

Leptocarposide: a new triterpenoid glycoside from *Ludwigia leptocarpa*

(Onagraceae)

Florence Declaire MABOU^a, Perrin Lanversin FONING TEBOU^a, David NGNOKAM^{a*},

Dominique HAKAKAT^b and Laurence VOUTQUENNE-NAZABADIOKO^c

^aFaculty of Science, Department of Chemistry, University of Dschang, P.O. Box 67. Dschang

Cameroon

^bService Commun d'Analyses, Institut de Chimie Moléculaire de Reims (ICMR), CNRS

UMR 7312, Bat. 18 B.P.1039, 51687 Reims Cedex 2, France

^cGroupe Isolement et Structure, Institut de Chimie Moléculaire de Reims (ICMR), CNRS

UMR 7312, Bat. 18 B.P.1039, 51687 Reims Cedex 2, France

*Corresponding author. Tel. (237)96932925. E-mail. dngnokam@yahoo.fr

ABSTRACT

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**) were 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 (¹H and ¹³C NMR, ¹H-¹H 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 DPPH and reveals non potent antioxidant activities, while compound **2** shows SC₅₀ of 0,038mM.

Key words: NMR; ¹H NMR; ¹³C 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 Africa¹. It is used in Nigerian folk medicine for the treatment of the rheumatism and the dysentery². Previous work on this species has revealed the presence of flavonoids³.

In the course of our continuing search for secondary metabolites of biological importance from Cameroonian medicinal plants, we investigate the MeOH extract of whole plant of *Ludwigia 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 ¹H-¹H COSY, TOCSY, HSQC-*J*mod, HMBC and ROESY were utilized in the structure elucidation and complete assignments of ¹H and ¹³C NMR spectra. The antioxidant activities of compounds **1** and **2** has been tested through DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging model.

RESULTATS AND DISCUSSION

The purification of the *n*-BuOH soluble extract from the methanolic extract gave a new compound namely leptocarposide (**1**) in addition to the known compounds: luteolin-8-*C*-glucoside (**2**)^{4,5} and 1-*O*- β -D-glucopyranosyl-(2*S*,3*R*,8*E*)-2-[(2'*R*)-2-hydroxypalmitoylamino]-8-octadecen-1,3-diol (**3**)^{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 C₆₆H₁₀₄O₃₂ (calcd. 1431.6408) and indicating fifteen degrees of unsaturation, and a 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.

Analysis of its ^1H 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^{8,9,10} (**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 δ_{C} 183.5 and 176.0. The ^1H - ^1H COSY showed cross peak correlations between H-2 (δ_{H} 4.33) and H-3 (δ_{H} 4.15) and between H-15 α (δ_{H} 1.67), H-15 β (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 (CH₃-24), 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 confirms the place of the substituent^{8,9,10}. The configuration of the 2 β , 3 β , 16 α -trihydroxy groups and α -orientation of the COOH at C-4 were confirmed from the ROESY experiments (**Figure 3**). The coupling constant ($J = 3.2$ Hz) between H-2 and H-3 are in accordance with the literature^{9,10}. On this basis, the aglycon moiety of compound **1** was established to be zanhic acid or 2 β ,3 β ,16 α -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 ^1H 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 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. 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 $^3J_{H1-H2}$ value of the anomeric proton signals and the chemical shift of anomeric carbon¹¹. The sugar units were confirmed by thin layer chromatography after hydrolysis, and the D or L-configurations were proved by 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 ($\delta_{C-4''}$ 82.7), and a 2,3,4-trisubstituted β -D-fucopyranose ($\delta_{C-2''}$ 72.8, $\delta_{C-3''}$ 80.8, $\delta_{C-4''}$ 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 1H 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 was 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 (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 \rightarrow 4)- α -L-rhamnopyranosyl(1 \rightarrow 2)-[α -L-arabinopyranosyl(1 \rightarrow 3)]-4-*O*-(3'-hydroxybutanoyloxy-3-hydroxybutanoyloxy)- β -D-fucopyranoside.

The structure of the compound **1** was further supported by a ¹H-¹H 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 basis of above information, the structure of this compound was elucidated as 3-*O*- β -D-glucopyranosyl-28-*O*- β -D-xylopyranosyl(1 \rightarrow 4)- α -L-

rhamnopyranosyl(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 moiety⁹. Saponins with two 3-hydroxybutanoic 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 previously⁵.

MATERIEL AND METHODS

General

The Melting points were recorded with a Reichert microscope and are uncorrected. IR spectra were recorded with a Shimadzu FT-IR-8400S spectrophotometer. ¹H (600 MHz) and ¹³C (150 MHz) NMR spectra were recorded on a Bruker Avance III 600 spectrometer equipped with a cryoplatfrom using CD₃OD 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 μ l min⁻¹. The Optical rotations were measured on a Bellingham & Stanley ADP 220 polarimeter. Column chromatography was run on Merck silica gel 60 (70-230 mesh) and gel permeation on Sephadex LH-20 while TLC was carried out on silica gel GF₂₅₄ pre-coated plates with detection accomplished by spraying with 50% H₂SO₄ followed

by heating at 100°C, or by visualizing with an UV lamp at 254 and 365 nm. GLC was carried out on a Perkin-Elmer Clarus 500 GC-MS instrument

Plant material

The whole plant of *Ludwigia leptocarpa* was collected in Foto village (Menoua Division, Wersten region of Cameroon), in April 2011. Authentication was done 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 three days, and the extract 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 G₁, G₂, G₃, G₄, and G₅. Fraction G₅ was purified over silica gel column eluted with the mixture EtOAc-MeOH-H₂O (7-2-1) to give the compound **1** (20 mg). Fraction G₂ 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 G₃ and G₄ were combined and purified over silica gel column eluted with the mixture EtOAc-MeOH-H₂O (18-1-1) 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 using 5 mm CPTCI 1H/13C/15N/D Zgrd operating at 600 MHz for ^1H 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 ^1H NMR and (δ (CD_3OD) = 49.0 ppm for ^{13}C NMR).

^1H and ^{13}C 1D spectra were acquired with relaxation delay $d1=1\text{s}$, 32K data points and 90° pulses were respectively $9.32\ \mu\text{s}$ at 3 dB and $12.0\ \mu\text{s}$ at -0.5 dB for ^1H and ^{13}C . Number of scans 16 for 1H and 6144 for 13C.

2D experiments were recorded with following parameters:

^1H - ^1H gradient COSY spectrum: relaxation delay $d1 = 1\ \text{s}$; 90° pulse, $9.32\ \mu\text{s}$ for ^1H at 3 dB; number of scans 2; 2K data points in t_2 ; spectral width 6.0 ppm in both dimensions; 512 experiments in t_1 ; zero-filling up to 1K in t_1 ; apodization with pure sine-bell in both dimensions prior to double Fourier transformation.

TOCSY spectrum: relaxation delay $d1 = 1\ \text{s}$; 90° pulse, $9.32\ \mu\text{s}$ for ^1H at 3 dB; number of scans 8; spin lock time, 200 ms using 90° pulse of $30\ \mu\text{s}$ at 14.21 dB; 2K data points in t_2 ; spectral width 6.0 ppm in both dimensions; 512 experiments in t_1 ; apodization with sine-bell (processing parameter $\text{SSB}=3$) in both dimensions; zero-filling with linear prediction up to 1K.

ROESY using Bruker library pulse sequence "roesyetgp": relaxation delay $d1 = 1\ \text{s}$; 90° pulse, $9.32\ \mu\text{s}$ for ^1H at 3 dB; number of scans 8; roesy spin lock pulse of 500 ms at 27.21 dB; 2K data points in t_2 ; spectral width 6.0 ppm in both dimensions; 512 experiments in t_1 ; apodization with squared cosine-bell in both dimensions; zero-filling up to 1K and 4K respectively in t_1 and t_2 .

HSQC-J modulated using Bruker library pulse sequence "hsqcedetgpsisp2.2": relaxation delay $d1 = 1\ \text{s}$; coupling constant $^1\text{J}(\text{H}-^{13}\text{C}) = 145\ \text{Hz}$ for $d4 = 1.72\ \text{ms}$; 90° pulse, $9.32\ \mu\text{s}$ at 3 dB for ^1H , $12\ \mu\text{s}$ at -0.5 dB for ^{13}C with gradient ratio $\text{GPZ1:GPZ2:GPZ3:GPZ4} = 80:20:11:-5$; 2K data

points in t₂; 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 1K.

HMBC 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 ¹J(¹H-¹³C) = 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; 2K 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 1K.

DPPH radical scavenging method

The sample was deposited on a TLC plate which was then developed in a suitably selected system. After 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 compound¹⁵.

The Scavenging activity (SC) of orientin (**2**) was calculated using the Cheng et al. method¹⁶ 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 1h 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 A₀ is the absorbance of the blank. The SC₅₀ 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-H₂O, 1:1, 2 ml) at 100°C for 2h. After dioxane was removed, the solution was extracted with EtOAc (2 ml x 3). Combined

remaining aqueous layer containing monosaccharides were 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 (HMDS-TMCS) (0.2 ml) at 60° for 2 h. The mixture was partitioned between *n*-hexane and H₂O (0.4 ml each) and the *n*-hexane extract was subjected to GC/MS analysis under the following conditions: capillary column, EQUITY™-1 (30 m x 0.25 mm x 0.25 μm), Supelco); column temperature, 230°C; injection temperature, 250°C; carrier N₂ gas; detection in EI mode, ionization potential, 70eV; ion-source temperature, 280°C. Absolute configurations of monosaccharides 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 literature¹⁷ 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; ¹H and ¹³C, see Table 1; [α]_D²⁰ -23° (*c* 0.06, CH₃OH) HRESIMS (positive-ion mode) *m/z*: 1431.6395 [M+Na]⁺ (calcd for C₆₆H₁₀₄O₃₂Na: 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-C₄H₈O₃]⁺; ESIMSⁿ (positive-ion mode): ESIMS¹ (1431.8) *m/z*: 751.4 [ester chain+Na]⁺, 647.4 [ester chain+Na-C₄H₈O₃]⁺, 473.3 [ester chain+Na-C₅H₈O₄-C₆H₁₀O₄]⁺, ESIMS² (751.3) *m/z*: 647.3 [ester chain+Na-C₄H₈O₃]⁺, 619.3 [ester chain+Na-C₅H₈O₄]⁺, 515.3 [ester chain+Na-C₄H₈O₃-C₅H₈O₄]⁺, 473.3 [ester chain+Na-C₅H₈O₄-C₆H₁₀O₄]⁺, 369.2 [ester chain+Na-C₅H₈O₄-C₆H₁₀O₄-C₄H₈O₃]⁺

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Table 1: ^1H (600 MHz) and ^{13}C (150 MHz) NMR data, for compound **1**, in CD_3OD

	δ_{C}	δ_{H} (<i>J</i> in Hz)		δ_{C}	δ_{H} (<i>J</i> in Hz)
Zanhic acid.	43.5	1.30 (m)	Glc 1'	103.2	4.42 (d, 7.8)
1		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-hydroxybutanoyl

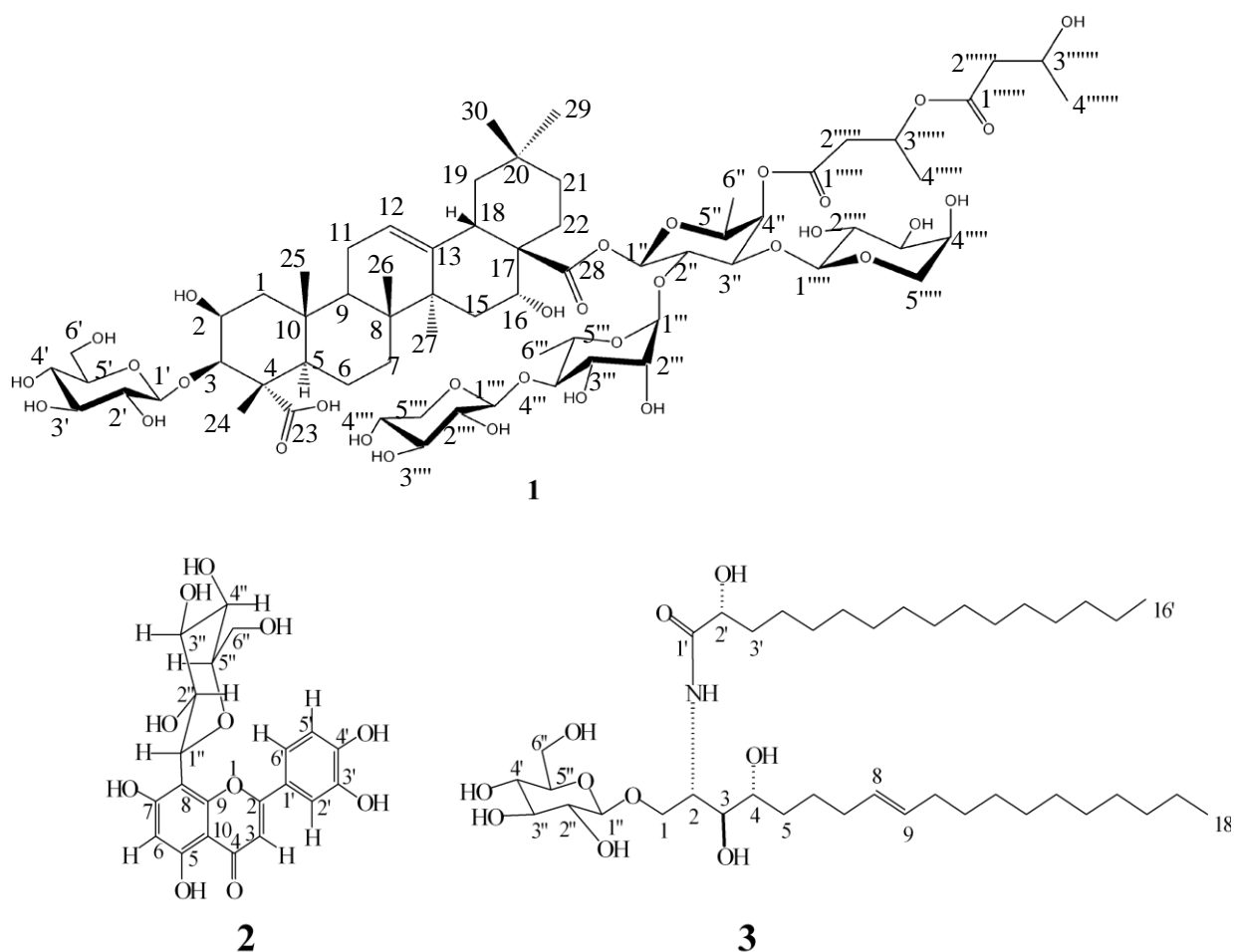


Figure 1: Structure of the isolated compounds

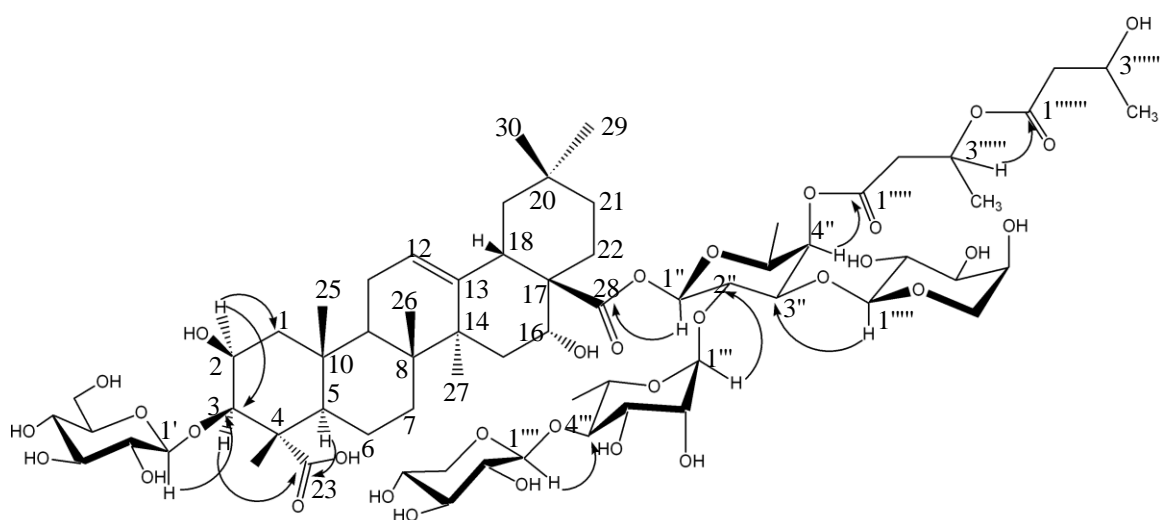


Figure 2: Selected HMBC Correlations for compound **1**.

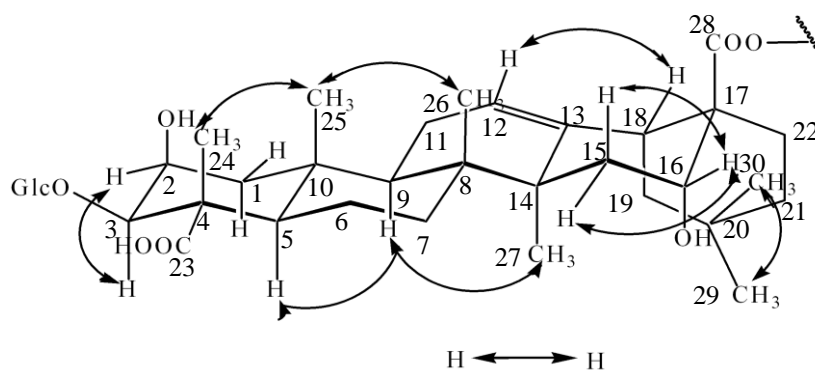


Figure 3: Selected ROESY Correlations Observed for the aglycon moiety of compound **1**