Original Article

Triterpenoid Saponins From the Stem Bark of *Pentaclethra eetveldeana*

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Abstract

Two previously undescribed triterpenoid saponins together with 4 known ones were isolated from the stem bark of *Pentaclethra eetveldeana* De Wild. & Th. Dur. Their structures were elucidated by spectroscopic methods including 1D and 2D NMR experiments in combination with mass spectrometry as 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyloleanolic acid and 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosylhederagenin.

Keywords

Pentaclethra eetveldeana, Fabaceae, triterpene saponins, 2D NMR, MS

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The genus *Pentaclethra* (Fabaceae) is represented by only 3 species (*Pentaclethra eetveldeana*, *P. macrophylla,* and *P. macroloba*).[1] *P. macroloba* is known to the local people in Brazil as antidote against snakebites[1] from which triterpenoid saponins have been isolated and characterized.[1,2] *P. eetveldeana* is a tree of 15 to 30 m of tropical African forest. It is used in Zairian traditional medicine for the treatment of hemorrhoids and various diseases such as malaria and epilepsy.[3] Previous phytochemical studies on *P. eetveldeana* showed the presence of saponins in the seeds[4] with oleanolic acid or hederagenin as aglycone and glucose, rhamnose, and arabinose as sugar units. In a continuation of our studies on natural saponins from the plants of the Fabaceae family,[5,6] we decided to examine the saponins from the stem bark of *P. eetveldeana* De Wild. & Th. Dur. In this paper, we report the isolation and structure elucidation of 2 previously undescribed triterpene saponins together with 4 known ones. Their structures were elucidated by spectroscopic methods including 600 MHz 1D and 2D experiments (^{1}H, ^{13}C, HSQC, HMBC, COSY, TOCSY, ROESY) in combination with HR-ESIMS and by comparison of their physical and spectral data with literature values.

The saponin fraction obtained from the 80% aqueous methanolic extract of the stem bark of *P. eetveldeana* was fractionated by vacuum liquid chromatography (VLC) on reverse-phase RP-18 silica gel and medium-pressure liquid chromatography (MPLC) yielding 2 previously undescribed saponins **1** and **2** (Figure 1) and 4 known ones. The known molecules were identified by comparison of their spectral data with literature values as 3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyloleanolic acid[7] and 3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosylhederagenin[8] widely distributed, 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyloleanolic acid,[2] and 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosylhederagenin[2] isolated from *P. macroloba*.

Compounds **1** and **2** were isolated as white amorphous powders. The monosaccharides were identified by extensive 2D NMR and GC analyses[9] (see the section "Experimental")

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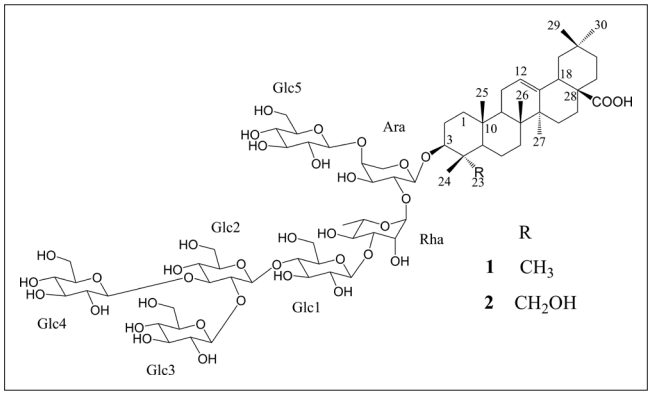

Figure 1. Structure of compounds **1** and **2**.

as β-D-glucopyranosyl (Glc), α-L-arabinopyranosyl (Ara), and α-L-rhamnopyranosyl (Rha) for **1** and **2**. The $^{3}J_{H\text{-}1,\ H\text{-}2}$ coupling constants (7.6-8.2 Hz) in the ^{1}H NMR spectrum for the glucose in its pyranose form indicated its β anomeric orientation and the large $^{1}J_{H\text{-}1,\ C\text{-}1}$ value of rhamnose (168 Hz) confirmed that the anomeric proton was equatorial in its α-pyranoid form. The doublet observed for the anomeric proton of arabinose in its pyranose form ($^{3}J_{H\text{-}1,\ H\text{-}2}$ 6.4, 7.7 Hz) is consistent with the $^{4}C_{1}$ conformation of Ara with an α-orientation of the anomeric center.

Compound **1** exhibited in the HR-ESIMS a quasimolecular ion peak at *m/z* 1567.7140 $[M+Na]^{+}$ (calcd. 1567.7144) compatible with the molecular formula $C_{71}H_{116}O_{36}Na$. Compound **1** showed in the ESIMS spectrum (positive-ion mode, Figure S7) an ion peak at *m/z* 1567 $[M+Na]^{+}$ indicating a molecular weight of 1544. The ^{1}H and ^{13}C NMR spectra due to the aglycone moiety displayed resonances characteristic of a triterpene with 7 angular methyl groups showing correlations in the HSQC spectrum at δ_H/δ_C 1.04 (s, H_3-23)/27.1, 0.85 (s, H_3-24)/15.6, 0.94 (s, H_3-25)/14.5, 0.82 (s, H_3-26)/16.4, 1.14 (s, H_3-27)/24.9, 0.89 (s, H_3-29)/32.2, and 0.93 (s, H_3-30)/22.6. Other characteristic signals were observed such as 1 olefinic proton at δ_H 5.22 (br t, H-12) and 1 oxygen-bearing methine proton signal at δ_H 3.11 (dd, J = 4.1, 11.1 Hz, H-3), showing correlations in the HSQC spectrum with δ_C 121.9 (C-12) and 89.1 (C-3), respectively. Extensive 2D NMR analysis confirmed the structure of the aglycone to be oleanolic acid (3β-hydroxyolean-12-en-28-oic acid) (Table 1), whose NMR data were in good agreement with literature values.[10]

The analysis of the ^{1}H NMR spectrum of the sugar part of **1** (Figure S1) showed the presence of 7 anomeric proton signals showing correlations in the HSQC spectrum at δ_H/δ_C 4.41 (d, J = 6.4 Hz)/104.4, 5.09 (d, J = 1.1 Hz)/100.7, 4.51 (d, J = 7.6 Hz)/104.2, 4.50 (d, J = 7.6 Hz)/101.7, 4.85 (d, J = 8.2 Hz)/102.1, 4.66 (d, J = 8.2 Hz)/102.8, and 4.45 (d, J = 7.6 Hz)/104.6 (Table 2). Extensive 2D NMR (Figures S2 and S3) and GC analyses (see the section "Experimental") allowed the characterization of 1 Ara, 5 Glc (Glc1-Glc5), and 1 Rha units. Extensive 2D NMR analysis allowed the identification of the partial sequence of **1** as Glc1-(1-3)-Rha-(1-2)-[Glc5-(1-4)]-Ara-(1-3)-oleanolic acid, which was previously characterized in the genus *Pentaclethra*.[1,2] The remaining 3 sugar units were identified as 3 Glc (Glc2, Glc3, and Glc4). Cross peaks were observed in the COSY spectrum at δ_H/δ_H 4.50 (Glc2 H-1)/3.68 (Glc2 H-2) and in the TOCSY spectrum at δ_H/δ_H 4.50 (Glc2 H-1)/3.74 (Glc2 H-3). The protons H-2 and H-3 give correlations with their carbon signals at δ_C 79.0 (Glc2 C-2) and 86.2 (Glc2 C-3), respectively, in the HSQC spectrum, suggesting a glycosylation at these positions. The HMBC correlations (Figures 2 and S2) at δ_H/δ_C 4.85 (Glc3 H-1)/79.0 (Glc2 C-2) and 4.66 (Glc4 H-1)/86.2 (Glc2 C-3) allowed to identify the final branched trisaccharide moiety as Glc3-(1→2)-[Glc4-(1→3)]-Glc2- which was confirmed by observation of the ROESY correlations at δ_H/δ_H 4.85 (Glc3 H-1)/3.68 (Glc2 H-2), 4.66 (Glc4 H-1)/3.74 (Glc2 H-3). Finally, the HMBC correlation at δ_H/δ_C 4.50 (Glc2 H-1)/80.3 (Glc1 C-4) allowed to link the

Table 1. ^{13}C NMR and ^{1}H NMR Spectroscopic Data of the Aglycone Moieties for Compounds **1** and **2**, in CD_3OD (δ in ppm)[a].

	1		**2**	
Position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	38.5	0.97 m, 1.61	38.2	0.96, 1.60
2	25.8	1.70, 1.82 m	25.0	1.74, 1.85
3	89.1	3.11 dd (4.1, 11.1)	81.0	3.62
4	38.9	-	42.5	-
5	55.7	0.78 br d (11.1)	46.7	1.26
6	17.9	1.40, 1.56	17.4	1.36, 1.49
7	32.6	1.30, 1.49	32.2	1.27, 1.61
8	39.1	-	39.1	-
9	47.6	1.58	47.8	1.63
10	36.5	-	36.2	-
11	23.1	1.88, 1.88	23.1	1.88, 1.88
12	121.9	5.22 br t	121.7	5.22 br t
13	144.0	-	144.0	-
14	41.5	-	41.6	-
15	27.4	1.08, 1.78	27.6	1.04 m, 1.81 m
16	22.7	1.59, 1.95 td (4.1, 13.4)	22.8	1.58, 1.96 td (4.1, 13.4)
17	46.0	-	nd	-
18	41.4	2.85 dd (3.8, 14.0)	41.6	2.86 dd (3.8, 14.0)
19	45.9	1.11 dd (2.2, 11.4), 1.68	46.2	1.10 dd (2.2, 11.4), 1.67 m
20	30.2	-	30.1	-
21	33.6	1.18, 1.38	34.0	1.17, 1.36
22	32.6	1.52, 1.73	32.6	1.52, 1.73
23	27.1	1.04 s	63.0	3.33, 3.61
24	15.6	0.85 s	12.2	0.70 s
25	14.5	0.94 s	14.9	0.96 s
26	16.4	0.82 s	16.5	0.83 s
27	24.9	1.14 s	25.0	1.15 s
28	nd	-	182.0	-
29	32.2	0.89 s	32.3	0.88 s
30	22.6	0.93 s	22.7	0.93 s

nd: not determined.
[a]Overlapped proton NMR signals are reported without designated multiplicity.

Table 2. ^{13}C NMR and ^{1}H NMR Spectroscopic Data of the Sugar Moieties for Compounds **1** and **2**, in CD_3OD (δ in ppm)[a].

	1		2	
Position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
3-*O*-				
Ara-1	104.4	4.41 d (6.4)	104.0	4.44 d (7.7)
2	76.5	3.64	76.3	3.58
3	72.0	3.73	72.5	3.68
4	78.3	3.89	78.9	3.89
5	63.9	3.53, 4.16 dd (4.1, 12.8)	64.3	3.52, 4.19 dd (3.5, 12.9)
Rha-1	100.7	5.09 d (1.1)	100.7	5.12 d (1.1)
2	69.7	4.25 br s	70.1	4.27 br s
3	81.5	3.86	81.5	3.89
4	71.0	3.56	71.0	3.56
5	68.7	3.88	68.8	3.91
6	16.7	1.22 d (5.8)	16.7	1.25 d (5.8)
Glc1-1	104.2	4.51 d (7.6)	104.2	4.52 d (8.2)
2	73.6	3.36	73.6	3.36
3	74.6	3.56	74.8	3.56
4	80.3	3.46	80.6	3.43
5	75.2	3.47	75.2	3.48 m
6	61.1	3.65, 3.86	61.1	3.65, 3.87
Glc2-1	101.7	4.50 d (7.6)	101.7	4.50 d (7.6)
2	79.0	3.68	78.9	3.68
3	86.2	3.74	86.2	3.74
4	68.7	3.40	68.7	3.40
5	76.8	3.36	76.8	3.36
6	61.2	3.66, 3.87	61.5	3.68, 3.85
Glc3-1	102.1	4.85 d (8.2)	102.7	4.85 d (7.7)
2	74.6	3.23 t (8.7)	73.9	3.23
3	76.8	3.36	76.5	3.36
4	69.9	3.30	70.1	3.33
5	76.5	3.28	76.6	3.28
6	61.1	3.67, 3.88	61.1	3.65, 3.87
Glc4-1	102.8	4.66 d (8.2)	102.8	4.66 d (7.6)
2	73.8	3.26	73.9	3.26
3	76.7	3.36	76.5	3.36
4	70.1	3.35	70.1	3.35
5	76.6	3.30	76.6	3.28
6	61.2	3.66, 3.86	61.1	3.65, 3.87
Glc5-1	104.6	4.45 d (7.6)	104.8	4.46 d (7.6)
2	74.2	3.28	73.9	3.28
3	76.2	3.38	76.1	3.40
4	69.9	3.30	70.1	3.30
5	76.5	3.30	76.7	3.29
6	61.1	3.66, 3.87	61.1	3.65, 3.87

[a]Overlapped proton NMR signals are reported without designated multiplicity.

trisaccharide moiety at position 4 of the Glc1, which was corroborated by the ROESY correlation at δ_H/δ_H 4.50 (Glc2 H-1)/3.46 (Glc1 H-4). Thus, the structure of **1** was elucidated as 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyloleanolic acid.

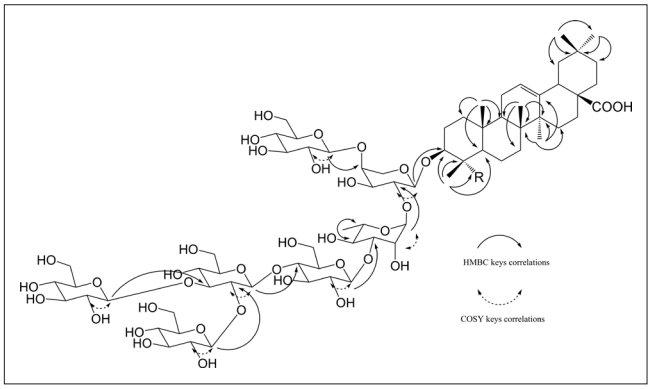

Figure 2. HMBC and COSY correlations for compounds **1** and **2**.

Compound **2** exhibited in the HR-ESIMS a quasimolecular ion peak at *m/z* 1583.7098 $[M+Na]^+$ (calcd. 1583.7093) compatible with the molecular formula $C_{71}H_{116}O_{37}Na$. Compound **2** showed in the ESIMS spectrum (positive-ion mode, Figures S7) a pseudomolecular ion peak at *m/z* 1583 $[M+Na]^+$ indicating a molecular weight of 1560. Extensive 1D and 2D NMR analysis (Tables 1 and 2, Figures S4-S6) revealed that compound **2** differed from compound **1** only by the aglycone moiety at C-23. The methyl group in **1** (δ_H/δ_C 1.04/27.1) was replaced by a primary alcoholic function CH_2OH in **2** (δ_H/δ_C 3.33, 3.61/63.0) allowing to characterize hederagenin (3β,23-dihydroxy-olean-12-en-28-oic acid) as aglycone, whose NMR data were in good concordance with literature values.[11] Thus, the structure of **2** was elucidated as 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosylhederagenin.

From the crude ethanolic extract of the stem bark of *P eetveldeana*, we isolated 2 previously undescribed monodesmosidic triterpenoid saponins and 4 known ones by successive MPLC. As it was reported, structural analogs were isolated from *P. macroloba*.[1,2] Some of them shared the same 6 sugar sequence Glc-(1→2)-Glc-(1→4)-Glc1-(1→3)-Rha-(1→2)-[Glc-(1→4)]-Ara-linked at C-3 of oleanolic acid and hederagenin as in **1** and **2,** which might be considered as a potential chemotaxonomic marker of the genus *Pentaclethra* in the Fabaceae family.

Experimental

General Procedures

Optical rotation values were recorded on AA-10R automatic polarimeter. NMR spectra were performed using a Varian INOVA 600 at the operating frequency of 600 MHz. For details, see the section Experimental part of Ref. 11.[11] HR-ESIMS (positive-ion mode) and ESIMS (positive-ion mode) were carried out on a Bruker micrOTOF mass spectrometer. GC analysis was carried out on a thermoquest gas chromatograph using a DB-1701 cap. column (30 m × 0.25

mm, i.d.) (J and W Scientific); detection by FID; detector temperature 250°C, injection temperature 230°C; initial temperature was maintained at 80°C for 5 minutes and then raised to 270°C at 15°C/min; carrier gas He. TLC and HPTLC are performed using silica gel plates (Merck) ($CHCl_3$-MeOH-H_2O, 70/30/5 and 60/32/7). The spray reagent for saponins was vanillin reagent (2% mixture of conc. H_2SO_4 soln. and 1% vanillin in EtOH). Isolations were carried out using an MPLC system (Alltech pump, Büchi column [460 × 15 mm], Büchi precolumn [110 × 15 mm], silica gel 60 [Merck, 15-40 μm]).

Plant Material

The stem bark of *P. eetveldeana* De Wild. & Th. Dur. (Fabaceae) was collected on the campus of the University of Kinshasa (Democratic Republic of Congo) in a small forest. It was identified by comparison with the reference Herbal P. Compere 698 kept in the Herbarium of INERA/University of Kinshasa.

Extraction and Isolation

A total of 221 g of dried and pulverized stem bark was macerated in 350 mL of 80% aqueous MeOH during 1 week and then refluxed for 1h 30. The extract was then filtered and washed with 300 mL of 80% aq. MeOH and left above a water bath to evaporate at 50°C in a ventilated atmosphere. The hydro-alcoholic extract was dissolved in MeOH (150 mL) and saponin was precipitated in Et_2O (450 mL), filtered, and dried. This procedure was repeated again, yielding 11 g of a whitish crude saponin mixture (CSM). An aliquot of CSM (1.5 g) was fractionated by VLC on normal phase silica gel (40-60 μm) using as eluent $CHCl_3$-MeOH-H_2O (60/32/7) to give 7 fractions Fr.1 to Fr.7 (150 mL ×7). Fr.2 (190 mg) was fractionated by MPLC over silica gel eluted with a $CHCl_3$-MeOH-H_2O gradient (70/30/5, 60/32/7), 2.5 mL/min, to give 12 fractions Fr.2.1 to Fr.2.12. Fr.2.11 (11 mg) was purified by VLC over RP-18 silica gel (75-200 μm) using as eluent H_2O/EtOH (linear gradient 100/0-0/100) to give compound **1** (4 mg) in the ethanolic fraction. Fr.4 (115 mg) was fractionated by VLC over RP-18 silica gel (75-200 μm) using the same gradient as above to give 2 fractions Fr.4.1 and Fr.4.2. Fr.4.2 (32 mg) was purified by MPLC over silica gel using as eluent $CHCl_3$-MeOH-H_2O (60/32/7), 2.5 mL/min, to give **2** (9.3 mg).

Acid Hydrolysis and GC Analysis

Each compound (3 mg) was hydrolyzed with 2 N aq. CF_3COOH (5 mL) for 3 hours 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. Furthermore, the residue of sugars was dissolved in anhydrous pyridine (100 μL), and L-cysteine methyl ester hydrochloride (0.06 mol/L) was added. The mixture was stirred at 60°C for 1 hour, then 150 μL of HMDS-TMCS (hexamethyl-disilazane-trimethylchlorosilane 3:1) was added, and the mixture was stirred at 60°C for another 30 minutes. The precipitate was centrifuged off, and the supernatant was concentrated under N_2 stream. The residue was partitioned between *n*-hexane and H_2O (0.1 mL each), and the hexane layer (1 μL) was analyzed by GC.[9] The absolute configurations were determined by comparing the retention times with thiazolidine derivatives prepared in a similar way from standard sugars (Sigma-Aldrich): L-rhamnose, D-glucose, and L-arabinose for **1** and **2** were characterized by co-injection of the silylated derivatives with standard silylated samples having t_R 13.1 minutes (L-rhamnose), 18.6 minutes (D-glucose), and 11.9 minutes (L-arabinose).

3-*O*-β-D-Glucopyranosyl-(1→2)-[β-D-Glucopyranosyl-(1→3)]-β-D-Glucopyranosyl-(1→4)-β-D-Glucopyranosyl-(1→3)-α-L-Rhamnopyranosyl-(1→2)-[β-D-Glucopyranosyl-(1→4)]-α-L-Arabinopyranosyloleanolic Acid (1)

White, amorphous powder.

$[\alpha]_D^{25}$: +2.1 (*c* 0.3, MeOH).

^1H NMR and ^{13}C NMR (CD_3OD, 600 and 150 MHz): Tables 1 and 2.

HR-ESIMS (positive-ion mode) *m/z* 1567.7140 $[M+Na]^+$ (calcd. 1567.7144 for $C_{71}H_{116}O_{36}Na$); ESIMS (positive-ion mode) *m/z* 1567 $[M+Na]^+$.

3-*O*-β-D-Glucopyranosyl-(1→2)-[β-D-Glucopyranosyl-(1→3)]-β-D-Glucopyranosyl-(1→4)-β-D-Glucopyranosyl-(1→3)-α-L-Rhamnopyranosyl-(1→2)-[β-D-Glucopyranosyl-(1→4)]-α-L-Arabinopyranosylhederagenin (2)

White, amorphous powder.

$[\alpha]_D^{25}$: +1.2 (*c* 0.1, MeOH).

^1H NMR and ^{13}C NMR (CD_3OD, 600 and 150 MHz): Tables 1 and 2.

HR-ESIMS (positive-ion mode) *m/z* 1583.7098 $[M+Na]^+$ (calcd. 1583.7093 for $C_{71}H_{116}O_{37}Na$); ESIMS (positive-ion mode) *m/z* 1583 $[M+Na]^+$.

Declaration of Conflicting Interests

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Supplemental Material

Supplemental material for this article is available online.

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