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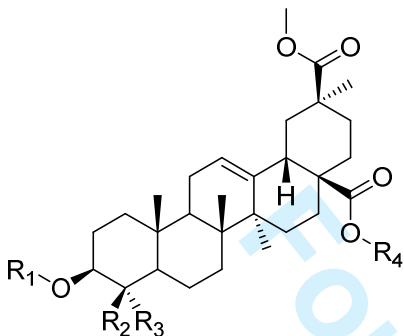
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The LC-SPE-NMR analysis of *Dendrobangia boliviana* roots extract revealed the presence of five new saponins that were not identified during a previous conventional phytochemical study.

New oleanane saponins from the roots of *Dendrobangia boliviana* identified by LC-SPE-NMR.

Short title: Saponins from *Dendrobangia boliviana* identified by LC-SPE-NMR

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ABSTRACT

A LC-SPE-NMR system efficiently contributed to the isolation and identification of five new oleanane saponins from the roots of *Dendrobangia boliviana* Rusby along with twelve known saponins previously isolated from this plant and of 3-*O*- β -D-glucuronopyranosylphytolaccagenic acid, a compound identified from *Diploclisia glaucescens*. Their structures were established on the basis of spectral data, mainly HR-ESI-MS, ^1H -NMR, HSQC, and ROESY, and by comparison with literature data. All but one of these oleanane saponins are glycosides of serjanic or phytolaccinic acid. The remaining one contained a new sapogenin, the 3 β ,24-dihydroxy-olean-12-en-28,30-dioic acid, 30-methyl ester or 24-hydroxy-serjanic acid, tentatively named dendrobangionic acid.

Keywords

LC-SPE-NMR, ^1H NMR, HSQC, ROESY, HR-ESI-MS, Saponins, *Dendrobangia boliviana*.

INTRODUCTION

Dendrobangia is a genus from the Cardiopteridaceae family,^[1] composed of 43 species grouped in 6 tropical genera. This genus is composed of three species, *D. multinerva* Ducke, *D. tenuis* Ducke, and *D. boliviana* Rusby, large trees growing in the tropical areas.

Dendrobangia boliviana Rusby is a tree widely distributed in the Neotropical region of Costa Rica and Panama, Brazil and Bolivia. It can be from 5 to 30 m high and up to 50 cm in diameter. It grows mainly in mountain forests and more rarely in floodplain forests, in an altitude of 100 to 1200 m. It tolerates a wide range of soils. In Colombia, the community of the region Hibito consumes the fine fruits in their dried form. The wood of this tree is very hard and therefore used in construction, for furnitures, sporting goods, tool handles and interior coverings.^[2] In order to discover new secondary metabolites of biological importance from Peru's Amazon rainforest, we investigated in a previous phytochemical study the roots of *D. boliviana* and isolated fourteen oleanane saponins which are glycosides of serjanic or phytolaccinic acid.^[3] In order too push our investigation further, the saponins containing fractions of interest were analyzed using a hyphenated LC-SPE-NMR system in order to identify as many compounds as possible. This methodology has been widely used for the structure elucidation of natural products from complex mixtures^[4-8]. The joint use of LC and solid-phase extraction (SPE) has considerably improved the sensitivity of NMR detection by its ability to concentrate the isolated compounds. It appeared as a timesaving method with a broad application range. As a result, the LC-SPE-NMR study allowed us to identify five new saponins, together with twelve known saponins already identified in these roots and another known saponin from another plant species. Their structural characterization has been carried out through NMR spectroscopy and mass spectrometry studies.^[3]

RESULTS AND DISCUSSION

The powdered roots of *Dendrobangia boliviana* were macerated with a mixture of CH₃OH-H₂O (8:2) to give a hydromethanolic extract which was fractioned by High Performance Flash Chromatography (HPFC) on C₁₈ reversed phase to afford thirteen fractions labelled DB-1 to DB-13. The saponin containing fractions, DB-8 to DB-11, were analysed by LC-SPE-NMR. An HPLC chromatographic method was optimized for each of these fractions before

entrapping the separated compounds in GP (General Phase/Polyvinyl-benzene) SPE cartridges. Then, each compound was recovered by desorption with acetonitrile-d₃ before 1D and 2D NMR analysis. Eighteen compounds were isolated and identified as five new compounds (**1-5**) (Figure 1), twelve known compounds that were previously isolated by chromatography (on silica gel or RP-18) from this plant^[3] and the 3-*O*-β-D-glucuronopyranosylphytolaccagenic acid known from *Diploclisia glaucescens*.^[9] The known compounds were identified by comparison of their NMR spectral data and High Resolution ElectroSpray Ionisation Mass Spectrometry (HR-ESI-MS) data to zebiriosides A-G and J-L, talunòmocide I, and 3-*O*-β-D-glucuronopyranosylserjanic acid.^[3] Comparing to the previous study, in which fourteen saponins were isolated by vacuum liquid chromatography and successive HPFC and preparative HPLC, in this study, the extract was fractionated in only one step (HPFC) affording eighteen saponins.

HR-ESI-MS in positive ion mode of compound **1** revealed a pseudomolecular ion at m/z 1169.5348 ($[M + Na]^+$, calcd for C₅₅H₈₆O₂₅Na, 1169.5356) indicating a molecular formula of C₅₅H₈₆O₅₅ (Figure S4). The ¹H NMR spectrum of **1** showed the characteristic elements of the serjanic acid with the olefinic proton H-12 at δ_H 5.31 (t, J = 3.7 Hz), the deshielded proton H-18 resonating as a doublet of doublets at δ_H 2.71 (J = 13.0, 3.8 Hz) and the signals of six methyl groups resonating as singlets between δ_H 0.7 and 1.6 in addition to a methoxy group at δ_H 3.70^{3, 10} (Table 1). We also detected the presence of four sugars by the resonance of their anomeric protons (Figure S1). The four sugars were identified by studying their chemical shifts after analysis of ROESY (Figure S2) and HSQC (Figure S3) spectra and by comparison with the spectra of zebiriosides A-C to a β-D-glucopyranose (δ_H 4.62, J = 7.7 Hz), a α-L-rhamnopyranose (δ_H 4.97, J = 1.8 Hz), a β-D-glucuronopyranose (δ_H 4.56, J = 8.1 Hz) and a second β-D-glucopyranose (δ_H 5.39, J = 8.1 Hz) (Table 2)^{3, 11}. The chemical shifts of the anomeric signals of the second β-D-glucopyranose (δ_H 5.39, δ_C 95.3) suggest an ester linkage on the aglycon via the carboxyl group at position 28, as in zebirioside B.^[3] This was confirmed by the presence of a fragment ion peak at m/z 685.6 corresponding to $[28-O-Glc-serjanic\ acid + Na]^+$ in the ESI-MS-MS spectrum. The study of ROESY correlations confirmed the structure of the four sugar units and allowed assignment of the other interglycosidic linkage. The glucuronic acid unit is attached to serjanic acid *via* its position 3 as indicated by the ROE between H-1' and H-3; this sugar was substituted at positions 2' and 3' by the β-D-glucopyranose and the α-L-rhamnopyranose units, respectively, as deduced

from the ROEs between H-1''' (δ_{H} 4.62) and H-2' (δ_{H} 3.71), and between H-1'' (δ_{H} 4.97) and H-3' (δ_{H} 3.67) (Figure S2). Thus, the trisaccharide chain was identical to the one in zebirioside C, and the disubstitution of glucuronic acid was confirmed by the ESI-MS-MS spectrum in which fragment ions were observed at m/z 507.3 [Rha-(Glc-)GlcA + Na]⁺, 361.2 [Glc-GlcA + Na]⁺ and 507.3 [Rha-GlcA + Na]⁺. This indicated that compound **1** is 3-*O*- β -D-glucopyranosyl-(1 \rightarrow 2)-[α -L-rhamnopyranosyl-(1 \rightarrow 3)]- β -D-glucuronopyranosyl-28-*O*- β -D-glucopyranosyl serjanic acid, named zebirioside M (**1**) (Figure 1).

The positive mode HR-ESI-MS spectra of compound **2** presented a pseudomolecular ion peak at m/z 1021.8984 ([M + Na]⁺ calcd for C₄₉H₇₆O₂₀Na, 1021.4992) corresponding to a molecular formula of C₄₉H₇₆O₂₀ (Figure S7). The NMR spectra were very similar to those of compound **1** with one less osidic unit and to zebirioside B.^[3] We observed the signals of three osidic units in addition to those of serjanic acid (Tables 1 and 2), with anomeric position signals at δ_{H} 5.40 (d, J = 8.1 Hz, δ_{C} 95.2), 5.06 (d, J = 1.8 Hz, δ_{C} 102.5) and 4.44 (d, J = 7.9 Hz, δ_{C} 106.5) that led to the identification of β -D-glucopyranose, α -L-rhamnopyranose and β -D-glucuronopyranose^{3,11} (Figure S5). The only difference with zebirioside B was the signal of a second methoxy group at δ_{H} 3.77 (δ_{C} 53.4) that showed a ROESY correlation with the glucuronopyranose anomeric proton, suggesting a methylation of the carboxyl group of this sugar unit. This was confirmed by the presence in the ESI-MS-MS spectrum of a fragment ion at m/z 377.0 [Rha-GlcA-Me + Na]⁺. We deduced the position of the β -D-glucopyranose on the C-28 carboxyl from the chemical shifts of the anomeric signals at δ_{H} 5.40 and δ_{C} 95.2 as in zebirioside B. The linkage of the two other sugars together and with the genin was determined by ROESY correlations and ¹³C NMR chemical shifts and were identical to those of zebirioside B.^[3] Thus, saponin **2**, zebirioside N, was elucidated as the previously undescribed 3-*O*- α -L-rhamnopyranosyl-(1-3)- β -D-glucuronopyranosyl methyl ester-28-*O*- β -D-glucopyranosyl serjanic acid (Figure 1).

A molecular formula of C₃₇H₅₈O₁₁ was assigned to compound **3** from its pseudomolecular ion at m/z 701.3885 ([M + Na]⁺ calcd for C₃₇H₅₈O₁₁Na, 701.3877) in the positive HR-ESI-MS spectrum (Figure S9). The ¹H NMR spectrum of compound **3** showed genin resonances similar to those of zebiriosides J-L.^[3] We observed the olefinic proton H-12 at δ_{H} 5.32 (t, J = 3.4 Hz), proton H-18 at δ_{H} 2.68 (dd, J = 13.5, 3.8 Hz), a methoxy group at δ_{H} 3.70 and five methyl groups instead of six in compounds **1** and **2** at δ_{H} 0.74 (Me-26), 0.77 (Me-24), 0.99 (Me-25), 1.17 (Me-29) and 1.19 (Me-27). The singlet corresponding to the methyl at C-23 in

serjanic acid was absent and C-24 was shielded due to environment change. An additional methylene at δ_{H} 3.50 (d, $J = 10.3$ Hz) and 3.29 (d, $J = 10.3$ Hz), and δ_{C} 65.0 indicated the presence of a hydroxyl group at C-23. Thus, the genin of this compound is phytolaccinic acid as in zebiriosides J-L.^[3, 7] We also observed a sugar unit with anomeric protons at δ_{H} 5.40 ppm (d, $J = 8.1$ Hz) and δ_{C} 95.3 (Figure S8). The analysis of ^1H and ^{13}C data from the HSQC spectrum allowed us to assign the complete spin system of a β -D-glucopyranose linked by an ester linkage (δ_{C} 95.3) to the carboxyl group at position 28 (Table 1).^[11] Consequently, the structure of saponin **3**, zebirioside O, was found to be 28- β -D-glucopyranosyl phytolaccinic acid (Figure 1).

The positive HR-ESI-MS study of compound **4** revealed a pseudomolecular ion at m/z 875.4415 ($[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{44}\text{H}_{68}\text{O}_{16}\text{Na}$, 875.4405) corresponding to a molecular formula of $\text{C}_{44}\text{H}_{68}\text{O}_{16}$ (Figure S13). These data indicated additional 14 uma when compared to zebirioside J and suggested a methyl instead of a proton in compound **4**. The fragment ions at m/z 377.0 [$\text{Rha-GlcA-Me} + \text{Na}]^+$ and 231.1 [$\text{GlcA-Me} + \text{Na}]^+$ suggested that this methyl was located as an ester on the carboxylic function of the glucuronic acid as in compound **2**. The ^1H and ^{13}C NMR spectra of compound **4** showed similarity with zebirioside J.^[3] We observed signals for the same genin, phytolaccinic acid, two sugar units identified as α -L-rhamnopyranose (δ_{H} 5.08 (d, $J = 1.8$ Hz), δ_{C} 102.6) and β -D-glucuronopyranose (δ_{H} 4.48 (d, $J = 7.8$ Hz), δ_{C} 105.5). The only difference laid in the presence of a supplementary signal corresponding to a methoxy group at δ_{H} 3.79 (δ_{C} 60.2) in **4** (Figure S10). The observed ROESY (Figure S11) correlations (H-3/H-1' (GlcA), H-1'' (Rha)-H-3' (GlcA)) allowed us to determine compound **4** as 3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 3)- β -D-glucuronopyranosyl methyl ester-phytolaccinic acid (Figure 1), named zebirioside P.

The positive ion mode HR-ESI-MS analysis of compound **5** showed a pseudomolecular ion at m/z 1037.4924 ($[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{50}\text{H}_{78}\text{O}_{21}\text{Na}$, 1037.4933) corresponding to the a molecular formula of $\text{C}_{50}\text{H}_{78}\text{O}_{21}$ (Figure S17). The ^1H , HSQC, and ROESY NMR spectra of the aglycone part of **5** indicated the presence of five methyl groups at δ_{H} 0.77 (s, Me-26, δ_{C} 18.2), 1.02 (s, Me-25, δ_{C} 17.6), 1.08 (s, Me-23, δ_{C} 14.5), 1.18 (s, Me-29, δ_{C} 29.0), and 1.20 (s, Me-27, δ_{C} 26.7), one olefinic proton at δ_{H} 5.32 ($J = 3.7$ Hz, H-12, δ_{C} 124.6), one oxygenated methine protons at δ_{H} 3.32 (m, H-3, δ_{C} 87.2) and the deshielded proton H-18 at δ_{H} 2.71 (δ_{C} 44.1), in the carbonyl anisotropy cone of C-28. The singlet corresponding to the methyl signal of C-24 was absent and C-23 was shielded due to environment change. Two additional

methylenes signals at δ_{H} 3.85 (d, $J = 10.5$ Hz) and 3.33 (d, $J = 10.5$ Hz), and δ_{C} 79.8 indicated the presence of an hydroxyl group on the C-24 (Table 1). A methoxy group was also observed at δ_{H} 3.70 (δ_{C} 53.2) as in serjanic and phytolaccinic acids (Figures S14-S16).^[3] These data, combined with observed 2D correlations, allowed us to identify a new genin as the 3 β ,24-dihydroxy-olean-12-en-28,30-dioic acid, 30-methyl ester or 24-hydroxy-serjanic acid, named by us dendrobangionic acid. The ^1H NMR spectra revealed also the presence of three sugar units with anomeric protons at δ_{H} 5.40 (d, $J = 8.2$ Hz), 4.85 (d, $J = 1.9$ Hz) and 4.64 (d, $J = 5.3$ Hz) bound to the corresponding carbons at δ_{C} 95.2, 104.7, and 103.9 (Table 2), suggesting three osidic units as in zebirioside C.³ A second methoxy group was observed (δ_{H} 3.74, δ_{C} 53.4) having a ROESY correlation with the anomeric proton of the glucuronic acid which indicated that the acid function of this sugar was esterified. Analysis of ROESY correlations allowed us to completely assign the spin systems of β -D-glucopyranose, α -L-rhamnopyranose and β -D-glucuronopyranose units as in zebirioside C and compound 2.^[3, 6] In addition, the ROE correlations between H-3/H-1' (GlcA), H-1'' (Rha)-H-3' allowed us to determine the structure of this molecule as being 3-O- α -L-rhamnopyranosyl-(1 \rightarrow 3)- β -D-glucuronopyranosyl methyl ester-dendrobangionic acid-28-O- β -D-glucopyranosyl ester, named zebirioside Q (5).

In conclusion, the use of a hyphenated LC-SPE-NMR system for the analysis of the hydromethanolic extract of the roots of *Dendrobangia boliviana* lead us to identify eighteen compounds in its saponin enriched fractions. Two new compounds, zebirioside M (1) and zebirioside Q (5) were isolated from fraction DB-8. Four known saponins and zebirioside M (1) were isolated from fraction DB-9. Three new saponins, zebirioside O (3), zebirioside P (4), and 3-O- β -D-glucuronopyranosylphytolaccagenic acid were isolated along with six known compounds from fraction DB-10. From fraction DB-11 were isolated zebirioside N (2) and six known compounds. Fraction DB-12 contained two known compounds. Their structure were established by ^1H -NMR, HSQC, and ROESY NMR spectra with the support of HR-ESI-MS and ESI-MS-MS data. ^1H and HSQC NMR spectra were enough discriminant to identify this class of compounds by the assignment of their characteristic signals (δ_{H} and δ_{C} of genin methyl groups and δ_{C} of sugar). In addition ROESY spectra was used to determine the stereochemistry of the genin and the nature of the interglycosidic linkages. Seventeen of these oleanane saponins are glycosides of serjanic or phytolaccinic acids and zebirioside Q (5) was a glycoside of a new genin, named dendrobangionic acid. This study provided a way to identify five new saponins (1-5), named zebiriosides M-Q, with twelve known saponins,

previously identified from the roots of *D. boliviana* by classical chromatographic technic, and 3-*O*- β -D-glucuronopyranosylphytolaccagenic acid, previously identified from *Diplocisia glaucescens*. However, two minor saponins (zebiriosides H and I), already identified in *D. boliviana* and containing five sugars units,^[3] were not recovered by LC-SPE-NMR. This was explained by the difficulty of trapping the minor peaks but also to chromatographic resolution problems. By comparison with the standard phytochemical tools, LC-SPE-NMR allowed us to save a considerable amount of time by optimizing the fractionation and purification steps. Therefore, we forecast that this strategy will be increasingly involved in our research group for the discovery of new natural products.

EXPERIMENTAL SECTION

General Experimental Procedures

All NMR experiments were performed on a Bruker Avance AVIII-600 NMR spectrometer equipped with a 5 mm TCI cryoprobe using the Bruker TopSpin 3.2 software (Rheinstetten, Germany). Static field gradient pulses were generated by a 10 A amplifier, so that the sample is submitted to a nominal 0.613 T m⁻¹ gradient. Temperature control was performed using a Bruker variable temperature (BVT) unit in combination with a Bruker cooling unit (BCU-05) to provide chilled air.

HR-ESI-MS and ESI-MS-MS experiments were performed using a Micromass Q-TOF micro-instrument (Manchester, UK) with an electrospray source (eV = 60 V, 80°C). The samples were introduced by direct infusion in a solution of MeOH at a rate of 5 μ L/min.

HPFC was performed on a Grace® Reveleris System using Grace® cartridges (RP-C₁₈, 40 g) at a flow rate of 40 mL/min. Elution was monitored by UV absorption measurement at 205 and 210 nm.

The LC part of the LC-SPE-NMR equipment consisted of an Agilent 1260 chromatographic chain made of a G1329B auto sampler, a G1311B quaternary pump with integrated degasser, a G1330B thermostated column compartment, and a G1315D photodiode-array detector. Separation was achieved using a silica-based ProntoSIL C₁₈ column (125 mm x 4.0 mm x 5 μ m) (Bischoff Chromatography, Leonberg, Germany). The HPLC-UV was connected to a Spark Prospekt 2 solid-phase extraction (SPE) device (Spark Holland, Emmen, The Netherlands) containing HySphere General Phase resin cartridges (polydivinylbenzene

material, 10 × 2 mm i.d., particule size 5-15 µm) to capture and collect the compounds. After the UV detector, water was added by a Knauer K-120 HPLC pump (Knauer K 120, Berlin, Germany) in order to decrease the organic solvent proportion of the eluent and to increase the retention of the separated compounds in the SPE cartridges. Each cartridge was cleaned with 500 µl of acetonitrile and equilibrated with 500 µl of water before use. The system was controlled by the HyStar 3.2 software (Bruker Biospin, Rheinstetten, Germany).

HPLC separations were carried out at a flow rate of 1.0 ml.min⁻¹. A binary gradient of H₂O with 0.1 % trifluoroacetic acid (TFA) (solvent A) and MeCN (solvent B) was applied.

Plant material

Dendrobangia boliviana roots were collected and identified in Iquitos, Loreto district, Peru, by C. Amasifusen, E. Rengifo and M. Haddad, in September 2011. A voucher specimen (No. CA3240) was prepared and deposited at the national Herbarium of the National University San Marcos in Lima, Peru (UNMSM).

Extraction and isolation

The powdered dried roots of *D. boliviana* (245 g) were extracted twice by maceration in MeOH/H₂O (8/2 v/v, 4 L) at room temperature for 24 h. After filtration and evaporation to dryness under reduced pressure, the crude MeOH extract (29.5 g) solubilized in H₂O and passed through an IRN77 (H⁺) Amberlite resin column (activation with HCl 1 N/H₂O 30/60 and elution with H₂O 100%) to give a protonated saponins fraction. This extract (1.4 g) was fractionated by HPFC on RP-18 (40 g cartridges) using a binary gradient of MeOH/H₂O (20/8 for 10 min, 6/4 for 10 min, 4/6 for 10 min, 2/8 for 10 min and 10/0 for 10 min) to give 13 fractions (DB-1 to DB-13).

LC-SPE-NMR Analysis

Fractions DB-8 to DB-12 were analyzed by LC-SPE-NMR on RP-18 column Gradient 1 (0 min: 10% B, 20 min: 70% B, 21 min: 100% B, 23 min: 100% B, 24.5 min: 10% B) was used for fractions DB-8, 10, 11 and 12. For the fraction DB-9, the following gradient elution profile was used: 0 min: 10% B, 10 min: 30% B, 15 min: 45% B, 18 min: 70% B, 18.1 min: 100% B, 19 min: 100% B, 19.5 min: 10% B. After UV detection, majority and minority peaks from each fraction were trapped on GP SPE cartridges. All loaded cartridges were dried in a stream of nitrogen for 30 min to remove protonated solvent residues. The adsorbed

compounds were transferred into 5 mm NMR tubes by injection of 550 μ l of MeCN- d_3 (99.8% D, Eurisotop) in the SPE cartridges.

LC-SPE-NMR Purification and identification

From the fraction DB-8 were isolated zebirioside M (**1**) and zebirioside Q (**5**) with retention times (t_R) of 10.50 and 10.87 min, respectively. From the fraction DB-9 were trapped five peaks at t_R (in min) 13.76 zebirioside M (**1**), 14.09 zebirioside B, 14.18 zebirioside K, 15.45 zebirioside L and 15.75 zebirioside J. From fraction DB-10 purified compounds are: zebirioside B (t_R 11.36 min), talunòmocide I (t_R 11.68 min), zebirioside E (t_R 11.70 min), zebirioside L (t_R 12.18 min), zebirioside J (t_R 12.51 min), zebirioside O (**3**) (t_R 12.80 min), 3-*O*- β -D-glucuronopyranosylphytolaccagenic acid (t_R 12.80 min), zebirioside F (t_R 12.98 min) and zebirioside P (**4**) (t_R 13.60 min). From fraction DB-11 were isolated zebirioside N (**2**) (t_R 11.39 min), zebirioside F (t_R 11.58 min), zebirioside C (t_R 11.74 min), zebirioside G (t_R 11.88 min), zebirioside D (t_R 12.09 min), zebirioside J (t_R 12.32 min) and zebirioside A (t_R 13.00 min). From fraction DB-12 two compounds were trapped and purified: zebirioside J (t_R 13.13 min) and 3-*O*- β -D-glucuronopyranosyl serjanic acid (t_R 13.73 min).

1D ^1H -NMR spectra were acquired with presaturation in order to eliminate the residual HDO signal and with WET solvent suppression to suppress the residual signal of CHD_2CN . Additionally, the pulse sequence used decoupling for the elimination of the ^{13}C satellites. The 1D spectra were recorded using relaxation delay $d_1=5\text{s}$, 32 K data points and 90° pulses of 7.7 μs at 8.3 W, SW = 14 ppm, SI = 32768.

No filtering was used prior to the Fourier transform. Spectra were calibrated so that the proton signal of residual acetonitrile- d_2 appeared at δ 1.94 ppm.

The 2D J-modulated HSQC spectra were recorded by means of the HSQCETGPIWT Bruker pulse sequence using the following parameters: relaxation delay $d_1 = 2\text{ s}$; coupling constant $^1J(^1\text{H}-^{13}\text{C}) = 145\text{ Hz}$ for $d_4 = 1.7\text{ ms}$; 90° pulse of 8.8 μs at 8.3 W for ^1H and of 13 μs at 99.6 W for ^{13}C ; GARP pulse decoupling of 55 μs at 5.5 W with gradient ratio GPZ1:GPZ2:GPZ21:GPZ22:GPZ23:GPZ24 = 80:20.1:80:40:20:10; spectral width 8.0 ppm in F2 and 160 ppm in F1; number of scans 24. Solvent suppression was carried out by WET using a selective excitation shape pulse to eliminate the residual of CD_3CN . A total of 1024 data points in f2 and 512 data points in f1 were recorded; apodization with pure cosine-bell in both dimensions; zero-filling with linear prediction up to 1K.

2D ROESY spectra were acquired using presaturation with shaped pulses to suppress the resonance of HDO. ROESY spectra were performed with: relaxation delay $d1 = 1$ s; 90° pulse of $7.7 \mu\text{s}$ at 8.3 W; number of scans 24; ROESY spin lock pulse of 500 ms at 0.060 W; 1K data points in $t2$; spectral width 8.0 ppm in both dimensions; 512 experiments in $t1$; apodization with squared cosine-bell in both dimensions; zero-filling up to 1K and 4K respectively in $t1$ and $t2$.

Mass data of compounds 1 to 5

3-O- β -D-glucopyranosyl-(1 \rightarrow 2)-[α -L-rhamnopyranosyl-(1 \rightarrow 3)]- β -D-glucuronopyranosyl-28-O- β -D-glucopyranosyl serjanic acid, zebirioside M (1)

HR-ESI-MS (positive-ion mode) m/z 1169.5348 $[\text{M} + \text{Na}]^+$ (calculated for $\text{C}_{55}\text{H}_{86}\text{O}_{22}\text{Na}$, 1169.5356). ESI-MS-MS: MS^1 (1170): m/z 1007.8 $[\text{M} + \text{Na} - \text{Glc}]^+$; 685.6 $[\text{M} + \text{Na} - \text{trisaccharidic chain}]^+$; 523.5 $[\text{serjanic acid} + \text{Na}]^+$; 507.3 $[\text{Rha}(\text{Glc})\text{-GlcA} + \text{Na}]^+$; 361.2 $[\text{Glc-GlcA} + \text{Na}]^+$; 343.2 $[\text{Rha-GlcA} + \text{Na}]^+$.

3-O- α -L-rhamnopyranosyl-(1 \rightarrow 3)- β -D-glucuronopyranosyl methyl ester-28-O- β -D-glucopyranosyl serjanic acid, zebirioside N (2)

HR-ESI-MS (positive-ion mode) m/z 1021.4992 $[\text{M} + \text{Na}]^+$ (calculated for $\text{C}_{50}\text{H}_{78}\text{O}_{20}\text{Na}$, 1021.4984); ESI-MS-MS: MS^1 (1021): m/z 859.2 $[\text{M} + \text{Na} - \text{Glc}]^+$, 377.0 $[\text{Rha-GlcA-Me} + \text{Na}]^+$.

28-O- β -D-glucopyranosyl phytolaccinic acid, zebirioside O (3)

HR-ESI-MS (positive-ion mode) m/z 701.3885 $[\text{M} + \text{Na}]^+$ (calculated for $\text{C}_{37}\text{H}_{58}\text{O}_{11}\text{Na}$, 701.3877); ESI-MS-MS: MS^1 (702): m/z 539.4 $[\text{phytolaccinic acid} + \text{Na}]^+$, 495.4 $[\text{phytolaccinic acid} + \text{Na} - \text{CO}_2]$, 185.1 $[\text{Glc} + \text{Na}]^+$.

3-O- α -L-rhamnopyranosyl-(1 \rightarrow 3)- β -D-glucuronopyranosyl methyl ester-28-O- β -D-glucopyranosyl phytolaccinic acid, zebirioside P (4)

HR-ESI-MS (positive-ion mode) m/z 875.4415 $[\text{M} + \text{Na}]^+$ (calculated for $\text{C}_{44}\text{H}_{68}\text{O}_{16}\text{Na}$, 875.4405); ESI-MS-MS: MS^1 (875): m/z 729.5 $[\text{GlcA-Me phytolaccinic acid} + \text{Na}]^+$, 377.0 $[\text{Rha-GlcA-Me} + \text{Na}]^+$, 231.1 $[\text{GlcA-Me} + \text{Na}]^+$.

3-O- α -L-rhamnopyranosyl-(1 \rightarrow 3)- β -D-glucuronopyranosyl methyl ester-dendrobangionic acid-28-O- β -D-glucopyranosyl ester, zebirioside Q (5)

HR-ESI-MS (positive-ion mode) m/z 1037.4924 $[M + Na]^+$ (calculated for $C_{50}H_{78}O_{21}Na$, 1037.4933). ESIMS-MS: MS^1 (1037): m/z 875.7 $[M + Na - Glc]^+$; 729.5 $[M + Na - Glc - Rha]^+$.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

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13 **FIGURE CAPTIONS**
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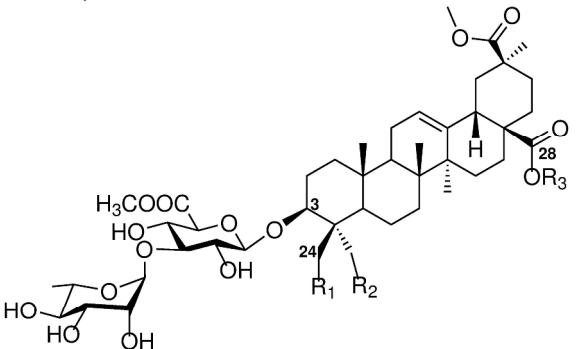
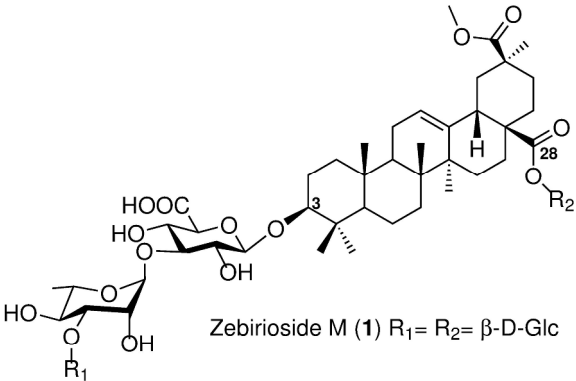
15 **Figure 1:** Structure of compounds **1-5**
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Table 1. ¹H (600 MHz) and ¹³C (151 MHz) NMR spectral data (δ in ppm) of aglycone parts of saponins 1-5 (MeOD)

Atoms No.	1		2		3		4		5	
	δC	δH (J in Hz)	δC	δH (J in Hz)	δC	δH (J in Hz)	δC	δH (J in Hz)	δC	δH (J in Hz)
1	40.0	0.99, m	39.7	1.00, dt (11.4-2.7)	39.4	0.98, m	39.5	1.00, m	39.9	0.91, dm (10.6)
		1.65, dt (11.7-3.5)		1.62, m		1.60, dt (11.7-3.5)		1.68, m		1.63, m
2	27.3	1.74, m	27.1	1.70, m	26.3	1.72, m	26.3	1.81, m	27.0	1.74, m
		1.89, m		1.87, m		1.86, m		1.93, m		1.87, m
3	91.3	3.16, dd (11.5-4.3)	90.8	3.17, dd (11.4-4.3)	87.8	3.28, dd (9.1-5.2)	83.9	3.65, dd (11.9-4.5)	87.2	3.32, m
4	ND		ND		ND		ND		ND	
5	56.8	0.81, dt (11.7-1.6)	56.7	0.83, dt (11.5-1.8)	48.1	1.24, m	48.5	1.19, m	52.8	0.91, dt (11.7-1.6)
6	19.3	1.41, m	19.4	1.43, td (13.6-3.7)	19.4	1.37, m	19.1	1.39, m	19.7	1.32, m
		1.53, m		1.56, m		1.51, m		1.52, m		1.43, td (13.6-3.5)
7	34.3	1.31, m	33.9	1.29, m	33.4	1.30, m	33.5	1.33, m	33.7	1.27, dt (11.7-1.6)
		1.54, td (13.6-3.9)		1.53, td (13.3-3.7)		1.54, m		1.66, m		1.50, m
8	ND		ND		ND		ND		ND	
9	48.9	1.61, t (8.9)	48.8	1.62, t (9.2)	47.0	1.63, m	48.9	1.68, m	49.4	1.70, m
10	ND		ND		ND		ND		ND	
11	24.7	1.93, dd (9.7-3.7)	24.5	1.93, dd (9.2-3.2)	24.7	1.91, m	24.8	1.96, m	24.8	1.95, m
12	124.2	5.31, t (3.7)	124.1	5.32, t (3.6)	124.1	5.32, t (3.4)	124.0	5.31, t (3.6)	124.6	5.32, t (3.7)
13	ND		ND		ND		ND		ND	
14	ND		ND		ND		ND		ND	
15	28.9	1.09, m	29.0	1.14, dt (14.8-4.0)	29.1	1.10, m	29.0	1.15, m	29.6	1.14, m
		1.78, m		1.78, m		1.75, m		1.75, m		1.75, m
16	24.7	1.67, m	24.0	1.68, td (14.3-2.9)	24.4	1.59, m	24.4	1.66, m	24.5	1.78, m
		2.02, m		2.08, td (14.3-5.6)		2.00, m		2.05, m		2.05, m
17	ND		ND		ND		ND		ND	
18	43.9	2.71, dd (13.7-3.8)	43.9	2.71, dd (13.5-3.4)	43.6	2.68, dd (13.5-3.8)	44.0	2.70, dm (13.8)	44.1	2.71, dd (13.7-3.8)
19	43.4	1.66, m	43.2	1.70, m	43.1	1.65, m	43.1	1.73, m	43.5	1.69, m
		1.95, m		1.93, m		1.87, m		1.92, m		1.95, m
20	ND		ND		ND		ND		ND	
21	31.3	1.37, m	31.3	1.43, m	31.1	1.38, m	31.4	1.43, td (13.7-4.2)	31.3	1.43, m
		2.00, m		2.01, m		1.95, m		2.00, m		1.93, m
22	35.4	1.57, m	34.4	1.55, m	34.7	1.58, m	35.0	1.66, m	35.1	1.54, m
		1.60, td (13.7-3.8)		1.71, m						1.70, td (13.7-3.8)
23	28.5	1.06, s	28.2	1.05, s	65.0	3.29, m	65.4	3.34, d (11.5)	14.5	1.08, s
						3.50, d (10.3)		3.53, d (11.5)		
24	17.2	0.86, s	17.2	0.86, s	17.8	0.77, s	13.5	0.75, s	79.8	3.33, d (10.5) 3.85, d (10.5)
25	16.3	0.97, s	16.3	0.97, s	16.7	0.99, s	16.7	1.02, s	17.6	1.02, s
26	17.9	0.77, s	17.9	0.77, s	13.7	0.74, s	18.1	0.82, s	18.2	0.77, s
27	26.5	1.19, s	26.3	1.20, s	26.3	1.19, s	26.6	1.22, s	26.7	1.20, s
28	ND		ND		ND		ND		ND	
29	28.7	1.17, s	28.6	1.18, s	28.8	1.17, s	28.8	1.19, s	29.0	1.18, s
30	ND		ND		ND		ND		ND	
31	52.7	3.70, s	52.8	3.70, s	52.8	3.70, s	52.7	3.71, s	53.2	3.70, s

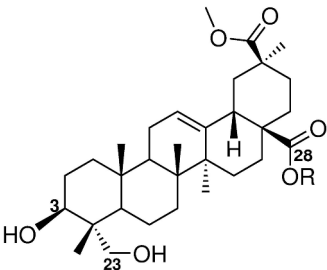
Table 2. ^1H (600 MHz) and ^{13}C (151 MHz) NMR spectral data (δ in ppm) of osidic parts of saponins **1-5** (MeOD)

	1		2		3		4		5	
3-O-	δC , Type	δH (J in Hz)	δC , Type	δH (J in Hz)	δC , Type	δH (J in Hz)	δC , Type	δH (J in Hz)	δC , Type	δH (J in Hz)
β -D-GlcA										
1'	104.8, CH	4.56, d (8.1)	106.5, CH	4.44, d (7.9)			105.5, CH	4.48, d (7.8)	103.9, CH	4.64, d (5.3)
2'	78.3, CH	3.71, dd (9.3, 7.3)	75.4, CH	3.33, dd (9.1, 7.9)			74.1, CH	3.33, dd (8.9, 7.4)	74.4, CH	3.32, m
3'	87.6, CH	3.67, dd (9.3, 6.3)	84.1, CH	3.76, t (9.0)			83.9, CH	3.53, t (8.9)	74.7, CH	3.76, m
4'	72.2, CH	3.61, t (9.3)	71.9, CH	3.59, t (9.4)			71.8, CH	3.60, m	71.2, CH	3.78, dd (9.5, 6.3)
5'	75.4, CH	3.85, d (9.3)	75.4, CH	3.87, d (9.6)			76.5, CH	3.89, d (9.7)	73.9, CH	4.24, d (6.3)
6'	nd, C		nd, C				nd, C		nd, C	
COOMe			53.4, CH ₃	3.77, s			60.2, CH ₃	3.79, s	53.4, CH ₃	3.74, s
α -L-Rha										
1''	103.4, CH	4.97, d (1.8)	102.5, CH	5.06, d (1.8)			102.6, CH	5.08, d (1.8)	104.7, CH	4.85, d (1.9)
2''	71.9, CH	4.06, dd (3.3, 1.9)	72.2, CH	3.89, dd (3.4, 1.8)			72.5, CH	3.91, dd (3.4, 1.8)	81.4, CH	3.96, dd (4.2, 2.9)
3''	71.9, CH	3.66, dd (6.7, 2.9)	72.3, CH	3.62, dd (9.4, 3.4)			72.4, CH	3.64, dd (9.2, 3.4)	72.8, CH	3.86, dd (5.4, 3.4)
4''	78.1, CH	3.37, dd (9.3, 7.1)	73.9, CH	3.32, t (9.4)			75.5, CH	3.34, m	73.0, CH	3.64, t (9.3, 5.4)
5''	70.9, CH	3.84, dq (9.3, 6.0)	70.1, CH	3.90, dq (9.4, 6.2)			70.1, CH	3.92, dq (9.5, 6.1)	75.7, CH	3.87, dq (9.8, 6.2)
6''	18.3, CH ₃	1.25, d (6.1)	18.4, CH ₃	1.22, t (6.2)			18.5, CH ₃	1.24, t (6.1)	18.8, CH ₃	1.25, d (6.2)
β -D-Glc										
1'''	103.8, CH	4.62, d (7.7)								
2'''	75.7, CH	3.13, dd (9.3, 7.7)								
3'''	77.5, CH	3.28, t (9.3)								
4'''	72.5, CH	3.21, dd (9.3, 8.9)								
5'''	78.3, CH	3.32, m								
6'''	63.6, CH ₂	3.59, dd (11.5, 2.9) 3.78, dd (11.5, 3.2)								
28-O- β -D-Glc										
1 ^{IV}	95.3, CH	5.39, d (8.1)	95.2, CH	5.40, d (8.1)	95.3, CH	5.40, d (8.1)			95.2, CH	5.40, d (8.2)
2 ^{IV}	73.8, CH	3.27, dd (9.6, 8.1)	74.0, CH	3.27, dd (8.8, 8.1)	74.2, CH	3.32, dd (8.9, 8.1)			74.5, CH	3.27, dd (9.0, 8.2)
3 ^{IV}	77.8, CH	3.36, m	78.1, CH	3.37, t (8.8)	77.5, CH	3.35, t (8.9)			78.6, CH	3.37, t (8.9)
4 ^{IV}	71.4, CH	3.30, t (8.6)	71.3, CH	3.31, t (9.3)	70.0, CH	3.30, m			71.9, CH	3.30, t (9.3)
5 ^{IV}	78.3, CH	3.32, m	78.3, CH	3.34, m	77.8, CH	3.30, m			78.9, CH	3.34, m
6 ^{IV}	62.8, CH ₂	3.60, dd (11.5, 3.3) 3.72, dd (11.5, 2.7)	62.7, CH ₂	3.60, dd (11.9, 5.1) 3.73, dd (11.9, 2.6)	62.8, CH ₂	3.60, dd (11.9, 4.9) 3.72, dd (11.9, 2.7)			63.4, CH ₂	3.66, dd (11.9, 5.1) 3.73, dd (11.9, 2.6)



Zebirioside P (4) $R_1 = \text{H}, R_2 = \text{OH}, R_3 = \beta\text{-D-Glc}$

Zebirioside Q (5) $R_1 = \text{OH}, R_2 = \text{H}, R_3 = \beta\text{-D-Glc}$



Structure of compounds 1-5

Figure 1

221x460mm (300 x 300 DPI)