Phytochemistry Letters 43 (2021) 190–195

Contents lists available at ScienceDirect

Phytochemistry Letters

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

Polythosides A and B, two new triterpenoid saponins from the roots of *Acacia polyacantha* Willd. (Mimosaceae)

Abdou Tchoukoua [a,*], Sandrine Carole Tomfeun Nganou [b], Bernard Dabole [a], Jun Yoshida [c], Yoshiaki Ito [d], Bonaventure Tchaleu Ngadjui [b,*], Yoshihito Shiono [e,*]

[a] Department of Chemistry, Faculty of Science, University of Maroua, P.O. Box 814, Maroua, Cameroon
[b] Department of Organic Chemistry, University of Yaounde 1, P.O. Box 812, Yaounde, Cameroon
[c] Center for Liberal Arts and Sciences, Iwate Medical University, 1-1-1 Idaidori, Yahaba, Iwate, Japan
[d] Department of Biological Chemistry and Food Science, Faculty of Agriculture, Iwate University, Morioka, Iwate, Japan
[e] Department of Food, Life, and Environmental Science, Faculty of Agriculture, Yamagata University, Tsuruoka, Yamagata, 997–8555, Japan

ARTICLE INFO

Keywords:
Acacia polyacantha
Mimosaceae
Triterpenoid saponins
Cytotoxicity
H4IIE hepatoma cell line

ABSTRACT

Two new oleanane-type triterpene saponins, named polythosides A and B (**1** and **2**), together with a known compound, silphioside E (**3**), were isolated from the roots of *Acacia polyacantha*. Their structures were elucidated by analysis of 1D and 2D-NMR experiments, and mass spectrometry (HR-ESITOF-MS) as oleanolic acid 3-*O*-*β*-D-galactopyranosyl-(1→2)-*β*-D-xylopyranosyl-(1→6)-[*β*-D-xylopyranosyl-(1→4)]-[*β*-D-xylopyranosyl-(1→2)]-*β*-D-galactopyranoside (**1**), 3-*O*-[*β*-D-galactopyranosyl-(1→2)-*β*-D-xylopyranosyl-(1→6)-[*β*-D-xylopyranosyl-(1→4)]-[*β*-D-xylopyranosyl-(1→2)]-*β*-D-galactopyranosyl] oleanolic acid-28-*O*-*β*-D-glucopyranosyl ester (**2**) and 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl] oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester (**3**). The cytotoxic effect of isolated saponins was evaluated on the H4IIE rat hepatoma cell line. The results show that none of the compounds (assayed at 100 μM) showed cytotoxicity against H4IIE cells.

1. Introduction

Acacia polyacantha Willd. (Mimosaceae) synomym of *Senegalia polyacantha* (Willd.) Seigler & Ebinger is a deciduous, thorny tree of about 10−25 m height found in Tropical Africa. The plant has been used in traditional medicine for the treatment of snakebite, livestock diseases (Koudoro et al., 2015), venereal diseases and stomach disorders (Daffalla et al., 2018).

Pharmacological studies of extracts and compounds from the plant included antibacterial and larvicidal activities (Mambe et al., 2019; Daffalla et al., 2018), and radical-scavenging activity (Koudoro et al., 2015). *A. polyacantha* has a good fatty acid profile, anti-oxidant, and phenolic contents (Saha Tchinda et al., 2020).

Previous phytochemical studies on *A. polyacantha* have demonstrated the presence of triterpenoid saponins (Fotso et al., 2019). As part of our continuing investigation on saponins from *Acacia* genus (Tchoukoua et al., 2017, 2018), two new oleanane saponins, polythosides A–B (**1**–**2**), were isolated from the roots of *A. polyacantha*. Herein, we report the isolation and structure elucidation of these new compounds. In addition, the cytotoxic activities of isolated saponins against H4IIE cells are also described.

2. Results and discussion

The dried roots of *Acacia polyacantha* were extracted with MeOH. The methanolic extract was successively partitioned with *n*-hexane, ethylacetate and *n*-butanol saturated with water. The *n*-BuOH extract was subjected to Diaion HP20 macroporous resin column eluted with H_2O, H_2O-MeOH and MeOH. Fractionation of the MeOH extract by a combination of gel filtration on Sephadex LH-20 (MeOH), Flash chromatography on silica gel ($CHCl_3$-MeOH-H_2O gradient) and CC on ODS (H_2O/MeOH, isochratic) afforded compounds **1**–**2** (Fig. 1) and compound **3** (Fig. S1, supplementary data). The structure of the known saponin, **3,** was identified as 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl] oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester (Silphioside E) (Davidyants et al., 1984; Park et al., 1999), by comparing its spectroscopic data with the literature.

Polythoside A (**1**) was isolated as a white amorphous powder. The HRESI-TOF-MS in the positive-ion mode showed a pseudo-molecular ion peak at *m/z* 1199.5593 $[M + Na]^+$ consistent with a molecular formula

* Corresponding authors.
E-mail addresses: tchouks2000@yahoo.fr (A. Tchoukoua), ngadjuibt@yahoo.fr (B.T. Ngadjui), yshiono@tds1.tr.yamagata-u.ac.jp (Y. Shiono).

https://doi.org/10.1016/j.phytol.2021.04.007
Received 20 December 2020; Received in revised form 15 April 2021; Accepted 16 April 2021
Available online 27 April 2021

of $C_{57}H_{92}O_{25}$.

The ^{1}H NMR spectrum of compound **1** (Table 1) showed signals for seven tertiary methyls groups at δ_H 0.79, 0.83, 0.88, 0.92, 0.93, 1.06, and 1.14 (each s), a trisubstituted olefinic proton at δ_H 5.22 (t-like, J =3.6 Hz), and a methine proton bearing oxygen at δ_H 3.18. These signals along with the resonances in the ^{13}C NMR spectrum for methyl groups at δ_C 14.6, 15.6, 16.4, 22.7, 25.2, 27.2, and 32.3, two olefinic carbons at δ_C 122.3 and 143.8, and one carboxylic acid group at δ_C 180.5 evidenced an olean-12-en-28-oic acid as aglycone of compound **1** (Nigam et al., 1997). The downfield shift of C-3 (δ_C 90.3) due to the glycosylation suggested that the saponin was a monodesmosidic saponin. The absolute configuration of C-3 was determined by NOESY cross-peak between δ_H 3.18 (H-3) and 0.75 (d, J = 11.4, H-5). Thus, the aglycone was identified as 3β-hydroxy-olean-12-en-28-oic acid.

The presence of five sugar moieties in **1** was evidenced by the ^{1}H NMR spectrum which displayed anomeric protons at δ_H 4.39 (d, J =7.8 Hz), 4.43 (d, J =7.8 Hz), 4.48 (d, J =7.2 Hz), 4.50 (d, J =7.2 Hz), 4.69 (d, J =7.2 Hz) (Table 2), giving correlations with the corresponding carbons at δ_C 106.7, 103.9, 102.4, 103.5, 103.0, respectively in the HMQC spectrum.

All proton signals due to the sugar moieties were identified by detailed analysis of the COSY, TOCSY and NOESY spectra, and the carbon signals were assigned based on HMQC and further confirmed by HMBC spectrum.

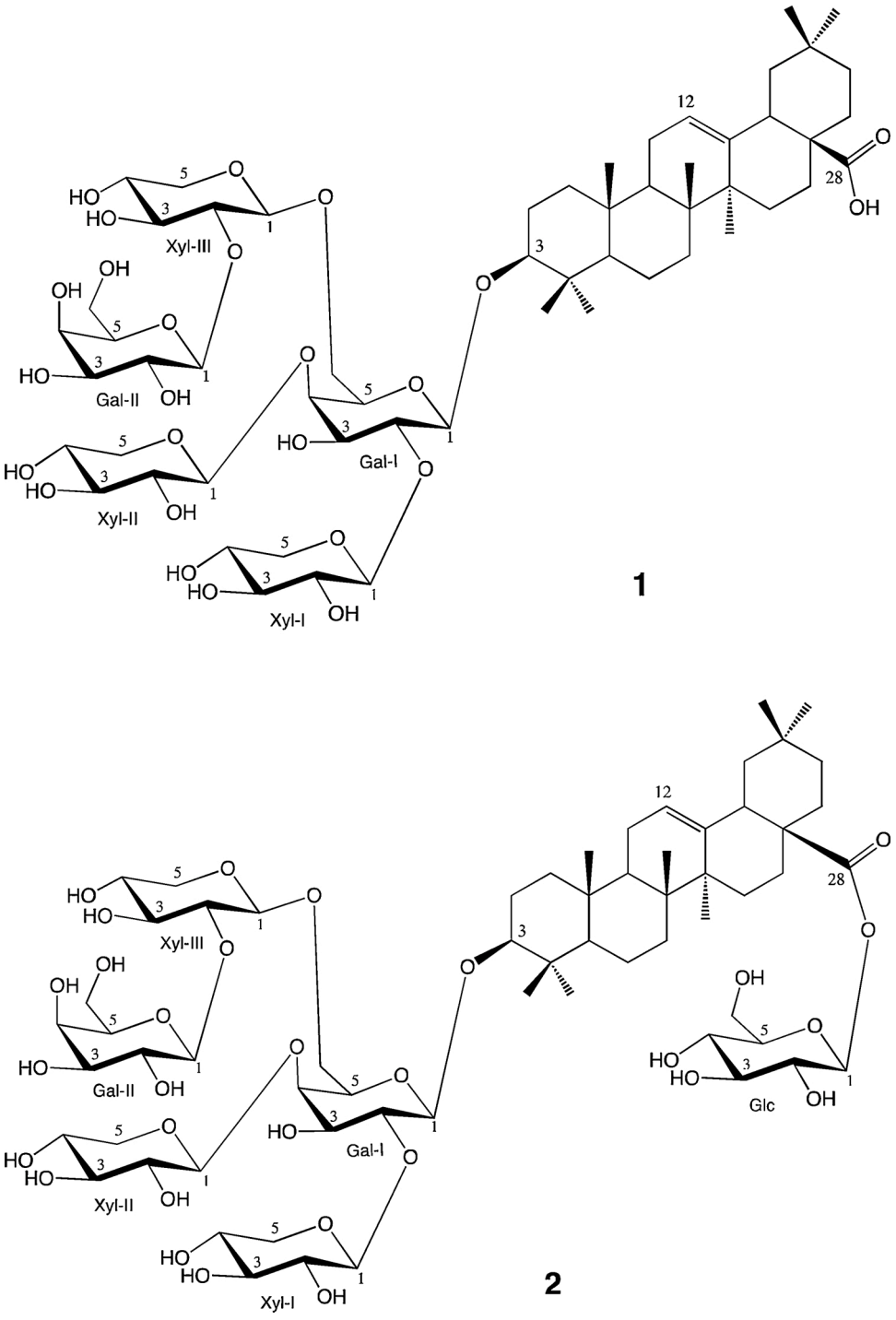

Fig. 1. Chemical structures of compounds **1**–**2**.

Table 1
^{1}H and ^{13}C NMR Data for the Aglycone Moieties of **1** and **2** (in MeOH-d_4, δ in ppm, J in Hz)[a].

	1		**2**	
Position	δ_C	δ_H	δ_C	δ_H
1	38.4	0.98, 1.59	38.4	0.98, 1.59
2	25.9	1.66, 1.88	25.9	1.66, 1.88
3	90.3	3.18	90.3	3.17
4	39.1	–	39.1	–
5	55.7	0.75 d (11.4)	55.7	0.75 d (11.4)
6	18.0	1.36, 1.55	18.0	1.35, 1.53
7	32.7	1.27, 1.47	32.6	1.27, 1.47
8	39.2	–	39.4	–
9	47.7	1.56	47.7	1.55
10	36.5	–	36.5	–
11	23.2	1.88	23.2	1.87
12	122.3	5.22 brt (3.6)	122.5	5.23 brt (3.6)
13	143.8	–	143.5	–
14	41.6	–	41.6	–
15	27.5	1.75	27.5	1.77
16	22.7	1.57, 1.98 dt (13.2, 3.6)	25.6	1.70, 2.02 dt (13.2, 3.0)
17	46.3	–	46.7	–
18	41.4	2.82 dd (13.8, 4.2)	41.2	2.83 dd (13.8, 3.6)
19	45.9	1.11, 1.66	45.9	1.12, 1.68
20	30.3	–	30.2	–
21	33.5	1.17, 1.38	33.6	
22	32.5	1.51, 1.73	32.6	1.28, 1.47
23	27.2	1.06, s	27.2	1.05, s
24	15.6	0.83, s	15.6	0.82, s
25	14.6	0.93, s	14.7	0.93, s
26	16.4	0.79, s	16.4	0.77, s
27	25.2	1.14, s	25.1	1.13, s
28	180.5	–	176.7	–
29	32.3	0.88, s	32.2	0.89, s
30	22.7	0.92, s	22.7	0.91, s

[a] Overlapping ^{1}H-NMR signals are reported without designated multiplicity.

Table 2
^{1}H and ^{13}C NMR Data for C-3 and C-28 Saccharide Portions of Saponins **1** and **2** (MeOH-d_4, δ in ppm, J in Hz) [a].

	1		**2**	
	δ_H	δ_C	δ_H	δ_C
3-*O*-β-D-galactose I (Gal-I)				
1	4.43 d (7.8)	103.9	4.43 d (7.8)	103.9
2	3.64	82.5	3.64	82.5
3	3.64	75.0	3.64	75.0
4	4.12	78.0	4.12	78.0
5	3.47	73.4	3.47	73.4
6	4.06 d (10.8) nd	66.6	4.06 d (9.6) nd	66.6
β-D-xylose I (at C-2 Gal-I)				
1	4.39 d (7.8)	106.7	4.39 d (7.8)	106.7
2	3.25	75.4	3.25	75.4
3	3.22	76.8	3.36	76.7
4	3.82	68.2	3.81	68.2
5	3.29, 3.83	65.7	3.29, 3.83	65.7
β-D-xylose II (at C-4 Gal-I)				
1	4.50 d (7.2)	103.5	4.50 d (7.8)	103.5
2	3.16	73.3	3.16	73.3
3	3.68	72.7	3.68	72.7
4	3.44	69.9	3.46	69.9
5	3.20, 3.99 dd (12.0, 5.4)	66.1	3.20, 3.99	66.1
β-D-xylose III (at C-6 Gal-I)				
1	4.48 d (7.2)	102.4	4.48 d (6.6)	102.4
2	3.64	78.6	3.64	78.6
3	3.22	77.0	3.32	77.3
4	3.15	70.7	3.15	70.7
5	3.51, 3.83	65.5	3.51, 3.83	65.5
β-D-galactose II (at C-2 Xyl-III)				
1	4.69 d (7.2)	103.0	4.69 d (7.2)	103.0
2	3.18	75.0	3.17	75.0
3	3.21	76.6	3.38	76.6
4	3.50	69.7	3.32	69.7
5	3.33	76.4	3.33	76.4
6	3.55, 3.79	61.8	3.55, 3.79	61.8
28-*O*-β-D-glucose				
1			5.36 d (8.4)	94.3
2			3.29	72.6
3			3.20	77.0
4			3.15	70.7
5			3.38	76.9
6			3.66, 3.80	61.0

[a] Overlapping ^{1}H-NMR signals are reported without designated multiplicity.

The acid hydrolysis of **1** afforded D-xylose, and D-galactose in a ratio of 3:2 confirmed by specific rotation using chiral detection in HPLC analysis (see experimental section) and oleanolic acid as the aglycone.

The relatively large $^{3}J_{H\text{-}1,H\text{-}2}$ values of the xylosyl and galactosyl moieties (7.2–7.8 Hz) indicated an β-anomeric orientation for the xylose and galactose units (Mimaki et al., 2004).

The sugar units with anomeric proton signals at δ_H 4.39 (d, J =7.8 Hz), 4.48 (d, J =7.2 Hz), and 4.50 (d, J =7.2 Hz), were attributed to three β-D-xyloses and those with anomeric proton signals at 4.43 (d, J =7.8 Hz) and 4.69 (d, J =7.2 Hz) were identified as two β-D-galactose units.

The sugar sequence and its linkage position to the aglycone were determined by detailed analysis of the HMBC and NOESY spectra. Finally, the $^{3}J_{C,H}$ correlations from each anomeric proton across the glycosidic bond to the carbon of another substituted monosaccharide and the aglycone revealed the sugar sequence.

In the HMBC spectrum, the anomeric proton signal at δ_H 4.43 (Gal-I) showed long-range correlation with C-3 of the aglycone at δ_C 90.3. In the ^{13}C NMR spectrum of **1**, the C-2, C-4 and C-6 positions of Gal-I were shifted to δ_C 82.5, 78.0, and 66.6, respectively, establishing the site of glycosylation. Analysis of NMR data allowed the sequential assignments of the proton and carbon resonances of a xylose unit (δ_H 4.39, Xyl-I) linked to Gal-I C-2, another one (δ_H 4.50, Xyl-II) linked to Gal-I C-4, and two sugar units chain (Gal-II and Xyl-III) linked to Gal-I C-6. The absence of any ^{13}C NMR glycosylation shift for the Xyl-I, Xyl-II, and Gal-II units suggested that these sugars were terminal sugar units (Nigam et al., 1997). However, glycosylation shift observed for Xyl-III C-2 (δ_C 78.6) suggested that the galactose (δ_H 4.69 Gal-II) was linked to Xyl-III C-2. The sequence of the saccharide chain was unambiguously defined by the HMBC experiment with cross-peaks between Gal-II H-1 (δ_H 4.69) and Xyl-III C-2, Xyl-III H-1 (δ_H 4.48) and Gal-I C-6 (δ_C 66.6), Xyl-I (δ_H 4.39) and Gal-I C-2 (δ_C 82.5), and between Xyl-II (δ_H 4.50) and Gal-I C-4 (δ_C 78.0). The sequencing deduced from HMBC spectrum was confirmed in NOESY spectrum by observation of cross-peaks between Gal-I H-1 (δ_H 4.43) and aglycone H-3 (δ_H 3.18), Xyl-III H-1 (δ_H 4.48) and Gal-I H-6 (δ_H 4.06), and between Xyl-II H-1 (δ_H 4.50) and Gal-I H-4 (δ_H 4.12). Finally, the structure of **1** was determined as oleanolic acid 3-*O*-β-D-galactopyranosyl-(1→2)-β-D-xylopyranosyl-(1→6)-[β-D-xylopyranosyl-(1→4)]-[β-D-xylopyranosyl-(1→2)]-β-D-galactopyranoside, named polythoside A.

Polythoside B (**2**) was isolated as an amorphous solid with a molecular formula of $C_{63}H_{102}O_{30}$, as determined from the positive-ion HRESI-TOF-MS (m/z 1361.6187 $[M+Na]^+$, and m/z 1339.6385 $[M+H]^+$). The molecular weight of **2** is 162 mass units higher than that of **1**, implying additional monosaccharide hexose residue. Other fragment ion peaks were observed at m/z 1177 $[M+H-162]^+$, 1045 $[M+H-162-132]^+$, 913 $[M+H-162-132-132]^+$, and 751 $[M+H-162-132-132-162]^+$, and 621 $[M+H-H_2O-162-3\times132-162]^+$, corresponding to the successive loss of one hexosyl, one pentosyl, one pentosyl, and one hexosyl moieties, respectively.

The ^{1}H NMR spectrum of **2** showed signals for seven tertiary methyl groups at δ_H 0.77, 0.82, 0.89, 0.91, 0.93, 1.05, and 1.13 (each s) and a trisubstituted olefinic proton at δ_H 5.23 (t-like, J =3.6 Hz), as observed in **1**. The ^{1}H and ^{13}C NMR data (Table 1) of the aglycone of **2** were also in good agreement with those of oleanolic acid. Besides, the ^{1}H NMR spectrum of **2** displayed signals for six anomeric protons at δ_H 4.39 (d, J =7.8 Hz), 4.43 (d, J =7.8 Hz), 4.48 (d, J =6.6 Hz), 4.50 (d, J =7.8 Hz),

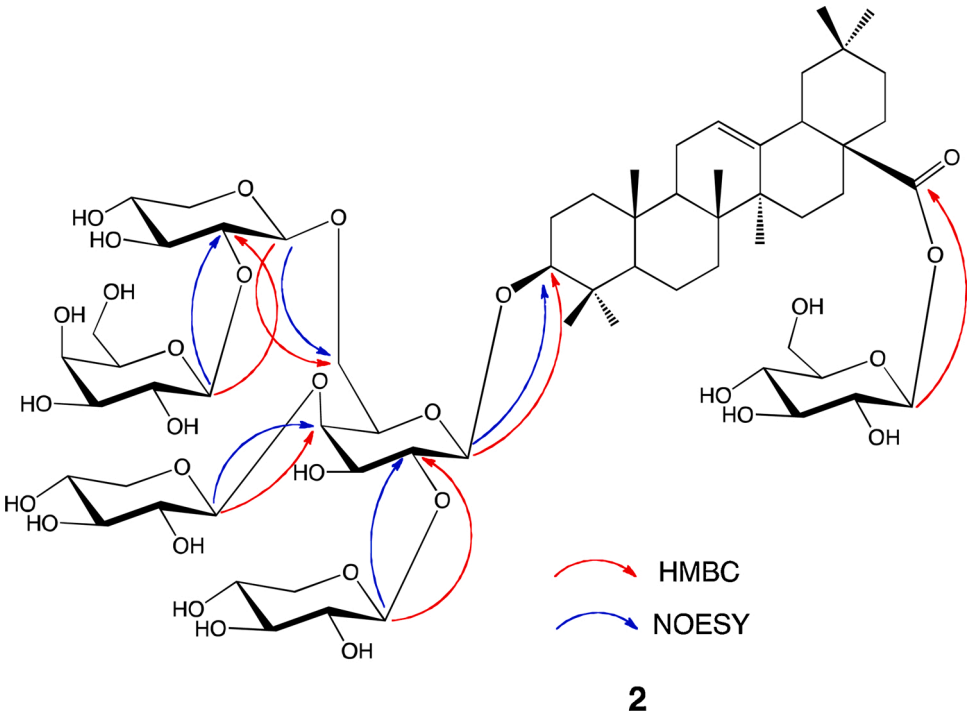

Fig. 2. Key HMBC and NOESY correlations of compound **2**.

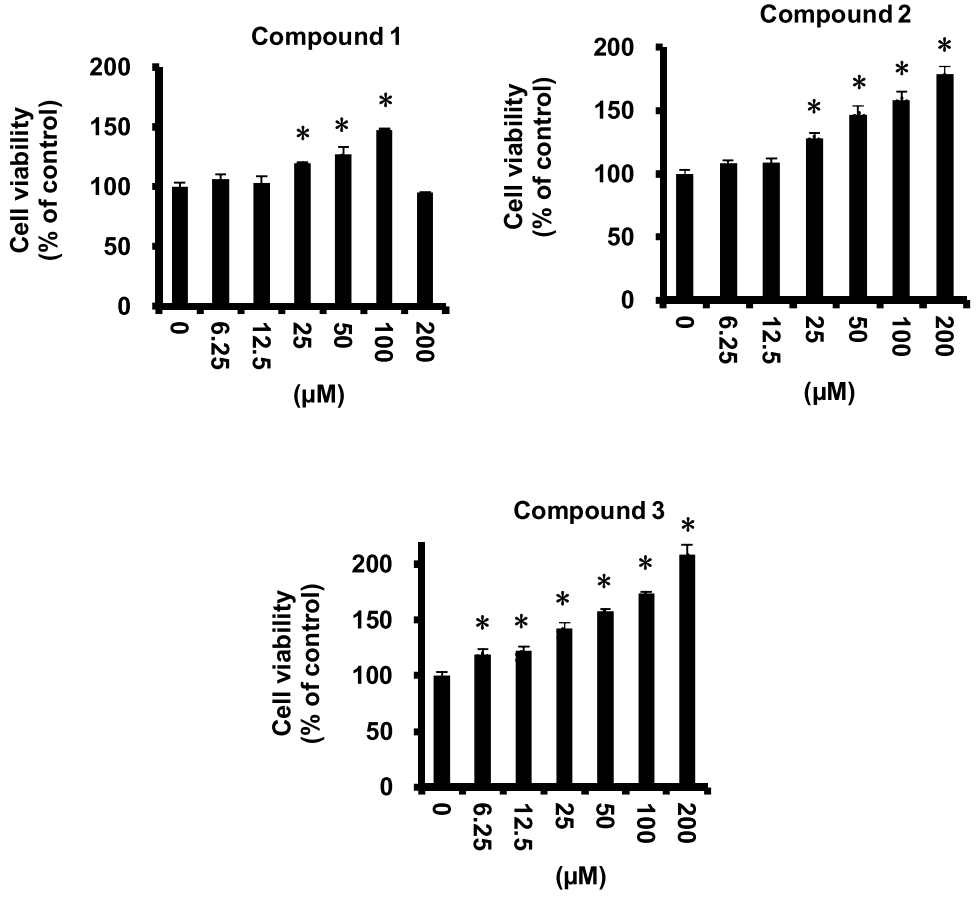

Fig. 3. Cytotoxicity of compounds **1**–**3** toward H4IIE cells. H4IIE cells were treated with compounds **1**–**3** at various concentrations for 48 h. Cell viability was measured by the MTT assay. Data are the means ± SD (n = 3). *$p <$ 0.05, **$p <$ 0.01, for a comparison versus the control (DMSO), as determined by the ANOVA/Dunnett test.

4.69 (d, J =7.2 Hz), and 5.36 (d, J =8.4 Hz), which correlated to the anomeric carbons at δ_C 106.7, 103.9, 102.4, 103.5, 103.0, and 94.3, respectively, in the HMQC spectrum (Table 2).

Acid hydrolysis of **2** yielded D-xylose, D-galactose, and D-glucose in a ratio of 3:2:1, which were identified by HPLC analysis and by using a combination of RI and optical rotation (OR) detection.

Comparison of the ^{1}H and ^{13}C NMR spectra of **2** with those of **1** revealed that **2** had a terminal β-D-glucopyranosyl moiety in addition to a pentaglycoside group attached to C-3 of the aglycone, which was identical to that of **1**. Since the C-28 carbonyl carbon signal of the aglycone of **2** was shifted upfield by 3.8 ppm in comparison with that of **1**, the additional glycosyl moiety was located at C-28 in an ester linkage form (Table 2). This was confirmed by HMBC correlation from the anomeric proton of Glc at δ_H 5.36 to C-28 of the aglycone at δ_C 176.7 (Fig. 2). Accordingly, the structure of **2** was determined as 3-*O*-[β-D-galactopyranosyl-(1→2)-β-D-xylopyranosyl-(1→6)-[β-D-xylopyranosyl-(1→4)]-[β-D-xylopyranosyl-(1→2)]-β-D-galactopyranosyl] oleanolic acid-28-*O*-β-D-glucopyranosyl ester, named polythoside B.

All the isolated saponins were evaluated for cytotoxic activities against H4IIE hepatoma cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. As a result, compounds **1**–**3** were not significantly cytotoxic to H4IIE cells even at 100 μM (Fig. 3).

3. Material and methods

3.1. General experimental procedures

Optical rotations were measured with a Horiba SEPA-300 polarimeter (HORIBA, Kyoto, Japan). The HRESIMS was taken on a JEOL HX110 mass spectrometer (JEOL, Tokyo, Japan). NMR spectra were recorded on a JEOL ECZ-600 at 600 MHz for ^{1}H and 150 MHz for ^{13}C (JEOL, Tokyo, Japan). The chemical shifts are given in ppm (δ), relative to TMS as internal standard, and coupling constants are in Hz. ^{1}H, ^{13}C, COSY, HMQC, HMBC, TOCSY and NOESY spectra were recorded using Jeol standard pulse sequences. Column chromatography (CC) was carried out on silica gel 60 (Kanto Chemical Co., Inc., Japan) and vacuum liquid chromatography (VLC) on reversed-phase materials (Lichroprep RP-18, 25–40 μm). Semi-preparative HPLC was carried out with Shimadzu pump and UV LC-10A detector (set at 206 nm) on Mightysil RP-18 column (250 × 6.0 mm i. d.) at the flow rate of 1.5 mL/min. TLC was performed on Merck precoated aluminum silica gel 60 F_{254} sheets (Merck, Darmstadt, Germany), and spots were detected using sulfuric acid spray reagent followed by heating.

3.2. Plant material

Acacia polyacantha (Mimosaceae) was collected at Tchere, a small locality about 30 km away from Maroua town, in the Far-North region of Cameroon, in November 2017 and identified by Mr. Nana, botanist at the National Herbarium (Yaounde, Cameroon) and a voucher specimen N° 58985/SFR/CAM has been deposited at the National Herbarium Cameroon.

3.3. Extraction and isolation

The air-dried and powdered roots of *A. polyacantha* (2.7 kg) were extracted with MeOH at room temperature. After filtration, the organic layer was evaporated to give 200 g of crude extract. The methanolic extract was dissolved in H_2O (400 mL), and successively partitioned with *n*-hexane (3 × 200 mL), EtOAc (3 × 200 mL), and *n*-BuOH (3 × 200 mL) saturated with H_2O. The *n*-BuOH extract (22.3 g) was dissolved in water, and passed through a column of Diaion HP-20 and washed with H_2O, 50 % MeOH-H_2O, and MeOH. The MeOH fraction (9.2 g) enriched in saponins is applied to ODS column with MeOH-H_2O (6:4) to give 5 main fractions (Frs. A1 ~ A5). Fr. A5 (4.1 g) was subjected to Flash chromatography by using a prepack silica gel cartridge, 80 g. Then eluted with $CHCl_3$-MeOH-H_2O mixtures (80:20:2 to 70:30:3) to yield 8 fractions (Fr. B1 ~ B8). Fr. B5 (80 mg) was applied to an open ODS column with solvent system MeOH-H_2O (8:2) to give **1** (43 mg) and **3** (9.8 mg). Fr. A2 ~ A4 (2.0 g) were combined and applied to repeated Flash chromatography by using a prepack silica gel cartridge, 50 g. Then eluted with $CHCl_3$-MeOH-H_2O mixtures (80:20:2 to 70:30:3) to yield 5 fractions (Fr. C1 ~ C5). Fr. C3 (94 mg) was applied to an open ODS column with solvent system MeOH-H_2O (8:2) to give **2** (20.7 mg).

3.4. Acid hydrolysis and HPLC analysis

Acid hydrolysis was done according to the method reported in literature (Tchoukoua et al., 2017). Briefly, each solution of **1**–**3** (6 mg) in 0.2 M HCl (dioxane-H_2O 1:1, 3 mL) was heated at 95 °C for 30 min under argon. After cooling, the mixture was neutralized by passage through an Amberlite-IRA-93ZU (Organo, Tokyo, Japan) column and chromatographed (Diaion HP-20, 40 % MeOH followed by Me_2CO-EtOH 1:1) to give aglycone fractions (2.7 mg) and a sugar fraction (1.8 mg). The aglycone was identified as oleanolic acid. After the sugar fraction was passed through a Sep-Pak-C_{18} cartridge (Waters, Milford, MA, USA; with 40 % MeOH) and Toyopak-IC-SP-M-cartridge (Tosoh; with 40 % MeOH), it was analyzed by HPLC (MeCN-H_2O 17:3, flow rate, 0.9 mL. min^{-1}; detection, refractive index (RI) and optical rotation (OR): 9.73 (D-xylose, positive OR). 15.37 (D-glucose, positive OR); 14.65 (D-galactose, positive OR).

3.5. Physical and spectral properties of isolates

3.5.1. Polythoside A (1)

White amorphous powder; $[\alpha]_D^{20}$ +0.71 (*c* 0.3, MeOH); ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; HRESI-TOF-MS (positive-ion mode) *m/z* 1199.5593 $[M + Na]^+$, molecular formula $C_{57}H_{92}O_{25}$.

3.5.2. Polythoside B (2)

White amorphous powder; $[\alpha]_D^{20}$ -8.0 (*c* 0.3, MeOH); ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; HRESI-TOF-MS (positive-ion mode) (*m/z* 1361.6187 $[M + Na]^+$, and *m/z* 1339.6385 $[M+H]^+$, molecular formula $C_{63}H_{102}O_{30}$.

3.6. Chemicals and reagents

Dulbecco's modified Eagle's medium (DMEM), phosphate buffered saline (PBS), and penicillin-streptomycin were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Fetal bovine serum albumin was purchased from BioWest (Vancouver, Canada). MTT was purchased from Dojindo Laboratories (Kumamoto, Japan). Isopropanol and dimethyl sulfoxide (DMSO) were purchased from Wako Pure Chemical Industries (Osaka, Japan).

3.7. Cell culture

H4IIE cells (American Type Culture Collection [ATCC], Manassas, VA, USA) were grown to sub-confluence in DMEM supplemented with 10 % heat-inactivated fetal bovine serum and penicillin-streptomycin at 37 °C under a 5% CO_2 atmosphere in a humidified incubator (Yoshida et al., 2017).

3.8. Cell viability assay

Cell viability was determined using the MTT assay. Briefly, H4IIE cells were seeded at 2 × 10^4 cells/well in a 96-well plate and were treated with test sample. After 48 h, 10 μL of MTT solution (5 mg/mL in PBS) was added and the mixture was incubated at 37 °C for 4 h. At the end of the incubation period, the resultant formazan crystals were

dissolved in 0.04 M HCl/isopropanol (100 μL) and the absorbance intensity was measured by using a microplate reader (Multiskan GO Microplate Spectrophotometer, Thermo Fisher Scientific) at 560 nm.

3.9. Statistical analysis

Data are presented as mean ± standard deviation (SD). One-way ANOVA followed by the Dunnett test was used. $p < 0.01$ and $p < 0.05$ indicated statistical significance.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

AT is grateful to the JSPS RONPAKU (Dissertation PhD, ID: R11516) Program for the financial support.

The authors acknowledge support from DAAD (German Academic Exchange Service) through the Yaounde-Bielefeld Graduate school of Natural Products with Anti-parasites and Antibacterial activities [YaBiNaPA project n°57316173] for providing research facilities for the extraction and fractionation of the crude extracts.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phytol.2021.04.007.

References

Daffalla, H.M., Ali, K.S.E., Tajelser, T., Hagr, T.E., Ahmed, N.S., Ahmed, R.H., 2018. Larvicidal and antibacterial activities of methanol extract of Acacia polyacantha Willd. J. Adv. Res. Pharm. Sci. Pharmacol. Interventions 2, 7–11.

Davidyants, E.S., Putieva, Zh.M., Bandyukova, V.A., Abubakirov, N.K., 1984. Triterpene glycosides of*Silphium perfoliatum*. III. Structure of silphioside E. Chem. Nat. Compd. 20, 708–710.

Fotso, W.G., Na-Iya, J., Mbaveng, T.A., Ango, Y.P., Demirtas, I., Kuete, V., Samuel, Y., Ngameni, B., Efferth, T., Ngadjui, T.B., 2019. Polyacanthoside A, a new oleanane-type triterpenoid saponin with cytotoxic effects from the leaves of *Acacia polyacantha* (Fabaceae). Nat. Prod. Res. 33, 3521–3526.

Koudoro, Y.A., Agbangnan, D.C.P., Yèhouénou, B., Tchobo, F.P., Alitonou, G.A., Avlessi, F., Akoègninou, A., Sohounhloué, K.C.D., 2015. Chemical characterization and biological activities of extracts from two plants (*Cissus quadrangularis* and *Acacia polyacantha*) used in veterinary medicine in Benin. J. Pharmacogn. Phytochem. 3, 91–96.

Mambe, F.T., Na-Iya, J., Fotso, G.W., Ashu, F., Ngameni, B., Ngadjui, B.T., Beng, V.P., Kuete, V., 2019. Antibacterial and antibiotic modifying potential of crude extracts, fractions, and compounds from *Acacia polyacantha* Willd. against MDR gram-negative bacteria. Evid. Based Complementary Altern. Med. 2019, 1–13.

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

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.

Park, S.-H., Oh, S.R., Jung, K.Y., Lee, I.S., Ahn, K.S., Kim, J.-Y., Lee, J.J., Lee, H.-K., 1999. Anticomplement activities of oleanolic acid monodesmosides and bisdesmosides isolated from *Tiarella polyphylla*. Arch. Pharm. Res. 22, 428–431.

Saha Tchinda, J.-B., Mbitnkeu Fetngna, T.T., Tchoukoua, A., Cheumani, Y.A.M., Fauconnier, M.L., Ndikontar, K.M., Richel, A., 2020. Fatty acid profiles, antioxidant, and phenolic contents of oils extracted from Acacia polyacantha and Azadirachta indica (Neem) seeds using green solvents. J. Food Process. Preserv. https://doi.org/10.1111/jfpp.15115.

Tchoukoua, A., Tabopda, T.K., Uesugi, S., Ohno, M., Kimura, K.-I., Kwon, E., Momma, H., Horo, I., Çalişkan, O.A., Shiono, Y., Ngadjui, B.T., 2017. Triterpene Saponins from the Roots of*Acacia albida* Del. (Mimosaceae). Phytochemistry 136, 31–38.

Tchoukoua, A., Tabopda, T.K., Usukhbayar, N., Kimura, K.-I., Kwon, E., Momma, H., Koseki, T., Shiono, Y., Ngadjui, B.T., 2018. New Triterpene saponins from the stem of *Acacia kamerunensis* (Mimosaceae). Phytochem. Lett. 23, 21–25.

Yoshida, J., Uesugi, S., Kawamura, T., Kimura, K., Hu, D., Xia, S., Toyooka, N., Ohnishi, M., Kawashima, H., 2017. (4Z,15Z)-Octadecadienoic acid inhibits glycogen synthase kinase-3β and glycose production in H4IIE cells. Lipids 53, 295–301.