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Triterpenoid Saponins From the Root Bark of *Haplocoelum congolanum*

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Abstract

Two undescribed triterpenoid saponins together with 5 known ones were isolated from the root bark of *Haplocoelum congolanum* Hauman. Their structures were elucidated by spectroscopic methods including one-dimensional and two-dimensional nuclear magnetic resonance experiments in combination with mass spectrometry as 3-*O*-(4-*O*-[3-hydroxy-3-methylglutaryl])-α-L-arabinopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyloleanolic acid and 3-*O*-α-L-arabinofuranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyloleanolic acid.

Keywords

Haplocoelum congolanum, Sapindaceae, triterpene saponins, 2D NMR, MS

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The Sapindaceae family includes more than 2 000 species distributed in 3 subfamilies as Sapindoideae, Dodonaeoideae, and Aceroideae.[1,2] These plants are known to contain triterpenoid saponins.[3] In a continuation of our studies on natural saponins from the plants of this family,[4,5] we decided to examine the saponins from the root bark of *Haplocoelum congolanum* Hauman, belonging to the Sapindoideae subfamily. No information was available about its therapeutic or medicinal properties. Previous chemical studies of *H. congolanum* led to the isolation and characterization of a triterpene saponin mixture having hederagenin or oleanolic acid as aglycone with glucose, arabinose, rhamnose, and xylose as sugars.[6] In the present paper, we report the isolation and structure elucidation of 2 undescribed triterpene saponins together with 5 known ones. Their structures were elucidated by spectroscopic methods including 600 MHz one-dimensional (1D) and two-dimensional (2D) experiments (^{1}H, ^{13}C, heteronuclear single-quantum coherence [HSQC], heteronuclear multiple bond correlation [HMBC], correlated spectroscopy [COSY], total correlated spectroscopy [TOCSY], rotating-frame Overhauser spectroscopy [ROESY]) in combination with high-resolution electroscopy ionization mass spectroscopy (HR-ESIMS) and by comparison of their physical and spectral data with literature values.

The saponin fraction obtained from the 80% aqueous ethanolic extract of the root bark of *H. congolanum* was fractionated by repeated medium-pressure liquid chromatography (MPLC) and semipreparative high-performance liquid chromatography (HPLC) on RP-18 silica gel yielding 2 undescribed compounds **1** and **2** (Figure 1). Furthermore, 5 known molecules were isolated and 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-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L- arabinopyranosyloleanolic acid[7] only isolated from Sapindaceae and Ranunculaceae families, 3-*O*-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L- arabinopyranosylhederagenin[9] previously isolated

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from *Anemone taipaiensis* (Ranunculaceae) and 3-*O*-α-L-arabinopyranosyl-(1→3)- α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L- arabinopyranosyloleanolic acid[10] isolated twice from *Anemone raddeana* (Ranunculaceae) and from *Serjania marginata* (Sapindaceae).[11]

Compounds **1** and **2** were isolated as white amorphous powders. The monosaccharides were identified by extensive 2D nuclear magnetic resonance (NMR) and gas chromatography (GC) analyses[12] (see the Experimental section) as β-D-glucopyranosyl (Glc), α-L-arabinopyranosyl (Ara), and α-L-rhamnopyranosyl (Rha) for **1** and Glc, Rha, Ara, and α-L-arabinofuranosyl (Araf) for **2**. The $^{3}J_{H\text{-}1,\,H\text{-}2}$ coupling constants (7.2-8.0 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 the rhamnose (168 Hz) confirmed that the anomeric proton was equatorial in its α-pyranoid form.

Compound **1** exhibited in the HR-ESIMS a quasi-molecular ion peak at *m/z* 1195.5872 $[M+Na]^+$ (calculated 1195.5876) compatible with the molecular formula $C_{58}H_{92}O_{24}$. Compound **1** showed in the ESIMS spectrum (positive-ion mode) an ion peak at *m/z* 1195 $[M+Na]^+$ indicating a molecular weight of 1172. The ^{1}H and ^{13}C NMR spectra due to the aglycone part displayed resonances characteristic of a triterpene with 7 angular methyl groups showing correlations in the HSQC spectrum at δ_H/ δ_C 1.03 (s, H_3-23)/27.2 (C-23), 0.85 (s, H_3-24)/15.7 (C-24), 0.94 (s, H_3-25)/14.8 (C-25), 0.80 (s, H_3-26)/16.3 (C-26), 1.15 (s, H_3-27)/25.0 (C-27), 0.90 (s, H_3-29)/32.2 (C-29), and 0.93 (s, H_3-30)/22.6 (C-30). Other characteristic signals were observed such as one olefinic proton at δ_H 5.23 ppm (br t, H-12) and one oxygen-bearing methine proton signal at δ_H 3.11 (dd, J = 4.1, 11.7 Hz, H-3), showing correlations in the HSQC spectrum with δ_C 122.1 (C-12) and 89.5 (C-3), respectively. Extensive 2D NMR spectroscopic analysis confirmed the structure of the aglycone to be oleanolic acid (Table 1).[13]

The analysis of the ^{1}H NMR spectrum of the sugar part of **1** showed the presence of 4 anomeric proton signals at δ_H 4.43 (d, J = 5.8 Hz), 4.43 (d, J = 7.6 Hz), 5.21 (br s), and 4.47 (d, J = 7.0 Hz) giving correlations with their anomeric carbons in the HSQC spectrum at δ_C 104.3, 104.3, 100.1, and 105.1, respectively (Table 2). Extensive 2D NMR and GC analyses (see the Experimental section) allowed the characterization of 2 Ara (Ara1 and Ara2), 1 Glc, and 1 Rha units. The HMBC correlation at δ_H / δ_C 4.43 (Ara1 H-1)/ 89.5 (C-3) showed that the Ara1 was linked at C-3 of the aglycone. The cross-peak at δ_H / δ_H 4.43 (Ara1 H-1)/ 3.73 (Ara1 H-2) in the COSY spectrum, δ_H/ δ_C 3.73 (Ara1 H-2)/ 75.5 (Ara1 C-2) in the HSQC spectrum, and δ_H / δ_C 5.21 (Rha H-1)/ 75.5 (Ara1 C-2) in the HMBC spectrum showed that the Rha was linked at C-2 of the Ara1. In the TOCSY spectrum the signal at δ_H 4.43 (Ara1 H-1) gives a correlation with δ_C 3.89 ppm, attributed to the H-4 of the Ara1,

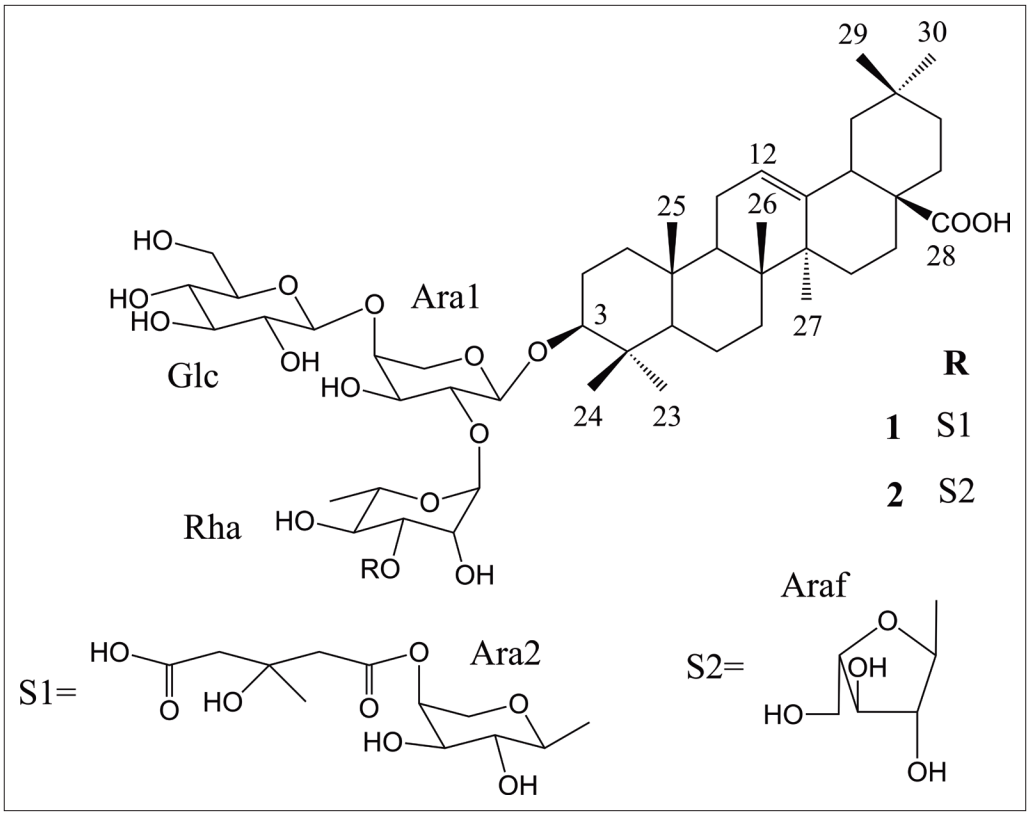

Figure 1. Saponins from root bark of *Haplocoelum congolanum*.

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].

Position	1		2	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	38.6	0.98 m, 1.62	38.5	0.98 m, 1.61
2	25.8	1.70, 1.81 m	25.6	1.70, 1.81
3	89.5	3.11 dd (4.1, 11.7)	89.2	3.10 dd (4.3, 11.4)
4	38.9	-	38.8	-
5	55.9	0.78 m	55.7	0.78 br d (11.8)
6	18.0	1.42 m, 1.56	17.9	1.42, 1.55
7	32.6	1.31, 1.52	32.6	1.31, 1.51
8	39.2	-	39.1	-
9	47.6	1.58	47.8	1.58
10	36.4	-	36.4	-
11	23.0	1.88, 1.88	23.1	1.88, 1.88
12	122.1	5.23 br t	121.7	5.23 br t
13	143.9	-	144.3	-
14	41.4	-	41.5	-
15	27.3	1.08 m, 1.77	27.5	1.08, 1.77
16	22.8	1.59, 2.00 m	22.8	1.59, 2.00
17	nd	-	46.6	-
18	41.3	2.84 br d (12.8)	41.5	2.84 dd (3.9, 14.0)
19	46.0	1.12, 1.69	46.1	1.12, 1.68
20	30.1	-	30.2	-
21	33.5	1.20, 1.39	33.5	1.20, 1.38
22	32.6	1.54, 1.74	32.6	1.52, 1.74
23	27.2	1.03 s	27.3	1.03 s
24	15.7	0.85 s	15.7	0.85 s
25	14.8	0.94 s	14.6	0.94 s
26	16.3	0.80 s	16.6	0.82 s
27	25.0	1.15 s	24.9	1.14 s
28	nd	-	nd	-
29	32.2	0.90 s	32.3	0.89 s
30	22.6	0.93 s	22.6	0.93 s

nd, not determined; NMR, nuclear magnetic resonance.
[a]Overlapped proton NMR signals are reported without designated multiplicity.

which correlates with δ_C 78.0 in the HSQC spectrum (Ara1 C-4). The HMBC correlation at δ_H / δ_C 4.43 (Glc H-1)/ 78.0 (Ara1 C-4) showed Glc to be linked at the C-4 of Ara1. Finally, the HMBC correlation at δ_H / δ_C 4.47 (Ara2 H-1)/ 81.2 (Rha C-3) showed that Ara2 was linked at C-3 of Rha. These data allowed to propose the sequence Ara2-(1→3)-Rha-(1→2)-[Glc-(1→4)]-Ara1- to be linked at C-3 of the aglycone. These linkages were confirmed by the ROESY cross-peaks at δ_H / δ_H 4.43 (Ara1 H- 1)/ 3.11 (H-3), 4.43 (Glc H-1)/ 3.89 (Ara1 H-4), 5.21 (Rha1 H-1)/ 3.73 (Ara1 H-2) and 4.47 (Ara2 H-1)/ 3.84 (Rha H-4).

Furthermore, the TOCSY spectrum showed a correlation at δ_H / δ_H 4.47 (Ara2 H-1)/ 5.05, which suggested an acylation. We observed the presence in 1D- and 2D-NMR spectra (Table 2) of additional signals of a 3-hydroxy-3-methylglutaryl moiety (dicrotalic acid), which were in good agreement with literature data.[14,15] The location of dicrotalic acid at C-4 position of Ara2 was ascertained by observation of a deshielded ^{13}C NMR chemical shift at δ_C71.2 (Ara2 C-4) instead of 67.5 to 68.7, or 69.2 for a free Ara 4-position as described in the literature.[5,16] Furthermore, all the NMR data of acylated Ara2 C-4 were in good agreement with literature values for an acylated Ara at C-4 position.[16] This result allowed to confirm that the dicrotalic acid was linked at the C-4 position of Ara2. Thus, the structure of **1** was elucidated as 3-*O*-(4-*O*-[3-hydroxy-3-methylglutaryl])-α-L-arabinopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyloleanolic acid.

Compound **2** exhibited in the HR-ESIMS a quasi-molecular ion peak at *m/z* 1051.2016 $[M+Na]^+$ (calculated 1051.2011) compatible with the molecular formula $C_{52}H_{84}O_{20}$. Compound **2** showed in the ESIMS spectrum (positive-ion mode) a pseudomolecular ion peak at *m/z* 1051 $[M+Na]^+$ indicating a molecular weight of 1028. Extensive 2D NMR analysis (Tables 1 and 2) revealed that compound **2** possesses oleanolic acid and 4 sugar units, which were identified as Ara1, Glc, Rha, and Araf units. Extensive 2D NMR analysis of **2** allowed the identification of a partial sequence Rha-(1→2)-[Glc-(1→4)]-Ara-(1→3)-oleanolic acid, which was the same as in **1** (Table 2). Both compounds differ only by the substitution pattern of Rha at C-3. Namely, the Araf in **2** was found to be linked at Rha C-3 by observation of an HMBC correlation at δ_H / δ_C 5.16 (Araf H-1)/ 78.1 (Rha C-3) and a ROESY cross-peak at δ_H / δ_H 5.16 (Araf H-1)/ 3.80 (Rha H-3). Thus, the structure of **2** was elucidated as 3-*O*-α-L-arabinofuranosyl-(1→3)-α-L-rhamnopyrano-syl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyloleanolic acid.

From the crude 80% ethanolic extract of the root bark of *H. congolanum*, we isolated 2 undescribed compounds and 5 known ones by successive MPLC and semipreparative HPLC. The sequences Rha-(1→2)-[Glc-(1→4)]-Ara- and Xyl-(1→3)-Rha-(1→2)-[Glc-(1→4)]-Ara- linked at C-3 of oleanolic acid or hederagenin was not considered as a chemotaxonomic marker in this family because they were also found in the Ranunculaceae family. All known triterpenoid saponins were also isolated from the genus *Anemone*, in the Ranunculaceae family. The group 3-hydroxy-3-methylglutaric acid was not unusual in the Sapindaceae family: it was encountered only once linked to a phenolic glycoside from *Eurycorymbus cavaleriei*,[17] but was found for the first time linked at a sugar of a triterpenoid saponin in this family. The

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].

Position	**1**			**2**	
	δ_C	δ_H (*J* in Hz)		δ_C	δ_H (*J* in Hz)
3-*O*-			3-*O*-		
Ara1-1	104.3	4.43 d (5.8)	Ara-1	104.0	4.45 d (5.7)
2	75.5	3.73 dd (5.8, 8.2)	2	75.6	3.72
3	72.3	3.78	3	72.1	3.79
4	78.0	3.89	4	77.9	3.89
5	63.6	3.54, 4.13	5	63.2	3.54 dd (1.7, 11.8), 4.12 dd (4.3, 12.3)
Glc-1	104.3	4.43 d (7.6)	Glc-1	104.5	4.44 d (7.4)
2	73.9	3.28	2	73.9	3.27
3	76.6	3.34 dd (8.2, 8.7)	3	76.4	3.35 dd (8.7, 8.7)
4	69.9	3.30	4	69.9	3.29
5	76.7	3.28	5	76.6	3.28
6	61.5	3.66, 3.85	6	61.2	3.65, 3.84
Rha-1	100.1	5.21 br s	Rha-1	100.2	5.16 br s
2	70.2	4.12 br s	2	70.4	4.10 br s
3	81.2	3.84	3	78.1	3.80
4	71.7	3.57	4	71.3	3.48 t (9.6)
5	68.4	3.92 m	5	68.8	3.90
6	16.7	1.23 d (6.4)	6	16.6	1.22 d (6.1)
Ara2-1	105.1	4.47 d (7.0)	Araf	109.9	5.16 br s
2	72.0	3.65	2	81.5	4.07 br s
3	71.7	3.69 dd (3.5, 9.3)	3	77.5	3.84
4	71.2	5.05 m	4	85.1	4.04 m
5	63.6	3.62, 3.96 dd (2.3, 13.4)	5	61.9	3.64, 3.73
Acyl-1	170.9	-			
2	45.6	2.61 d (15.2), 2.70			
3	69.9	-			
4	45.6	2.52 d (15.2), 2.71			
5	nd	-			
6	26.5	1.36 s			

nd, not determined; NMR, nuclear magnetic resonance.
[a]Overlapped proton NMR signals are reported without designated multiplicity.

sequence Araf-^{3}Rha-^{2}Ara-^{3}oleanolic acid in compound **2** has previously been reported in *Sapindus mukorossi*.[18] Therefore, additional studies of other Sapindaceae species are necessary to draw some chemotaxonomic conclusions.

Experimental

General Procedures

Optical rotation values were recorded on AA-10R automatic polarimeter. NMR spectra: Spectra were performed using a Varian INOVA 600 at the operating frequency of 600 MHz. For details, see experimental part.[5] 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. Thin-layer chromatography (TLC) and high-performance TLC employed precoated Si gel plates 60 F_{254} (Merck) ($CHCl_3$–MeOH–H_2O, 70/30/5 and 60/32/7). The spray reagent for saponins was vanillin reagent (2% mixture of concentrated H_2SO_4 solution and 1% vanillin in EtOH). Isolations were carried out using a MPLC system (Alltech pump, Büchi column [460 × 15 mm], Büchi precolumn [110 × 15 mm], Silica gel 60 [Merck, 15-40 µm]). HPLC was performed on a 1260 Agilent instrument, equipped with a degasser, a quaternary pump, an autosampler, an UV detector at 210 nm. Semipreparative separation was carried out on a C-18 column (250 mm × 10 mm id,

5 µm; Phenomenex LUNA) at room temperature and protected by a guard column. Eluent: (A) 0.01% (*v*/*v*) aqueous trifluoroacetic acid and (B) acetonitrile, 3 mL/min, detection at 210 nm. Gradient: 35% B to 45% B for 20 minutes, 45% B for 10 minutes.

Plant Material

Haplocoelum congolanum Hauman. root bark was collected from Yangambi (Democratic Republic of Congo) and identified by H. Breyne. A voucher specimen (No. 2845) is deposited in the herbarium of the Laboratory of Botanic of National University of Zaïre in Kinshasa and the National Botanical Garden in Brussels.

Extraction and Isolation

Dried powdered root bark (800 g) of *H. congolanum* was macerated in a 80% ethanolic solution and refluxed for 3 hours. After evaporation of the solvent under vacuum, the obtained residue was treated by successive protocols as dissolution in methanol, filtration, precipitation in diethyl ether, dialysis, and treatment by charcoal[6] to give 8.5 g of a crude saponin mixture. An aliquot (4 × 150 mg) of this residue was subjected to MPLC (1.5 × 46 cm, 2.5 mL/min) over silica gel using a $CHCl_3$-MeOH-H_2O gradient (80/20/2, 70/30/5, 60/32/7) to give 22 fractions Fr. 1 to Fr. 22. Fr. 3 and Fr. 4 were combined to give **2** (2.4 mg). Fr. 19 to Fr. 22 were combined (14.1 mg) and were fractionated by semipreparative HPLC (see the General procedures section) to give 13 fractions. Fr. 11 (2.8 mg) was pure, **1**.

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 aqueous 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 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.[12] 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*-(4-*O*-[3-Hydroxy-3-Methylglutaryl])-α-L-Arabinopyranosyl-(1→3)-α-L-Rhamnopyranosyl-(1→2)-[β-D-Glucopyranosyl-(1→4)]-α-L-Arabinopyranosyloleanolic Acid (1)

White, amorphous powder.
$[\alpha]^{25}_D$: +23 (*c* 0.3, MeOH)
^{1}H NMR and ^{13}C NMR (CD_3OD, 600 MHz and 150 MHz): Tables 1 and 2.
HR-ESIMS (positive-ion mode) *m/z* 1195.5872 $[M+Na]^+$ (calculated for $C_{58}H_{92}O_{24}Na$, 1195.5876) ; ESIMS (positive-ion mode) *m/z* 1195 $[M+Na]^+$.

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

White, amorphous powder.
$[\alpha]^{25}_D$: – 12 (*c* 0.1, MeOH)
^{1}H NMR and ^{13}C NMR (CD_3OD, 600 MHz and 150 MHz): Tables 1 and 2.
HR-ESIMS (positive-ion mode) *m/z* 1051.2016 $[M+Na]^+$ (calculated for $C_{52}H_{84}O_{20}Na$, 1051.2011) ; ESIMS (positive-ion mode) *m/z* 1051 $[M+Na]^+$.

Declaration of Conflicting Interests

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