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Two new triterpenoid saponins from *Pittosporum senacia* Putterlick (Pittosporaceae)

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From the branches of *Pittosporum senacia* Putterlick (Pittosporaceae), two new triterpenoid saponins, senaciapittosides A and B (1, 2), were isolated. Their structures were elucidated by extensive analysis of one- and two-dimensional nuclear magnetic resonance spectroscopy, high-resolution electrospray ionization mass spectrometry (HR-ESIMS) and chemical evidence as 3-*O*-[β-D-glucopyranosyl-(1 → 2)]-[α-L-arabinopyranosyl-(1 → 3)]-[α-L-arabinofuranosyl-(1 → 4)]-β-D-glucuronopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl ester (1) and 3-*O*-[β-D-glucopyranosyl-(1 → 2)]-[α-L-arabinopyranosyl-(1 → 3)]-[α-L-arabinofuranosyl-(1 → 4)]-β-D-glucuronopyranosyl-22-*O*-α-L-arabinopyranosyl-21-acetoxy R1-barrigenol (2). Compound 2 presents an unusual glycosylation at C-22 of its aglycone. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: NMR; ^{1}H; ^{13}C; 2D NMR; triterpene saponins; Pittosporaceae; *Pittosporum senacia*

Introduction

The Pittosporaceae family is represented by nine genera, which contains approximately 250 species, distributed in tropical and subtropical regions.[1] The use for local traditional medicine from species of the Pittosporaceae family has been reported in multiple cases.[2–5] The presence of triterpene saponins in the genus *Pittosporum* has been previously reported.[6–8] Some different endemic subspecies of *Pittosporum senacia* are known to have an indigenous medicinal role in tropical regions for treatment of rheumatism and throat infections.[4,5] A previous work on the volatile constituents of the leaves of *P. senacia* var. *coursii* Cufodontis was reported,[9] whereas triterpene saponins have never been characterized in this species. In this article, we report about the isolation and characterization of two new saponins from *P. senacia* Putterlick. The structures were elucidated mainly by a 600-MHz nuclear magnetic resonance (NMR) analysis including one-dimensional (1D) and two-dimensional (2D) NMR (^{1}H, ^{13}C NMR, COSY, TOCSY, ROESY, HSQC and HMBC) spectroscopy and mass spectrometry.

Results and Discussion

The dried and powdered branches of *P. senacia* Putterlick were refluxed in MeOH. After evaporation of the solvent, the dried MeOH extract was submitted to several liquid chromatographic steps [vacuum liquid chromatography (VLC), medium pressure liquid chromatography (MPLC)] on normal-(SiO_2) and reversed-phase (RP-18 SiO_2) affording two new triterpene saponins, senaciapittosides A (**1**) and B (**2**).

The high-resolution electrospray ionization mass spectrometry (HR-ESIMS, positive-ion mode) spectrum of **1** showed a pseudo-molecular ion peak at *m/z* 1243.5729 $[M+Na]^+$ (calculated 1243.5724) ascribable to the molecular formula $C_{58}H_{92}O_{27}$. The ESIMS spectrum (negative ion-mode) showed a quasi-molecular ion peak at *m/z* 1219 $[M-H]^-$, indicating a molecular weight of 1220. Another fragment ion was observed at 1087 $[(M-H)-132]^-$ corresponding to the loss of one pentosyl moiety.

The ^{1}H NMR spectrum of the aglycone part of **1** showed seven tertiary methyl groups as singlets at δ_H 0.69, 0.75, 0.86, 0.87, 0.88, 0.97 and 1.08, one olefinic proton at δ_H 5.16 (br s, H-12) and one oxygen bearing methine proton at δ_H 2.99 (H-3), characteristic of a triterpene skeleton. Furthermore, the shielded chemical shift in the ^{13}C NMR spectrum at δ_C 175.1 (C-28) in comparison with a free carboxyl group (δ_C 180.0) suggested an ester function. The extensive analysis of 1D and 2D NMR spectra (^{1}H, ^{13}C NMR, ROESY, HSQC and HMBC) led to the identification of the aglycone part of **1** as oleanolic acid (=3-β-hydroxy-olean-12-en-28-oic acid) and was in full agreement with literature data.[10] The ^{1}H NMR spectrum of **1** showed five anomeric proton signals at δ_H 5.24 (d, J = 8.1 Hz), 5.06 (br s), 4.82 (d, J = 6.9 Hz), 4.71 (d, J = 7.3 Hz) and 4.28 (d, J = 6.9 Hz), which correlated in the HSQC spectrum with five anomeric carbons at δ_C 94.0, 107.1, 101.7, 101.3 and 103.6, respectively, and indicated the presence of five sugar moieties. The bidesmosidic structure has been revealed by the chemical shift of C-3 (δ_C 88.4) and C-28 (δ_C 175.1). Complete assignments of each glycosidic proton system were achieved by COSY, TOCSY and ROESY experiments starting from the readily identifiable anomeric protons. Thus, one β-glucuronopyranosyl acid (GlcA), two β-glucopyranosyl (Glc I and Glc II), one α-arabinopyranosyl (Ara) and one α-arabinofuranosyl (Ara*f*) units were identified (see Table 2). The evidence of the sugar linkages to the aglycone at C-28 and C-3 was in particular given by

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the HMBC cross peaks δ_H 5.24 (d, J = 8.1 Hz, Glc II H-1)/δ_C 175.1 (Agly C-28) and δ_H 4.28 (d, J = 6.9 Hz, GlcA H-1)/δ_C 88.4 (Agly C-3), respectively. Furthermore, the linkage of GlcA to the aglycone at C-3 was confirmed by the ROESY cross peak at δ_H 4.28 (d, J = 6.9 Hz, GlcA H-1)/δ_H 2.99 (Agly H-3). The sequence of the 3-*O*-oligosaccharidic moiety was assigned by HMBC and ROESY experiments. HMBC cross peaks at δ_H 3.73 (GlcA H-2)/δ_C 101.3 (Glc I C-1) and at δ_H 3.68 (GlcA H-3)/δ_C 101.7 (Ara C-1) suggested a Glc I-(1 → 2)-[Ara-(1 → 3)]-GlcA sequence. This was confirmed by the ROESY cross peaks at δ_H 4.71 (d, J = 7.3 Hz, Glc I H-1)/δ_H 3.73 (GlcA H-2) and δ_H 4.82 (d, J = 6.9 Hz, Ara H-1)/δ_H 3.68 (GlcA H-3). A strong ROESY cross peak δ_H 5.06 (br s, Ara*f* H-1)/δ_H 3.55 (GlcA H-4) showed the additional Ara*f*-(1 → 4)-GlcA linkage. The absolute configurations of the sugars were determined to be D for glucose and glucuronic acid and L for arabinose by gas chromatography (GC) analysis (see Experimental section). On the basis of the previous results, the structure of compound **1** was elucidated as 3-*O*-[β-D-glucopyranosyl-(1 → 2)]-[α-L-arabinopyranosyl-(1 → 3)]-[α-L-arabinofuranosyl-(1 → 4)]-β-D-glucuronopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl ester, named *senaciapittoside A* (Fig. 1).

Compound **2** was obtained as a white amorphous powder. The molecular formula of compound **2** was deduced as $C_{59}H_{94}O_{30}$ on the basis of HR-ESIMS (positive-ion mode) exhibiting the pseudo-molecular peak at *m/z* 1305.5731 $[M+Na]^+$ (calculated 1305.5728). The ESIMS (negative-ion mode) showed a quasi-molecular ion peak at *m/z* 1281 $[M-H]^-$, indicating a molecular weight of 1282. The ^{1}H NMR spectrum corresponding to the aglycone part of **2** showed as for compound **1** seven methyl groups as singlets at δ_H 0.74, 0.76, 0.88, 0.90, 0.91, 0.98 and 1.27 and one olefinic proton at δ_H 5.32 (1H, br s, H-12), characteristic of an oleanene-type skeleton. Furthermore, the presence of five oxygen bearing methine protons at δ_H 3.02 (1H, H-3), 3.36 (1H, H-16), 3.58 (1H, H-15), 4.03 (1H, H-22) and 5.48 (1H, d J = 9.3 Hz, H-21), and one primary alcoholic function at δ_H 3.12, 3.33 (2H, H_2-28) suggested a hexahydroxy-3,15,16,21,22,28-oleanene-type skeleton. Cross peaks in the HMBC spectrum at δ_H 5.48 (^{1}H, d J = 9.3 Hz, H-21)/δ_C 19.6 (C-30), 29.1 (C-29), 35.1 (C-20), 78.5 (C-22) and 170.3, respectively (Fig. 2), allowed us to determine the secondary alcoholic function to be esterified at C-21 by an acetyl group. Furthermore, the COSY cross peak at δ_H 5.48 (1H, d J = 9.3 Hz, H-21)/δ_H 4.03 (1H, H-22) assigned unambiguously the location of a further

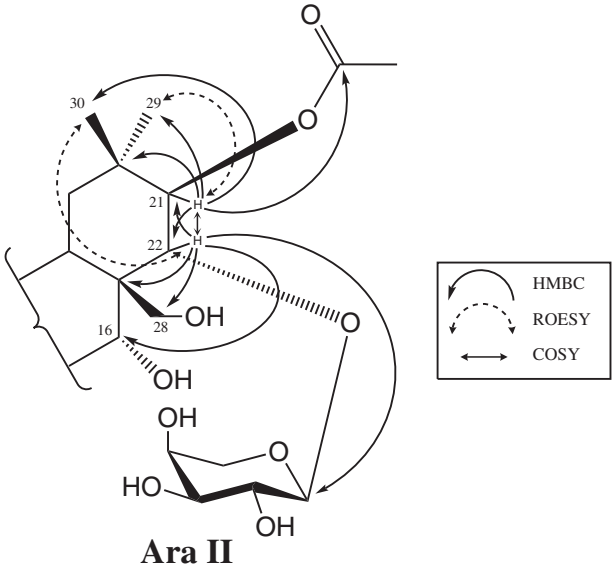

Figure 2. Correlations of the aglycone part of compound **2** characterized by HMBC, ROESY and COSY.

secondary alcoholic function at C-22. The HMBC connectivities δ_H 4.03 (1H, H-22)/δ_C 78.3 (C-21), 71.8 (C-16), 61.7 (C-28) and 47.8 (C-17) (Fig. 2) and HMBCs between the protons of the methyl group at δ_H 1.27 (3H, s, H_3-27) and the carbon at δ_C 66.1 (C-15) confirmed the position of the secondary alcoholic functions at C-15 and C-16. The stereochemistry was verified by ROESY experiments. Namely, correlations between δ_H 3.02 (1H, H-3) and 0.72 (1H, H-5) and δ_H 0.98 (3H, s, H_3-23), between 3.58 (1H, H-15) and 0.91 (3H, s, H_3-26) and between 3.36 (1H, H-16) and 3.12 (1H, H_2-28) suggested the absolute configuration of the R1-barrigenol at C-3, C-15 and C-16.[6] Furthermore, ROESY cross peaks at δ_H 0.74 (3H, s, H_3-29)/5.48 (1H, d J = 9.3 Hz, H-21) and 0.88 (3H, s, H_3-30)/4.03 (1H, H-22) revealed the β-axial orientation of the H-22, and the multiplicity as a doublet and the coupling constant of 9.3 Hz suggested an α-axial orientation of H-21. This extensive 2D NMR analysis confirmed the identification of the aglycone of compound **2** as the already known sapogenol called *R1-barrigenol* (olean-12-ene-3β,15α,16α,21β,22α,28-hexol),[6,7,11–16] which is esterified at C-21

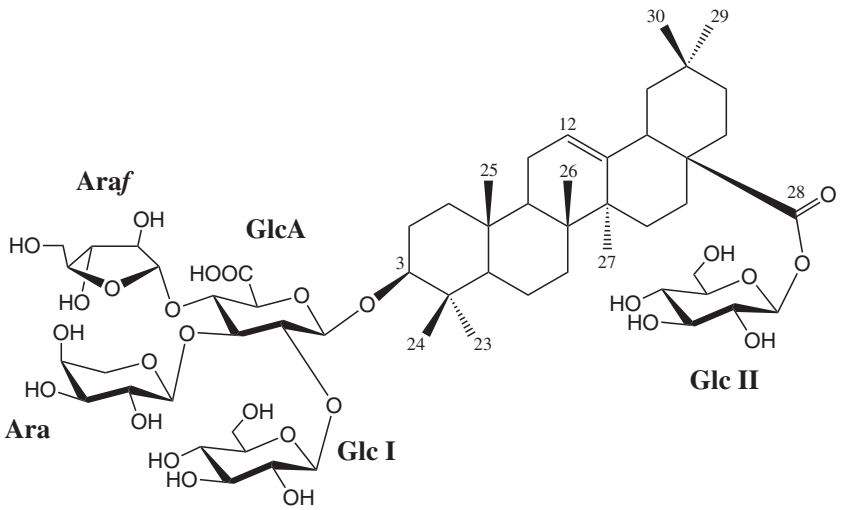

Figure 1. Structure of compound **1**.

by an acetyl function. In the ^{1}H NMR spectrum of compound **2**, signals of five anomeric protons were shown at δ_H 5.06 (br s), 4.81 (d, J = 6.0 Hz), 4.72 (d, J = 7.3 Hz), 4.28 (d, J = 6.9 Hz) and 4.14 (d, J = 5.7 Hz), which correlated in the HSQC spectrum with five carbons at δ_C 106.8, 101.9, 101.4, 103.6 and 104.6, respectively, indicating the presence of five sugar moieties. Complete assignments were achieved by 1D and 2D NMR spectra. The evaluation of chemical shifts and spin-spin couplings allowed the identification of one β-glucuronopyranosyl acid (GlcA), one β-glucopyranosyl (Glc), two α-arabinopyranosyl (Ara I and Ara II) and one α-arabinofuranosyl (Ara*f*) units (see Table 2). As for compound **1**, we have here to deal with a bidesmosidic structure, showing the sugar linkages to the aglycone at C-22 and C-3, which were given by the HMBC cross peaks at δ_H 4.14 (d, J = 5.7 Hz, Ara II H-1)/δ_C 78.5 (Agly C-22) and δ_H 4.28 (d, J = 6.9 Hz, GlcA H-1)/δ_C 88.3 (Agly C-3), respectively. Furthermore, the linkages of GlcA to C-3 and Ara II to C-22 were confirmed by the ROESY cross peaks at δ_H 4.28 (d, J = 6.9 Hz, GlcA H-1)/δ_H 3.02 (Agly H-3) and δ_H 4.14 (d, J = 5.7 Hz, Ara II H-1)/δ_H 4.03 (d, J = 8.5 Hz, Agly H-22). The sugar sequence investigated by extensive 2D NMR experiments revealed that the ^{1}H and ^{13}C NMR signals of the oligosaccharide chain at C-3 were almost superimposable with those of (3-*O*-[Glc-(1 → 2)]-[Ara-(1 → 3)]-[Ara*f*-(1 → 4)]-GlcA) characterized in compound **1**. The absolute configurations of the sugars were determined to be D for glucose and glucuronic acid and L for arabinose by GC analysis (see Experimental section). Throughout this information, the structure of compound **2** was elucidated as 3-*O*-[β-D-glucopyranosyl-(1 → 2)]-[α-L-arabinopyranosyl-(1 → 3)]-[α-L-arabinofuranosyl-(1 → 4)]-β-D-glucuronopyranosyl-22-*O*-α-L-arabinopyranosyl-21-acetoxy R1-barrigenol, named *senaciapittoside B* (Fig. 3).

Esterified R1-barrigenol derivatives have already been characterized as glycosylated compounds at C-3 from *Pittosporum tobira*,[6] *Pittosporum viridiflorum*[7] and *Pittosporum brevicalyx*.[17] Therefore, from a chemotaxonomic point of view, it was not surprising to find a structural analog in another species of this genus, *P. senacia*. Usually, the position at C-22 of R1-barrigenol is substituted by an acyl moiety (tigloyl, angeloyl, 2-acetoxy-2-methylbutanoyl, 3,3-dimethylacryloyl and 2-methylbutanoyl units[6,7,12,14,15]). To the best of our knowledge, the glycosylation at C-22 of R1-barrigenol is unusual.

Experimental

General

The materials and methods used were as follows: VLC, silica gel 60 (15–40 µm; Merck); MPLC, silica gel RP-18 Spherical C18 (300 Å, 75–200 mm; Silicycle); Pump Module C-601 (Büchi); fraction collector, C-660 (Büchi); Büchi glass column (460 × 25 mm and 460 × 15 mm); Büchi precolumn (110 × 15 mm); rotary evaporator, Rotavapor R-210 (Büchi); GC, Termoquest gas chromatograph; DB-1701 capillary column (30 m × 0.25 mm) (J & W Scientific); detection, Free Induction Decay (FID); detector temperature, 80 °C for 5 min and raised to 270 °C at the at 15 °C/min; carrier gas, He; TLC, silica plate F-254 (60 Å, Silicycle); HPTLC, silica gel 60 F254 (Merck); solvent systems, $CHCl_3$/MeOH/H_2O 65:32:6.5; detection with vanillin reagent, that is, 1% 4-hydroxy-3-methoxybenzaldehyd in EtOH with 2% of a 96% H_2SO_4, followed by heating; optical rotation, AA-OR automatic polarimeter. NMR spectra were measured on a Varian VNMR-S 600 MHz spectrometer equipped with 3 mm triple resonance inverse and 3 mm dual broadband probe heads. Spectra are recorded in 150 µl DMSO-d_6. Solvent signals were used as internal standard (DMSO-d_6: δ_H = 2.50, δ_C = 39.5 ppm); all spectra are recorded at T = 35 °C. Pulse sequences were taken from Varian pulse sequence library and used with following conditions (compounds **1** and **2**): relaxation delay in 2D spectra have been 1.5 s (**1**) or 2 s (**2**); gCOSY with 1 (**1**) or 4 (**2**) transients per 512 increments, data were transformed using sinebell square apodization in f1 and f2 to 4096 × 4096 points and spectral width was 5656 × 5656 Hz (**1**) or 5952 × 5952 Hz (**2**); TOCSY spectra are acquired using DIPSI spin lock and 150 ms missing time with 1 (**1**) or 8 (**2**) transients per 1024 increments, data were transformed using Gaussian apodization in f1 and f2 to 4096 × 4096 points and spectral width was 5896 × 5896 Hz (**1**) or 5841 × 5841 Hz (**2**); ROESY acquisition with 300 ms mixing time and 16 (**1**) or 32 (**2**) transients per 1024 (**1**) or 512 (**2**) increments, data transformation with Gaussian apodization to 4096 × 4096 (**1**) or 8192 × 2048 (**2**) points and spectral width was 5981 × 5981 Hz (**1**) or 13298 × 13298 Hz (**2**); gHSQCAD with 2 (**1**) or 8 (**2**) transients per 1024 increments, data transformation with Gaussian apodization in f1 and f2 to 4096 × 4096 points and f1 × f2 spectral width was

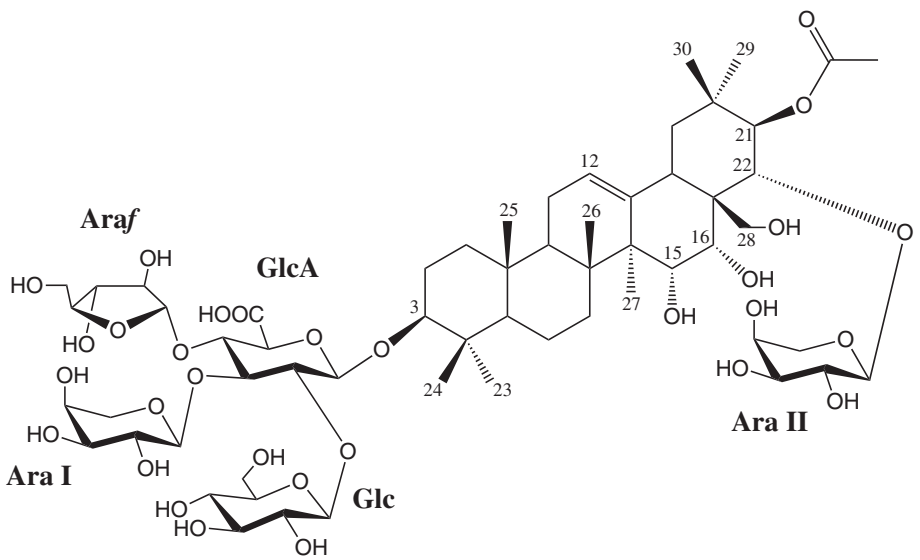

Figure 3. Structure of compound **2**.

27137 × 5760 Hz (**1**) or 27137 × 5483 Hz (**2**); gHMBCAD with 8 (**1**) or 64 (**2**) transients per 1024 increments, data transformation with Gaussian apodization in f1 and f2 to 4096 × 4096 points and f1 × f2 spectral width was 36199 × 5924 Hz (**1**) or 36199 × 5760 (2). Carbon type (CH_3, CH_2 and CH): DEPT experiments. ESI− (negative) and HR-ESI-MS (positive): Q-TOF-1-Micromass spectrometer in *m/z*.

Plant material

The branches of *P. senacia* Putterlick were collected near Antsiranana (Madagascar) in 2009 and identified by the botanist Natalie Rafalimanana (University of Antsiranana, Madagascar). A voucher specimen (no. 26062010) was deposited in the Herbarium of the Laboratory of Pharmacognosy, Burgundy University, Dijon, France.

Extraction and isolation

The dried and powdered branches of *P. senacia* (800 g) were refluxed with 70% MeOH for 1 h at 70–80 °C (2 × 3 l). After filtration, the solvent was removed by rotary evaporation yielding 30.12 g of extract. An aliquot of this MeOH extract (8.00 g) was subjected to VLC (silica gel, $CHCl_3$/MeOH/H_2O 65:32:6.5 and 100% MeOH) giving 19 main fractions: Fr.a-Fr.s. An aliquot of Fr.j (105.3 mg) was

Table 1. ^{13}C NMR (150 MHz) and ^{1}H NMR (600 MHz) data of the aglycones of compounds **1** and **2** in DMSO-d_6 from 1D and 2D NMR experiments (δ in ppm, *J* in Hz)[a]

	1		**2**	
No.	δ_C	δ_H	δ_C	δ_H
1	38.1	0.88, 1.50	38.3	0.89, 1.51
2	25.4	1.51, 1.78	25.6	1.53, 1,81
3	88.4	2.99	88.3	3.02
4	38.5	—	38.5	—
5	55.0	0.70	54.7	0.72
6	17.6	1.27, 1.46	17.8	1.44, ND
7	32.2	1.21, 1.38 m	35.5	1.70 m, ND
8	38.9	—	40.5	—
9	47.0	1.47	46.1	1.46
10	36.4	—	36.2	—
11	22.8	1.02 m, 1.79	23.0	0.87, 1.81
12	121.6	5.16 br s	124.0	5.32 br s
13	143.3	—	143.2	—
14	41.2	—	46.4	—
15	27.1	0.94, 1.71	66.1	3.58
16	22.4	1.58, 1.95	71.8	3.36
17	47.7	—	47.8	—
18	41.2	2.74 dd (13.4, 3.6)	41.8	2.41
19	45.4	1.08, 1.62	45.8	1.05, 2.41
20	30.2	—	35.1	—
21	33.1	1.15 m, 1.33 m	78.3	5.48 d (9.3)
22	31.5	1.49, 1.57		
22			78.5	4.03
23	27.5	0.97 s	27.2	0.98 s
24	16.0	0.75 s	16.1	0.76 s
25	15.1	0.86 s	15.4	0.89 s
26	16.6	0.69 s	16.8	0.91 s
27	25.4	1.08 s	20.1	1.27 s
28	175.1	—	61.7	3.12, 3.33
29	32.6	0.88 s	29.1	0.74 s
30	23.2	0.87 s	19.6	0.88 s
at C-21				
$CH_3\underline{CO}$			170.3	—
$\underline{CH_3}CO$			21.1	1.95 s

ND, not determined.
[a]Overlapped proton NMR signals are reported without designated multiplicity.

Table 2. ^{13}C NMR (150 MHz) and ^{1}H NMR (600 MHz) data of sugar moieties of compounds **1** and **2** in DMSO-d_6 from 1D and 2D NMR experiments (δ in ppm, *J* in Hz)[a]

	1		**2**	
No.	δ_C	δ_H	δ_C	δ_H
GlcA				
1	103.6	4.28 d (6.9)	103.6	4.28 d (6.9)
2	78.5	3.73 t (7.7)	78.5	3.73 t (8.9)
3	77.9	3.68 m	77.7	3.68
4	75.1	3.55	74.8	3.57
5	77.7	3.38	77.0	3.42
6	171.6		171.8	
Glc I				
1	101.3	4.71 d (7.3)	101.4	4.72 d (7.3)
2	74.6	3.00	74.6	3.02
3	76.5	3.15	76.3	3.16
4	78.5	3.71	71.8	3.36
5	76.6	3.08 m	76.5	3.08
6	61.0	3.43, 3.65 m	61.2	3.45, 3.65
Glc II				
1	94.0	5.24 d (8.2)		
2	72.3	3.11		
3	76.7	3.22 lt (8.5)		
4	69.5	3.13		
5	77.7	3.14		
6	60.6	3.44, 3.60		
Ara I				
1	101.7	4.82 d (6.9)	101.9	4.81 d (6.0)
2	71.0	3.43	70.5	3.43
3	72.3	3.35	72.6	3.32
4	67.4	3.62	67.6	3.62
5	64.6	3.37, 3.75 m	65.0	3.34, 3.76
Ara II				
1			104.6	4.14 d (5.7)
2			70.9	3.32
3			72.4	3.32
4			67.6	3.61
5			65.4	3.32, 3.63
Araf				
1	107.1	5.06 br s	106.8	5.06 br s
2	80.2	3.73	80.1	3.78 m
3	76.9	3.58	76.9	3.58
4	84.8	4.04 m	85.0	4.05 m
5	61.7	3.40, 3.48 m	61.7	3.42, 3.48 m

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

submitted to MPLC (RP-18 silica gel, H_2O containing increasing amounts of MeOH, 5 ml/min) to give eight subfractions, one of them containing the pure compound **1**, senaciapittoside A (8.7 mg). In the same way, after another purification of an aliquot of Fr.j (151.1 mg) by MPLC in the same conditions as previously mentioned, 11 subfractions were obtained, one of them containing the pure compound **2**, senaciapittoside B (7.3 mg).

Compound 1 (senaciapittoside A): white amorphous powder. $[\alpha]_D^{25}$ −25.0 ($c = 0.03$, MeOH). For ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2. HR-ESIMS *m/z* 1243.5729 $[M + Na]^+$ (calculated 1243.5724). EISMS (negative mode) *m/z* 1219 $[M–H]^-$ and the fragment 1087 $[(M–H)–132]^-$.

Compound 2 (senaciapittoside B): white amorphous powder. $[\alpha]_D^{25}$ −9.5 ($c = 0.05$, MeOH). For ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2. HR-ESIMS *m/z* 1305.5731 $[M + Na]^+$ (calculated 1305.5728). EISMS (negative mode) *m/z* 1281 $[M–H]^-$.

Acid hydrolysis and GC analysis

Each compound (3 mg) was hydrolyzed with 2 N aqueous TFA (5 ml) for 3 h 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. The trimethylsilyl thiazolidine derivatives of the sugar residue of each compound were prepared and analyzed by GC.[18] The absolute configuration was determined by comparing the retention times with thiazolidine derivatives prepared in a similar way from standard sugars (Sigma-Aldrich). L-Arabinose, D-glucose and D-glucuronic acid were detected by coinjection of the derivatives with standard silylated samples with t_R 11.8, 18.6 and 15.2, respectively, from compounds **1** and **2**.

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