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Triterpenoid saponins and others glycosides from the stem barks of *Pancovia turbinata* Radlk

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ABSTRACT

In our continuing search of saponins from the plant of Sapindaceae family, phytochemical investigation of the stem barks of *Pancovia turbinata* Radlk., led to the isolation and structural characterization of two new triterpenoid saponins, named turbinatosides A-B (**1**–**2**), one new farnesyl glycoside, named turbinoside A (**3**), one new coumarin glucoside, named panturboside A (**4**), together with a known saponin (**5**). The structures of the new compounds were established, using extensive analysis of NMR techniques, mainly 1D NMR (^{1}H, ^{13}C, and DEPT) and 2D NMR (COSY, NOESY, HSQC, HSQC-TOCSY and HMBC) experiments, HRESIMS and by comparison with the literature data, as 3-*O*-*β*-D-xylopyranosyl-(1 → 3)-*α*-L-arabinopyranosyl-(1 → 4)-*β*-D-glucopyranosyl-(1 → 3)-*α*-L-rhamnopyranosyl-(1 → 2)-*α*-L-arabinopyranosylhederagenin 28-*O*-*β*-D-glucopyranosyl ester (**1**), 3-*O*-*α*-L-arabinopyranosyl-(1 → 4)-*β*-D-glucopyranosyl-(1 → 3)-*α*-L-rhamnopyranosyl-(1 → 2)-*α*-L-arabinopyranosylhederagenin 28-*O*-*β*-D-xylopyranosyl-(1 → 4)-*β*-D-glucopyranosyl ester (**2**), 1-*O*-{*β*-D-glucopyranosyl-(1 → 3)-*α*-L-rhamnopyranosyl-(1 → 2)-[*α*-L-rhamnopyranosyl-(1 → 6)]-*β*-D-glucopyranosyl}-(*2E,6E*)-farnes-1,12-diol (**3**), and 5-*O*-*β*-D-glucopyranosyl-5,6,7-trihydroxy-8-methoxycoumarin (**4**), respectively. Our findings highlight the presence of -^{3}Rha-^{2}Ara-^{3}hederagenin oligosaccharidic sequence usually described in saponins from Sapindoideae subfamily of Sapindaceae family, as well as farnesol glycosides, and represent therefore a valuable contribution to the chemotaxonomy of the Sapindoideae subfamily.

1. Introduction

The Sapindaceae family, mostly distributed in tropical and subtropical regions [1], is described as trees, shrubs and herbaceous plants, containing about 1900 species divided into three subfamilies namely, Sapindoideae, Dodonaeoideae and Aceroideae, and 144 genera [2]. Plants of *Pancovia* genus (Sapindoideae subfamily) are used in Cameroonian's traditional medicine for the treatment of several ailments such as skin diseases, dysentery and rheumatism [3,4]. Previous phytochemical investigations of this genus revealed the presence of ellagic acid derivatives from *P. pedicellaris* with antibacterial potential [5], and ceramide and cerebroside from *P. laurentii* with antiprotozoal properties [6], while triterpenoid saponins are described as major constituents of the whole subfamily [7–16]. *Pancovia turbinata* Radlk. is a small tree, widely distributed in West Africa and in Cameroon [17]. To the best of our knowledge, no ethnomedicinal and phytochemical study are reported for this species. As part of our continuing search of saponins from Cameroonian's Sapindaceae family [15,16], we have investigated the saponins content of the stem barks of *P. turbinata*.

In this paper, we report the isolation and structure characterization

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of two new triterpenoid saponins (**1**–**2**), one new glycoside of farnesyl (**3**), one new coumarin glucoside (**4**), together with a known saponin (**5**) having hederagenin as aglycone (Fig. 1). The structures of the new isolated compounds were established by extensive analysis of 1D and 2D NMR experiments, chemical evidences and mass spectrometry.

2. Results and discussion

The air-dried powdered of stem barks of *P. turbinata* were extracted with *aq*-EtOH 70% using a sonicator apparatus. After evaporation of the solvent, the resulting dark residue was suspended in water and partitioned against *n*-BuOH saturated with water. The *n*-BuOH phase was then evaporated to dryness affording a brown gum which was submitted to column chromatography (CC) using Diaion HP-20 resin yielding enriched saponins fractions which were submitted to VLC using silica gel to give four main subfractions. Purification of the eluated subfractions by Semiprep-HPLC afforded two new triterpenoid saponins (**1**–**2**), one new glycoside of farnesyl (**3**), one new coumarin glucoside (**4**), together with a known saponin (**5**) having hederagenin as aglycone (Fig. 1).

All the isolated compounds **1**–**5**, were obtained as white amorphous powders. The monosaccharides obtained by acid hydrolysis of each compound were identified by comparison on TLC with authentic samples as arabinose, xylose, rhamnose and glucose. The absolute configurations were determined by UHPLC/MS analysis to be D for glucose and xylose and L for rhamnose and arabinose (See **Experimental Section**) [18]. The ^{1}H and ^{13}C NMR data of the monosaccharide residues were assigned starting, either from the anomeric protons, or from the CH_3-proton doublet of rhamnose unit, by means of COSY, HSQC-TOCSY, HSQC, NOESY, and HMBC spectra obtained for each compound. Data from these above NMR experiments indicated that the sugar residues were in their pyranose form, and relatively large $^{3}J_{H\text{-}1,\ H\text{-}2}$ values 6.3–8.1 Hz of Glc, Xyl and Ara in their pyranose form evidenced a

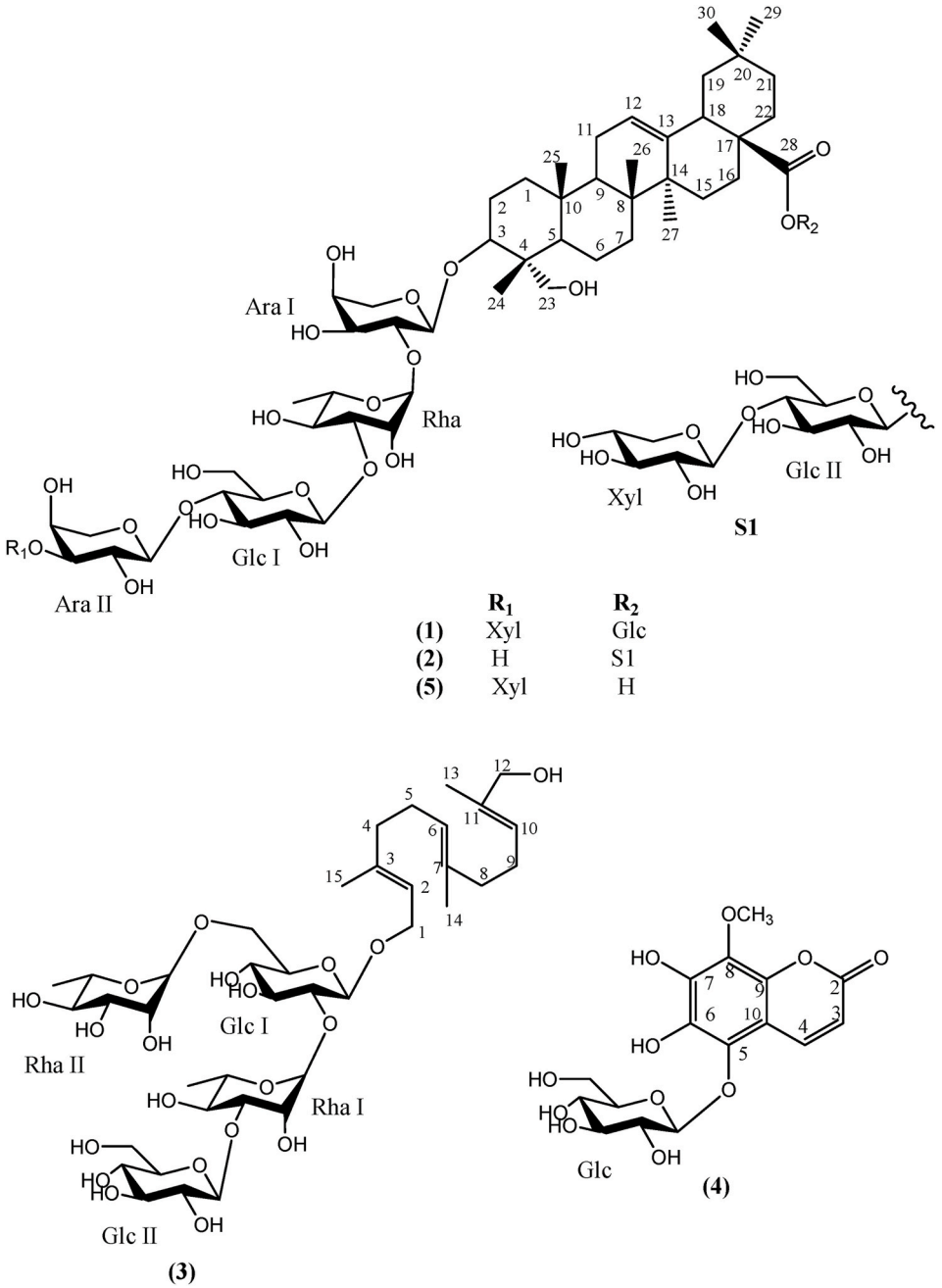


Fig. 1. Structures of isolated compounds **1**–**5**.

β-anomeric orientation for Glc and Xyl, and an α-anomeric configuration for Ara [19]. The α-anomeric configuration of the L-rhamnose unit was judged by the broad singlet of its anomeric proton and the chemical shift value of C-5 [20].

HR-ESIMS of turbinatoside A (**1**), indicated a $C_{63}H_{102}O_{30}$ molecular formula, as deduced from the protonated molecular ion peak at *m/z* 1339.6538 $[M + H]^+$ (calcd for $C_{63}H_{103}O_{30}$ 1339.6534). The ^{1}H NMR and ^{13}C NMR data of **1** displayed resonances due to the aglycone part characteristic for hederagenin unit (Table 1), a common triterpene of triterpene glycosides, which were in full agreement with literature data [13–16,21].

The ^{1}H NMR spectrum of **1** showed six anomeric protons at δ_H 4.81 (d, $J = 6.8$ Hz), 5.88 (br s), 5.08 (d, $J = 7.8$ Hz), 4.79 (d, $J = 7.6$ Hz), 4.92 (d, $J = 8.0$ Hz), and 6.00 (d, $J = 8.1$ Hz), which correlated with six anomeric carbon atom signals at δ_C 104.3, 100.9, 105.7, 104.6, 106.1 and 95.2, respectively, in the HSQC spectrum. Complete assignments of each sugar moiety were achieved by extensive analyses of 1D and 2D NMR experiments and UHPLC/MS analysis (see Experimental Section) allowing the characterization of two α-L-arabinopyranosyl (Ara I and Ara II), one α-L-rhamnopyranosyl (Rha), two β-D-glucopyranosyl (Glc I and Glc II), and one β-D-xylopyranosyl (Xyl) units (Table 2). The sequence of the oligosaccharide chain was established from the HMBC and NOESY experiments.

For the sugars chain attached at C-3 of the aglycone, the correlations observed in the HMBC spectrum between H-1 of Ara I (δ_H 4.81) and C-3 of the aglycone (δ_C 80.8), and in the NOESY spectrum between H-1 of Ara I (δ_H 4.81) and H-3 of the hederagenin moiety (δ_H 4.01), suggested that Ara I was directly attached to C-3 of the aglycone. Moreover, the HMBC correlation observed between H-1 of Rha (δ_H 5.88) and C-2 of Ara I (δ_C 75.1) established the connectivity between the two sugar units, which was confirmed by the NOESY correlation observed between H-2 of Ara I (δ_H 4.22) and H-1 of Rha (δ_H 5.88). In addition, the HMBC correlations observed between H-1 of Glc I (δ_H 5.08) and C-3 of Rha (δ_C 82.5), confirmed by the NOESY correlation observed between H-3 of Rha (δ_H 4.44) and H-1 of Glc I (δ_H 5.08) allowed us to locate Glc I at C-3 of Rha (Fig. 2a). On the other hand, the HMBC correlation observed between H-1 of Ara II (δ_H 4.79) and C-4 of Glc I (δ_C 79.6) established the connectivity between the two sugar units, which was confirmed by the NOESY correlation observed between H-4 of Glc I (δ_H 4.04) and H-1 of Ara II (δ_H 4.79). Moreover, the HMBC correlation observed between H-1 of Xyl (δ_H 4.92) and C-3 of Ara II (δ_C 82.8), confirmed by the NOESY correlation observed between H-3 of Ara II (δ_H 3.92) and H-1 of Xyl (δ_H 4.92) allowed us to locate Xyl at C-3 of Ara II. Thus, the pentasaccharide β-D-xylopyranosyl-(1 → 3)-α-L-arabinopyranosyl-(1 → 4)-β-D-glucopyranosyl(1 → 3)-α-L-rhamnopyranosyl(1 → 2)-α-L-arabinopyranoside moiety was established to be linked at C-3 of the aglycone (Fig. 2a).

The observation of the signals (δ_H/δ_C 6.00/95.2) of Glc II, indicated that this sugar should be directly attached to C-28 of the aglycone. This

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].

N°	1		2		3			4	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H		δ_C	δ_H
1	38.7	0.91 1.45	38.9	0.94 1.48	66.1	4.31; 4.59 m		–	–
2	25.9	1.83 2.03	26.1	1.86 2.09	121.2	5.64 (br s)		161.6	–
3	80.8	4.01 (dd, $J = 11.4, 5.0$)	81.0	4.09 (dd, $J = 10.3, 5.6$)	141.4	–		110.5	6.23 (d, $J = 9.5$)
4	43.1	–	43.3	–	40.3	2.05; nd		140.4	8.23 (d, $J = 9.5$)
5	47.1	1.51	47.3	1.55	27.1	2.14; 2.25		127.3	–
6	17.8	1.20 1.53	17.9	1.25 1.57	125.1	5.22 (br t, $J = 7.0$)		145.7	–
7	32.2	1.20 1.60	32.3	1.22 1.64	135.7	–		145.8	–
8	39.5	–	39.7	–	40.3	2.11; nd		133.3	–
9	47.8	1.60	48.0	1.65	27.1	2.14; 2.25		145.7	–
10	36.5	–	36.7	–	124.6	5.70 (br t, $J = 7.1$)		102.8	–
11	23.5	1.78 nd	23.6	1.83 nd	136.8	–	-OMe	60.8	3.85 (s)
12	122.5	5.28 m	122.7	5.32 m	68.4	4.31; nd			
13	143.8	–	144.0	–	14.4	1.82 (s)			
14	41.8	–	42.0	–	16.5	1.65 (s)			
15	27.9	0.98 2.07	28.1	0.99 2.14	17.0	1.70 (s)			
16	23.0	2.07 nd	23.3	1.81 1.97					
17	46.7	–	46.8	–					
18	41.4	2.97 (dd, $J = 13.7, 4.5$)	41.5	3.06 (dd, $J = 13.8, 4.6$)					
19	45.9	1.11 1.60	46.0	1.15 1.65					
20	30.4	–	30.6	–					
21	33.7	1.00 1.23	33.8	1.02 1.26					
22	32.3	1.15 1.48	32.5	1.19 1.52					
23	63.5	3.64 3.99	63.7	3.71 4.07					
24	13.5	0.88 (s)	13.8	0.95 (s)					
25	15.7	0.83 (s)	15.9	0.87 (s)					
26	17.1	0.92 (s)	17.3	0.98 (s)					
27	25.6	1.06 (s)	25.8	1.10 (s)					
28	176.3	–	176.4	–					
29	32.6	0.79 (s)	32.8	0.82 (s)					
30	23.2	0.77 (s)	23.4	0.80 (s)					

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

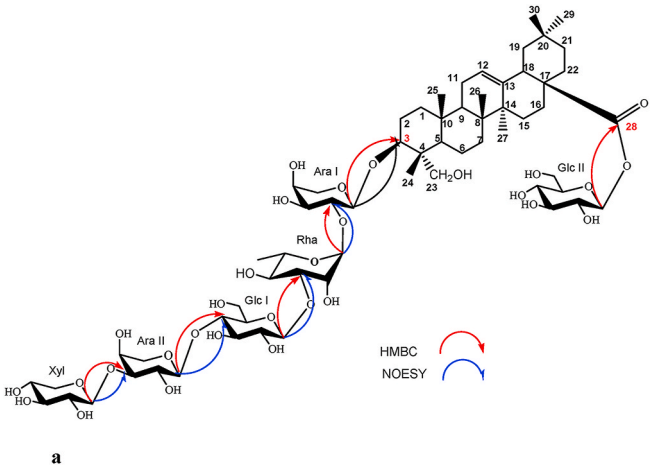
Table 2
NMR spectroscopic data (500 MHz for ^{1}H and 125 MHz for ^{13}C) for the sugar moieties attached at C-3 and C-28 of compounds **1**–**4** (δ in ppm and J in Hz) in pyridine-d_5.

Position	1		2		3		4	
	δ_C	δ_H (J *in Hz*)	δ_C	δ_H (J *in Hz*)	δ_C	δ_H (J *in Hz*)	δ_C	δ_H (J *in Hz*)
3-*O*-sugars					**1-*O*-sugars**		**5-*O*-sugar**	
Ara I					Glc I		Glc	
1	104.3	4.81 (d, J = 6.8)	104.5	4.87 (d, J = 6.6)	101.6	4.78 (d, J = 7.7)	108.3	5.41 (d, J = 6.3)
2	75.1	4.22	75.2	4.30	78.4	4.15	75.6	4.25
3	74.1	3.82	74.4	3.86	77.5	4.15	78.2	4.25
4	69.2	3.95 m	69.4	4.00 m	71.6	4.18	71.5	4.25
5	65.6	3.54 (brd, J = 11.9); 4.07	65.9	3.59 (brd, J = 11.5); 4.12	75.4	3.98	79.2	4.01
					67.6	4.32; 4.71 m	62.8	4.32; 4.50 m
Rha					Rha I			
1	100.9	5.88 (brs)	101.1	5.98 (brs)	102.6	6.13 (d, J = 1.5)		
2	70.9	4.64	71.2	4.72	72.6	4.71		
3	82.5	4.44	82.8	4.54	82.6	3.98		
4	72.2	4.13	72.5	4.23	73.1	4.52		
5	69.1	4.40	69.4	4.48	70.4	4.77		
6	17.8	1.38 (d, J = 6.1)	18.1	1.44 (d, J = 6.2)	19.1	1.75 (d, J = 6.2)		
Glc I					Glc II			
1	105.7	5.08 (d, J = 7.8)	106.0	5.18 (d, J = 8.1)	105.7	4.93 (d, J = 7.9)		
2	74.8	3.81	75.1	3.87	75.3	3.98		
3	75.5	3.94	75.8	4.02	78.6	4.18		
4	79.6	4.04	79.9	4.09	71.4	4.21		
5	76.1	3.67	76.4	3.73	78.8	3.97		
6	60.7	4.21; nd	61.0	4.26; 4.30	62.6	4.31; 4.47 m		
Ara II					Rha II			
1	104.6	4.79 (d, J = 7.6)	105.3	4.80 (d, J = 7.6)	102.6	5.63 (d, J = 1.6)		
2	70.8	4.28	72.5	4.22	72.7	4.62		
3	82.8	3.92	74.1	3.92	72.7	4.73		
4	68.6	4.20 m	70.6	4.10 (d, J= 11)	74.4	4.28		
5	67.0	3.66 (brd, J = 11.8); 4.04 (dd, J = 11.8; 3.1)	67.5	3.69 (brd, J = 12.2); 4.13	70.5	4.36		
					19.1	1.68 (d, J = 6.2)		
Xyl I								
1	106.1	4.92 (d, 8.0)						
2	76.1	3.68						
3	77.3	3.84						
4	70.4	3.88						
5	66.6	3.47 (dd, J = 12.1; 2.5); 4.10 (dd, J = 12.1; 1.4)						
	104.3	4.81 (d, J = 6.8)						
28-*O*-Sugars								
Glc II								
1	95.2	6.00 (d, J = 8.1)	95.2	6.09 (d, J = 8.1)				
2	73.5	3.89	73.7	3.97				
3	78.1	3.99	78.40	4.08				
4	70.4	3.99	81.0	4.08				
5	78.6	3.76	78.9	3.84				
6	61.6	4.12; 420	61.8	4.20; 4.29				
Xyl II								
1			105.2	4.89 (d, J = 7.5)				
2			74.5	3.75				
3			78.4	4.07				
4			70.4	3.94				
5			67.0	3.51 (brd, J = 10.3); 4.11				

Assignments were based on the HMBC, HSQC, COSY, TOCSY, NOESY, and DEPT experiments. [a)]Overlapped proton NMR signals are reported without designated multiplicity.

was confirmed by the HMBC correlation observed between H-1 of Glc II (δ_H 6.00) and C-28 of the aglycone (δ_C 176.3) permitting us to link Glc II to C-28 of hederagenin moiety. Hence, the structure of turbinatoside A (**1**), was established as 3-*O*-β-D-xylopyranosyl-(1 → 3)-α- L-arabinopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosylhederagenin-28-*O*-β-D-glucopyranosyl ester.

The HRESIMS spectrum ($[M+ H]^+$ at *m/z* 1339.6511) of turbinatoside B (**2**), was identical to that obtained for compound **1**, suggesting that these compounds were isomers with $C_{63}H_{103}O_{30}$ formula. Extensive 2D NMR analysis of saponin **2** revealed that it has also hederagenin as aglycone. Comparison of the NMR spectra of **2** with those of **1**, showed that compound **2** differs from compound **1** only by the substitution pattern of the sugar chains at C-3 and C-28 of the aglycone. Indeed, instead of a pentasaccharide moiety at C-3, and a

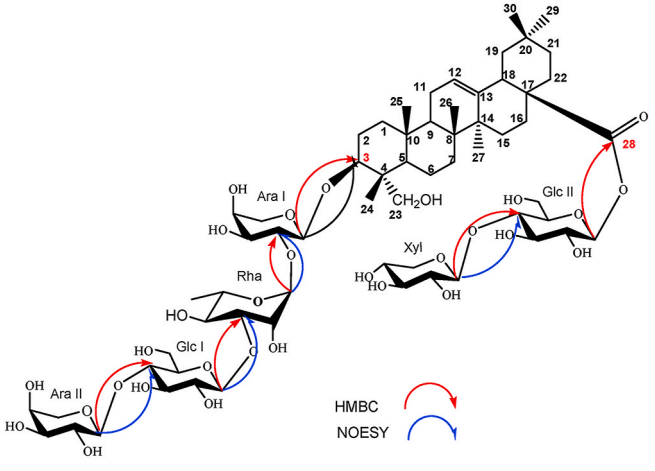


a

Fig. 2a. Key HMBC and NOESY correlations observed for Compound 1.

monosaccharide moiety at C-28 of hederagenin moiety, respectively, as observed in compound **1**, compound **2** displayed signals for a tetrasaccharide moiety at C-3, and a disaccharide moiety at C-28 of the aglycone, respectively, according to the analysis of 2D NMR experiments (Table 2). The observation of the signal of C-4 of Ara II at δ_C 74.1 (shield, −8.2 ppm) in **2** in compararaison to Ara II (δ_C 82.8) in **1**, and of C-4 of Glc II at δ_C 81.0 (deshield, +10.6 ppm) in **2** in comparaison to Glc II (δ_C 70.4) in **1**, indicated that Ara II was terminal in **2** and Glc II substituted at its C-4. In addition, the observation of the HMBC correlation observed between H-1 of Xyl (δ_H 4.89) and C-4 of Glc II (δ_C 81.0), and confirmed by the NOESY correlation observed between H-1 of Xyl (δ_H 4.89) and H-4 of Glc II (δ_H 4.08) allowed us to attach Xyl at C-4 of Glc II. Therefore, the sequences of the sugar chains at C-3 and C-28 of hederagenin, established by extensive analysis of 2D NMR experiments, were determined as *α*-L-arabinopyranosyl-(1 → 4)-*β*-D-glucopyranosyl-(1 → 3)-*α*-L-rhamnopyranosyl-(1 → 2)-*α*-L-arabinopyranoside, and *β*-D-xylopyranosyl-(1 → 4)-*β*-D-glucopyranoside, respectively (Fig. 2b). Consequently, the structure of turbinatoside B was established as 3-*O*-*α*-L-arabinopyranosyl-(1 → 4)- *β*-D-glucopyranosyl-(1 → 3)-*α*-L-rhamnopyranosyl-(1 → 2)-*α*-L-arabinopyranosylhederagenin-28-*O*-*β*-D-xylopyranosyl-(1 → 4)-*β*-D-glucopyranosyl ester (**2**).

The HRESIMS (negative-ion mode) of Turbinoside A (**3**) exhibited a protonated molecular ion peak at *m/z* 855.4222 ($[M+H]^+$, calcd for $C_{39}H_{67}O_{20}$ 855.4297) indicating a molecular formula of $C_{39}H_{66}O_{20}$. The ^{1}H NMR spectrum of compound **3** revealed the presence of three methyl



b

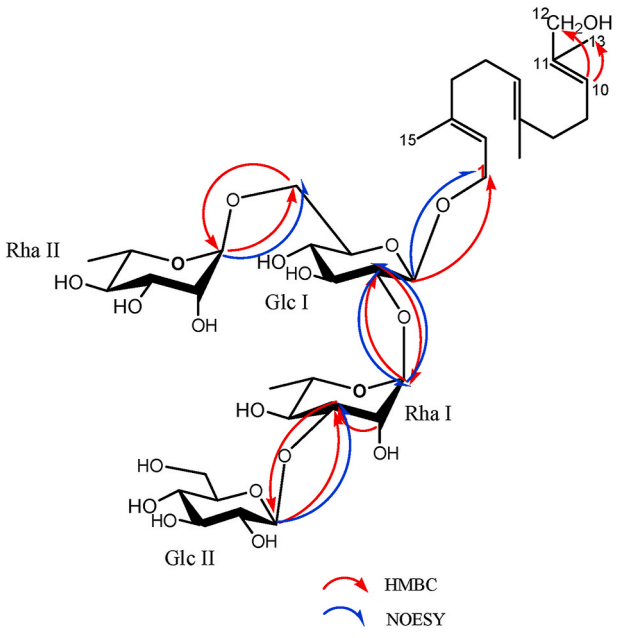
Fig. 2b. Key HMBC and NOESY correlations observed for Compound **2**.

groups at δ_H 1.82 (s, H-13), 1.65 (s, H-14) and 1.70 (s, H-15), showing correlations in the HSQC spectrum with carbon signals at δ_C 14.4 (C-13), 16.5 (C-14) and 17.0 (C-15), respectively. Three olefinic proton signals at δ_H 5.64 (brs, H-2), 5.22 (brt, H-6) and 5.70 (brt, H-10), showing correlations in the HSQC spectrum with carbon atom at δ_C 121.2, 125.1 and 124.6, respectively, were also observed, as well as six methylene carbon signals at δ_C 66.1 (C-1), 40.3 (C-4), 27.1 (C-5), 40.3 (C-8), 27.1 (C-9) and 68.4 (C-12). In addition, two oxymethylene proton groups at δ_H 4.31, 4.59 (m, H-1a, H-1b), and at δ_H 4.31(m, H-12a), showing correlations in the HSQC spectrum with carbon atom signals at δ_C 66.1 (C-1) and δ_C 68.4 (C-12), respectively, were also observed. Furthermore, in the ^{13}C NMR spectrum, three other carbon signals were observed as quaternary carbon at δ_C 141.4 (C-3), 135.7 (C-7) and 136.8 (C-11). These resonances, due to the sesquiterpene moiety, are characteristic of farnes-1,12-diol aglycone (Table 1), which was recognized to be (2*E*, 6*E*)-farnes-1, 12-diol [(*2E,6E*)-3,7,11-trimethyl-2,6,10-dodecatriene-1,12-diol] by ^{1}H NMR and ^{13}C NMR analyses using the correlations observed in COSY, HSQC, and HMBC spectra, and was in full agreement with literature data [22,23]. The chemical shifts of methyl carbons, C-14, C-15 at δ_C 16.5, 17.0, respectively, and those of two methylenes C-4 and C-8 at δ_C 40.3 and 40.3, respectively, were observed at almost the same resonances as those reported for (2*E*, 6*E*)-12-hydroxy-farnesol, while the terminal methylene C-12 appeared at δ_C 68.4 ppm, which is particularly indicative of the *E* stereochemistry of the double bonds in the 12-hydroxy-all-*trans*-farnesyl unit was also evidenced [22,24]. The absence of signals in the ^{13}C NMR spectrum in the region between 30 and 33 ppm allowed us to eliminate the other possible configurations (2*E*, 6*Z*), (2*Z*, 6*Z*), or (2*Z*, 6*E*) for this 12-hydroxy-farnesol unit.

The deshielded shift of C-1 of the 12-hydroxy-farnesol unit (δ_C 66.1) suggested this carbon as the point of linkage of the sugars chain. The ^{1}H NMR spectrum of the sugar portion of compound **3** showed four anomeric signals at δ_H 4.78 (d, *J*=7.7 Hz), 4.93 (d, *J* = 7.9 Hz), 6.13 (d, *J* = 1.5 Hz) and 5.63 (d, *J* = 1.6 Hz), which correlated with four anomeric carbon atom resonances at δ_C 101.6, 105.7, 102.6 and 102.6, respectively in the HSQC spectrum (Table 2). Complete assignments of each sugar moiety were achieved by extensive analyses of 1D and 2D NMR experiments and UHPLC/MS analysis (see Experimental Section) allowing the characterization of two *α*-L-rhamnopyranosyl (Rha I and Rha II) and two *β*-D-glucopyranosyl (Glc I and Glc II) units (Table 2).

The sequencing of the sugars chain was achieved by analysis of HMBC and NOESY experiments. HMBC correlations observed between H-1 of Glc I (δ_H 4.78) and C-1 of Aglycone (δ_C 66.1) allowed us to attach Glc I to the agly at its C-1. The HMBC correlations observed between H-1 of Rha I (δ_H 6.13) and C-2 of Glc I (δ_C 78.4) and between H-1 (δ_H 5.63) of Rha II and C-6 of Glc I (δ_C 67.6) permitted us to attach Rha I, and Rha II to C-2 and C-6 of Glc I, respectively. In addition, HMBC correlations observed between H-1 of Glc II (δ_H 4.93) and C-3 of Rha I (δ_C 82.6) suggested us to connect Glc II to C-3 of Rha I. These connectivities were supported by NOESY cross-peaks correlations observed between H-1 of Glc I (δ_H 4.78) and H-1 of Agly (δ_H 4.59), between H-1 of Rha I (δ_H 6.13) and H-2 of Glc I (δ_H 4.15), between H-1 of Rha II (δ_H 5.63) and H-6 of Glc I (δ_H 4.71), and between H-1 of Glc II (δ_H 4.93) and H-3 of Rha I (δ_H 3.98) (Fig. 2c). Hence, the sugar chain at C-1 of the aglycone was established as *β*-D-glucopyranosyl-(1 → 3)-*α*-L-rhamnopyranosyl-(1 → 2)-[*α*-L-rhamnopyranosyl-(1 → 6)]-*β*-D-glucopyranosyl moiety and the structure of **3** was elucidated as 1-*O*-{*β*-D-glucopyranosyl-(1 → 3)-*α*-L-rhamnopyranosyl-(1 → 2)-[*α*-L-rhamnopyranosyl-(1 → 6)]-*β*-D-glucopyranosyl}-(*2E,6E*)-farnes-1,12-diol (**3**).

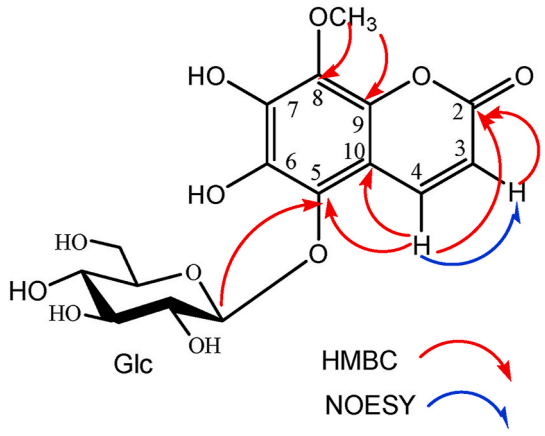
The HRESIMS (positive-ion mode) of Panturboside (**4**) exhibited a protonated molecular ion peak at *m/z* 387.0949 ($[M+H]^+$, calcd for $C_{16}H_{19}O_{11}$ 387.0962) indicating a molecular formula of $C_{16}H_{18}O_{11}$. The ^{13}C NMR spectrum of compound **4** exhibited sixteen carbon signals, from which, six were suggested to a hexosyl moiety and the remaining ten, assignabled to the aglycone. For the aglycone signals, seven quaternary and two tertiary carbon signals were observed. Among these, one conjugated lacton carbonyl was observed at δ_C 161.6 (C-2), six



c

Fig. 2c. Key HMBC and NOESY correlations observed for Compound **3**.

quaternary aromatic carbon signals at δ_C 102.8 (C-10), 127.3 (C-5), 145.7 (C-9), 145.7 (C-6), 145.8 (C-7) and 133.3 (C-8), and two carbone signals of a conjugated ethylenic group at δ_C 110.5 (C-3) and 140.4 (C-4). The ^{1}H NMR spectrum of compound **4** revealed two olefinic protons as two 1,2-cis-coupled doublets at δ_H 6.23 (H-3, d, $J = 9.5$ Hz) and 8.23 (H-4, d, $J = 9.5$ Hz), an AB system characteristic of the protons H-3 and H-4 of a coumarin skeleton. A singlet of three protons at δ_H 3.85 (3H, s) was assigned to the protons of a methoxyl group which correlated with a carbone signal at δ_C 60.8. Considering the NMR data, trihydroxycoumarin methylether was suggested as the only plausible structure of the aglycone of **4** (Table 1) [25]. The HMBC correlations observed between H-4 (δ_H 8.23) and C-5 (δ_C 127.3) of the aglycone and on the other hand between the methoxyl protons (δ_H 3.85) and C-8 (δ_C 133.3) of the aglycone revealed not only the site of glucosidation and the linking position of the methoxyl group (Fig. 2d), but also excluded the C-6 and C-7 positions, and allowed the unambiguous assignment of all other carbons of the aglycone.



d

Fig. 2d. Key HMBC and NOESY correlations observed for Compound **4**.

The ^{1}H NMR spectrum of compound **4** showed one anomeric signal at δ_H 5.41 (d, $J = 6.3$ Hz), which correlated with one anomeric carbon atom resonance at δ_C 108.3, in the HSQC spectrum (Table 2). Complete assignment of this sugar moiety was achieved by extensive analyses of 1D and 2D NMR experiments and UHPLC/MS analysis (see Experimental Section) allowing the characterization of a β-D-glucopyranosyl (Glc) unit, which was linked at C-5 of the aglycone as argumentated above. Consequently, the structure of **4** was elucidated as 5-*O*-β-D-glucopyranosyl-5,6,7-trihydroxy-8-methoxycoumarin (**4**).

Compound **5** was identified as 3-*O*-β-D-xylopyranosyl-(1 → 3)-α-L-arabinopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosylhederagenin, previously isolated from *Blighia unijugata*, by comparison of its spectra data with those reported in the literature [14].

3. Conclusion

Our findings highlight the presence of three main classes of secondary metabolites often described in the Sapindaceae family, namely saponins, farnesyl glycosides and coumarin derivatives. The isolated saponins **1**–**2**, **5** are all hederagenin glycosides, sharing the -^{3}Rha-^{2}Ara-^{3}hederagenin oligosaccharidic sequence most often encountered in the saponins from Sapindoideae [7–14].

Concerning the farnesyl glycosides, previous studies from the Sapindoideae subfamily revealed their presence in *Sapindus mukurossi* [21], *Sapindus delavayi* [22], *Guioa crenulate* [26], and recently in *Eriocoelum microspermum* [13]. It is worthy to note that our farnesyl glycoside (**3**) is a 12- hydroxyl-(*2E,6E*)-farnesol glycoside, so far isolated only from *Sapindus mukurossi* and *Sapindus delavayi*.

In the case of coumarin glucoside (**4**), we have observed that coumarin derivatives are common in Sapindoideae subfamily. They have been previously identified from *Xanthoceras sorbifolia* [27], *Eurycorymbus cavaleriei* [28], *Cardiospermum corundum* [29], and recently from *Paullinia pinnata* [30]. Panturboside A (**4**) is to the best of our knowledge, the first report on a trihydroxycoumarin methyl ether glycoside, so far isolated from Sapindoideae subfamily. Our findings represent therefore a valuable contribution to the chemotaxonomy of Sapindoideae subfamily.

4. Experimental Section

4.1. General experimental procedures

Optical rotations were measured on a Jasco P-2000 polarimeter. NMR experiments were performed at 298 K in pyridine-d5 and DMSO-d_6 on a Bruker AVANCE 500 spectrometer equipped with a 5 mm Z-gradient TCI cryoprobe. HRESIMS spectra were recorded using a UHPLC-DAD-LTQ Orbitrap XL instrument (Thermo Fisher Scientific, UK) equipped with an electrospray ionization source (ESI). Semi-preparative high performance liquid chromatography (HPLC) was carried out using a LaChrom Merck Hitachi system consisting of a LaChrom L-7100 pump, an L-7455 DAD, and a D-7000 interface and employing a Phenomenex Luna 5 µm C18(2) 100 Å, 250 × 10 mm^2 column. Vacuum-liquid chromatography (VLC) was carried out using RP-18 silica gel 60 (25–40 µm). Thin layer chromatography (TLC) was performed on pre-coated silica gel plates (60 F254, Merck) using the system solvent *n*-BuOH-AcOH-H_2O, 60:15:25 as eluent. The spray reagent for saponins was vanillin reagent (10% mixture of conc. H_2SO_4 soln. and 1% vanillin in EtOH).

4.2. Plant material

The barks of *Pancovia turbinata* Radlk. were harvested at Nkolbisson, Yaoundé peripheral quarter, in Cameroon in October 2016 under the guidance of Mr. Victor Nana, botanist of the National Herbarium of Cameroon (NHC), where a voucher specimen (37637/HNC) was

deposited.

4.3. Extraction and isolation

The air-dried, powdered stems barks of *P. turbinata* (300 g) were extracted with aq-EtOH 70% using a sonicateur apparatus for 1 h three times. The resulting hydroalcoholic solution was then evaporated to dryness under reduce pressure to yield brown residue (25.4 g). This residue was dissolved in ethyl acetate (300 mL), and the insoluble residue (19.52 g) was suspended in 10 mL of H_2O and partitioned in with *n*-BuOH sat. H_2O (3 x 300 mL). The *n*-BuOH soluble phase was evaporated to dryness affording 5.36 g of brown gum residue. Part of this residue (3 g) was suspended in 100 mL of water and then submitted to column chromatography (CC) using Diaion HP-20 resin, eluting with H_2O, 50% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, and 100% MeOH, successively giving four main fractions (PTE1—PTE4) after TLC monitoring. The 80%–100% MeOH fraction (PTE4) was evaporated to dryness yielding a crude saponin mixture (1.8 g) that was then submitted to VLC using silica gel 60 (15–40 μm), eluted with CH_2Cl_2–MeOH (80:20) and CH_2Cl_2–MeOH–H_2O (70:30:5, 60:32:6.5) to give four main subfractions (PTE41— PTE44). Subfraction PTE43 (296.7 mg) was purified by semi preparative HPLC using gradient system of CH_3CN–H_2O (30 mL/min) to yield compounds **1** (t_R, 16.8 min, 6.2 mg), **2** (t_R, 19.3 min, 9.8 mg), and **5** (t_R, 12.5 min, 4.2 mg), while subfraction PTE44 yielded compounds **3** (t_R, 10.5 min, 3.7 mg) and **4** (t_R, 11.6 min, 5.3 mg).

4.3.1. Turbinatoside A (1)

White amorphous powder; $[\alpha]^{25}_D$ −43.7 (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*: 1339.6538 $[M + H]^+$ (calcd for $C_{63}H_{103}O_{30}$, 1339.6635).

4.3.2. Turbinatoside B (2)

White amorphous powder; $[\alpha]^{25}_D$ −18.5 (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*: 1339.6511 $[M + H]^+$ (calcd for $C_{63}H_{103}O_{30}$, 1339.6635).

4.3.3. Turbinoside A (3)

White amorphous powder; $[\alpha]^{25}_D$ −27.2 (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*: 855.4222 $[M + H]^+$ (calcd for $C_{39}H_{67}O_{20}$, 855.4297).

4.3.4. Panturboside A (4)

White amorphous powder; ^{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*: 387.0949 $[M + H]^+$ (calcd for $C_{16}H_{19}O_{11}$, 387.0962).

4.4. Acid hydrolysis of isolated compounds

Each compound (2 mg) was refluxed in 2 N aqueous CF_3COOH (3 mL) for 2 h at 100 °C. The reaction mixture was diluted with H_2O (10 mL) and extracted with CH_2Cl_2 (3 x 5 mL). The combined CH_2Cl_2 extracts were washed with water to give after evaporation the aglycone moiety. After extraction, the aqueous layer was repeatedly evaporated to dryness with MeOH until it reached a neutral pH. The sugars were first analyzed by TLC over silica gel ($CHCl_3$–MeOH–H_2O, 8:5:1) by comparison with standard samples. The following sugars were detected: glucose, arabinose, xylose and rhamnose. Furthermore, the absolute configuration of the sugar moiety was determined as previously described by Wang et al. (2012), with slight modifications [18]. Briefly, the residue of sugars was dissolved in 120 μL of a solution of L-cysteine methyl ester in pyridine (0.3 M) and incubated for 1 h at 90 °C. Then 160 μL of a solution of phenyl isothiocyanate in pyridine (0.69 M) was added, and incubated for 1 h at 90 °C. The reaction mixture was allowed to cool and diluted 20-fold with CH_3CN and filtered before UHPLC/MS analysis. Each sugar standard (2 mg of L-glucose, D-glucose, L-rhamnose, D-rhamnose, L-xylose, D-xylose, L-arabinose and D-arabinose) was subjected to the same protocol, with 240 μL of the L-cysteine methyl ester solution and 320 μL of the phenyl isothiocyanate solution. The reaction mixtures were diluted 60-fold with CH_3CN and filtered before UHPLC/MS analysis. The observed retention times were 11.63, 15.17, 13.91 and 12.76 min for the samples obtained from **1**–**2**, 11.63 and 15.17 from **3**, to 11.63 for the sample obtained from **4**, respectively. These retention times were similar with those observed for D-glucose (11.64), L-rhamnose (15.19 min), D-xylose (13.93 min) and L-arabinose (12.77 min).

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.

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