0	1.58, 1.23, m	33.2	1.64, 1.27, m	33.4	1.60, 1.23, m	33.0	1.58, 1.26, m	32.9	1.61, 1.29, m
2	30.5	2.10, 1.69, m	30.3	2.10, 1.69, m	30.3	2.12, 1.71, m	30.5	2.10, 1.69, m	30.2	1.98, 1.70, m	30.6	1.77, 1.64, m
3	89.9	3.27, dd (11.3, 4.0)	90.7	3.58, dd (12.2, 3.5)	89.7	3.29, dd (11.3, 4.0)	89.6	3.27, dd (11.3, 5.6)	89.7	3.23, dd (11.3, 4.0)	89.7	3.24, dd (11.3, 4.0)
4	42.9	–	43.7	–	42.9	–	43.3	–	42.8	–	42.3	–
5	54.6	1.39, d (9.7)	55.0	1.39, d (9.7)	54.7	1.44, d (9.7)	54.8	1.39, d (9.7)	53.4	1.56, d (9.6)	52.9	1.54, d (9.7)
6	69.2	3.48, ddd (9.7, 9.7, 4.5)	69.5	3.47, ddd (9.7, 9.7, 4.5)	69.1	3.51, ddd (9.7, 9.7, 3.2)	69.4	3.48, ddd (9.7, 9.7, 3.2)	81.3	3.45, ddd (9.7, 9.7, 3.2)	81.2	3.46, ddd (9.7, 9.7, 3.2)
7	38.6	1.47, 1.37, m	38.5	1.50, 1.37, m	38.7	1.50, 1.40, m	38.8	1.49, 1.36, m	35.6	1.86, 1.41, m	35.5	1.86, 1.40, m
8	48.4	1.86, dd (11.3, 4.0)	49.0	1.83, dd (13.1, 3.5)	47.3	1.93, dd (12.0, 4.0)	48.4	1.87, dd (12.0, 4.0)	47.5	1.86, dd (12.0, 4.0)	47.6	1.86, dd (12.0, 4.0)
9	21.2	–	22.1	–	21.0	–	21.6	–	21.6	–	21.5	–
10	29.9	–	29.4	–	30.0	–	30.1	–	29.8	–	29.8	–
11	26.8	2.05, 1.24, m	26.7	2.04, 1.24, m	26.5	2.05, 1.24, m	27.2	2.05, 1.24, m	26.9	2.05, 1.24, m	26.5	2.05, 1.24, m
12	33.0	1.79 (2H) m	33.6	1.80, (2H) m	32.9	1.80, 1.70, m	33.7	1.84, 1.72, m	33.9	1.83, 1.72, m	33.9	1.85, 1.73, m
13	46.9	–	47.3	–	46.6	–	47.3	–	47.3	–	47.3	–
14	47.4	–	47.7	–	43.0	–	47.8	–	47.8	–	47.7	–
15	46.9	2.25, dd (13.7, 8) 1.38 dd (12.9, 5.6)	46.8	1.98,dd (12.3,8.0) 1.39, dd (12.3, 5.6)	51.8	2.05, brs	49.2	2.06, dd (13.0, 8.0) 1.54, dd (13.0, 4.0)	49.5	2.05, dd (13.0, 8.0) 1.56, dd (13.0, 5.6)	49.3	2.06, dd (12.9, 8.0) 1.57, dd (12.9, 5.6)
16	77.9	5.49, ddd (8.0, 8.0, 5.6)	78.2	5.49, ddd (8.0, 8.0, 5.3)	222.2	–	74.1	4.62, ddd (7.3, 7.3, 5.6)	74.5	4.61, ddd (7.8, 7.8, 5.2)	74.4	4.61, ddd (7.8, 7.8, 5.2)
17	58.4	2.59, d (8.0)	59.0	2.33, d (8.0)	66.9	2.98, s	57.1	2.27, d (7.3)	57.1	2.28, d (7.8)	56.9	2.29, d (7.2)
18	21.1	1.36, s	21.2	1.35, s	20.4	1.28, s	21.1	1.44, s	21.2	1.44, s	21.2	1.44, s
19	31.8	0.58, d (4.8) 0.39, d (4.8)	32.0	0.59, d (4.8) 0.41, d (4.8)	31.4	0.60, d (4.4) 0.41, d (4.4)	31.9	0.59, d (4.4) 0.41, d (4.4)	31.4	0.60, d (4.4) 0.42, d (4.4)	31.3	0.60, d (4.8) 0.42, d (4.8)
20	86.8	–	86.4	–	86.3	–	87.3	–	88.5	–	88.8	–
21	28.4	1.33, s	28.5	1.48, s	25.0	1.25	28.4	1.36, s	28.8	1.36, s	29.0	1.35, s
22	37.0	2.38, 1.64, m	47.7	3.09, 1.67, m	35.9	2.40, 1.78, m	38.9	2.40, 1.80, m	39.1	2.39, 1.80, m	39.0	2.40, 1.80, m
23	26.8	2.05, (2H) m	74.5	4.47, ddd (8.8, 6.0, 3.5)	27.0	2.05, (2H) m	25.9	1.98, 1.85, m	25.9	1.94, 1.84, m	26.0	1.99, 1.86, m
24	83.3	3.77 dd (8.2, 6.0)	89.3	3.57, d (6.0)	83.2	3.79, dd (10.5, 5.2)	88.5	3.84 dd (10.5, 5.2)	88.5	3.85, dd (10.5, 5.2)	88.4	3.84, dd (10.5, 5.2)
25	72.3	–	72.6	–	72.5	–	72.0	–	71.9	–	71.7	–
26	25.9	1.14, s	25.7	1.19, s	25.5	1.13, s	25.1	1.17, s	25.5	1.17, s	25.4	1.17, s
27	25.9	1.16, s	27.8	1.29, s	27.7	1.24, s	26.1	1.22, s	26.2	1.22, s	26.2	1.22, s
28	28.6	1.33, s	28.0	1.29, s	28.1	1.33, s	28.7	1.33, s	28.7	1.21, s	29.0	1.14, s
29	16.4	1.05, s	16.5	1.05, s	16.6	1.06, s	16.6	1.07, s	16.9	1.05, s	16.3	0.98, s
30	20.7	1.06, s	20.2	1.01, s	19.8	1.21, s	20.3	0.99, s	20.4	1.00, s	20.5	1.00, s
$COCH_2OH$	175.7	–	177.0	–								
$COCH_2OH$	61.7	4.14, 4.07, brs	61.3	4.15, 4.08, brs								

Table 3 ^{13}C and ^{1}H NMR data (*J* in Hz) of the sugar portions of compounds **7, 9**, and **10** (600 Mz, *δ* ppm, in CD_3OD).

	7[a]		9		10	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
	β-D-GlcA (at C-3)		*β*-D-GlcA (at C-3)		*β*-D-Glc (at C-3)	
1	105.6	4.44, d (7.5)	105.6	4.43, d (7.5)	105.2	4.26, d (7.5)
2	78.8	3.58, dd (7.5, 9.0)	78.6	3.58, dd (7.5, 9.0)	75.3	3.22, dd (7.5, 9.0)
3	78.8	3.65, dd (9.0, 9.0)	78.6	3.64, dd (9.0, 9.0)	77.9	3.28, dd (9.0, 9.0)
4	74.1	3.43, dd (9.0, 9.0)	74.2	3.43,dd (9.0, 9.0)	71.6	3.29, dd (9.0, 9.0)
5	77.0	3.57, d (9.0)	76.9	3.57, d (9.0)	78.3	3.38, ddd (3.5, 4.5, 9.0)
6	177.1	–	177.2	–	62.8	3.90, dd (3.5, 12) 3.69, dd (4.5, 12)
	β-D-Xyl (at C-2_{glcA})		*β*-D-Xyl (at C-2_{glcA})			
1	103.3	4.82, d (7.5)	103,2	4.82, d (7.5)		
2	79.3	3.41, dd (7.5, 9.2)	79.4	3.40, dd (7.5, 9.2)		
3	77.4	3.52, dd (9.2, 9.2)	76.5	3.57, dd (9.2, 9.2)		
4	71.0	3.53, m	71.0	3.54, m		
5	66.6	3.83, dd (5.2, 11.7) 3.11, t (11.7)	66.6	3.84, dd (5.2, 11.7) 3.11, t (11.7)		
6						
	α-L-Rha (at C-2_{xyl})		*α*-L-Rha (at C-2_{xyl})			
1	102.3	5.23, d (1.2)	102.3	5.23, d (1.2)		
2	72.3	3.96, dd (1.2, 3.2)	72.0	3.97, dd (1.2, 3.2)		
3	72.2	3.76, dd (3.2, 9.7)	72.1	3.78, dd (3.2, 9.7)		
4	74.1	3.42, t (9.7)	74.2	3.43, t (9.7)		
5	69.7	4.15, m	69.7	4.16, m		
6	17.6	1.26, d (6.5)	17.5	1.25, d (6.5)		
			α-L-Ara (at C-22)			
1			102.0	4.27, d (3.7)		
2			74.2	3.60, dd (8.5, 3.7)		
3			72.2	3.58, dd (8.5, 3.0)		
4			69.0	3.86, m		
5			65.7	3.89 dd (11.9, 2.0) 3.52, dd (11.9, 3.0)		

[a] The chemical shift values of the sugar portion of **8** were superimposable with those reported for **7**

2; ESI-MS (pos. ione mode) *m/z* 659 $[M + Na]^+$, MS/MS *m/z* 513 $[M + Na - 146]^+$; HRMALDITOFMS *m/z* 659.4139 $[M + Na]^+$ (calcd. for $C_{36}H_{60}O_9Na$, 659.4135).

Compound **7**: Amorphous white solid; $[\alpha]_D^{25}$ − 10.9 (*c* 0.1 MeOH); IR (KBr) ν_{max} 3455, 2945, 1666, 1252, and 1052 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the sugar portion, see **Table 3**; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the aglycone moiety, see **Table 4**; ESI-MS (pos. ione mode) *m/z* 967 $[M + Na]^+$, MS/MS *m/z* 821 $[M + Na - 146]^+$, *m/z* 689 $[M + Na - 146 - 132]$; HRMALDITOFMS *m/z* 967.4881 $[M + Na]^+$ (calcd. for $C_{47}H_{76}O_{19}Na$, 967.4879).

Compound ***8***: Amorphous white solid; $[\alpha]_D^{25}$ + 7.12 (*c* 0.1 MeOH); IR (KBr) ν_{max} 3451, 2937, 1735, 1658, 1248, and 1048 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the sugar portion are superimposable with those reported for **7**; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the aglycone moiety, see **Table 4**; ESI-MS (pos. ione mode) *m/z* 965 $[M + Na]^+$, MS/MS *m/z* 819 $[M + Na - 146]^+$, *m/z* 687 $[M + Na - 146 - 132]$; HRMALDITOFMS *m/z* 965.4726 $[M + Na]^+$ (calcd. for $C_{47}H_{74}O_{19}Na$, 965.4722).

Compound ***9***: Amorphous white solid; $[\alpha]_D^{25}$ − 8.54 (*c* 0.1 MeOH); IR (KBr) ν_{max} 3446, 2930, 1730, 1648, 1245, and 1052 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the sugar portion, see **Table 3**; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the aglycone moiety, see **Table 3**; ESI-MS (pos. ione mode) *m/z* 1067 $[M + Na]^+$, MS/MS *m/z* 921 $[M + Na - 146]^+$, *m/z* 789 $[M + Na - 146 - 132]$, *m/z* 613 $[M + Na - 146 - 132 - 176]^+$; HRMALDITOFMS *m/z* 1067.5406 $[M + Na]^+$ (calcd. for $C_{52}H_{84}O_{21}Na$, 1067.5403).

Compound ***10***: Amorphous white solid; $[\alpha]_D^{25}$ + 27.7 (*c* 0.1 MeOH); IR (KBr) ν_{max} 3440, 2933, 1645, 1240, and 1044 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the sugar portion, see **Table 3**; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data of the aglycone moiety, see **Table 4**; ESI-MS (pos. ione mode) *m/z* 659 $[M + Na]^+$, MS/MS *m/z* 439 $[M + Na - 180]^+$; HRMALDITOFMS *m/z* 659.4139 $[M + Na]^+$ (calcd. for $C_{36}H_{60}O_9Na$, 659.4135).

Acid hydrolysis

The configurations of the sugar units were established after hydrolysis of **1**–**10** 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. [20].

The peaks of the hydrolysate of **1** were detected at 10.72 (L-rhamnose) and at 14.73 min (D-glucose). The peaks of the hydrolysate of **5** were detected at 10.73 (L-rhamnose) and 10.98 and 12.02 (D-xylose). The peaks of L-arabinose (8.94 and 9.81 min), L-rhamnose (10.72), D-xylose (10.98 and 12.01 min), and D-glucuronic acid (15.83 min) were detected in the hydrolysate of **9**. For the hydrolysate of **10** a peak at 14.73 min (D-glucose) was detected. 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), 10.70 (L-rhamnose),

Table 4 ^{13}C and ^{1}H NMR data (*J* in Hz) of the aglycone moieties of compounds **7–10** (600 Mz, δ ppm, in CD_3OD).

	7		8		9		10	
	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)
1	39.7	1.64, 1.04, m	39.7	1.66, 1.04, m	39.5	1.67, 1.04, m	39.8	1.71, 1.01, m
2	26.7	2.18, 1.80, m	26.8	2.18, 1.80, m	26.8	2.18, 1.82, m	28.4	2.18, 1.82, m
3	92.4	3.35, dd (11.5, 4.5)	92.6	3.36, dd (11.5, 4.2)	91.9	3.35, dd (11.5, 4.2)	81.6	3.36, dd (11.5, 4.2)
4	44.0	–	44.4	–	44.4	–	43.8	–
5	57.5	0.97, m	57.5	0.98, m	57.0	0.96, m	57.3	0.89, m
6	19.0	1.67, 1.41	19.1	1.66, 1.39, m	19.0	1.67, 1.41, m	19.4	1.68, 1.42, m
7	33.8	1.61,1.38, m	34.7	1.60,1.43, m	33.7	1.60,1.43, m	34.8	1.56, 1.43, m
8	41.0	–	40.4	–	40.0	–	40.5	–
9	48.7	1.62, m	48.7	1.60, m	48.3	1.60, m	49.0	1.59, m
10	37.5	–	37.5	–	36.7	–	37.6	–
11	24.6	1.92, (2H) m	24.5	1.90, (2H) m	24.5	1.90, (2H) m	24.5	1.91, (2H) m
12	123.7	5.27, t (3.5)	124.1	5.31, t (3.5)	123.8	5.31, t (3.5)	124.1	5.31, t (3.5)
13	146.0	–	145.5	–	145.1	–	145.4	–
14	43.1	–	43.5	–	42.5	–	43.0	–
15	27.3	1.90, 1.02, m	26.3	1.80, 1.06, m	26.1	1.79, 1.05, m	26.5	1.78, 1.06, m
16	30.4	1.91, 1.00, m	29.9	1.80, 1.31, m	29.2	1.80, 1.38, m	29.9	1.78, 1.39, m
17	39.7	–	38.2	–	36.8	–	38.4	–
18	43.9	2.29, m	46.4	2.02, m	46.9	2.12, m	45.9	2.12, m
19	41.3	2.12, 0.99, m	42.8	2.38, 1.18, m	47.0	1.78, 1.00, m	42.0	1.89, 0.90, m
20	41.5	–	44.4	–	31.1	–	36.2	–
21	70.7	3.83, brs	38.8	2.08, 1.45, m	36.8	1.57, 1.37, m	36.8	1.72, 1.34, m
22	80.8	3.36, d (3.5)	77.3	3.52, brs	83.3	3.44, brs	77.1	3.50, brs
23	22.8	1.24, s	22.6	1.24, s	22.8	1.25, s	23.0	1.24, s
24	64.2	4.12, d (11.5) 3.23, d (11.5)	63.6	4.11, d (11.4) 3.23, d (11.4)	63.4	4.12, d (11.4) 3.23, d (11.4)	65.3	4.14, d (11.4) 3.41, d (11.4)
25	15.9	0.90, s	15.9	0.91, s	16.0	0.90, s	16.6	0.99, m
26	17.2	1.00, s	17.5	1.00, s	17.5	1.00, s	17.1	1.01, m
27	26.8	1.23, s	24.9	1.18, s	25.4	1.16, s	25.0	1.15, m
28	21.9	0.98, s	19.8	0.83, s	21.1	0.93, s	19.9	0.85, m
29	71.8	3.45, d (10.5) 3.12, d (10.5)	26.2	1.24, s	32.5	0.94, s	80.2	3.68, d (9.7) 3.17, d (9.7)
30	16.8	1.03	187.8	–	28.8	1.06, s	24.4	1.08, s

10.98 and 12.00 min (D-xylose), 14.71 min (D-glucose), and 15.81 min (D-glucuronic acid).

Cancer cell lines

Human cervical (Hela), human lung (H-446), and human colon (HT-29) 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), and 1% penicillin/streptomycin (all from Cambrex Bioscience); human leukemic monocyte lymphoma (U937) cells were cultured in RPMI medium (Cambrex Bioscience) supplemented with 2 mM L-glutamine, 10% FBS, and 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.

MTT bioassay

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 (purity > 97%, 1–50 µM) were added to the cells and incubated for 48 h. Etoposide (purity = 98%) purchased from Sigma was used as the positive control. Cell viability was assessed through the MTT assay as previously reported [21].

The optical density (OD) of each well was measured with a microplate spectrophotometer (Titertek Multiskan MCC/340) equipped with a 620-nm filter.

Supporting information

NMR data for the new compounds **1–10** are available as Supporting Information.

Results and Discussion

A detailed comparison of the NMR (^{1}H, ^{13}C, 1D-TOCSY, HSQC, HMBC, DQF-COSY) and ESIMS data of the sugar portion of compounds **1–4** showed that the four compounds possessed the same disaccharide chain. In particular, the ^{1}H NMR spectrum of compound **1** exhibited two anomeric proton doublets at δ 4.44 (*J* = 7.5 Hz) and 5.42 (*J* = 1.2 Hz) (**Table 1**). In the HSQC spectrum, these protons correlated to carbons at δ 105.7 and 102.0, respectively (**Table 1**). Complete assignments of the ^{1}H and ^{13}C NMR signals allowed us the identification of an α-rhamnopyranosyl (δ 5.42) unit and a β-glucopyranosyl (δ 4.44) unit. The glycosidation site on the aglycone of **1** as well as the position of the interglycosidic linkage were determined by HMBC experiment, which showed long-range correlations between the anomeric proton signal at δ 4.44 ($H\text{-}1_{glc}$) and the carbon resonance at δ 89.9 (C-3), and between the anomeric proton signal at δ 5.42 ($H\text{-}1_{rha}$) and the carbon resonance at δ 79.7 ($C\text{-}2_{glc}$). The configurations of glucose and rhamnose units were established as D for glucose unit and L for rhamnose unit after hydrolysis of **1** with 1 N HCl, trimethylsilation, and determination of retention time by GC [20]. Thus, the sugar

sequence of compounds **1** – **4** was established as 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl].

The ESIMS spectrum of compound **1** showed the $[M + Na]^+$ ion at *m/z* 879. The MS/MS spectrum of this ion showed peaks at *m/z* 803 $[M + Na - 76]^+$, due to the loss of a hydroxyacetate unit, at *m/z* 657 $[M + Na - 76 - 146]^+$, corresponding to the subsequent loss of a deoxyhexose unit and at *m/z* 477 $[M + Na - 76 - 146 - 180]^+$, ascribable to the loss of a hexose unit.

The ^{1}H NMR spectrum showed for the aglycone moiety signals due to a cyclopropane methylene at δ 0.58 and 0.39 (each 1H, d, *J* = 4.8 Hz), seven tertiary methyl groups at δ 1.36, 1.33 (6H), 1.16, 1.14, 1.06, 1.05 and four methine protons at δ 5.49 (ddd, *J* = 8.0, 8.0, 5.6 Hz), 3.77 (dd, *J* = 8.2, 6.0 Hz), 3.48 (ddd, *J* = 9.7, 9.7, 4.5 Hz), and 3.27 (dd, *J* = 11.3, 4.0 Hz), indicative of secondary alcoholic functions, along with signals at δ 4.14 and 4.07 (each, br s) corresponding to a primary alcoholic function (**Table 2**). On the basis of DQF-COSY, HSQC, and HMBC spectra and by comparison of these data with those of cycloastragenol [22], it was observed that the aglycone of compound **1** differed from cycloastragenol only in the presence of a $-COCH_2OH$ group. The HMBC correlation between the proton signal at δ 5.49 (H-16) and the carbon resonance at δ 175.7 ($-\underline{C}OCH_2OH$) confirmed the location of the $-COCH_2OH$ group at C-16. Thus the aglycone of **1** was identified as 16-*O*-hydroxyacetoxy-3β,6α,16β,25-tetrahydroxy-20(*R*),24(*S*)-epoxycycloartane, and, consequently, the structure of compound **1** was established as 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl]-16-*O*-hydroxyacetoxy-3β,6α,16β,25-tetrahydroxy-20(*R*),24(*S*)-epoxycycloartane (**Fig. 1**).

The ^{1}H and ^{13}C NMR spectroscopic data of the aglycone portion of **2** were similar to those of **1** except for the presence of signals due to an additional secondary alcoholic function (δ 4.47, ddd, *J* = 8.8, 6.0, 3.5 Hz; δ_C 74.5) (**Table 2**). In the HMBC spectrum, cross-peaks between the proton signals at δ 4.47, 1.19 (Me-26), and 1.29 (Me-27) with the carbon at δ 72.6 (C-25) suggested the placement of this additional secondary alcoholic function at C-23. The α-orientation of the hydroxyl group at C-23 was deduced from the *J* values of the signal corresponding to H-23 (8.8, 6.0, 3.5) in comparison with literature values [23]. Therefore, the structure of compound **2** was established as 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl]-16-*O*-hydroxyacetoxy-3β,6α,16β,23α,25-pentahydroxy-20(*R*),24(*S*)-epoxycycloartane (**Fig. 1**).

The NMR data of the aglycone portion of **3** differed from those of cycloastragenol [22] only in the absence of the signal due to a secondary alcoholic function and the occurrence of a typical resonance of a keto group (δ 222.2) in the ^{13}C NMR spectrum (**Table 2**). The carbon resonances of the D ring and Me-18 in **3** suggested that the keto group was located at C-16. This hypothesis was confirmed by the HMBC experiment, which showed diagnostic long-range correlations between the proton signal at δ 1.28 (Me-18) and the carbon resonance at δ 66.9 (C-17), and between the proton signals at δ 2.98 (H-17) and 2.05 (H_2-15) with the carbon resonance at δ 222.2. From this evidence, in combination with the data of the sugar moiety reported above, the structure of **3** was established as 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl]-3β,6α,25-trihydroxy-20(*R*),24(*S*)-epoxycycloartane-16-one (**Fig. 1**).

The full assignments of the proton and carbon signals of the aglycone moiety of **4**, secured by DQF-COSY, HSQC, and HMBC spectra, were in good agreement with those of cycloastragenol [22], except for the chemical shift of C-24 (δ 88.5), suggesting a change of the absolute configuration at this center. The *R* configurations of C-20 and C-24 were derived from the ROESY spectrum, which showed key correlation peaks between Me-18 (δ 1.44) and H-23β (δ 1.85), which in turn correlated with H-24 (δ 3.84) suggesting a β orientation for H-24. Further ROESY correlations between H-17 (δ 2.27), Me-21 (δ 1.36), and Me-30 (δ 0.99) were observed. These data confirmed that the aglycone of **4** was the C-24 epimer of cycloastragenol. 20,24-Epoxycycloartanes represent the largest group belonging to the class of cycloartane triterpenoids. Of the four possible side-chain stereoisomers [20*R*,24*S*, 20*S*,24*R*, 20*R*,24*R* and 20*S*,24*S*], to the authors' knowledge, at present only the first two have been found in *Astragalus* spp. [24]. Therefore, this is the first report of a 20,24-epoxycycloartane with a 20(*R*),24(*R*) configuration. On the basis of these data, the structure of the new compound **4** was established as 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl]-3β,6α,16β,25-tetrahydroxy-20(*R*),24(*R*)-epoxycycloartane (**Fig. 1**).

The ESIMS spectrum of **5** showed the $[M + Na]^+$ ion at *m/z* 791. The MS/MS of this ion showed peaks at *m/z* 645 $[M + Na - 146]^+$, due to the loss of a deoxyhexose unit, at 495 $[M + Na - 146 - 150]^+$, corresponding to the loss of a xylopyranosyl unit.

A detailed analysis of the NMR data (^{1}H, ^{13}C, HSQC, HMBC, COSY) of compound **5** in comparison with those of **4** showed the same aglycone moiety in the two compounds.

Moreover, the ^{1}H NMR spectrum of **5** showed two anomeric proton doublets at δ 4.78 (*J* = 1.2 Hz) and 4.28 (*J* = 7.5 Hz) in the downfield region (**Table 1**). The determination of the sequence and linkage sites was obtained from the HMBC correlations between the proton signals at δ 4.78 ($H\text{-}1_{rha}$) and the carbon resonance at δ 81.3 (C-6), and between the proton signal at δ 4.28 ($H\text{-}1_{xyl}$) and the carbon resonance at δ 89.7 (C-3). Thus, compound **5** was identified as 3-*O*-β-D-xylopyranosyl-6-*O*-α-L-rhamnopyranosyl-3β,6α,16β,25-tetrahydroxy-20(*R*),24(*R*)-epoxycycloartane (**Fig. 1**).

The positive ESIMS spectrum of **6** showed the $[M + Na]^+$ ion at *m/z* 659. Its MS/MS fragmentation showed a peak at *m/z* 513 $[M + Na - 146]^+$ due to the loss of a deoxyhexose unit. A detailed analysis of the NMR data (^{1}H, ^{13}C, HSQC, HMBC, COSY) of compound **6** revealed that while the aglycone moiety was the same as in **4**, the sugar portion corresponded to an α-rhamnopyranose unit (**Table 1**). In the HMBC spectrum a key correlation peak was observed between the anomeric proton signal at δ 4.80 and the carbon resonance at δ 81.2 (C-6). Therefore, the structure of **6** was established as 6-*O*-α-L-rhamnopyranosyl-3β,6α,16β,25-tetrahydroxy-20(*R*),24(*R*)-epoxycycloartane (**Fig. 1**).

A detailed comparison of NMR (^{1}H, ^{13}C, HSQC, HMBC, COSY) and ESIMS data of compounds **7** – **8** showed that the sugar chain was identical in the two compounds. In particular, for the sugar portion, compound **7** showed signals corresponding to three anomeric protons at δ 5.23 (d, *J* = 1.2 Hz), 4.82 (d, *J* = 7.5 Hz), and 4.44 (d, *J* = 7.5 Hz) (**Table 3**). On the basis of 2D NMR data, one α-rhamnopyranosyl (δ 5.23), one β-xylopyranosyl (δ 4.82), and one β-glucuronic acid (δ 4.44) were identified. The determination of the sequence and linkage sites was obtained from the HMBC correlations between the proton signal at δ 5.23 ($H\text{-}1_{rha}$) and the carbon resonance at δ 79.3 ($C\text{-}2_{xyl}$), the proton signal at δ 4.82 ($H\text{-}1_{xyl}$) and the carbon resonance at δ 78.8 ($C\text{-}2_{glcA}$), and the proton signal at δ 4.44 ($H\text{-}1_{glcA}$) and the carbon resonance at δ 92.4 (C-3). Thus, the sugar sequence of compounds **7** – **8** was established as 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-xylopyranosyl-(1 → 2)-β-D-glucuronopyranoside. Moreover, the aglycone of compounds **7** – **10** was recognized as an oleanane-type triterpene by ^{1}H NMR and ^{13}C NMR analyses (**Table 4**) [25,26].

The ESIMS spectrum of **7** showed the $[M + Na]^+$ ion peak at *m/z* 967. The MS/MS spectrum of this ion showed peaks at *m/z* 821 $[M + Na - 146]^+$, due to the loss of a deoxyhexose unit and at *m/z* 689 $[M + Na - 146 - 132]^+$, corresponding to the loss of a xylopyranosyl unit. The ^{1}H NMR spectrum of **7** showed signals for six methyl groups at δ 1.24, 1.23, 1.03, 1.00, 0.98, 0.90, one olefinic proton at δ 5.27 (H-12, d, J = 3.5 Hz), three oxygen-bearing methine protons at δ 3.35 (H-3, dd J = 11.5, 4.5 Hz), 3.83 (H-21, brs), 3.36 (H-22, dd J = 3.5) and signals for two primary alcoholic functions at δ 3.23 and 4.12 (H_2-24, d J = 11.5), and 3.12 and 3.45 (H_2-29, d, J = 10.5) (**Table 4**). Detailed NMR studies allowed us to identify the aglycone of **7** as kudzusapogenol A [27]. The β-orientation of the hydroxyl group at C-21 and the α-orientation of the hydroxyl group at C-22 were derived from the ROESY spectrum, which showed key correlation peaks between H-21 (δ 3.83) and the proton signals H-29 (3.45) and H-19α (2.12), and between H-22 (δ 3.36) and Me-28 (0.98). Thus, compound **7** was identified as the new 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-xylopyranosyl-(1 → 2)-β-D-glucuronopyranosyl]-3β,21β,22α,24,29-pentahydroxyolean-12-ene (**Fig. 2**).

The ^{1}H NMR spectrum of **8** displayed signals for six tertiary methyl groups at δ 1.24 (6H), 1.18, 1.00, 0.91, 0.83, for an olefinic proton at δ 5.31 (t, J = 3.5 Hz), two oxygen-bearing methine protons at δ 3.36 (H-3, dd, J = 11.5, 4.5 Hz) and 3.52 (H-22, brs), and one primary alcoholic function at δ 4.11 and 3.23 (H_2-24, d, J = 11.5 Hz) (**Table 4**). A combination of 1D and 2D NMR techniques led to the assignment of ^{1}H and ^{13}C NMR signals due to A, B, C, and D rings of a 3β,24-dihydroxyolean-12-ene sapogenol unit [28], glycosylated at C-3. On the basis of NMR data, the E ring exhibited an oxymethine at C-22 (δ_C 77.3, δ_H 3.52 brs) and a -COOH group at C-30 (δ_C 187.8). Thus, the structure of compound **8** was elucidated as 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-xylopyranosyl-(1 → 2)-β-D-glucuronopyranosyl]-3β,22β,24-trihydroxyolean-12-en-29-oic acid (**Fig. 2**).

The ^{1}H NMR spectrum of **9** displayed signals for seven tertiary methyl groups at δ 1.25, 1.16, 1.06, 1.00, 0.94, 0.93, and 0.90, and for an olefinic proton at δ 5.31 (1H, t, J = 3.5 Hz) (**Table 4**). On the basis of NMR data in comparison with those of astragaloside VIII [29], it clearly appeared that compound **9** differed from astragaloside VIII only in the presence of an additional α-arabinopyranosyl unit (H-1_{ara} δ 4.27, d, J = 3.7 Hz), placed at position C-22, on the basis of the HMBC correlation between the proton signal at δ 4.27 (H-1_{ara}) and the carbon resonance at δ 83.3 (C-22). Therefore, the structure of **9** was established as 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-xylopyranosyl-(1 → 2)-β-D-glucuronopyranosyl]-22-*O*-α-L-arabinopyranosyl-3β,22β,24-trihydroxyolean-12-ene (**Fig. 2**).

The ESIMS spectrum of **10** showed the $[M + Na]^+$ ion at *m/z* 659. The MS/MS of this ion showed a peak at *m/z* 439 $[M + Na - 180]^+$ due to the loss of a hexose unit. A combination of 1D and 2D NMR techniques led to the assignment of ^{1}H and ^{13}C NMR signals due to the A, B, C, D rings of a 3β,24-dihydroxyolean-12-ene sapogenol unit [30]. The E ring was shown to contain an oxymethine at C-22 (δ_C 77.1, δ_H 3.50 brs) and a glycosylated hydroxymethyl group at C-29 (δ_C 80.2, δ_H 3.17 and 3.68, each d, J = 9.7 Hz). The occurrence of a 29-CH_2OR function was derived from the resonance of Me-30 at δ 24.4, superimposable to that of abrisapogenol B [30]. 1D and 2D NMR data also indicated the presence of a terminal β-D-glucopyranosyl moiety, which was located at C-29 on the basis of the HMBC correlation between H-1_{glc} (δ 4.26) and C-29 (δ 80.2) signals. Thus, the structure of **10** was elucidated as 29-*O*-β-D-glucopyranosyl-3β,22β,24,29-tetrahydroxy-olean-12-ene (**Fig. 2**).

Additionally, three known cycloartane-type glycosides, 25-*O*-glucopyranosylcycloastragenol (**11**) [29], cycloaraloside D (**12**) [31], and 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl]-25-*O*-β-D-glucopyranosyl-20(*R*),24(*S*)-epoxy-3β,6α,16β,25-tetrahydroxycycloartane (**13**) [11], and two known oleanane glycosides, astrajanoside A (**14**) [4] and astragaloside VIII (**15**) [29], were isolated.

On the basis of the cytotoxic activities exhibited by cycloartane- and oleanane-type glycosides isolated from *Astragalus* species [15, 19] and on the basis of cancer chemopreventive effects reported for the natural and semisynthetic cycloartane-type and related triterpenoids [32], the antiproliferative activity of compounds **1** – **15** was tested in different cancer cell lines including Hela (human cervical cancer cells), H-446 (human lung cancer cells), HT-29 (human colon carcinoma cells), and U-937 (human leukemia cells). In a range of concentrations between 1 and 50 µM, none of the tested compounds, except compound **8**, caused a significative reduction of the cell number when compared with controls. Compound **8** displayed weak cytotoxicity against HeLa and HT-29 cell lines with IC_{50} values of 36 and 50 µM, respectively. Etoposide, used as a positive control, showed IC_{50} values ranging from 2 µM (U-937 cells) to 12 µM (H-446 cells).

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Conflict of Interest

The authors declare that there is no conflict of interest.

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