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Cycloartane glycosides from *Astragalus gombo*

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ABSTRACT

Six new cycloartane-type triterpene glycosides named 3-*O*-[β-D-glucopyranosyl(1 → 2)-β-D-xylopyranosyl]-3β,16β,23(*R*),24(*R*),25-pentahydroxycycloartane (**1**), 3-*O*-[β-D-glucopyranosyl(1 → 2)-β-D-xylopyranosyl]-3β,16β,23(*R*),24(*R*)-tetrahydroxy-25-dehydrocycloartane (**2**), 3-*O*-[β-D-xylopyranosyl]-6α-acetoxy-23α-methoxy-16β,23(*R*)-epoxy-24,25,26,27-tetranorcycloartane (**3**), 3-*O*-[β-D-xylopyranosyl]-6α-acetoxy-23α-butoxy-16β,23(*R*)-epoxy-24,25,26,27-tetranorcycloartane (**4**), 3-*O*-[β-D-glucopyranosyl(1 → 2)]-β-D-xylopyranosyl]-6α-acetoxy-23α-methoxy-16β,23(*R*)-epoxy-24,25,26,27-tetranorcycloartane (**5**), 3-*O*-[β-D-glucopyranosyl(1 → 2)]-β-D-xylopyranosyl]-23α-methoxy-16β,23(*R*)-epoxy-4,25,26,27-tetranorcycloartane (**6**), in addition to three known secondary metabolites consisting of another cycloartane triterpene glycoside and two flavonol glycosides, were isolated from the aerial parts of *Astragalus gombo* Coss. & Dur. (Fabaceae). The structures of the isolated compounds were established by spectroscopic methods, including 1D and 2D-NMR, mass spectrometry and comparison with literature data.

1. Introduction

The genus *Astragalus* contains about 3000 species and represents one of the largest genus in the family of Fabaceae (Heywood, 1978). It is widely distributed throughout the temperate regions of the world and located principally in Europe, Asia and North America (Davis, 1982; Rios and Waterman, 1997). This genus is represented in Algeria by about 40 species, including *Astragalus gombo* Coss. & Dur. This endemic perennial plant grows in sandy arid and desert pastures of Algeria (Quezel and Santa, 1963).

Several *Astragalus* species are used in traditional medicine as antiperspirants, diuretics, tonics, in treatment of nephritis, diabetes, leukemia and uterine cancer (Avunduk et al., 2008; Choudhary et al., 2008). The genus *Astragalus* is known for the presence of two major classes of biologically active compounds, polysaccharides and saponins (Bedir et al., 2000; Li, 2000; Rios and Waterman, 1997). Previous phytochemical studies on *Astragalus* saponins have shown the importance of cycloartane-type triterpene glycosides as major compounds (Barbić et al., 2010; Choudhary et al., 2008; Polat et al., 2010). They are known for their interesting biological properties, including immunostimulating (Bedir et al., 2000; Çaliş et al., 1997; Yesilada et al., 2005), antiprotozoal (Özipek et al., 2005), antiviral (Gariboldi et al., 1995), wound healing (Sevimli-Gur et al., 2011), anti-inflammatory (Lee et al., 2013) and cytotoxic activities (Radwan et al., 2004).

In a continuation of our work on the chemical constituents of *Astragalus* species growing in Algeria (Benchadi et al., 2013; Maamria et al., 2014), the aerial parts of *A. gombo* were investigated. We reported in the present study the isolation of six new cycloartane-type triterpene glycosides (**1**–**6**) (Fig. 1) from the *n*-butanol extract, along with three known compounds (**7**–**9**). Structures of the isolated compounds have been determined on the basis of 1D and 2D homo- and heteronuclear NMR and mass spectrometry, as well as by comparison with reported literature data.

2. Results and discussion

The *n*-BuOH extract of the aerial parts of *A. gombo* was subjected to vacuum liquid chromatography (VLC) and different chromatographic steps to yield six new saponins (**1**–**6**) (Fig. 1), along with three known compounds (**7**–**9**). The known compounds were

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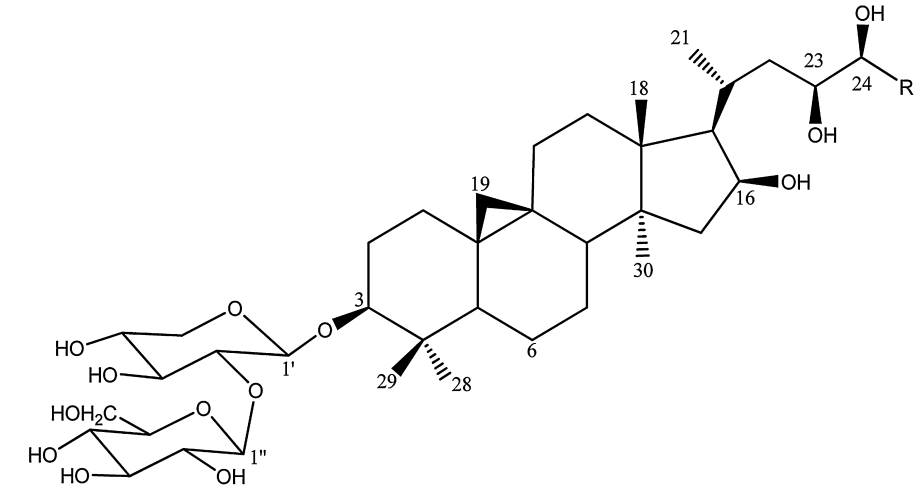
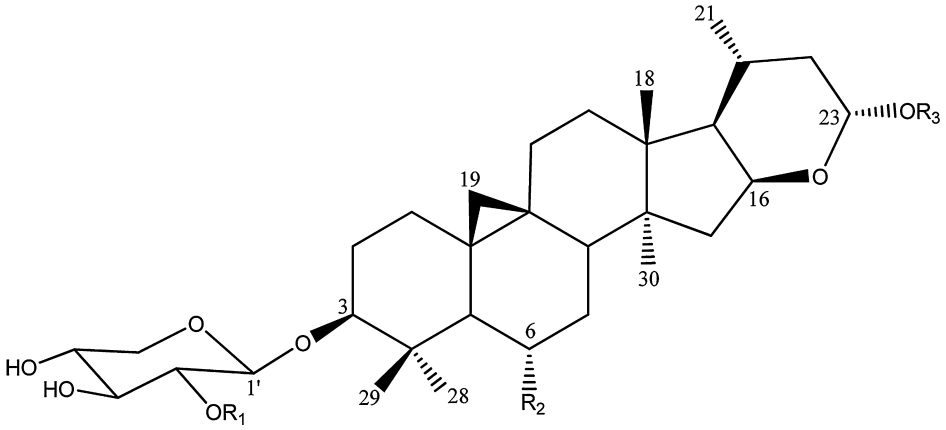


1 R = $(CH_3)_2{}^{25}C(OH)$
2 R = $^{26}(CH_3)^{25}C=^{27}CH_2$



3	R_1= H	R_2= OAc	R_3= $^{1"}CH_3$
4	R_1= H	R_2= OAc	R_3= $^{1"}CH_2{}^{2"}CH_2{}^{3"}CH_2{}^{4"}CH_3$
5	R_1= Glc	R_2= OAc	R_3= CH_3
6	R_1= Glc	R_2= H	R_3= CH_3

Fig. 1. Structures of new compounds **1**–**6**.

identified as tomentoside II (**7**) (Abdallah et al., 1994), kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-[6-*O*-(3-hydroxy-3-methyl-glutaryl)-*β*-D-galactopyranoside] (**8**) (Montoro et al., 2013; Porter et al., 2012) and kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-galactopyranoside (**9**) (Yasukawa and Takido, 1987).

The HRESIMS mass spectrum of **1** (*m*/*z* 809.5589 $[M+Na]^+$, calcd. for $C_{41}H_{70}O_{14}Na$, 809.4658) supported a molecular formula of $C_{41}H_{70}O_{14}$. The ESI-MS mass spectrum showed the major ion peak at *m*/*z* 809 which was assigned to $[M+Na]^+$. The ^1H NMR spectrum showed for the aglycon moiety, signals due to a cyclopropane methylene at δ_H 0.62 and 0.40 (each 1H, d, *J* = 4.0 Hz) characteristic for cycloartane-type triterpenes, six tertiary methyl groups at δ_H 0.91, 0.95, 1.09, 1.22, 1.24, and 1.25, and a secondary methyl group at δ_H 1.05 (d, *J* = 6.5 Hz). Additionally, four methine proton signals at δ_H 4.70 (ddd, *J* = 7.6, 7.6, 5.2 Hz), 3.71 (ddd, *J* = 10.5, 8.2, 2.3 Hz), 3.24 (dd, *J* = 8.1, 4.2 Hz), and 3.10 (d, *J* = 8.2 Hz), were indicative of secondary alcoholic functions (Table 1). Furthermore, the ^1H NMR spectrum of **1** showed two anomeric protons at δ_H 4.47 (d, *J* = 7.9 Hz) and 4.68 (d, *J* = 7.7 Hz) in the downfield region, indicative of two *β*-linked sugar moieties (Table 1). The chemical shifts of all individual protons of the two sugar units were ascertained from COSY spectral analysis, and the ^{13}C NMR chemical shifts of their attached carbons could be assigned unambiguously from the HSQC spectrum (Table 1). These data showed the presence of one *β*-xylopyranosyl unit (δ_H 4.47) and one *β*-glucopyranosyl unit (δ_H 4.68). The sites of attachment of sugar moieties on the aglycon of **1** were determined by HMBC experiment, which showed long-range correlations between the anomeric proton signal at δ_H 4.47 (H-1′) and the carbon resonance at δ_C 89.3 (C-3) and between the second anomeric proton signal at δ_H 4.68 (H-1″) and the carbon resonance at δ_C 79.6 (C-2′) indicating the presence of a disaccharide unit at C-3. The chemical shifts of protons and carbons of the aglycon moiety (Table 1), established mainly by COSY H–H, HSQC and HMBC, are similar to those reported for signals of cycloorbigenin C (Mamedova et al., 2003; Perrone et al., 2008), except for the upfield shifts of CH_2-6 resonances (δ_H 1.65, 0.85, δ_C 20.6), suggesting the absence of the hydroxyl group at this position. Based on the fact that the chemical

Table 1
^{1}H and ^{13}C NMR data of compounds **1** and **2** in CD_3OD.

	1		**2**	
	δ_H (m, *J* Hz)	δ_C	δ_H (m, *J* Hz)	δ_C
1	1.56, 1.30 (m)	31.7	1.53, 1.25 (m)	33.2
2	1.96, 1.72 (m)	29.1	1.94, 1.71 (m)	30.6
3	3.24 (dd 8.1, 4.2)	89.3	3.23 (dd 11.6, 4.4)	90.8
4	–	40.8	–	42.4
5	1.35 dd (12.5, 4.2)	47.4	1.32[a]	49.0
6	1.65, 0.85 (m)	20.6	1.59, 0.83 (m)	22.2
7	1.39, 1.15 (m)	25.9	1.35, 1.12 (m)	27.4
8	1.65[a]	48.3	1.62 (dd 13.5, 4.2)	49.9
9	–	19.6	–	21.2
10	–	25.9	–	27.5
11	2.05, 1.15 (m)	25.9	2.02, 1.12 (m)	27.4
12	1.7 (m)	32.4	1.63 (m)	34.0
13	–	46.5	–	47.1
14	–	46.5	–	48.0
15	2.03 (dd 12.4, 7.6) 1.40 (dd 12.4, 5.2)	46.9	2.00, 1.37 (m)	48.3
16	4.7 (ddd 7.6, 7.6, 5.2)	72.0	4.45 (m)	73.6
17	1.75 (dd 11.3, 7.6)	56.7	1.68 (dd 11.3,7.2)	58.6
18	1.22 (s)	18.4	1.18 (s)	19.5
19	0.62 (d 4.0) 0.40 (d 4.0)	29.8	0.60 (d 3.9) 0.38 (d 3.9)	31.3
20	2.04 (m)	26.4	2.01 (m)	28.5
21	1.05 (d 6.5)	18.7	0.99 (d 6.7)	20.2
22	1.66 (m)	41.4	1.57,1.32 (m)	40.0
23	3.71 (ddd 10.5, 8.2, 2.3)	71.7	3.64 (dd 11.4, 5.7)	72.2
24	3.10 (d 8.2)	79.6	3.88 (d 5.7)	80.3
25	–	73.5	–	146.7
26	1.25 (s)	22.5	1.76 (s)	19.0
27	1.24 (s)	26.5	4.98, 4.90 (br s)	113.2
28	1.09 (s)	24.5	1.05 (s)	26.0
29	0.91 (s)	14.0	0.88 (s)	15.5
30	0.95 (s)	19.0	0.93 (s)	20.6
1′	4.47 (d 7.9)	104.5	4.41 (d 7.5)	106.1
2′	3.59 (t 7.9)	79.6	3.56 (t 7.5)	81.2
3′	3.54 (t 7.9)	76.5	3.48 (t 7.5)	71.2
4′	3.51 (m)	69.7	3.22 (ddd 10.8, 7.5, 4.6)	73.0
5′	3.86 (dd 11.5, 4.7) 3.21 (m)	65.1	3.84 (dd 10.8, 4.6) 3.20 (t 10.8)	66.7
1″	4.68 (d 7.7)	103.2	4.66 (d 7.9)	104.8
2″	3.25 (dd 8.6, 7.7)	74.8	3.19 (t 7.9)	76.4
3″	3.38 (t 8.6)	76.5	3.36 (t 7.9)	78.0
4″	3.24 (t 8.6)	70.4	3.51(t 7.9)	78.1
5″	3.28 (ddd 8.6, 6.5, 5.9)	77.0	3.24 (ddd 7.9, 6.0, 2.3)	78.5
6″	3.85 (dd 11.2, 6.5) 3.66 (dd 11.2, 5.9)	61.6	3.82 (dd 11.5, 2.3) 3.63 (dd 11.5, 6.0)	63.2

[a] Signal patterns are unclear due to overlapping.

shift values of C-23 (δ_C 71.7) and C-24 (δ_C 79.6), the large homonuclear coupling constant $^{3}J_{H\text{-}23\text{–}H\text{-}24}$ = 8.2 Hz, and the ROE effects (Fig. 2) observed in the ROESY spectrum of compound **1** recorded in pyridine-d_5, particularly between Me-21 (δ_H 1.21)/H-23 (δ_H 4.35), OH-23 (δ_H 6.85, d, *J* = 2.2 Hz)/H-20 (δ_H 2.60) and H-24 (δ_H 3.78)/H-22b (δ_H 2.20), are completely similar to those depicted in the case of eremophiloside C previously isolated from *Astragalus eremophilus* (Perrone et al., 2008), it seems evident that the new cycloartane triterpene glycoside **1** possesses the same *R* configuration at carbons C-23 and C-24. From all these elements, compound **1** was elucidated to be 3-*O*-[β-D-glucopyranosyl (1 → 2)-β-D-xylopyranosyl]-3β,16β,23(*R*),24(*R*),25-pentahydroxycycloartane and named gomboside A.

The ESI$^+$ mass spectrum of compound **2** showed a quasi-molecular ion signal at *m*/*z* 791 [M+Na]$^+$, indicating a molecular mass of 768 uma. The molecular formula was confirmed as $C_{41}H_{68}O_{13}$ by HRESIMS (*m*/*z* 791.5493 [M+Na]$^+$, calcd. for $C_{41}H_{68}O_{13}Na$, 791.4552). The ^{1}H and ^{13}C NMR spectral data of **2** (Table 1) were closely similar to those of compound **1**. The difference is that compound **2** possessed an exocyclic methylene attached to the carbon C-25 [δ_H 4.98 (1H, br s, H-27a), δ_H 4.90 (1H, br s, H-27b), δ_C 113.2 (C-27), δ_C 146.7 (C-25)]. However, the presence of the methylene group induced different chemical shifts for protons and carbons CH-24 (δ_H 3.88, δ_C 80.3), CH-23 (δ_H 3.64, δ_C 72.2) and CH_3-26 (δ_H 1.76, δ_C 19.0). The points of attachment of sugar moieties to the aglycon were confirmed by HMBC experiment, which showed long-range correlations between the xyl H-1′ at δ_H 4.41 and the carbon resonance at δ_C 90.8 (C-3), and between the glc H-1″ at δ_H 4.66 and the carbon resonance at δ_C 81.2 (C-2′$_{xyl}$). Thus, the structure of compound **2** was elucidated as 3-*O*-[β-D-glucopyranosyl(1 → 2)-β-D-xylopyranosyl]-3β,16β,23(*R*),24(*R*)-tetrahydroxy-25-dehydrocycloartane and named gomboside B.

The ESI$^+$ mass spectrum of compound **3** gave a quasi-molecular ion peak at *m*/*z* 629 [M+Na]$^+$ indicating a molecular mass M = 606 corresponding to the formula $C_{34}H_{54}O_9$ which was supported by HRESIMS (*m*/*z* 629.4482 [M+Na]$^+$, calcd. for $C_{34}H_{54}O_9Na$, 629.3660). For the aglycon portion, the ^{1}H NMR spectrum (Table 2) showed two characteristic cyclopropane methylene at δ_H 0.60 and 0.31 (each 1H, d, *J* = 4.5 Hz), four tertiary methyl groups at δ_H 0.91, 0.95, 0.97 and 1.07, a secondary methyl group at δ_H 0.92 (d, *J* = 6.5 Hz), four methine proton signals at δ_H 4.75 (m), 4.71 (t, *J* = 7.2 Hz), 4.23 (q, *J* = 7.6 Hz) and 3.23 (dd, *J* = 11.6, 4.4 Hz), indicative of secondary alcoholic functions. The ^{1}H NMR spectrum of **3** revealed also the presence of one β-linked sugar unit with signal of one anomeric proton doublet at δ_H 4.49 (*J* = 5.8 Hz) and a signal for one acetyl methyl group (δ_H 1.99) which showed in HSQC spectrum correlation with carbon signal at δ_C 22.1 (Table 2). The chemical shifts of all individual protons of the sugar unit were determined from COSY spectrum, and the ^{13}C NMR chemical shifts of their attached carbons were assigned unambiguously from the HSQC spectrum (Table 2). These data showed the presence of one β-xylopyranosyl unit. The determination of the linkage site was obtained from the HMBC correlation (Fig. 3) between the anomeric proton signal (δ_H 4.49) and the carbon resonance at δ_C 88.7 (C-3). The ^{1}H NMR and ^{13}C NMR data of **3** are found closely similar to those of tomentoside II (**7**) isolated in this work and previously from *Astragalus tomentosus* (Abdallah et al., 1994). The only difference was the presence in compound **3** of a methoxy group at δ_H 3.30 and δ_C 55.0 linked at C-23 instead of the

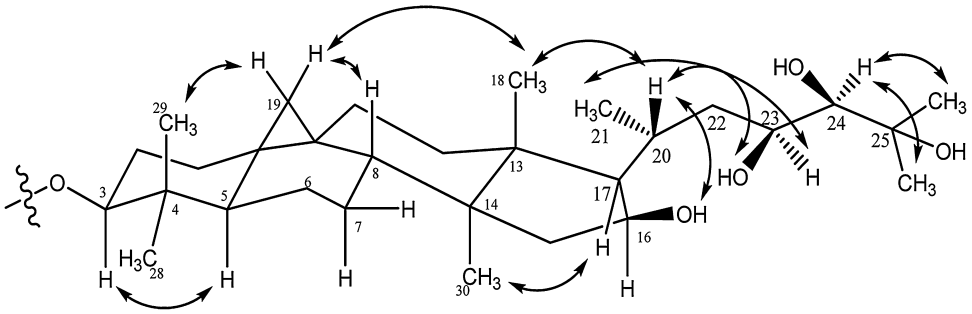


Fig. 2. Key ROESY correlations of aglycon moiety (compound **1**).

Table 2
^{1}H and ^{13}C NMR data of compounds **3** and **4** in $CDCl_3$.

	3		**4**	
	δ_H (m, *J* Hz)	δ_C	δ_H (m, *J* Hz)	δ_C
1	1.58, 1.30 (m)	31.8	1.60, 1.32 (m)	31.8
2	1.99, 1.72 (m)	29.1	2.01, 1.74 (m)	29.1
3	3.23 (dd 11.6, 4.4)	88.7	3.2 (dd,11.5, 4.6)	88.7
4	–	41.8	–	41.8
5	1.69 (d 9.4)	49.9	1.71 (d 9.4)	49.9
6	4.75 (m)	70.3	4.75 (m)	70.4
7	1.61, 1.33(m)	33.1	1.63, 1.35 (m)	33.1
8	1.97(m)	44.3	1.97 (m)	44.3
9	–	21.1	–	21.1
10	–	27.9	–	28.0
11	1.83, 1.35(m)	26.1	1.87, 1.35 (m)	26.1
12	1.61, 1.51 (m)	32.8	1.63,1.55 (m)	32.1
13	–	44.8	–	44.8
14	–	45.9	–	45.9
15	1.78 (dd 12.5, 7.9)	43.0	1.79 (dd 12.3, 7.9)	43.0
	1.40[a]		1.42[a]	
16	4.23 (q 7.9)	70.5	4.27 (q 7.9)	70.4
17	1.52[a]	56.5	1.55[a]	56.5
18	1.07 (s)	19.8	1.09 (s)	19.8
19	0.60 (d 4.5)	28.1	0.61 (d 4.8)	28.3
	0.31 (d 4.5)		0.33 (d 4.8)	
20	1.66 (m)	25.5	1.67 (m)	25.5
21	0.92 (d 6.5)	20.6	0.94 (d 6.1)	20.6
22	1.83, 1.12 (m)	37.8	1.86, 1.15 (m)	38.0
23	4.71 (t 7.2)	100.4	4.83 (t 7.2)	99.1
28	0.97 (s)	26.7	0.99 (s)	26.8
29	0.95 (s)	16.4	0.97 (s)	16.4
30	0.91 (s)	19.3	0.93 (s)	19.3
1′	4.49 (d 5.8)	104.9	4.51 (d 5.7)	104.9
2′	3.52 (dd 8.9, 5.8)	72.4	3.55 (dd 9.3, 5.7)	72.4
3′	3.63 (dd 9.7, 8.9)	73.9	3.64 (dd, 11.6, 9.3)	73.9
4′	3.76 (ddd 9.7, 7.5, 4.1)	69.6	3.78 (ddd 11.6, 7.3, 4.0)	69.9
5′	4.08 (dd 11.9, 4.1)	63.9	4.10 (dd 12.0, 4.0)	63.9
	3.38 (dd 11.9, 7.5)		3.40 (dd 12.0, 7.3)	
1″	3.30 (s)	55.0	3.69 (dt, 9.8, 6.8)	67.3
			3.40 (m)	
2″	–	–	1.54 (m)	32.9
3″	–	–	1.38 (m)	19.6
4″	–	–	0.92 (t 7.1)	14.1
$OCOCH_3$	1.99 (s)	22.1	2.02 (s)	22.1
CO	–	170.6	–	170.6

[a] Signal patterns are unclear due to overlapping

hydroxyl group in the case of **7**. The precise location of this methoxy at C-23 and the glucopyranosyl moiety at C-3 was confirmed from the observation of appropriate long-range CH correlations (Fig. 3) in the HMBC spectrum. The stereochemistry at C-6, C-16, C-17, C-20 and C-23 for compound **3** was confirmed by analysis of the ROESY spectrum. The β-orientation of H-6 (δ_H 4.75) induced a ROE effect with H-19β (δ_H 0.60). The ROE effects between Me-30α (δ_H 0.91) and H-16 (δ_H 4.23) and H-17 (δ_H 1.52) indicated that these protons were on the same side and involved in a α configuration. The ROE effect between H-17α and Me-21 (δ_H 0.92) proved the α orientation of CH_3-21. The value of coupling constant of H-23 (δ_H 4.71, *J* = 7.2 Hz) and the absence of ROE effects between H-16α and H-23 confirmed the β orientation of H-23. Thus, the structure of **3** was characterized as 3-*O*-[*β*-D-xylopyranosyl]-6*α*-acetoxy-23*α*-methoxy-16*β*,23(*R*)-epoxy-24,25,26,27-tetranorcycloartane named gomboside C.

The ESI mass spectrum in positive mode of compound **4** showed quasi-molecular ion peak at *m*/*z* 671 $[M+Na]^+$, indicating a molecular mass of 648 uma. The formula was confirmed as $C_{37}H_{60}O_9$ by HRESIMS (*m*/*z* 671.5006 $[M+Na]^+$, calcd. for $C_{37}H_{60}O_9Na$, 671.4130). The NMR data of **4** (Table 2), established mainly by COSY H–H, HSQC, HMBC and ROESY, in comparison to those of **3** showed that **4** differed only by the presence of butoxy group at C-23 [δ_H 3.69 (1H, dt, *J* = 9.8, 6.8 Hz, H-1″a), 3.40 (1H, m, H-1″b), 1.54 (1H, m, H-2″), 1.38 (1H, m, H-3″), 0.92 (1H, t, *J* = 7.1 Hz, H-4″) and δ_C 67.3 (C-1″), δ_C 32.9 (C-2″), δ_C 19.6 (C-3″), δ_C 14.1 (C-4″)] instead of methoxy group for **3**. This was confirmed by the HMBC experiment which showed correlation between CH_2-1″ and C-23 (δ_C 99.1). The point of attachment of the xylopyranosyl moiety to the aglycon was evident from a HMBC correlation between the Xyl H-1′ (δ_H 4.51) and C-3 (δ_C 88.7) signals. Therefore, the structure of compound **4** was established as 3-*O*-[*β*-D-xylopyranosyl]-6*α*-acetoxy-23*α*-butoxy-16*β*,23(*R*)-epoxy-24,25,26,27-tetranorcycloartane and named gomboside D.

The HRESIMS mass spectrum of **5** exhibited a major ion peak at *m*/*z* 791.5141 $[M+Na]^+$ which corresponded to the molecular formula $C_{40}H_{64}O_{14}$ (calcd. for $C_{40}H_{64}O_{14}Na$, 791.4188). The ^{1}H NMR spectrum (Table 3) displayed two characteristic cyclopropane methylene at δ_H 0.61 and 0.37 (each 1H, d, *J* = 4.8 Hz), four tertiary methyl groups at δ_H 0.96, 0.98, 1.05, and 1.12, a secondary methyl group at δ_H 0.94 (d, *J* = 6.3 Hz), four methine proton signals at δ_H 4.74 (m), 4.70 (t, *J* = 7.1 Hz), 4.23 (q, *J* = 7.6 Hz) and 3.23 (dd, *J* = 10.6, 4.3 Hz), attributed to secondary alcoholic functions (Table 3). A detailed comparison of the aglycon moiety NMR data (^{1}H, ^{13}C, HSQC, HMBC and COSY) of compounds **5** and **3** indicated that the aglycon moiety was similar in the two compounds. Furthermore, the ^{1}H NMR spectrum of **5** showed two anomeric protons doublets at δ_H 4.66 (*J* = 7.9 Hz) and 4.41 (*J* = 7.5 Hz) in the downfield region, corresponding to two *β*-linked sugar units (Table 3). The chemical shifts of all individual protons and carbons of the two sugar units were ascertained from COSY and HSQC experiments (Table 3). These data showed the presence of *β*-xylopyranosyl (δ_H 4.41) and *β*-glucopyranosyl (δ_H 4.66) units. The sites of attachment and sequence of sugar moieties on the aglycon were determined by HMBC experiment, which showed long-range CH correlations between the anomeric proton signal resonating at δ_H 4.41 (H-1′$_{xyl}$) and the carbon resonance at δ_C 89.8 (C-3), and

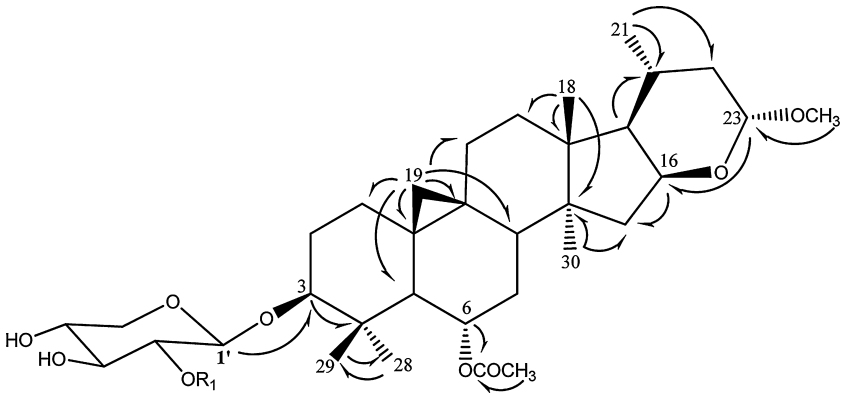


Fig. 3. Selected HMBC correlations of compound **3** (arrows from H to C).

Table 3
^{1}H and ^{13}C NMR data of compounds **5** (CD_3OD) and **6** (DMSO-d_6).

	5		**6**	
	δ_H (m, *J* Hz)	δ_C	δ_H (m, *J* Hz)	δ_C
1	1.60, 1.31 (m)	32.9	1.43, 1.17 (m)	31.3
2	1.98, 1.70 (m)	30.5	1.79, 1.50 (m)	29.1
3	3.23 (dd 10.6, 4.3)	89.8	3.09 (dd 11.7, 4.1)	87.4
4	–	43.2	–	40.4
5	1.72 (d 9.4)	51.2	1.24 (dd 11.8, 3.7)	46.7
6	4.74 (m)	71.9	1.49, 0.78 (m)	20.2
7	1.66, 1.58 (m)	34.2	1.29, 1.02 (m)	25.7
8	1.95 (dd 10.1, 5.2)	46.1	1.54[a]	46.8
9	–	22.2	–	19.2
10	–	29.4	–	25.9
11	1.91, 1.39 (m)	27.0	1.91, 1.12 (m)	25.6
12	1.66, 1.58 (m)	34.0	1.52 (m)	32.6
13	–	45.9	–	44.0
14	–	47.1	–	45.6
15	1.79 (dd, 11.9, 8.3) 1.42 (dd 11.9, 6.5)	44.0	1.78, 1.28[a]	43.0
16	4.23 (q 7.6)	71.9	4.11 (q 7.6)	69.7
17	1.57[a]	57.8	1.48[a]	55.8
18	1.12 (s)	20.4	1.08 (s)	20.1
19	0.61 (d 4.8) 0.37 (d 4.8)	29.3	0.54 (d 3.5) 0.28 (d 3.5)	29.5
20	1.66 (m)	26.6	1.51 (m)	24.9
21	0.94 (d 6.3)	20.9	0.87 (d 5.9)	20.1
22	1.85, 1.12 (m)	38.7	1.76, 1.01 (m)	37.0
23	4.70 (t 7.1)	101.7	4.65 (t 7.2)	99.3
28	1.05 (s)	27.2	0.89 (s)	25.0
29	0.98 (s)	16.6	0.77 (s)	14.5
30	0.96 (s)	19.8	0.83 (s)	19.2
1′	4.41 (d 7.5)	106.1	4.29 (d 6.9)	104.1
2′	3.56 (t 7.5)	81.2	3.30 (t 6.9)	81.2
3′	3.48 (t 7.5)	71.2	3.30 (t 6.9)	69.4
4′	3.22 (t 7.5)	73.0	3.12 (m)	69.7
5′	3.84 (dd 10.8, 4.6) 3.20 (t 10.8)	66.7	3.66 (dd 11.3, 3.2) 3.03 (t 11.3)	65.2
1″	4.66 (d 7.9)	104.8	4.42 (d 7.7)	104.0
2″	3.19 (t 7.9)	76.4	2.99 (t 7.7)	75.2
3″	3.36 (t 7.9)	78.0	3.14 (t 7.7)	76.1
4″	3.51 (t 7.9)	78.1	3.30 (t 7.7)	76.1
5″	3.24 (ddd 7.9, 6.0, 2.3)	78.5	3.03 (m)	76.8
6″	3.82 (dd 11.5, 2.3) 3.63 (dd 11.5, 6.0)	63.2	3.59 (m) 3.48 (dd 11.4, 4.3)	60.8
OCH_3	3.31 (s)	55.2	3.19 (s)	54.1
$OCOCH_3$	1.99 (s)	22.0	–	–
CO	–	172.6	–	–

[a] Signal patterns are unclear due to overlapping.

between the second anomeric proton signal detected at δ_H 4.66 (H-1″$_{glc}$) and the carbon resonance at δ_C 81.2 (C-2′$_{xyl}$) indicating the presence of a disaccharide unit at C-3. On the basis of all these evidence, the structure of compound **5** was elucidated as 3-*O*-[β-D-glucopyranosyl(1 → 2)]-β-D-xylopyranosyl]-6α-acetoxy-23α-methoxy-16β,23(*R*)-epoxy-24,25,26,27-tetranorcycloartane named gomboside E.

The molecular formula of compound **6** was determined to be $C_{38}H_{62}O_{12}$ on the basis of HRESIMS data (*m*/*z* 733.5020 $[M+Na]^+$, calc. for $C_{38}H_{62}O_{12}Na$, 733.4133). The NMR data (^{1}H, ^{13}C, COSY, HSQC, HMBC and ROESY) of **6** in comparison to those of **5** suggested that **6** differed only by the absence of acetyl group in the carbon C-6 (CH_2-6, δ_H 1.49, 0.78, δ_C 20.2). The sequence and linkage sites were confirmed from the HMBC spectrum, which showed long-range correlations between the anomeric proton signal at δ_H 4.29 (H-1′$_{xyl}$) and the carbon resonance at δ_C 87.4 (C-3) and between the second anomeric proton signal at δ_H 4.42 (H-1″$_{glc}$) and the carbon resonance at δ_C 81.2 (C-2′$_{xyl}$). Thus, the structure of **6** was characterized as 3-*O*-[β-D-glucopyranosyl(1 → 2)]-β-D-xylopyranosyl]-23α-methoxy-16β,23(*R*)-epoxy-4,25,26,27-tetranorcycloartane and was named gomboside F.

3. Conclusions

These results are in agreement with previous studies performed on *Astragalus* species (Özipek et al., 2005; Pistelli et al., 2003). The phytochemical investigation of *A. gombo* confirms the occurrence of cycloartane triterpene glycosides in the *Astragalus* genus. In fact, the present study reports the isolation of seven cycloartane glycosides of which six compounds (gombosides A–F) are described for the first time. However, it is not excluded that compound **4** (gomboside D) could be an artifact formed during the butanolic extraction. To the best of our knowledge, kaempferol 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[6-*O*-(3-hydroxy-3-methyl-glutaryl)-β-D-galactopyranoside] was previously identified only in *Astragalus gombiformis* (Montoro et al., 2013) while this is the first report of kaempferol 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranoside in the *Astragalus* genus.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured on a Perkin Elmer model 241 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance II spectrometer equipped with a cryoprobe and Bruker Advance 600 spectrometer at 500 and 600 MHz for ^{1}H, 125 and 150 MHz for ^{13}C. Complete assignments were performed on the basis of 2D NMR experiments (COSY, HSQC, HMBC and ROESY). Positive ESI mass spectra were produced on ion trap Bruker Esquire-LC. HRESIMS spectra were performed on a Bruker Micromass Q-TOF. A pre-packed C_{18} reversed-phase column (XTerra RP-18, 19 mm × 250 mm, 10 μm) was used for semi-preparative HPLC with a binary gradient elution (solvent A: H_2O and solvent B: MeCN) at 25 °C, a flow rate of 20 ml min^{-1} and the chromatogram was monitored at 205 nm and 250 nm. Column chromatography was carried out using Merck Kieselgel 60 (70–230 mesh), Merck Lobar Lichroprep RP-18 (40 μm × 63 μm) and Sephadex LH-20. Analytical TLC was carried out in silica gel plates (Merck Kieselgel 60 F_{254S}).

4.2. Plant material

The plant material (aerial parts) was collected in April 2009 in the Biskra area (Algeria) and was identified by Pr Bachir Oudjehih, Agronomic institute of the University of Batna, where a voucher specimen has been deposited (N° 663/LCCE).

4.3. Extraction and isolation

Part of the air-dried and powdered plant material of *A. gombo* (aerial parts; 1 kg) was macerated two times (10 l × 2, each 48 h) with EtOH–H_2O (70:30) at room temperature. After filtration, the filtrate was concentrated under vacuum at room temperature to obtain 500 mL. The solution was submitted to liquid–liquid fractioning using solvents (petroleum ether, EtOAc and *n*-butanol) with increasing polarities (each solvent, 500 mL × 3). Filtration and evaporation of different solvents produced 2.5 g of petroleum ether, 9.5 g of EtOAc and 25 g of *n*-butanol extracts.

The *n*-BuOH extract (14 g) was separated over a VLC chromatography (vacuum liquid chromatography) using reverse-phase material. Elution was performed with H_2O–MeOH (100:0 to 0:100) to give fourteen fractions (F1–F14). Fraction F10 (679 mg) was subjected to silica gel column chromatography with the solvent system CH_2Cl_2–MeOH (100:0 to 60:40) to give 8 sub-fractions (F10-1 to F10-8). The sub-fraction F10-1 (166 mg) was chromatographed by semi-preparative HPLC using a gradient of H_2O–acetonitrile (50:50, 50:50, 0:100, 0:100) to yield compounds

3 (R_t = 9.521 min, 80.7 mg) and **4** (R_t = 12.346 min, 32.4 mg). Sub-fraction F10-3 (95 mg) was purified by semi-preparative HPLC using a gradient of H_2O-acetonitrile (75:25, 44:56, 0:100) to yield compounds **1** (R_t = 5.851 min, 2 mg), **2** (R_t = 6.678 min, 2.5 mg), **5** (R_t = 7.743 min, 2.9 mg) and **6** (R_t = 8.728 min, 5.9 mg). Fraction F11 (300 mg) was subjected to CC on Sephadex LH-20 in $CHCl_3$ giving 5 sub-fractions (F11-1 to F11-5). Preparative TLC of sub-fraction F11-2 (50 mg), developed with $CHCl_3$–MeOH (90:10), afforded compound **7** (15 mg). Fraction F4 (379 mg) was applied to silica gel CC eluting with $CHCl_3$–MeOH (100:0, 90:10, 80:20, 75:25, 70:30), to obtain ten sub-fractions (F4-1 to F4-10). The sub-fraction F4-8 (30 mg) was chromatographed by semi-preparative HPLC using a gradient of H_2O–acetonitrile (80:20, 70:30) to give **8** (5 mg) and **9** (7 mg).

4.3.1. Gomboside A (**1**)

Amorphous powder; $[\alpha]_D^{20}$ + 30.0 (*c* 0.33, MeOH); ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), see Table 1; HRESIMS *m/z* [M+Na]$^+$ 809.5589 (calcd. for $C_{41}H_{70}O_{14}Na$, 809.4658); ESI-MS *m/z* 809 [M+Na]$^+$.

4.3.2. Gomboside B (**2**)

Amorphous powder; $[\alpha]_D^{20}$ – 2.4 (*c* 1.66, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz), see Table 1; HRESIMS *m/z* [M+Na]$^+$ 791.5493 (calcd. for $C_{41}H_{68}O_{13}Na$, 791.4552); ESI-MS *m/z* 791 [M+Na]$^+$.

4.3.3. Gomboside C (**3**)

Amorphous powder; $[\alpha]_D^{20}$ – 20.8 (*c* 2.5, CH_2Cl_2/MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; HRESIMS *m/z* [M+Na]$^+$ 629.4482 (calcd. for $C_{34}H_{54}O_9Na$, 629.3660); ESI-MS *m/z* 629 [M+Na]$^+$.

4.3.4. Gomboside D (**4**)

Amorphous powder; $[\alpha]_D^{20}$ – 5.5 (*c* 4.0, MeOH);); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; HRESIMS *m/z* [M+Na]$^+$ 671.5006 (calcd. for $C_{37}H_{60}O_9Na$, 671.4130); ESI-MS *m/z* 671 [M+Na]$^+$.

4.3.5. Gomboside E (**5**)

Amorphous powder; $[\alpha]_D^{20}$ – 14.5 (*c* 1.93, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz), see Table 3; HRESIMS *m/z* [M+Na]$^+$ 791.5141 (calcd. for $C_{40}H_{64}O_{14}Na$, 791.4188); ESI-MS *m/z* 791 [M+Na]$^+$.

4.3.6. Gomboside F (**6**)

Amorphous powder; $[\alpha]_D^{20}$ – 28.5 (*c* 2.95, CH_2Cl_2/MeOH); ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 3; HRESIMS *m/z* [M+Na]$^+$ 733.5020 (calcd. for $C_{38}H_{62}O_{12}Na$, 733.4133); ESI-MS *m/z* 733 [M+Na]$^+$.

4.4. Acid hydrolysis

Methanolic solutions of compounds **1** (1.5 mg), **2** (2 mg), **3** (20 mg), **4** (10 mg), **5** (2.5 mg) and **6** (5 mg) were refluxed separately with 5 ml of 3% H_2SO_4 in dry MeOH for 5 h. The solutions were neutralized with Na_2CO_3 and then extracted with EtOAc to give aqueous layers which were combined and repeatedly evaporated to dryness with MeOH. Two sugars were identified as xylose and glucose, by comparison with authentic samples on TLC in CH_2Cl_2–MeOH–H_2O (6:4:1) and *n*-BuOH–HOAc–H_2O (5:5:1). The purification of the two sugars was performed by preparative TLC using $CHCl_3$–MeOH–H_2O (70:30:3) as eluting solvent. The D configuration of xylose ($[\alpha]_D^{20}$ + 13.2) and glucose ($[\alpha]_D^{20}$ + 32.8) was established by comparison of their optical rotation values with reported literature data (Denizli et al., 2014).

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