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Leptocarposide: a new triterpenoid glycoside from *Ludwigia leptocarpa* (Onagraceae)

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A new triterpenoid bidesmoside (leptocarposide) possessing an acyl group in their glycosidic moiety (1), together with the known luteolin-8-*C*-glucoside (2) and 1-*O*-*β*-D-glucopyranosyl-(2*S*,3*R*,8*E*)-2-[(2′*R*)-2-hydroxypalmitoylamino]-8-octadecen-1,3-diol (3) was isolated from the *n*-butanol-soluble fraction of whole plant of *Ludwigia leptocarpa* (Nutt) Hara (Onagraceae). Structure of compound 1 has been assigned on the basis of spectroscopic data (^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, HSQC, HMBC, and ROESY), mass spectrometry, and by comparison with the literature. This compound was further screened for its potential antioxidant properties by using the radical scavenging assay model 2,2-diphenyl-1-picrylhydrazyl and reveals non-potent antioxidant activities, while compound 2 shows SC_{50} of 0,038 mM. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: NMR; ^{1}H NMR; ^{13}C NMR; 2D NMR; *Ludwigia leptocarpa*; Onagraceae; triterpenoid glycoside; bidesmoside; leptocarposide; NMR complete assignments

Introduction

Ludwigia leptocarpa (Nutt) Hara (Onagraceae) is a pantropical genus that is also well represented in North America and in tropical Africa.[1] It is used in Nigerian folk medicine for the treatment of rheumatism and dysentery.[2] Previous work on this species has revealed the presence of flavonoids.[3]

In the course of our continuing search for secondary metabolites of biological importance from Cameroonian medicinal plants, we investigate the MeOH extract of the whole plant of *L. leptocarpa*. In the present paper, we report the isolation and structural elucidation of new leptocarposide isolated from this plant, using chemical and spectroscopic methods. 2D NMR techniques, including ^{1}H-^{1}H COSY, TOCSY, HSQC-*Jmod*, HMBC, and ROESY, were utilized in the structure elucidation and complete assignments of ^{1}H and ^{13}C NMR spectra. The antioxidant activities of compounds **1** and **2** have been tested through DPPH (2,2-diphenyl-1-picryhydrazyl) radical scavenging model.

Results and Discussion

The purification of the *n*-BuOH soluble extract from the methanolic extract gave a new compound; namely, leptocarposide (i) in addition to the known compounds, luteolin-8-*C*-glucoside (ii),[4,5] and 1-*O*-*β*-D-glucopyranosyl-(2*S*,3*R*,8*E*)-2-[(2′*R*)-2-hydroxypalmitoylamino]-8-octadecen-1,3-diol (iii)[6,7] (Figure 1).

The high resolution time of flight electrospray ionisation mass spectrometry (HR-TOF-ESI-MS) of compound **1** exhibited a pseudo-molecular ion peak at *m/z* 1431.6395 $[M+Na]^+$ consistent with a molecular formula $C_{66}H_{104}O_{32}$ (calcd. 1431.6408) and indicating 15° of unsaturation and an ion fragment at *m/z* 703.3499 due to the loss a saccharidic ester chain. In ESI-MS, an ion fragment was observed at *m/z* 751.3 corresponding to the saccharidic ester chain. The MS/MS of this ion gave other fragment-ion peaks at *m/z* 647.3, 619.3, 515.3, 473.2, 369.2, and 323.2 suggesting the elimination of 3-hydroxybutyric acid, one pentosyl, 3-hydroxybutyric + one pentosyl, one pentosyl + one desoxyhexosyl, 3-hydroxybutyric acid + one pentosyl + one desoxyhexosyl, and two pentosyl + one desoxyhexosyl moieties, respectively.

The analysis of its ^{1}H NMR spectrum (Table 1) indicated the presence of six tertiary methyl protons at δ_H 0.80, 0.90, 0.98, 1.31, 1.39, and 1.41, one olefinic proton at δ_H 5.36, and three *O*-bearing methine protons at δ_H 4.15, 4.33, and 4.49. Its DEPT spectrum exhibited the methyl signals at δ_C 13.2, 16.1, 16.5, 23.6, 25.8, and 32.0, the ethylenic carbons at δ_C 121.9 (CH) and 143.5 (C), attributable to an olean-12-ene skeleton[8–10] (Table 1), in which three hydroxy groups were located at δ_C 69.9, 85.3, and 73.3 together with two carboxylic groups at $_C$ 183.5 and 176.0. The ^{1}H-^{1}H COSY showed cross-peak correlations between H-2 (δ_H 4.33) and H-3 (δ_H 4.15) and between H-15α (δ_H 1.67), H-15β (δ_H 1.47), and H-16 (δ_H 4.49) indicating the hydroxylation in positions 2 and 16 of the genine. The cross peak correlations observed in HMBC spectrum (Figure 2) between the signal of the carbonyl at δ_C 183.5 (C-23) and the protons at δ_H 4.15 (H-3), δ_H 1.64 (H-5), and δ_H 1.39 (CH_3-24), and between the signal of ester carbonyl at C-28 (δ_C 176.0) and the oxymethine proton H-16 at δ_H 4.49 confirm the place of the substituent.[8–10] The configuration of the 2β, 3β, 16α-trihydroxy groups, and

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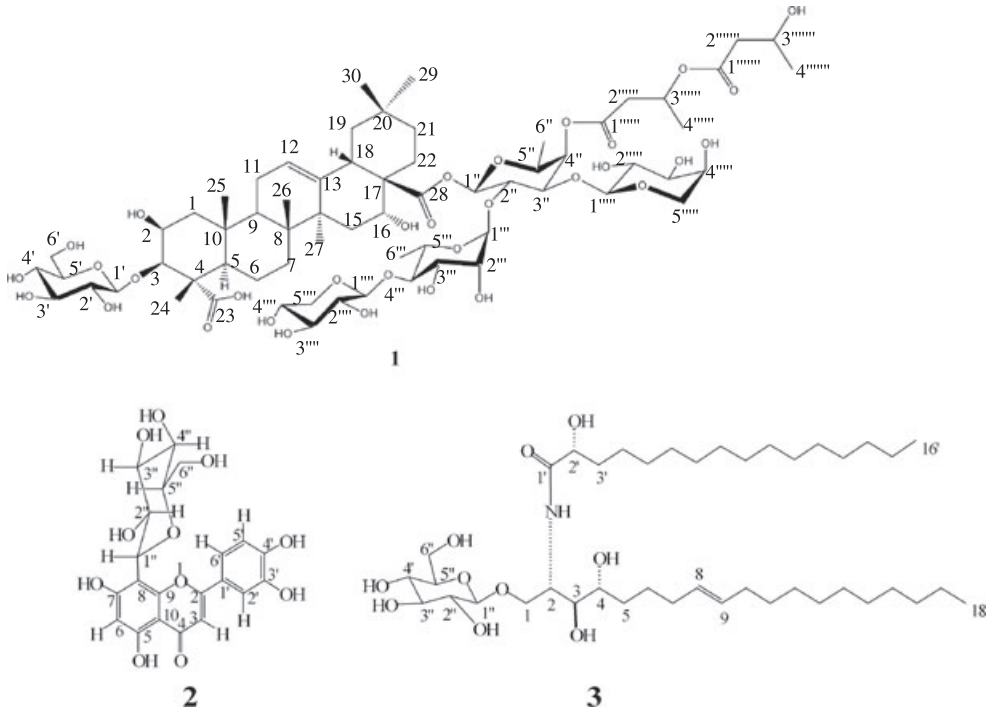

Figure 1. Structure of the isolated compounds.

α-orientation of the COOH at C-4 was confirmed from the ROESY experiments (Figure 3). The coupling constant ($J = 3.2$ Hz) between H-2 and H-3 is in accordance with the literature.[9,10] On this basis, the aglycone moiety of compound **1** was established to be zanhic acid or 2β,3β,16α-trihydroxyolean-12-en-23, 28-dioic acid. Deshielding ^{13}C NMR chemical shift at δ_C 85.3 (C-3) and shielding ^{13}C NMR chemical shift at δ_C 176.0 (C-28) suggested that this saponin was a bidesmosidic saponin with glucosidic linkages at C-3 through an *O*-heterosidic bond and at C-28 through an ester bond.[9,10] The ^{1}H NMR spectrum showed five anomeric protons signal at δ_H 4.41 (d, $J = 6.7$ Hz, H-1‴), 4.42 (d, $J = 7.8$ Hz, H-1′), 4.50 (d, $J = 7.6$ Hz, H-1⁗), 5.39 (d, $J = 1.7$ Hz, H-1″), and 5.41 (d, $J = 8.1$ Hz, H-1″). Their corresponding anomeric carbons were detected at δ_C 104.4 (C-1⁗), 103.2 (C-1′), 105.6 (C-1‴), 100.1 (C-1″), and 93.6 (C-1‴) after the analysis of HSQC spectrum indicating the presence of five sugar residues. Two methyl carbons at δ_C 15.5 (C-6″) and 17.0 (C-6″) indicate the presence of two 6-desoxyhexoses and the three oxymethine carbons at δ_C 60.9 (C-6′), 65.9 (C-5‴), and δ_C 65.6 (C-5⁗) suggest the presence of one hexose and two pentoses. The analysis of COSY, TOCSY, and ROESY spectra allowed the full indication of the spin systems of one rhamnopyranose and one fucopyranose from the anomeric signals at δ_H 5.39 (d, $J = 1.7$ Hz, H-1″) and δ_H 5.41 (d, $J = 8.1$ Hz, H-1″). A glucopyranose moiety was identified starting from the anomeric proton at δ_H 4.42 (d, $J = 7.8$ Hz, H-1′). One arabinopyranose and one xylopyranose were assigned starting from the anomeric protons at δ_H 4.41 (d, $J = 6.7$ Hz, H-1⁗) and 4.50 (d, $J = 7.6$ Hz, H-1‴). The anomeric configurations of glucose, fucose, and xylose in this saponin were all determined to be β, and that of arabinose and rhamnose to be α from the $^3J_{H1\text{-}H2}$ value of the anomeric proton signals and the chemical shift of anomeric carbon.[11] The sugar units were confirmed by thin layer chromatography (TLC) after hydrolysis, and the D or L-configurations were proved by gas chromatography-mass spectrometry (GC-MS) after derivatization. The carbons of each monosaccharides were attributed by analysis of HSQC spectrum and indicated the presence of a terminal β-D-glucopyranose, a terminal β-D-xylopyranose, a terminal α-L-arabinopyranose, a 4-substituted α-L-rhamnopyranose ($\delta_{C\text{-}4''}$ 82.7), and a 2,3,4-trisubstitued β-D-fucopyranose ($\delta_{C\text{-}2''}$ 72.8, $\delta_{C\text{-}3''}$ 80.8, and $\delta_{C\text{-}4''}$ 73.6) (**Table** 1). The downfield shift of protons H-4″ (δ_H 5.30 (d, $J = 2.8$ Hz) suggested an esterification of the fucose unit in this C-4″ position.

The ^{1}H NMR spectrum showed also two oxymethine groups at 4.18 (m, H-3⁗) and at 5.33 (m, H-3⁗) and two methyl at 1.23 (d, $J = 6.2$ Hz, H-4⁗) and at 1.36 (d, $J = 6.3$ Hz, H-4⁗), which suggested the presence of two 3-hydroxybutanoic acid (HBA)[12] In the[13]C NMR spectrum, resonances of two other ester carbonyl at δ_C 170.1 and 171.1 corresponding, respectively, to HBA C-1⁗ and HBA′ C-1⁗′, two methyl carbons at δ_C 18.6 (HBA C-4⁗) and 21.9 (HBA′ C-4⁗′), and two oxymethine carbons at δ_C 64.1 (HBA C-3⁗) and δ_C 67.3 (HBA′ C-3⁗′) confirmed the presence of two HBA.[13,14] The HMBC cross peaks observed between the signal of ester carbonyl at δ_C 170.1 (HBA C-1⁗) and three protons at δ_H 2.81 (HBA H-2⁗a), δ_H 2.73 (HBA H-2⁗b) for methylene protons and δ_H 5.33 (HBA H-3⁗); and between the signal of carbonyl at δ_C 171.1 (HBA′ C-1⁗′) and two oxymethine protons at δ_H 5.33 (HBA H-3⁗) and 4.18 (HBA′ H-3⁗′), and the methylene at δ_H 2.45 (HBA′ H-2⁗′a) and δ_H 2.50 (HBA′ H-2⁗′b) suggest that the two HBA were attached together.[13,14]

The sequences and linkage sites of the different monosaccharide units were determined with the aid of key HMBC correlations observed between the signal of ester carbonyl at C-28 (δ_C 176.0) and the anomeric proton at δ_H 5.41 (Glc H-1″) and between the signal of C-3 (δ_C 85.3) and anomeric proton at δ_H 4.42 (Fuc H-1″). This spectrum exhibited also cross peaks between the signals of Fuc C-2″at δ_C 72.8 and anomeric proton at δ_H 5.39 (Rha H-1‴), between Fuc C-3″ (δ_C 80.8) and anomeric proton at δ_H 4.41

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) nuclear magnetic resonance data, for compound **1**, in CD_3OD

	δ_C	δ_H (*J* in Hz)		δ_C	δ_H (*J* in Hz)
Zanhic acid. 1	43.5	1.30 (m)	Glc 1′	103.2	4.42 (d, 7.8)
		2.15 (dd, 14.9, 3.2)			
2	69.9	4.33 (q, 3.2)	2′	74.0	3.24 (dd, 8.7, 7.8)
3	85.3	4.15 (d, 3.2)	3′	76.4	3.38 (m)
4	52.5	-	4′	69.8	3.37 (t, 8.8)
5	51.8	1.64 (m)	5′	76.4	3.29 (m)
6	20.5	1.26 (m)	6′	60.9	3.71 (dd, 11.8, 4.8)
		1.63 (m)			3.83 (dd, 11.8, 2.3)
7	32.6	1.41 (m)	Fuc 1″	93.6	5.41 (d, 8.1)
		1.61 (m)			
8	39.7	—	2″	72.8	3.95 (dd, 9.5, 8.1)
9	47.1	1.69 (m)	3″	80.8	4.04 (dd, 9.5, 2.8)
10	36.0	—	4″	73.6	5.30 (d, 2.8)
11	23.2	1.97 (m)	5″	69.9	3.88 (m)
		2.02 (dd, 11.9, 2.5)			
12	121.9	5.36 (t, 3.3)	6″	15.5	1.08 (d, 6.4)
13	143.5	—	Rha 1‴	100.1	5.39 (d, 1.7)
14	41.5	—	2‴	70.4	3.97 (m)
15	35.0	1.47 (dd, 14.9, 2.5)	3‴	70.8	3.83 (m)
		1.67 (dd, 14.9, 3.2)			
16	73.3	4.49 (t, 2.9)	4‴	82.7	3.57 (t, 9.3)
17	48.9	—	5‴	67.7	3.82 (m)
18	40.9	2.95 (dd, 13.8, 3.9)	6‴	17.0	1.36 (d, 6.3)
19	46.8	1.08 (dd, 13.8, 3.9)	Xyl 1⁗	105.6	4.50 (d, 7.6)
		2.32 (t, 13.8)			
20	29.9	—	2⁗	74.8	3.26 (dd, 8.6, 7.6)
21	35.2	1.20 (m)	3⁗	76.9	3.32 (t, 8.6)
		1.98 (m)			
22	30.9	1.80 (td, 14.7, 4.4)	4⁗	69.5	3.52 (m)
		1.98 (m)			
23	183.5	—	5⁗	65.9	3.21 (t, 10.4)
					3.85 (dd, 10.4, 3.1)
24	13.2	1.39 (s)	Ara 1⁗′	104.4	4.41 (d, 6.7)
25	16.1	1.31 (s)	2⁗′	71.1	3.56 (m)
26	16.5	0.80 (s)	3⁗′	73.0	3.52(m)
27	25.8	1.41 (s)	4⁗′	68.3	3.81 (t, 2.1)
28	176.0	—	5⁗′	65.6	3.54 (d, 11.7)
					3.84 (d, 12.7)
29	32.0	0.90 (s)	HBA 1⁗″	170.1	-
30	23.6	0.98 (s)	2⁗″	40.0	2.73 (dd, 16.1, 5.6)
					2.81 (dd, 16.1, 7.3)
			3⁗″	67.3	5.33 (m)
			4⁗″	18.6	1.36 (d, 6.3)
			HBA′ 1⁗″′	171.1	-
			2⁗″′	43.6	2.45 (dd, 15.0, 5.6)
					2.50 (dd, 15.0, 7.5)
			3⁗″′	64.1	4.18 (m)
			4⁗″′	21.9	1.23 (d, 6.2)

Glc, *β*-D-glucopyranosyl; Fuc, *β*-D-fucopyranosyl; Xyl, *β*-D-xylopyranosyl; Ara, *α*-L-arabinopyranosyl; Rha, *α*-L-rhamnopyranosyl; HBA, 3-hydroxylbutanoyl.

(Ara H-1⁗), between Rha C-4‴ (δ_C 82.7) and anomeric proton at δ_H 4.42 (Xyl H-1⁗), and the signal of ester carbonyl at δ_C 170.1 (HBA C-1⁗″) and Fuc H-4″ at δ_H 5.30. These correlations suggested that the sugar moieties are 3-*O*-β-D-glucopyranoside and 28-*O*-*β*-D-xylopyranosyl(1 → 4)-*α*-L-rhamnopyranosyl(1 → 2)-[*α*-L-arabinopyranosyl(1 → 3)]-4-*O*-(3′-hydroxybutanoyloxy-3-hydroxybutanoyloxy)-*β*-D-fucopyranoside.

The structure of the compound **1** was further supported by a ^{1}H-^{1}H ROESY experiment, which revealed cross peak correlations between Rha H-1‴ (δ_H 5.39) and Fuc H-2″ (δ_H 3.95), Fuc H-3″ (δ_H 4.04), and Ara H-1⁗′ (δ_H 4.41) and between Xyl H-1⁗ (δ_H 4.50) and Rha H-4‴ (δ_H 3.57). On the basis of aforementioned information, the structure of this compound was elucidated as 3-*O*-*β*-D-glucopyranosyl-28-*O*-*β*-D-xylopyranosyl(1 → 4)-*α*-L-rhamnopyranosyl

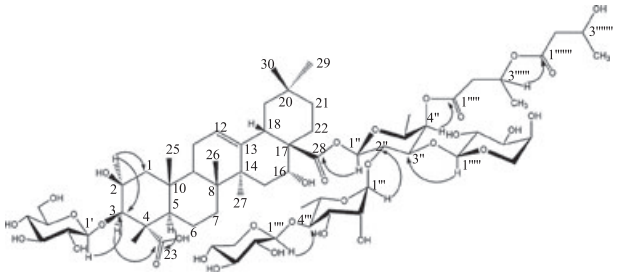

Figure 2. Selected heteronuclear multiple-bond correlation correlations for compound **1**.

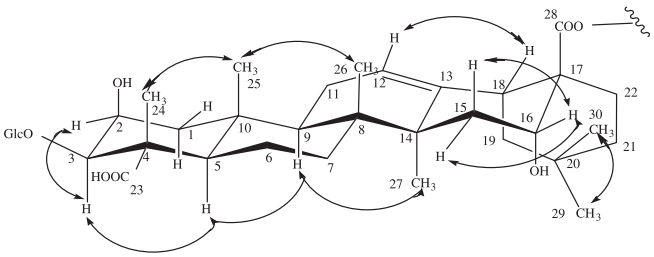

Figure 3. Selected rotating frame nuclear overhauser effect spectroscopy correlations observed for the aglycone moiety of compound **1**.

(1 → 2)-[α-L-arabinopyranosyl (1 → 3)]-4-*O*-(3′-hydroxybutanoyloxy-3- hydroxybutanoyloxy)-β-D-fucopyranosyl zanhic acid named leptocarposide.

Very similar structure was previously isolated from *Filicium decipiens* with two nilic acids linked each other in position 4 of the fucose moiety.[9] Saponins with two 3-hydroxy butanoic acids linked to the fucose moiety in position 4 were also previously isolated from *Solidago virgaurea* but with polygalacic acid as genin.[13,14] To our knowledge, this is the first report of zanhic acid saponin esterified by 3-hydroxybutanoic acid. The antioxidant activities of compounds **1** and **2** were studied using DPPH radical scavenging assay, and only orientin (**2**) showed radical scavenging activity (SC_{50} = 0.038 mM) as observed previously.[5]

Materials and Methods

General

The melting points were recorded with a Reichert microscope (Reichert Technologies, Depew, New York USA) and are uncorrected. IR spectra were recorded with a Shimadzu FT-IR-8400S (Shimadzu, France) spectrophotometer. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectra were recorded on a Bruker Avance III 600 spectrometer (Bruker, Wissembourg, France) equipped with a cryoplatform using CD_3OD with TMS as the internal standard. TOF-ESIMS and HR-TOF-ESI experiments were performed using a Micromass Q-TOF micro instrument (Manchester, UK) with an electrospray source. The samples were introduced by direct infusion in a solution of MeOH at a rate of 5 μl min^{-1}. The optical rotations were measured on a Bellingham & Stanley ADP 220 polarimeter (Bellingham + Stanley Ltd, United Kingdom). Column chromatography was run on Merck silica gel (VWR, France) 60 (70–230 mesh) and gel permeation on Sephadex LH-20 (VWR, France), while TLC was carried out on silica gel GF_{254} pre-coated plates with detection accomplished by spraying with 50% H_2SO_4 followed by heating at 100 °C or by visualizing with a UV lamp at 254 and 365 nm. GC-MS analysis was carried out on a Perkin-Elmer Clarus 500 GC-MS system and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: capillary column EQUITY^{TM}-1 (30 m × 0.25 mm × 0.25 μm, Supelco Dex phase, composed of 25% 2,3-*di*-O-acetyl-6-0-TBDMS-β-cyclodextrin embedded in SPB-20 poly(20% phenyl, 80% dimethylsiloxane). Column temperature, 230 °C; injection temperature, 250 °C; carrier N_2 gas; detection in Electronic impact (EI) mode, ionization potential, 70 eV; ion-source temperature, 280 °C.

Plant material

The whole plant of *L. leptocarpa* was collected in Foto village (Menoua Division, Western region of Cameroon), in April 2011. Authentication was performed by Victor Nana, a botanist of the Cameroon National Herbarium, Yaoundé, where a voucher specimen (N° 38782/HNC) has been deposited.

Extraction and isolation

The dried whole plant of *L. leptocarpa* (4 kg) was extracted with MeOH at room temperature for 3 days, and the extract was concentrated to dryness under reduced pressure. Part of residue obtained (97 g) was subjected to silica gel column chromatography and eluted with hexane containing increasing EtOAc, then with EtOAc containing increasing MeOH. Seven fractions were obtained: A (hexane), B (hexane–EtOAc : 9–1), C (hexane–EtOAc : 8–2), D (hexane–EtOAc : 6–4), E (hexane–EtOAc : 4–6), F (hexane–EtOAc : 9–1), and G (EtOAc–MeOH : 1–1). Fraction G was suspended in water and successively extracted with ethyl acetate and *n*-butanol to obtain after evaporation of solvent 5.20 g and 13.76 g, respectively. Part of butanol-solute extracts (13.76 g) was eluted with EtOAc containing increasing MeOH (10%, 20%, 30%, 40%, and 50%). Five under fractions were obtained G_1, G_2, G_3, G_4, and G_5. Fraction G_5 was purified over silica gel column eluted with the mixture EtOAc–MeOH–H_2O (7–2–1) to give the compound **1** (20 mg). Fraction G_2 was purified over silica gel column eluted with the mixture EtOAc–MeOH (85–15) to give the compound **2 (**white amorphous powder, 13 mg). Fractions G_3 and G_4 were combined and purified over silica gel column eluted with the mixture EtOAc–MeOH–H_2O 8–1–1) to give the compound **3** (yellow amorphous powder, 30 mg).

NMR data

The NMR spectra were recorded at 298 K using a BRUKER Avance DRX 600 spectrometer (Bruker, Wissembourg, France) using 5 mm CPTCI 1H/13C/15 N/D Zgrd operating at 600 MHz for ^{1}H and 150 MHz for ^{13}C. 1D and 2D NMR experiments (COSY, TOCSY, ROESY, HSQC-*Jmod*, and HMBC) were performed using standard Bruker pulse programs (XWinNMR version 2.1).

Sample was dissolved in CD_3OD. Chemical shifts were referenced to the solvent signal (δ (CD_3OD) = 3.33 ppm for ^{1}H NMR and [δ (CD_3OD) = 49.0 ppm for ^{13}C NMR].

^{1}H and ^{13}C 1D spectra were acquired with relaxation delay d1 = 1 s, 32 K data points and 90° pulses were, respectively, 9.32 μs at 3 dB and 12.0 μs at −0.5 dB for ^{1}H and ^{13}C. The number of scans is 16 for 1H and 6144 for 13C.

2D experiments were recorded with the following parameters:

^{1}H-^{1}H gradient COSY spectrum: relaxation delay d1 = 1 s; 90° pulse, 9.32 μs for ^{1}H at 3 dB; number of scans 2; 2 K data points in t2; spectral width 6.0 ppm in both dimensions; 512 experiments

in t1; zero-filling up to 1 K in t1; apodization with pure sine-bell in both dimensions prior to double Fourier transformation.

Total correlation spectroscopy spectrum: relaxation delay d1 = 1 s; 90° pulse, 9.32 μs for ^{1}H at 3 dB; number of scans 8; spin lock time, 200 ms using 90° pulse of 30 μs at 14.21 dB; 2 K data points in t2; spectral width 6.0 ppm in both dimensions; 512 experiments in t1; apodization with sine-bell (processing parameter SSB = 3) in both dimensions; zero-filling with linear prediction up to 1 K.

Rotating frame NOE spectroscopy using Bruker library pulse sequence "roesyetgp": relaxation delay d1 = 1 s; 90° pulse, 9.32 μs for ^{1}H at 3 dB; number of scans 8; ROESY spin lock pulse of 500 ms at 27.21 dB; 2 K data points in t2; spectral width 6.0 ppm in both dimensions; 512 experiments in t1; apodization with squared cosine-bell in both dimensions; zero-filling up to 1 and 4 K, respectively, in t1 and t2.

Heteronuclear single-quantum correlation-J modulated using Bruker library pulse sequence "hsqcedetgpsisp2.2": relaxation delay d1 = 1 s; coupling constant $^{1}J(^{1}H\text{-}^{13}C) = 145$ Hz for d4 = 1.72 ms; 90° pulse, 9.32 μs at 3 dB for ^{1}H, 12 μs at −0.5 dB for ^{13}C with gradient ratio GPZ1 : GPZ2 : GPZ3 : GPZ4 = 80 : 20 : 11 : −5; 2 K data points in t_2; spectral width 6 ppm in F2 and 160 ppm in F1; number of scans 2; 512 experiments in t1; apodization with pure cosine-bell in both dimensions; zero-filling with linear prediction up to 1 K.

Heteronuclear multiple-bond correlation using Bruker library pulse sequence "hmbcetgpl3nd": relaxation delay d1 = 1 s; same pulse calibration as HSQC; delay of the low-pass J-filter d2 = 3.44 ms (corresponding to $^{1}J(^{1}H\text{-}^{13}C) = 145$ Hz); delay for evolution of long-range coupling d6 = 62.5 ms; gradient ratio GPZ1 : GPZ3 : GPZ4 : GPZ5 : GPZ6 = 80:14 : −8 : −4 : −2; 2 K data points in t2; spectral width 6.0 ppm in F2 and 220 ppm in F1; number of scans 14; 512 experiments in t1; apodization with pure sine-bell in both dimensions; zero-filling with linear prediction up to 1 K.

DPPH radical scavenging method

The sample was deposited on a TLC plate, which was then developed in a suitably selected system. After the migration of compound and evaporation of solvent, this plate was revealed by a methanolic solution of DPPH to 2% (2 mg/100 ml). The appearance with visible of yellow-pale spots on purple bottom (or crimson) testifies to the activity of the compound.[15]

The scavenging activity (SC) of orientin (**2**) was calculated using the Cheng *et al.* method[16] with slight modification. Sample in various concentrations (5, 10, 50, and 100 μg/ml) was prepared in DMSO. In 96 well plates, 95 μl of 158 μM of DPPH solution in EtOH was added and the 5 μl of the sample. Ascorbic acid at 5 μg/ml was used as positive control. Absorbance was read at 515 nm during 1 h at 37 °C. The SC of the samples was calculated using the following formula: $SC\ (\%) = \{1-(A_s/A_0)\} \times 100$ where A_s is the absorbance of the sample and A_0 is the absorbance of the blank. The SC_{50} value was determined from their respective linear regression curves.

Acid hydrolysis and determination of absolute configuration of monosaccharide

Compound **1** (5 mg) was heated in 1 M HCl (dioxane-H_2O, 1 : 1, 2 ml) at 100 °C for 2 h. After dioxane was removed, the solution was extracted with EtOAc (2 ml × 3). Combined remaining aqueous layer containing monosaccharides was concentrated under reduced pressure to dryness, to give a residue. The residue was dissolved in pyridine (0.1 ml), to which 0.1 M L-cysteine methyl ester hydrochloride in pyridine (0.20 ml) was added. The mixture was heated at 60° for 2 h, dried *in vacuo*, and trimethylsilylated with hexamethyldisilazane-trimethylchlorosilane (0.2 ml) at 60° for 2 h. The mixture was partitioned between *n*-hexane and H_2O (0.4 ml each), and the *n*-hexane extract was subjected to GC-MS analysis. Absolute configurations of monosoccharides in compound **1** were determined as D-glucose, D-fucose, D-xylose, L-arabinose and, L-rhamnose by comparison of the retention times of their derivatives with those of literature[17] and with those of D-glucose, D-fucose, D-xylose, L-arabinose, and L-rhamnose derivatives prepared in the same way, which showed retention times of 10.32, 7.76, 6.32, 6.37, and 7.53.

Leptocarposide (1)

White amorphous powder; ^{1}H and ^{13}C, see Table 1; $[\alpha]_D^{20}$ −23° (*c* 0.06, CH_3OH) HRESIMS (positive-ion mode) *m/z*: 1431.6395 $[M + Na]^+$ (calcd. for $C_{66}H_{104}O_{32}Na$: 1431.6408), 703.3499 [M + Na-ester chain]$^+$; ESIMS (positive-ion mode) *m/z*: 1431.8 $[M + Na]^+$. 751.3 [ester chain + Na]$^+$, 647.3 [ester chain + Na-$C_4H_8O_3$]$^+$; $ESIMS^n$ (positive-ion mode): $ESIMS^1$ (1431.8) *m/z*: 751.4 [ester chain + Na]$^+$, 647.4 [ester chain + Na-$C_4H_8O_3$]$^+$, 473.3 [ester chain + Na-$C_5H_8O_4$-$C_6H_{10}O_4$]$^+$, $ESIMS^2$ (751.3) *m/z*: 647.3 [ester chain + Na-$C_4H_8O_3$]$^+$, 619.3 [ester chain + Na-$C_5H_8O_4$]$^+$, 515.3 [ester chain + Na-$C_4H_8O_3$-$C_5H_8O_4$]$^+$, 473.3 [ester chain + Na-$C_5H_8O_4$-$C_6H_{10}O_4$]$^+$, 369.2 [ester chain + Na-$C_5H_8O_4$-$C_6H_{10}O_4$-$C_4H_8O_3$]$^+$

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