Phytochemistry Letters 11 (2015) 37–42

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Triterpenoid saponins from *Albizia boromoensis* Aubrév. & Pellegr

Olivier Placide Noté [a,b,*], Dong Jihu [c], Cyril Antheaume [d], Dominique Guillaume [e], Dieudonné Emmanuel Pegnyemb [b], Marie Claude Kilhoffer [c], Annelise Lobstein [a]

[a] *Pharmacognosie et Molécules Naturelles Bioactives, Laboratoire d'Innovation Thérapeutique, UMR 7200, CNRS-Université de Strasbourg, Faculté de Pharmacie, 74 route du Rhin, F-67401 Illkirch Cedex, France*
[b] *Laboratoire de Pharmacochimie des Substances Naturelles, Département de Chimie Organique, Faculté de Sciences, Université de Yaoundé, BP 812, Yaoundé, Cameroon*
[c] *Chimie-Biologie Intégrative, Laboratoire d'Innovation Thérapeutique, UMR 7200, CNRS-Université de Strasbourg, Faculté de Pharmacie, 74 route du Rhin, F-67401 Illkirch Cedex, France*
[d] *Service Commun d'Analyse, UMR 7200, CNRS-Université de Strasbourg, Faculté de Pharmacie, 74 route du Rhin, F-67401 Illkirch Cedex, France*
[e] *Laboratoire de Chimie Thérapeutique, UMR 6229, 51 rue Congnacq-Jay, 51100 Reims, France*

ARTICLE INFO

Article history:
Received 23 August 2014
Received in revised form 5 November 2014
Accepted 14 November 2014
Available online 26 November 2014

Keywords:
Mimosaceae
Albizia boromoensis
Triterpenoid saponins
Boromoenosides
NMR

ABSTRACT

As part of our search of new bioactive saponins from Cameroonian medicinal plants, phytochemical investigation of the roots of *Albizia boromoensis* led to the isolation of four new oleanane-type saponins, named boromoenosides A–D (**1–4**). Their structures were established by direct interpretation of their spectral data, mainly HRESIMS, 1D NMR (^{1}H, ^{13}C NMR, and DEPT) and 2D NMR (COSY, NOESY, HSQC and HMBC), and by comparison with the literature data. All isolated saponins were assayed for their cytotoxicity against U-87 MG human glioblastoma cell lines and TG1 glioblastoma stem-like cells with no positive activity detected.

1. Introduction

Albizia boromoensis Aubrév. & Pellegr. (Mimosaceae) is a large emergent tree of the legume family. It is found growing naturally in Senegal, Soudan, and Cameroon where it is harvested for timber (Arbonnier, 2009). No ethnomedicinal use of this plant is documented. Its chemical constituents have not been previously investigated but phytochemical studies on related species of *Albizia* genus revealed the presence of triterpenoid saponins (Abdel-Kader et al., 2001; Haddad et al., 2003; Liu et al., 2009; Krief et al., 2005; Zheng et al., 2006; Melek et al., 2007; Cao et al., 2007; Noté et al., 2009; Miyase et al., 2010). As a part of our continuing studies on saponins from Cameroonian medicinal plants (Noté et al., 2013), we have investigated the saponins content of the roots of *A. boromoensis*.

In the present paper, we report the isolation and structure characterization of four new triterpenoid saponins, named boromoenosides A–D (**1–4**) having oleanolic acid as aglycone.

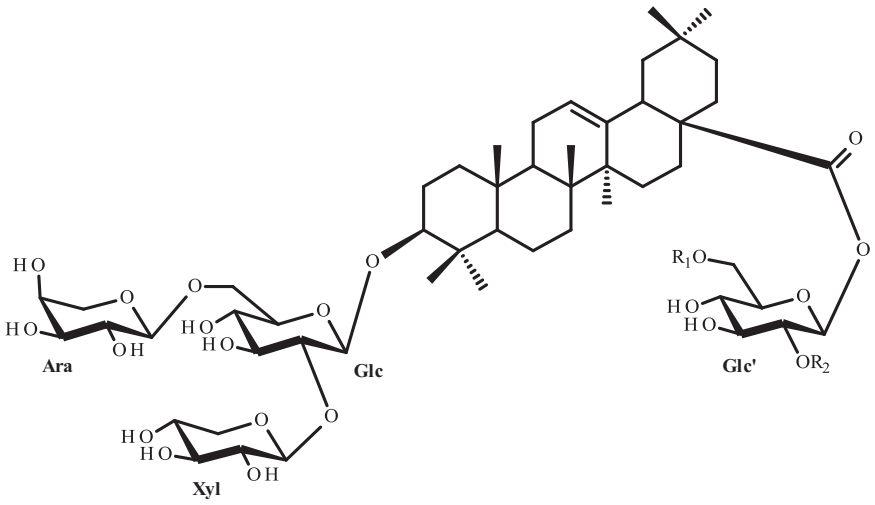
2. Results and discussion

The air-dried powdered roots of *A. boromoensis* (200 g) were extracted with 70% EtOH in soxhlet and after evaporation of the solvent, the hydroalcoholic 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 a VLC on silica gel. Purification of the eluted fractions by semipreparative high pressure liquid chromatography (HPLC) afforded compounds **1** (7.9 mg), **2** (8.5 mg), **3** (7.2 mg), and **4** (5.3 mg). Their structures (Fig. 1) were established by extensive analysis of NMR techniques, mainly 1D NMR (^{1}H, ^{13}C NMR, and DEPT) and 2D NMR (COSY, NOESY, HSQC and HMBC) experiments, and HRESIMS and by comparison with the literature data.

Boromoenoside A (**1**), obtained as a white amorphous powder, was assigned a $C_{64}H_{104}O_{31}$ molecular formula, as deduced from the $[M+NH_4]^+$ ion at *m/z* 1386.6789 in the positive ion mode HRESIMS.

* Corresponding author at: Laboratoire de Pharmacochimie des Substances Naturelles, Département de Chimie Organique, Faculté de Sciences, Université de Yaoundé, BP 812, Yaoundé, Cameroon. Tel.: +237 72700015; fax: +237 22239588.
E-mail address: oliviernote1@yahoo.fr (O.P. Noté).

http://dx.doi.org/10.1016/j.phytol.2014.11.004
1874-3900/



	R_1	R_2
(**1**)	Glc	Glc
(**2**)	H	Glc(1→6)Glc-
(**3**)	H	Glc
(**4**)	Glc	H

Fig. 1. Structures of isolated compounds **1–4**.

The ^{1}H NMR and ^{13}C NMR data of boromoenoside A displayed resonances due to the aglycone part characteristic for oleanolic acid (Table 1), a common triterpene of triterpene glycosides, which were in full agreement with literature data (Nigam et al., 1997; Mimaki et al., 2004). The sugar moieties of **1** were determined to be D-glucose (Glc), L-arabinose (Ara) and D-xylose (Xyl) by acid hydrolysis followed GC analysis of their trimethylsilylated derivatives by comparison with authentic samples (Section 3). The sugar portion of compound **1** exhibited in the ^{1}H NMR spectrum, six anomeric proton signals at δ 4.84 [d, J = 7.6 Hz,

Table 1
NMR spectroscopic data (500 MHz for ^{1}H and 125 MHz for ^{13}C) for the aglycone moieties of compounds **1–4** (δ in ppm and J in Hz).[a]

No.	1		2		3		4	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	38.9	0.98; 1.51	39.0	0.97; 1.50	38.9	0.97; nf	38.9	0.97; 1.49
2	27.0	1.88; 2.33	27.0	1.88; 2.32	27.0	1.87; 2.33	27.0	1.92; 2.34
3	89.5	3.16 (brd, 11.8)	89.7	3.15	89.4	3.17 (brd, 12.6)	89.7	3.20 (d, 11.5)
4	39.5	–	39.5	–	39.5	–	39.8	–
5	56.1	0.62 (brd, 11.5)	56.1	0.63 (brd, 11.8)	56.1	0.63 (brd, 11.8)	56.1	0.64 (brd, 9.8)
6	18.8	1.26; 1.46	18.7	1.21; 1.44	18.9	1.21; 1.45	18.7	1.22; 1.40
7	33.4	1.44; 1.60	33.2	1.41; 1.53	33.5	1.44; 1.56	33.4	1.37; 1.53
8	42.2	–	42.4	–	42.4	–	42.3	–
9	48.3	1.59 (d-like, 9.3)	48.2	1.58 (d-like, 8.8)	48.2	1.58 (t-like, 9.8; 8.2)	48.2	1.57 (t-like, 9.8; 8.2)
10	37.2	–	37.1	–	37.2	–	37.1	–
11	24.0	1.89	24.1	1.87	24.1	0.94; 1.90	24.0	1.87
12	122.9	5.41 (brs)	129.9	5.40 (brs)	122.6	5.41 (brs 5.7)	123.3	5.39 (brs)
13	144.7	–	144.7	–	144.7	–	144.6	–
14	42.4	–	42.4	–	42.4	–	42.3	–
15	29.4	1.39; 2.27	29.4	1.37; 2.26	29.4	1.40; nf	29.4	nf; nf
16	23.3	2.12; 2.33	23.3	2.12; 2.33	23.3	nf; nf	23.3	nf; nf
17	47.3	–	47.3	–	47.3	–	47.3	–
18	42.0	3.19 (d, 11.5)	42.0	3.17	42.1	3.19	41.9	3.20
19	46.6	1.26; 1.77	46.7	1.26; 1.79	46.6	1.28; nf	46.6	1.27; 1.76
20	31.0	–	31.0	–	31.0	–	31.0	–
21	34.4	1.07; 1.33	34.3	1.08; 1.33	34.4	1.07; 1.39	34.2	1.12; 1.36
22	32.6	1.74; 1.86	32.5	1.75; 1.86	32.5	1.79; nf	32.8	1.78; 1.92
23	28.5	1.18 (s)	28.4	1.27 (s)	28.5	1.20 (s)	28.4	1.28 (s)
24	17.1	1.04 (s)	17.1	1.12 (s)	17.1	1.02 (s)	17.1	1.11 (s)
25	15.8	0.80 (s)	15.8	0.75 (s)	15.8	0.79 (s)	15.9	0.74 (s)
26	17.7	1.01 (s)	17.7	0.99 (s)	17.7	1.03 (s)	17.8	1.01 (s)
27	26.4	1.22 (s)	26.4	1.21(s)	26.4	1.23 (s)	26.4	1.21 (s)
28	176.9	–	176.9	–	176.8	–	176.8	–
29	33.4	0.89 (s)	33.4	0.89 (s)	33.5	0.92 (s)	33.4	0.92 (s)
30	24.1	0.88 (s)	24.0	0.88 (s)	24.1	0.90 (s)	24.0	0.91 (s)

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

glucose (Glc)], 4.93 [d, J = 6.5 Hz, arabinose (Ara)], 5.38 [d, J = 7.4 Hz, xylose (Xyl)], 6.16 [d, J = 8.2 Hz, glucose (Glc′)], 5.71 [d, J = 7.7 Hz, glucose (Glc″)], and 5.02 [d, J = 7.7 Hz, glucose (Glc‴)]. Their associated ^{13}C resonances assigned from HSQC and HMBC experiments were at δ 104.9, 105.6, 106.3, 93.8, 104.9, and 105.6, respectively. All proton signals due to the sugar moieties were identified by careful analysis of COSY, TOCSY and NOESY spectra, and the carbon signals were assigned based on HSQC and further confirmed by HMBC spectrum (Table 2). Data from the above experiments indicated that these six sugar residues were in their pyranose form. The relative large $^{3}J_{H\text{-}1,H\text{-}2}$ values of these sugar moieties (6.1–8.2 Hz) in their pyranose form indicated a β anomeric configuration for all Glc and Xyl moieties, and an α anomeric configuration for Ara (Mimaki et al., 2004; Tené et al., 2011). The observation of the ^{13}C NMR signals at δ 89.5 (C-3 of aglycone) and 176.9 (C-28 of the aglycone) demonstrated that **1** is a bidesmosidic glycoside of oleanolic acid (Sahu and Achari, 2001; Tené et al., 2011) with sugar chains linked to C-3 and C-28 of the aglycone through an ether and ester bond, respectively. The linkage points of the sugar units to each other and to C-3 of the aglycone were established from the HMBC correlations of signals at δ 4.84 (H-1 of Glc1) with δ 89.5 (C-3 of the aglycone), δ 4.93 (H-1 of Ara) with δ 70.1 (C-6 of Glc) and δ 5.38 (H-1 of Xyl) with δ 83.3 (C-2 of Glc). This was supported by the NOESY correlations of signals at δ 4.84 (H-1 of Glc) with δ 3.16 and δ 1.18 (H-3 and H_3-23 of the aglycone, respectively), δ 4.24 (H-6a of Glc) with δ 4.93 (H-1of Ara) and δ 5.38 (H-1 of Xyl) with δ 4.19 (H-2 of Glc). Accordingly, the sugar arrangement in the 3-*O*-saccharide chain was established as α-L-arabinopyranosyl-(1→6)-[β-D-xylopyranosyl-(1→2)]-β-D-glucopyranosyl (Fig. 2). The sequence and binding sites of the sugar units at C-28 were determined by following HMBC correlations: δ 6.16 (H-1 of Glc′) with δ 176.9 (C-28 of the aglycone), δ 5.71 (H-1 of Glc″) with δ 69.3 (C-6 of Glc′) and δ 5.02 (H-1 of Glc‴) with δ 78.5 (C-2 of Glc′). Therefore, the sugar sequence at C-28 of the aglycone was determined as β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl. Thus, the structure of boromoenoside A (**1**), was assigned as 3-*O*-β-[α-L-arabinopyranosyl-(1→6)-[β-D-xylopyranosyl-(1→2)]-β-D-glucopyranosyl]-28-*O*-[β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl]-oleanolic acid.

The analysis of ^{13}C NMR spectra showed that the trisaccharide chain was common to all four saponins and placed at C-3 of the aglycone. It will not be further discussed in the structural elucidation of compounds **2**–**4**.

The HRESIMS spectrum ($[M+NH_4]^+$ at m/z 1386.6575) of boromoenoside B (**2**), obtained as a white amorphous powder, was identical to that obtained for compound **1**, suggesting that these compounds were isomers with $C_{64}H_{104}O_{31}$ formula. Comparison of their NMR spectra showed that the sugar directly

Table 2
NMR spectroscopic data (500 MHz for ^{1}H and 125 MHz for ^{13}C) for the sugar moieties of compounds **1–5** (δ in ppm and J in Hz).[a]

Position	**1**		**2**		**3**		**4**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
3-O-sugars								
Glc-1	104.9	4.84 (d, 7.6)	105.0	4.84 (d, 7.4)	105.0	4.84 (d, 7.4)	105.0	4.84 (d, 7.3)
2	83.3	4.19	83.2	4.19	83.3	4.19	83.3	4.19
3	77.7	4.14	77.7	4.11	77.7	4.14	77.7	4.14
4	72.2	4.08	72.2	4.09	72.2	4.08	72.2	4.08
5	76.7	4.06	76.7	4.07	76.7	4.06	76.7	4.06
6	70.1	4.24; 4.85 (t, 10.9; 7.6)	69.9	4.25; 4.84 (brd, 10.4)	70.0	4.25; 4.86	70.0	4.26; 4.84
Ara-1	105.6	4.93 (d, 6.5)	105.6	4.93 (d, 6.5)	105.6	4.94 (d, 6.5)	105.6	4.94 (d, 6.4)
2	72.5	4.49	72.4	4.48	72.5	4.49	72.5	4.49
3	71.9	4.08	71.6	4.09	71.9	4.08	71.9	4.08
4	74.6	4.20	74.6	4.20	74.7	4.20	74.6	4.20
5	64.2	4.43; 4.74 (d, 9.8)	64.2	4.43; 4.72 (d, 10.3)	64.1	4.44; 4.71	64.2	4.43; 4.74
Xyl-1	106.3	5.38 (d, 7.4)	106.2	5.37 (d, 7.7)	106.3	5.37 (d, 7.5)	106.3	5.39 (d, 7.5)
2	73.1	4.15	73.1	4.11	73.2	4.15	73.1	4.15
3	78.7	4.04	78.4	4.04	78.2	4.03	78.7	4.04
4	71.8	4.08	71.8	4.09	71.8	4.08	71.8	4.08
5	66.7	4.32; 3.78 (d, 11.0)	66.6	4.33; 3.77 (brd, 10.3)	66.6	4.34; 3.78 (d, 10.3)	66.6	4.34; 3.78
28-O-Sugars								
Glc′-1	93.8	6.16 (d, 8.2)	93.7	6.14 (d, 8.2)	93.9	6.24 (d, 8.2)	93.7	6.22 (d, 8.0)
2	78.5	4.42	78.5	4.39	79.2	4.52	75.4	4.04
3	77.6	4.21	77.6	4.30	77.8	4.14	78.2	4.10
4	72.0	4.24	70.7	4.26	70.9	4.30	71.6	4.24
5	76.0	4.06	77.8	3.95	78.7	3.95	76.6	4.06
6	69.3	4.35; 4.65 (brd, 11.0)	62.9	4.37; 4.49	62.2	4.38; 4.45	69.7	4.37; 4.73
Glc″-1	104.9	5.71 (d, 7.7)	104.9	5.69 (d, 7.7)	104.9	5.77 (d, 7.8)	104.9	5.77 (d, 7.4)
2	75.5	4.09	75.7	4.08	73.4	4.09	75.7	4.08
3	78.4	4.27	78.2	4.27	78.4	4.27	78.2	4.30
4	72.1	4.09	71.4	4.15	69.3	4.35	69.3	4.15
5	78.7	3.91	76.2	4.04	79.4	3.94	78.7	3.91
6	62.9	4.31; 4.43	69.3	4.35; 4.65 (brd, 10.7)	63.0	4.37; 4.51	62.9	4.38; 4.50
Glc‴-1	105.6	5.02 (d, 7.7)	105.5	5.02 (d, 7.7)				
2	75.2	4.04	75.2	4.02				
3	78.7	4.20	78.4	4.18				
4	69.3	4.34	69.3	4.35				
5	78.3	4.00	78.1	3.98				
6	63.0	4.37; 4.53	62.9	4.39; 4.52				

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

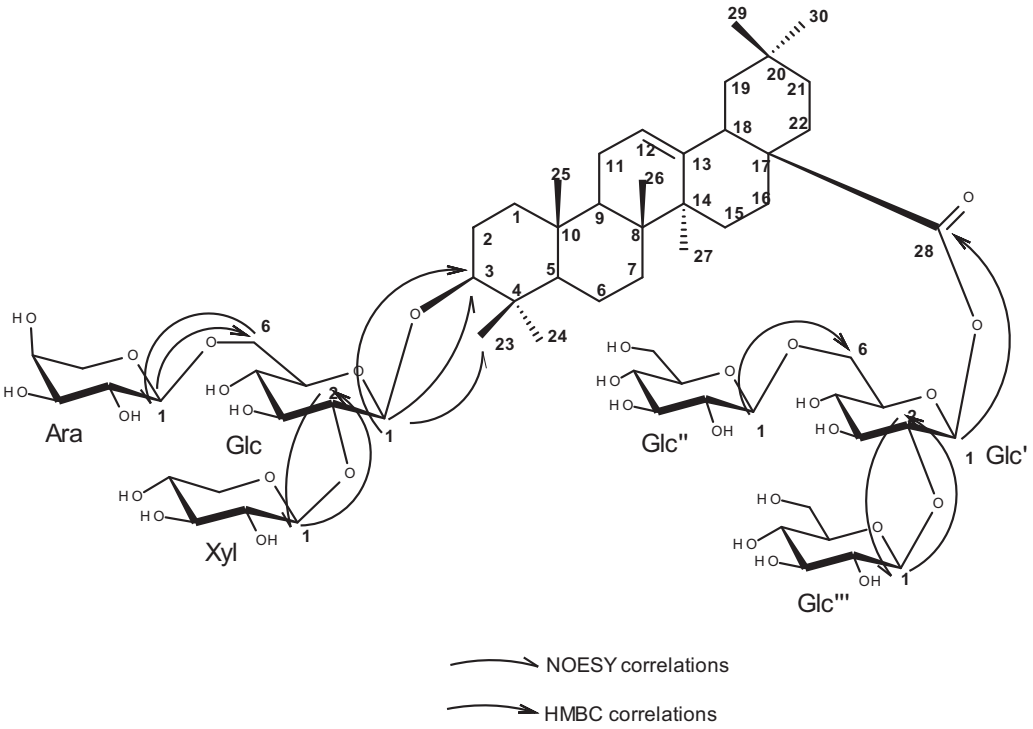


Fig. 2. Key HMBC and NOESY correlations for compound **1**.

attached at C-28 (Glc′) in **2** was monosubstituted at its C-2 position, instead of been disubstituted at its C-2 and C-6 positions as described in the case of **1** (Table 1). The presence of only one glycosylation shift at δ 78.5 (C-2 of Glc′), the absence of glycosylation shift at δ 62.9 (C-6 of Glc′) and the presence of one glycosylation shift at δ 69.3 (C-6 of Glc″) and the absence of any glycosylation shift for Glc‴ were evidences for the linear sequence established for the sugar moiety attached at C-28 of the aglycone for compound **2**. Therefore, the sugar sequence at C-28 of the aglycone was determined as β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl. Consequently, the structure of compound **2** was elucidated as 3-O-β-[α-L-arabinopyranosyl-(1→6)-[β-D-xylopyranosyl-(1→2)]-β-D-glucopyranosyl]-28-O-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-oleanolic acid.

HRESIMS of boromoenoside C (**3**), obtained as a white, amorphous powder, indicated a $C_{58}H_{94}O_{26}$ molecular formula, as deduced from the $[M+NH_4]^+$ ion at m/z 1224.6789 in the positive ion mode. The difference between compounds **2** and **3** (162 amu lower) was assigned to one glucose moiety to account for the similarities between their ^{1}H and ^{13}C NMR spectra (Table 2). Instead of a trisaccharide moiety at C-28 of oleanolic acid, compound **3** displayed signals for a disaccharide unit attached at C-28 of the aglycone according to the analysis of 2D NMR experiments. Accordingly, boromoenoside C (**3**) was elucidated to be 3-*O*-β-[α-L-arabinopyranosyl-(1→6)-[β-D-xylopyranosyl-(1→2)]-β-D-glucopyranosyl]-28-O-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-oleanolic acid.

The HRESIMS spectrum ($[M+NH_4]^+$ at m/z 1386.6575) of boromoenoside D (**4**), obtained as a white amorphous powder, was identical to that obtained for compound **3**, suggesting that the compounds were isomers with $C_{58}H_{94}O_{26}$ formula. Comparison of their NMR spectra showed that compound **4** differs from compound **3** only by the substitution pattern of the sugar chain at C-28 of the aglycone. This difference was evidenced by the carbon signal at δ 69.7 (C-6 of Glc′) in **4**, which was downfield shifted of+7.2 ppm compared to the carbon signal at δ 62.2 (C-6 of Glc′) as assigned in the case of **3**. Therefore, the sugar sequence at C-28 of the aglycone was determined as β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl. Consequently, the structure of boromoenoside D was elucidated as 3-O-β-[α-L-arabinopyranosyl-(1→6)-[β-D-xylopyranosyl-(1→2)]-β-D-glucopyranosyl]-28-O-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-oleanolic acid.

Compounds **1–4** were evaluated for their inhibitory effect on the survival of high grade human brain tumor cells, including the U-87 MG human glioblastoma cell line and the TG1 glioblastoma cancer stem-like cells isolated from a patient. None of the isolated compounds showed positive activity in our assay. In addition, to the best of our knowledge, our study is the first phytochemical study of *A. boromoensis* and represents a valuable contribution to the chemotaxomy of *Albizia* genus, which is known to be a rich source of triterpenoid saponins.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Jasco P-2000 polarimeter. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded at room temperature in pyridine-d_5 using a Bruker AVANCE III 500 spectrometer. Chemical shifts are given in δ (ppm) value relative to TMS as internal standard. HRESIMS spectra were recorded on a microTOF ESI-TOF mass spectrometer (Agilent) operating in positive mode. Analytical HPLC was performed on Varian 920-LC apparatus equipped with an autosampler, a pump, a diode array detector (DAD), and Galaxie software. Semipreparative HPLC was performed on a Gilson apparatus equipped with Trilution LC software using a Nucleodur 100-5 C18ec (21 mm × 250 mm, 5 μm) column purchased from Machery-Nagel (Germany). Thin layer chromatography (TLC) was performed on precoated silica gel plates (60 F_{254}, Merck) using the system solvent *n*-BuOH–AcOH–H_2O, 65:15:25 as eluent. The spots were observed after spray with Komarowsky reagent, a (5:1) mixture of *p*-hydroxybenzaldehyde (2% in MeOH) and (50%

H_2O in EtOH). Vacuum–liquid chromatography (VLC) was carried out using RP-18 silica gel 60 (25–40 μm) and silica gel 60 (15–40 μm and 40–63 μm).

3.2. Plant material

The roots of *A. boromoensis* were harvested at Nkolbisson, Yaoundé peripheral quarter, in Cameroon in October 2011 under the guidance of Dr. Paul Nana, botanist of the National Herbarium of Cameroon (NHC), where one specimen (47196/HNC) was deposited.

3.3. Extraction and isolation

The air-dried, powdered roots of *A. boromoensis* (200 g) were extracted with 70% EtOH in soxhlet apparatus. The resulting hydroalcoholic solution was then evaporated to dryness under reduce pressure to yield brown residue (4.83 g). This residue was suspended in 200 mL of H_2O and partitioned with *n*-BuOH sat. H_2O (3 × 300 mL). The *n*-BuOH soluble phase was evaporated to dryness affording 6.14 g of brown gum residue which was taken in a minimum of water (10 mL) and then submitted to vacuum–liquid chromatography (VLC) using RP-18 silica gel 60 (25–40 μm) eluted with a gradient of MeOH–H_2O (100% H_2O → 100%MeOH). The 70–85% MeOH fraction (600.5 mg) was subjected to VLC using silica gel 60 (15–40 μm) eluted with $CHCl_3$–MeOH–H_2O (80:20:2, 70:30:5, 60:33:7, and 60:40:10) to give four main subfractions (Bfr1–Bfr4). Subfraction Bfr4 (126.3 mg) was purified by semi-preparative HPLC using gradient system of CH_3CN–H_2O (20 mL/min) to yield compounds **1** (t_R, 13.20 min, 7.9 mg), **2** (t_R, 13.68 min, 8.5 mg), **3** (t_R, 16.16 min, 7.2 mg), and **4** (t_R, 16.63 min, 5.3 mg).

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

White amorphous powder; $[\alpha]_D^{25}$ −14.4 (c 0.01, MeOH); ^{1}H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) data, see Tables 1 and 2; HRESIMS *m/z*: 1386.6789 $[M+NH_4]^+$ (calcd. for $C_{64}H_{104}O_{31}$, 1386.6905).

3.5. 3-O-β-[α-L-arabinopyranosyl-(1→6)-[β-D-xylopyranosyl-(1→2)]-β-D-glucopyranosyl]-28-O-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-oleanolic acid (**2**)

White amorphous powder; $[\alpha]_D^{25}$ −17.5 (c 0.01, MeOH); ^{1}H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) data, see Tables 1 and 2; HRESIMS *m/z*: 1386.6787 $[M+NH_4]^+$ (calcd. for $C_{64}H_{104}O_{31}$, 1386.6905).

3.6. 3-O-β-[α-L-arabinopyranosyl-(1→6)-[β-D-xylopyranosyl-(1→2)]-β-D-glucopyranosyl]-28-O-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-oleanolic acid (**3**)

White amorphous powder; $[\alpha]_D^{25}$ −2.2 (c 0.01, MeOH); ^{1}H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) data, see Tables 1 and 2; HRESIMS *m/z*: 1224.6789 $[M+NH_4]^+$ (calcd. for $C_{58}H_{94}O_{26}$, 1224.6869).

3.7. 3-O-β-[α-L-arabinopyranosyl-(1→6)-[β-D-xylopyranosyl-(1→2)]-β-D-glucopyranosyl]-28-O-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-oleanolic acid (**4**)

White amorphous powder; $[\alpha]_D^{25}$ −5.3 (c 0.01, MeOH); ^{1}H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) data, see Tables 1 and 2; HRESIMS *m/z*: 1224.6787 $[M+NH_4]^+$ (calcd. for $C_{58}H_{94}O_{26}$, 1224.6869).

3.8. Acid hydrolysis of compounds and determination of the absolute configuration of the sugar residues

Each saponin (2 mg) was hydrolyzed with 2 mL of 2 M HCl at 85 °C during 2 h. After cooling, the solvent was removed under reduced pressure. The sugar mixture was extracted from the aqueous phase (10 mL) and washed with CH_2Cl_2 (3 × 5 mL). The combined CH_2Cl_2 extracts were washed with water to give after evaporation the aglycone moiety. The sugars were first analyzed by TLC over silica gel ($CHCl_3$–MeOH–H_2O, 8:5:1) by comparison with standard samples. The absolute configuration of each monosaccharide was determined from GC–MS analysis of their trimethylsilylated derivatives by comparison with authentic samples using the method previously described (Chaabi et al., 2010). The following sugars were detected: D-glucose, D-xylose, and L-arabinose.

3.9. Evaluation of inhibitory effect of saponins

Effect of chemical compounds on glioblastoma cells' ATP level was assessed using the CellTiter Glo reagent (Promega). Cells were seeded in 50 μL of their respective media (between 30 000 and 50 000 cells/well) into 96-well opaque bottom plates (Greiner, Courtaboeuf, France). 50 μL of pure compounds **1–4** (or 50 μL of Tamoxifen) dissolved at a given concentration in the respective cell culture medium, were added to the cell suspension. Cell were incubated at 37 °C, 5% CO_2 and ATP levels were measured 24 h later according to the manufacturer's instructions. Luminescence was measured using a Victor^{TM}3 plate reader (PerkinElmer). Relative ATP levels in each well were determined by calculating the percentage of luminescent signal in the well with respect to the average signal measured in negative control wells (without compounds). Tamoxifen was used as positive control.

Acknowledgement

The authors are grateful to Dr. Victor Nana of the National Herbarium of Cameroon (NHC) for the identification and collection of plant.

References

Abdel-Kader, M., Hoch, J., Berger, J.M., Evans, R., Miller, J.S., Wisse, J.H., Mamber, S.W., Dalton, J.M., Kingston, D.G., 2001. Two bioactive saponins from *Albizia subdimidiata* from the Suriname rainforest. J. Nat. Prod. 64, 536–539.

Arbonnier, M., 2009. Arbres arbustes et lianes des zones d'Afrique de l'Ouest. MNHN, Quæ, Paris, pp. 383.

Cao, S., Norris, A., Miller, J.S., Ratovoson, F., Razfitsalama, J., Andriantsiferana, R., Rasamison, V.E., Tendyke, K., Suh, T., Kingston, D.G.I., 2007. Cytotoxic triterpenoid saponins of *Albizia gummifera* from Madagascar rain forest. J. Nat. Prod. 70, 361–366.

Chaabi, M., Chabert, P., Vonthron-Sénécheau, C., Weniger, B., Ouattara, M., Corstjens, H., Sente, I., Declercq, L., Lobstein, A., 2010. Acylated flavonol pentaglycosides from *Baphia nitida* leaves. Phytochem. Lett. 3, 70–74.

Haddad, M., Miyamoto, T., Laurens, V., Lacaille-Dubois, M.A., 2003. Two new biologically active triterpenoidal saponins acylated with salicylic acid from *Albizia adianthifolia*. J. Nat. Prod. 66, 372–377.

Krief, S., Thoison, O., Sevenet, T., Wrangham, R.W., Lavaud, C., 2005. Triterpenoid saponins anthranilates from *Albizia grandibracteata* leaves ingested by primates in Uganda. J. Nat. Prod. 68, 897–903.

Liu, R., Ma, S., Yu, S., Pei, Y., Zhang, S., Chen, X., Zhang, J., 2009. Cytotoxic oleanane triterpene saponins from *Albizia chinensis*. J. Nat. Prod. 72, 632–639.

Melek, F.R., Miyase, T., Ghaly, N.S., Nabil, M., 2007. Triterpenoid saponins with N-acetyl sugar from the bark of *Albizia procera*. Phytochemistry 68, 1261–1266.

Mimaki, Y., Yokosuka, A., Hamanaka, M., Sakuma, C., Yamori, T., Shibata, Y., 2004. Triterpene saponins from the roots of *Clematis chinensis*. J. Nat. Prod. 67, 1511–1516.

Miyase, T., Melek, F.R., Ghaly, N.S., Warashina, T., El-Kady, M., Nabil, M., 2010. Echinocystic acid 3,16-O-bisglycosides from *Albizia procera*. Phytochemistry 71, 1375–1380.

Nigam, S.K., Gopal, M., Uddin, R., Yoshikawa, K., Kawamoto, M., Arihara, S., 1997. Pithedulosides A-G, oleanane glycosides from *Pithecellobium dulce*. Phytochemistry 44, 1329–1334.

Noté, O.P., Mitaine-offer, A.C., Miyamoto, T., Paululat, T., Mirjolet, J.F., Duchamp, O., Pegnyemb, D.E., Lacaille-Dubois, M.A., 2009. Cytotoxic acacic acid glycosides from the roots of *Albizia coriaria*. J. Nat. Prod. 72, 1725–1730.

Noté, O.P., Tapondjou, A., Mitaine-offer, A., Miyamoto, T., Pegnyemb, D.E., Lacaille-Dubois, M.A., 2013. Triterpenoid saponins from *Piptadeniastrum africanum* (Hook F.) Brenan. Phytochem. Lett. 6, 505–510.

Sahu, N.P., Achari, B., 2001. Advances in structural determination of saponins and terpenoid glycosides. Curr. Org. Chem. 5, 315–334.

Tené, M., Chabert, P., Noté, O., Kenla, N.J.T., Tane, P., Lobstein, A., 2011. Triterpenoid saponins from *Cylicodiscus gabunensis*. Phytochem. Lett. 4, 89–92.

Zheng, L., Zheng, J., Zhao, Y., Wang, B., Wu, L., Liang, H., 2006. Three anti-tumor saponins from *Albizia julibrissin*. Bioorg. Med. Chem. Lett. 16, 2765–2768.