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Triterpenoid saponins from *Piptadeniastrum africanum* (Hook. f.) Brenan

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ABSTRACT

One new triterpenoid saponin, named piptadeniaoside (**1**), along with two known saponins (**2**–**3**) have been isolated from the stem bark of *Piptadeniastrum africanum*. After previous isolation of flavone derivatives from this plant, new phytochemical investigations were performed for its saponin content. Their structures were established by direct interpretation of their spectral data, mainly HRESIMS, 1D NMR (^{1}H, ^{13}C NMR, DEPT) and 2D NMR (COSY, NOESY, HSQC and HMBC), and by comparison with the literature data.

1. Introduction

Piptadeniastrum africanum Hook.f. Brenan or *Piptadenia africana* Hook.f. (Mimosaceae), is a large emergent tree up to 50 m high widely distributed in the West and Central Africa (Burkill, 1995). In Cameroon it is used for the management of constipation (Noumi and Yomi, 2001), anaemia, lumbago, meningitis, convulsion and wound treatment (Betti, 2002). It is also reported to induce abortion by its oxytocic effects (Noumi and Tchakonang, 2001). Previous phytochemical studies have shown the presence of macrolactone and flavone derivatives (Mbouangouere et al., 2007, 2008). As a part of our continuing studies on saponins from Cameroonian medicinal plants (Tapondjou et al., 2002, 2003, 2005, 2006; Mitaine-Offer et al., 2004; Noté et al., 2009a, 2009b, 2010), we have investigated the stem bark of *P. africanum*.

In this paper, we report the isolation and structure elucidation of one new triterpene glycoside, named piptadeniaoside (**1**) along with two known saponins, 3-*O*-*β*-[*α*-L-arabinopyranosyl-(1→2)-*α*-L-arabinopyranosyl-(1→3)-*β*-D-glucopyranosyl]maslinic acid-28-[*β*-D-glucopyranosyl-(1→2)-*α*-L-rhamnopyranosyl] ester (**2**), and 3-*O*-*β*-[*α*-L-arabinopyranosyl-(1→2)-*α*-L-arabinopyranosyl-(1→3)-*β*-D-glucopyranosyl]maslinic acid-28-[*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→2)-*α*-L-rhamnopyranosyl] ester (**3**) (Tchivounda et al., 1991). To the best of our knowledge, this should be the first report on the saponin content of this plant.

2. Results and discussion

The air-dried powdered stem barks of *P. africanum* (300 g) were extracted with MeOH in soxhlet and after evaporation of the solvent, the methanol extract was partitioned against *n*-BuOH saturated with water. The *n*-BuOH fraction was then submitted to vacuum-liquid chromatography (VLC) on reversed phase silica gel yielding a methanol fraction that was subjected to VLC on silica gel. Purification of the eluted fractions by repetitive medium-pressure liquid chromatography (MPLC) over silica gel or/and RP-18 afforded compounds **1** (12.6 mg), **2** (4.8 mg) and **3** (5.5 mg). Their structures (Fig. 1) were elucidated by extensive NMR techniques, mainly 1D and 2D NMR (^{1}H, ^{13}C NMR, DEPT, COSY, HSQC and HMBC) experiments, and HRESIMS and by comparison with the literature data.

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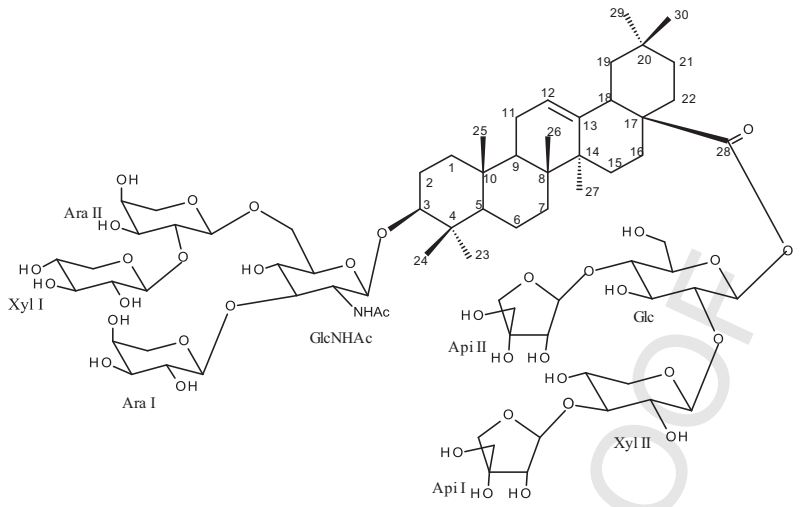

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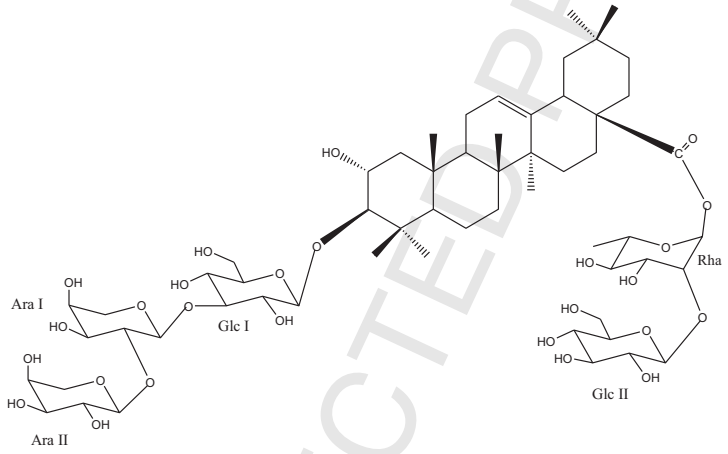

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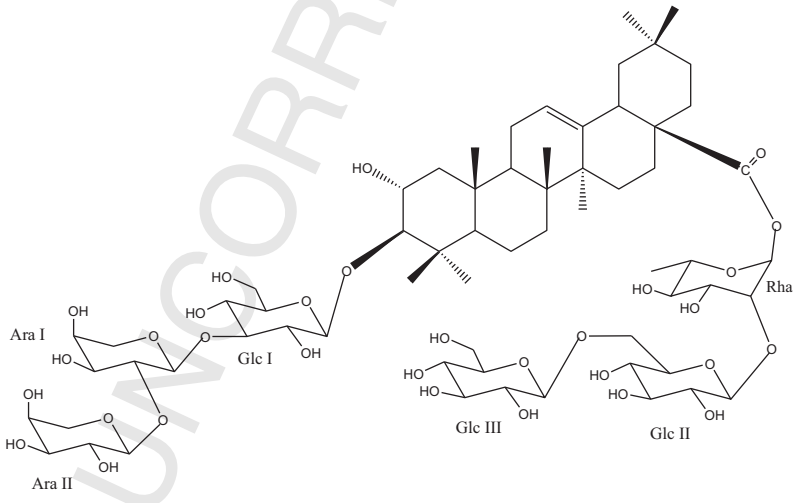

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Fig. 1. Structures of compounds 1–3.

Piptadeniaoside (**1**), isolated as white amorphous powder, exhibited in its HRESIMS (positive-ion mode) a pseudo-molecular ion peak at *m*/*z* 1636.7352 $[M+Na]^+$ (calcd for $C_{74}H_{119}O_{37}NNa$ 1636.7359), in accordance with a molecular formula of $C_{74}H_{119}O_{37}N$. Its FABMS (negative-ion mode) showed a quasi-molecular ion peak at *m*/*z* 1612 $[M-H]^-$, indicating a molecular weight of 1613. Other significant fragment ion peaks were observed at *m*/*z* 1481 $[(M-H)-132]^-$, 1349 $[(M-H)-132-132]^-$,

which indicate of the loss of two pentosyl units. Extensive analysis of 1D and 2D NMR spectra (^{1}H NMR, ^{13}C NMR, DEPT, COSY, NOESY, HSQC and HMBC) indicated the presence of seven tertiary methyl groups at δ 0.70, 0.82, 0.83, 0.86, 0.87, 1.18, and 1.19, an olefinic broad triplet proton at δ 5.31 (brt, *J* = 3.5 Hz, H-12) coupled to a carbon at δ 122.5 (C-12), a quaternary carbon at δ 143.8 (C-13), one oxymethine protons at δ 3.26 (brd, *J* = 7.6 Hz, H-3) which are typical signals of an olean-12-ene skeleton. The aglycone moiety of **1** was thus recognized to be oleanolic acid by ^{1}H NMR and ^{13}C NMR analyses (Table 1) using the correlations observed in COSY, NOESY, HSQC, and HMBC spectra, and was in full agreement with literature data (Carpani et al., 1989; Nigam et al., 1997; Mimaki et al., 2004). The chemical shifts of C-3 (δ 89.2) and C-28 (δ 176.4) (Table 1) indicated that **1** is a bidesmosidic glycoside (Woldemichael and Wink, 2001; Sahu and Achari, 2001) of oleanolic acid with sugar chains linked to C-3 and C-28 of the aglycone through an ether and ester bond, respectively.

The ^{1}H NMR spectrum of compound **1** showed eight anomeric signals at δ 4.97 (d, *J* = 7.1 Hz), 4.92 (d, *J* = 7.6 Hz), 4.99 (d, *J* = 2.9 Hz), 4.83 (d, *J* = 7.6 Hz), 5.96 (d, *J* = 8.1 Hz), 5.28 (d, *J* = 7.6 Hz), 6.12 (d, *J* = 2.1 Hz), and 5.91 (d, *J* = 3.8 Hz), which correlated with eight anomeric carbon atom resonances at δ 104.0, 104.8, 102.6, 105.9, 93.0, 104.3, 110.8, and 110.2, respectively in the HSQC spectrum (Table 2). From the anomeric proton of each monosaccharide moiety, all the protons within each spin system were assigned by means of COSY, NOESY, HSQC, and HMBC experiments. Units of one 2-acetamido-2-deoxy-β-glucopyranosyl (GlcNAc), one β-glucopyranosyl (Glc), two β-xylopyranosyl (Xyl I and Xyl II), two β-apiofuranosyl (Api I and Api II), and one α- and β-arabinopyranosyl (Ara I and Ara II, respectively), were identified (Table 2). The anomeric protons of Ara I were determined to have the α-orientation based on its relatively large $^3J_{H\text{-}1,\ H\text{-}2}$ value of 7.6 Hz, whereas Ara II was β-orientated based on its relatively small $^3J_{H\text{-}1,\ H\text{-}2}$ value of 2.9 Hz (Tene et al., 2011). The absolute configuration of these sugar moieties were determined to be D for GlcNAc, Api, and Xyl, and L for Ara by GC analysis (Section 3). The sequencing of the glycoside chains were achieved by analysis of HMBC and NOESY experiments. The cross peak correlations observed in the HMBC spectrum between H-1 (δ 4.97) of GlcNAc and C-3 (δ 89.2) of the aglycone, and in the NOESY spectrum between H-1 (δ 4.97) of GlcNAc and H-3 (δ 3.26) of oleanolic acid, suggested that GlcNAc was directly attached to C-3 of the aglycone. Moreover, the HMBC correlation observed between H-1 (δ 4.92) of Ara I and C-3 (δ 79.5) of GlcNAc established the connectivity between the two sugar units, which was confirmed by the reverse HMBC correlation observed between H-3 (δ 4.34) of GlcNAc and C-1 (δ 104.8) of Ara I. On the other hand, the HMBC correlation observed between H-1 (δ 4.99) of Ara II and C-6 (δ 67.8) of GlcNAc allowed us to locate Ara II at C-6 of GlcNHAc. This was supported by the NOESY correlation observed between H-1 (δ 4.99) of Ara II and H-6a (δ 4.10) of GlcNAc. This Ara II was substituted at its C-2 by Xyl I, as evidenced by the direct and reverse correlations observed in the HMBC spectrum between H-2 (δ 4.38) of Ara II and C-1 (δ 105.9) of Xyl I, and between H-1 (δ 4.83) of Xyl I and C-2 (δ 80.5) of Ara II (Fig. 2). The terminal positions of Xyl I and Ara I was evidenced by the absence of any ^{13}C NMR glycosylation shifts for these sugar moieties. Thus, the tetrasaccharide β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-[α-L-arabinopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranosyl moiety was established to be linked at C-3 of the aglycone (Fig. 2). Furthermore, the cross peak observed in the HSQC spectrum at δ 5.96/δ 93.0 (Glc H-1/C-1) suggested that this sugar should be directly attached to C-28 through an ester bond. The correlations observed in the HMBC spectrum between H-2 (δ 4.20) of Glc and C-1 (δ 104.3) of Xyl II, and in the NOESY spectrum between H-2 (δ 4.20) of Glc and H-1 (δ 5.28) of Xyl II allowed us to locate Xyl II at C-2 of Glc. Moreover, the NOESY correlation observed between H-3 (δ 4.04) of Xyl II and H-1 (δ 6.12) of Api I, and the HMBC correlation observed between H-1 (δ_H 5.91) of Api II and C-4 (δ_C 79.5) of Glc, were useful to attach Api I and Api II at C-3 and C-4 of Xyl II and Glc, respectively. In the same way, the absence of any ^{13}C NMR glycosylation shifts for Api I and Api II supported their terminal positions. Thus, the tetrasaccharide β-D-apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→2)-[β-D-apiofuranosyl-(1→4)]-β-D-glucopyranosyl moiety was established to be linked at C-28 of the aglycone (Fig. 2). Based on the above evidences, the structure of **1** was established as 3-*O*-β-[β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-[α-L-arabinopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranosyl]-28-*O*-[β-D-apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→2)-[β-D-a1piofuranosyl-(1→4)]-β-D-glucopyranosyl]-oleanolic acid.

Table 1
NMR spectroscopic data (600 MHz for ^{1}H and 150 MHz for ^{13}C) for the aglycone moieties of compounds **1**and **2** (δ in ppm and *J* in Hz)[a].

	1		**2**	
No C	δ_C	δ_H	δ_C	δ_H
1	38.4	1.39[b]	46.4	1.05; 2.12 (d, 4.0)
2	25.9	1.64; 2.03	66.8	3.62 (ddd, 11.2, 9.2, 4.0)
3	89.2	3.26 (brd, 7.6)	94.6	3.20 (dd, 9.2, 11.2)
4	38.8	–	40.5	–
5	55.5	0.64	55.0	0.64
6	18.0	1.22; 1.46	18.3	1.12; 1.36
7	32.8	1.39[b]	32.8	1.36; 1.46
8	38.8	–	39.4	–
9	47.6	1.53	47.6	1.56
10	36.5	–	37.3	–
11	23.1	1.83[b]	23.8	1.84
12	122.5	5.31 (brt, 3.5)	123.0	5.34 (brt, 3.2)
13	143.8	–	143.2	–
14	42.0	–	41.9	–
15	28.2	1.11; 2.06	36.0	1.03; 1.83
16	23.1	1.79	27.5	0.82
17	46.7	–	47.1	–
18	41.6	3.03 (d, 11.6)	41.7	3.06
19	45.8	1.13; 1.67	45.6	1.12; 1.66
20	30.3	–	30.5	–
21	32.8	1.39[b]	33.3	[b]
22	31.6	1.57; 1.81	32.8	1.36; 1.46
23	27.8	1.18 (s)	27.9	1.22 (s)
24	16.6	0.87 (s)	17.8	0.81 (s)
25	15.0	0.70 (s)	15.2	0.77 (s)
26	16.6	0.86 (s)	17.5	0.79 (s)
27	25.9	1.19 (s)	25.6	1.12 (s)
28	176.4	–	176.3	–
29	32.8	0.83 (s)	32.7	0.86 (s)
30	23.2	0.82 (s)	23.1	0.82 (s)

Assignments were based on the HMBC, HSQC, COSY and DEPT experiments.
[a]Overlapped ^{1}H NMR signals are reported without designated multiplicity.
[b]not determined.

By extensive analysis of their NMR data (^{1}H, ^{13}C NMR, DEPT, COSY, NOESY, HSQC and HMBC) and mass spectrometry, compounds **2** and **3** were identified as 3-*O*-β-[α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→3)-β-D-glucopyranosyl]maslinic acid-28-[β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl] ester and 3-*O*-β-[α-L-arabinopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→3)-β-D-glucopyranosyl]maslinic acid-28-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl] ester, respectively (Tchivounda et al., 1991).

In conclusion, the present study is the first report on the saponin content of *P. africanum*, and the presence of the two known compounds (**2** and **3**) in this species, previously isolated from *Cylicodiscus gabunensis* (Tchivounda et al., 1991) may indicate a close relationship between the two species of Mimosaceae subfamily.

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Table 2
NMR spectroscopic data (600 MHz for ^{1}H and 150 MHz for ^{13}C) for the sugar moieties of compounds **1–3** (δ in ppm and J in Hz)[a].

Position	**1**		**2**		**3**	
3-O-Sugars	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
Glc I or GlcNAc 1	104.0	4.97 (d, 7.1)	104.9	5.09 (d, 7.6)	105.0	5.07 (d, 7.6)
2	57.0	4.40	73.9	4.28	73.9	4.27
3	79.5	4.34	86.9	4.28	87.0	4.25
4	73.0	4.05	70.8	4.37	70.6	4.35
5	77.0	3.93	77.9	4.04	77.5	3.95
6	67.8	4.10; 4.22	62.0	4.44; 4.62	62.0	4.43; 4.58
$COCH_3$	23.2	2.17				
Ara I 1	104.8	4.92 (d, 7.6)	105.2	5.46 (d, 7.6)	105.2	5.48 (d, 7.6)
2	71.8	4.34	76.7	4.70	76.8	4.73
3	73.7	4.08	72.5	4.30	72.6	4.32
4	67.8	4.22	69.2	4.44	69.5	4.46
5	65.2	4.18 (d, 9.2); 3.66	66.9	3.90 (d, 11.6); 4.18	66.8	3.92 (d, 11.8); 4.16
Ara II 1	102.6	4.99 (d, 2.9)	100.2	6.16 (d, 3.2)	100.3	6.13 (d, 3.3)
2	80.5	4.38	69.9	4.87	69.5	4.85
3	74.2	4.18	72.9	4.52	72.6	4.53
4	67.8	4.18	69.9	4.83	70.0	4.86
5	66.2	3.56; 4.28 (d, 9.2)	64.1	4.20 (d, 10.4); 5.21	64.2	4.22 (d, 10.5); 5.18
Xyl I 1	105.9	4.83 (d, 7.6)				
2	75.1	4.00				
3	77.0	3.92				
4	70.4	4.14				
5	66.7	3.40 (t 11.2, \) 4.47				
28-O-Sugars						
Rha 1			93.9	6.92 (brs)	93.8	6.94 (brs)
2			80.5	4.83	80.6	4.58
3			72.5	4.30	72.1	4.44
4			72.9	4.52	73.1	4.24
5			71.9	4.48	71.9	4.22
6			18.2	1.82 (d, 5.9)	18.2	1.56
Glc II 1	93.0	5.96 (d, 8.1)	106.4	5.17 (d, 7.7)	106.3	5.19 (d, 7.6)
2	78.0	4.20	75.0	4.30	75.0	3.95
3	75.9	4.14	77.7	4.22	77.4	4.10
4	79.5	4.34	70.8	4.37	70.5	4.29
5	76.6	3.70	77.9	4.07	76.6	3.90
6	60.5	4.18; 4.39	61.8	4.40; 4.73	69.5	4.16; 4.60
Xyl II or Glc III 1	104.3	5.28 (d,7.6)			104.8	4.85 (d, 7.6)
2	74.3	3.92			74.5	3.90
3	84.6	4.04			77.4	4.12
4	70.4	4.11			70.9	4.06
5	66.2	3.56; 4.28 (d, 9.2)			77.5	3.80
Api I 1	110.8	6.12 (d, 2.1)			61.8	4.12; 4.45
2	77.5	4.71				
3	80.0	–				
4	74.6	4.68; 4.28				
5	64.7	3.99; 4.10				
Api II 1	110.2	5.91 (d, 3.8)				
2	77.0	4.59				
3	79.5	–				
4	74.2	4.52; 4.19				
5	63.5	3.92; 4.02				

Assignments were based on the HMBC, HSQC, COSY and DEPT experiments.
[a]Overlapped ^{1}H NMR signals are reported without designated multiplicity.

3. Experimental

3.1. General experimental procedure

Optical rotations were measured with a AA-10R automatic polarimeter. For 1D and 2D NMR spectra, and mass spectrum, see Noté et al. (2009a). Thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC) were performed on precoated silica gel plates (60 F_{254}, Merck) (system solvent: $CHCl_3$–MeOH–H_2O (60:32:7)). Vacuum-liquid chromatography (VLC) was carried out using RP-18 silica gel 60 (25–40 μm). Medium-pressure liquid chromatography (MPLC) was carried out using silica gel 60 (15– 40 μm) with Gilson pump M305 and Büchi columns (46 × 2.5 cm and 46 × 1.5 cm) for purifications. Further purifications were achieved using RP-18 silica gel 60 (25–40 μm) with Büchi Pump Manager C-605 having two pumps (2x Büchi Pump Module C-601) and one Büchi Fraction Collector C-660.

3.2. Plant material

The stem bark of *P. africanum* Hook. f. Brenan was collected at Eloundem, Yaoundé peripheral quarter, in Cameroon in September 2007 under the guidance of Mr. Victor Nana, a botanist of the National Herbarium of Cameroon (NHC), where one voucher specimen (No. 09566/HNC) was deposited.

3.3. Extraction and isolation

Air-dried finely powdered stem bark (300 g) of *P. africanum* was extracted with MeOH in soxhlet apparatus. The methanolic solution was then evaporated to dryness under reduced pressure to give 11.22 g of brown residue. This residue was suspended in 200 mL of water and partitioned against *n*-BuOH sat. H_2O (3 × 200 mL). The *n*-BuOH soluble phase was evaporated to dryness affording 9.97 g of a brown gum which was taken in a

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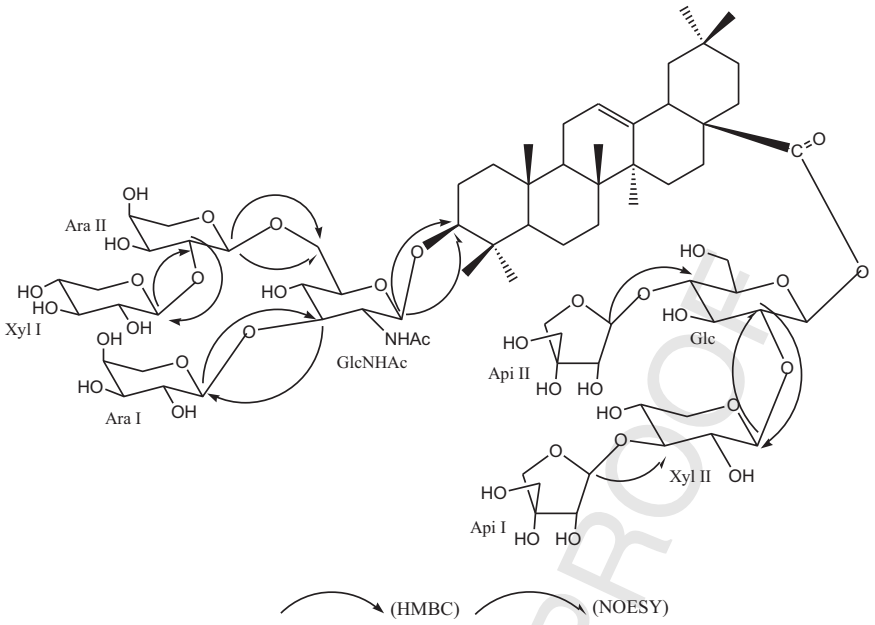

Fig. 2. Key HMBC and NOESY correlations for compound **1**.

minimum of water (10 mL) and then submitted to vacuum-liquid chromatography (VLC) using RP-18 (25–40 μm) eluting with H_2O, 50% MeOH, and 100% MeOH. The 100% MeOH eluated was evaporated to dryness affording 8.97 g of crude saponin mixture. Part of this saponin mixture (2.83 g) was then submitted to VLC using silica gel 60 (15–40 μm) eluted with $CHCl_3$–MeOH–H_2O (80:20:2; 70:30:5; 60:32:7) to give 15 fractions (I–XVI). Part of fraction VI (30.0 mg) was purified by repeated MPLC using RP-18 (25–40 μm) eluted with a gradient of MeOH–H_2O (60:30 → 80:20) affording **1** (12.6 mg). Part of fractions XV (27.0 mg) and XVI (24.9 mg) were submitted to MPLC using RP-18 (25–40 μm) eluted with a gradient of MeOH–H_2O (70:30 → 100:0) affording subfractions XVb (17.0 mg) and XVIc (15.4 mg) which were purified by MPLC using silica gel eluted with $CHCl_3$–MeOH–H_2O (60:32:7) to give **2** (4.8 mg) and **3** (5.5 mg), respectively.

3.4. 3-O-[β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-[α-L-arabinopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranosyl]-28-O-[β-D-apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→2)-[β-D-apiofuranosyl-(1→4)]-β-D-glucopyranosyl]-oleanolic (**1**)

Amorphous white powder; $[\alpha]^{25}_D$–22.3 (MeOH; *c* 0.15); ^{1}H NMR (C_5D_5N, 600 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz), see Tables 1 and 2; HRESIMS *m/z*: 1636.7352 $[M+Na]^+$ (calcd for $C_{74}H_{119}O_{37}NNa$ 1636.7359); FABMS (negative-ion mode) *m/z*: 1612 $[M-H]^-$, 1481 $[(M-H)-132]^-$, 1349 $[(M-H)-132-132]^-$.

3.4. Acid hydrolysis of compound **1**

Compound **1** (2 mg) was hydrolyzed with 2 N aq. CF_3COOH (5 mL) for 3 h at 95 °C. After extraction with CH_2Cl_2 (3 × 5 mL), the aq. layer was repeatedly evaporated to dryness with MeOH until neutral, and then analyzed by TLC over silica gel ($CHCl_3$–MeOH–H_2O, 8:5:1) by comparison with authentic samples. The trimethylsilyl thiazolidine derivatives of the sugar residue of each compound were prepared and analyzed by GC (Haddad et al., 2003). The absolute configurations were determined by comparing the retention times with thiazolidine derivatives prepared in a similar way from standard sugars (Sigma–Aldrich). The following sugars were detected: 2-amino-2-deoxy-D-glucose, L-arabinose, D-glucose, D-apiose, and D-xylose.

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