SHORT REPORT

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Soulieoside R: A New Cycloartane Triterpenoid Glycoside from *Souliea vaginata*

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Abstract: A new cycloartane triterpenoid glycoside, named soulieoside R, was isolated from the rhizomes of *Souliea vaginata*. Its structure was characterized by comprehensive analyses of ^{1}H, ^{13}C NMR, COSY, HSQC, HMBC, NOESY spectroscopic, and HRESIMS mass spectrometric data, as well as chemical methods. The new compound showed weak inhibitory activity against three human cancer cell lines.

Keywords: *Souliea vaginata*; cycloartane triterpenoid; soulieoside R; cytotoxicity.

1. Plant Source

In the course of phytochemical studies of Chinese herbal medicines, a new cycloartane triterpenoid glycoside was obtained from the rhizomes of *Souliea vaginata* (Figure 1).

The rhizomes of *S. vaginata* were collected in August 2016 from Wen County, Gansu Province, China and identified by Prof. Junshan Yang, Institute of Medicinal Plant Development, Chinese Academy

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of Medical Sciences and Peking Union Medical College. A voucher specimen (No. 20160822SQ) was deposited at the herbarium of the Institute of Medicinal Plant Development.

2. Previous Studies

Souliea vaginata is a perennial herb distributed in southwest and northwest China and is used to treat conjunctivitis, stomatitis, pharyngitis, and enteritis [1]. Previous chemical investigations on this plant have disclosed the presence of cycloartane triterpenoid glycosides as the main bioactive components [2~10].

3. Present Study

The air-dried and powdered rhizomes of *Souliea vaginata* (Maxim.) Franch (1.2 kg) were extracted three times with 95% ethanol (3 × 10 L) at room temperature. Removal of the ethanol under reduced pressure yielded the extract (110 g). The extract was suspended in distilled water and then the suspension was partitioned with EtOAc and *n*-BuOH, successively. The *n*-BuOH soluble fraction (45 g) was subjected to silica gel (100 ~ 200 mesh) column chromatography using a CH_2Cl_2-MeOH gradient (from 50:1 to 1:1) as eluent, to yield seven fractions (Fr. A–G). Fr. B (6.8 g) was applied to a silica gel (200 ~ 300 mesh) column eluting with CH_2Cl_2-MeOH gradient (from 25:1 to 5:1) to afford five fractions (Fr. B1–B5). Fr. B2 (1.2 g) was chromatographed over silica gel (300 ~ 400 mesh) eluting with CH_2Cl_2-MeOH (15:1-2:1) to obtain four sub-fractions, Fr. B2A~D. Sub-fraction B2C was separated by preparative thin layer chromatography using CH_2Cl_2-MeOH-Formic Acid (9:1:0.1) as mobile phase to yield **1** (3.9 mg).

*Soulieoside R (**1**):* White amorphous solid; $[\alpha]_D^{20}$ −10 (*c* 0.13, MeOH); IR ν_{max} (MeOH): = 3413, 2968, 2935, 1654, 1459, 1384, 1045 cm^{-1}; ^1H NMR (600 MHz, pyridine-d_5): δ (ppm) = 1.22 (1H, m, H-1α), 1.54 (1H, m, H-1β), 1.95 (1H, m, H-2α), 2.31 (1H, m, H-2β), 3.44 (1H, dd, *J* = 11.4, 4.2 Hz, H-3), 1.54 (1H, m, H-5), 1.22 (1H, m, H-6α), 1.96 (1H, m, H-6β), 3.78 (1H, m, H-7), 2.07 (1H, d, *J* = 9.0 Hz, H-8), 1.26 (1H, m, H-11α), 1.92 (1H, m, H-11β), 1.70 (1H, m, H-12α), 1.82 (1H, m, H-12β), 2.40 (1H, dd, *J* = 13.8, 5.4 Hz, H-15α), 2.76 (1H, dd, *J* =13.8, 7.8 Hz, H-15β), 4.87 (1H, d, *J* = 7.8 Hz, H-16), 2.21 (1H, d, *J* = 7.8 Hz, H-17), 1.76 (3H, s, CH_3-18), 0.24 (1H, d, *J* = 4.2 Hz, H-19α), 0.78 (1H, d, *J* = 4.2 Hz, H-19β), 1.36 (3H, s, CH_3-21), 1.68 (1H, m, H-22α), 2.53 (1H, m, H-22β), 1.89 (1H, m, H-23α), 2.25 (1H, m, H-23β), 3.95 (1H, t, *J* = 7.2 Hz, H-24), 1.50 (3H, s, CH_3-26), 1.28 (3H, s, CH_3-27), 1.26 (3H, s, CH_3-28), 1.24 (3H, s, CH_3-29), 1.11 (3H, s, CH_3-30), 4.84 (1H, d, *J* = 7.8 Hz, H-1'), 4.28 (1H, t, *J* = 8.4 Hz, H-2'), 4.18 (1H, m, H-3'), 4.15 (1H, m, H-4'), 3.67 (1H, t, *J* = 10.2 Hz, H-5'a), 4.32 (1H, dd, *J* = 10.2, 5.4 Hz, H-5'b), 6.58 (1H, s, H-1"), 4.87 (1H, br s, H-2"), 4.67 (1H, dd, *J* = 9.0, 3.0 Hz, H-3"), 4.36 (1H, t, *J* = 9.6 Hz, H-4"), 4.80 (1H, m, H-5"), 1.70 (3H, d, *J* = 6.0 Hz, H_3-6"); ^{13}C NMR (150 MHz, pyridine-d_5): δ (ppm) = 15.9 (CH_3, C-29), 19.2 (CH_3, C-6"), 20.4 (C, C-9), 20.6 (CH_3, C-30), 21.2 (CH_3, C-18), 24.8 (CH_2, C-23), 26.0 (CH_3, C-28), 26.9 (CH_3, C-21), 27.3 (C, C-10), 27.3 (CH_3, C-27), 27.4 (CH_2, C-11), 28.6 (CH_3, C-26), 29.3 (CH_2, C-19), 30.4 (CH_2, C-2), 32.4 (CH_2, C-1,6), 34.1 (CH_2, C-12), 38.0 (CH_2, C-22), 41.4 (C, C-4), 47.0 (CH, C-5), 47.2 (C, C-14), 47.7 (C, C-13), 51.4 (CH, C-15), 55.4 (CH, C-8), 56.5 (CH, C-17), 67.4 (CH_2, C-5'), 70.2 (CH, C-5"), 70.8 (CH, C-7,25), 72.0 (CH, C-4'), 73.0 (CH, C-2", 3"), 73.5 (CH, C-16), 74.5 (CH, C-4"), 78.2 (CH, C-2'), 80.2 (CH, C-3'), 85.3 (C, C-24), 87.1 (C, C-20), 88.4 (CH, C-3), 102.4 (CH, C-1"), 106.4 (CH, C-1'); HR-ESI-MS: *m/z* 791.4518 $[M + Na]^+$ (calcd. 791.4558 $[M + Na]^+$ for $C_{41}H_{68}O_{13}Na^+$).

Cytotoxic assays: The cytotoxicity of compound **1** was assessed against HepG2, MCF-7, and HT-29 human cancer cell lines by the MTT method using 5-FU as the positive control. HT-29 cells were grown in RPMI 1640 medium, HepG2 and MCF-7 cells in DMEM, supplemented with 10% v/v FBS and 1% penicillin–streptomycin solution, and were cultured at a density of 2×10^4 cells/ml per well in a 96-well microtiter plate (Corning, Suzhou, China) and incubated overnight at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Then, five different concentrations (6.25, 12.5, 25, 50, 100 μM/ml) of each compound dissolved in dimethyl sulfoxide (DMSO) were added to each well. Each concentration was tested in triplicate. The cells were treated with test samples for 24 h. After removing the supernatant of each well, a total of 10 μL of MTT solution were added to each well at the time of incubation for 4 h. The formazan crystals in each well were dissolved in lysis buffer for overnight at 37 °C. The absorbance at 550

nm was measured by an Infinite M200 Pro spectrophotometer (Tecan, Switzerland). The data are expressed as the percentage of the control optical density (OD) values for each experiment.

The dried and powdered rhizomes of *S. vaginata* were extracted with 95% ethanol. The crude extract obtained after evaporation of the solvent was subjected to conventional purification procedures and resulted in the isolation of one new cycloartane triterpenoid glycoside, soulieoside R (**1**) (Figure 1).

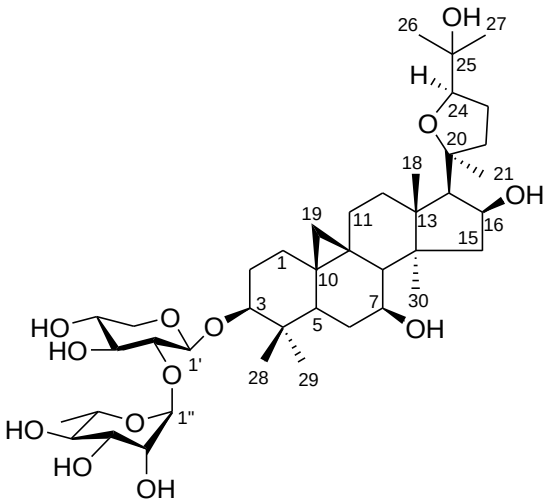

Figure 1. Structure of soulieoside R (**1**) isolated from *S. vaginata.*

Compound **1** was obtained as a white amorphous powder and exhibited a positive Libermann–Burchard reaction. Its molecular formula was deduced as $C_{41}H_{68}O_{13}$ from its pseudo-molecular ion peak at *m/z* 791.4518 $[M + Na]^+$ (calcd for $C_{41}H_{68}O_{13}Na$ 791.4558) in an HR-ESI-MS experiment, consistent with eight degrees of unsaturation. The IR spectrum absorption revealed the presence of hydroxyl (3413 cm^{-1}) groups. The ^1H NMR spectrum (Figure S2) of **1** exhibited the signals of two characteristic cyclopropane protons at δ = 0.24 and 0.78 (1H each, d, J = 4.2 Hz), seven singlet methyl groups at δ 1.11 (H-30), 1.24 (H-29), 1.26 (H-28), 1.28 (H-27), 1.36 (H-21), 1.50 (H-26), and 1.76 (H-18), as well as four oxygenated methine ascribable to H-3 (δ 3.44, dd, J = 11.4, 4.2 Hz), H-7 (δ 3.78, m), H-16 (δ 4.87, d, J = 7.8 Hz), and H-24 (δ 3.95, t, J = 7.2 Hz). In addition, the resonances of two anomeric protons at δ = 4.84 (d, J = 7.2 Hz, H-1') and 6.58 (s, H-1") were observed in the downfield region. The ^{13}C APT NMR spectrum (Figure S3) of **1** displayed 41 carbon resonances including methylene carbon of cyclopropane ring at δ = 29.3 (C-19), four oxygenated methine carbons at δ = 88.4 (C-3), 70.8 (C-7), 73.5 (C-16), and 85.3 (C-24), and two oxyquaternary carbons at δ = 87.1 (C-20) and 70.8 (C-25), and two anomeric carbon at δ = 106.4 (C-1') and 102.4 (C-1"). All carbon-bound protons were assigned based on HSQC and ^1H–^1H correlation spectra. The ^1H and ^{13}C NMR spectroscopic data of **1** confirmed that the compound was a cycloartane triterpene glycoside [11-14] and was similar to cycloalpioside D [15] with the differences that there was an extra sugar in **1**. On the basis of chemical shifts, the multiplicity of the signals and the coupling constants, the two sugar residues were identified as β-xylopyranose and α-rhamnopyranose, respectively [16]. The sugar was identified as xylose and rhamnose by acid hydrolysis followed by comparison with an authentic sample by TLC. The common L-configuration for α-rhamnopyranose and D-configuration for β-xylopyranose were assumed, according to those most often encountered among the plant glycosides in each case. The HMBC experiment (Figure 2 and S6) was performed to establish the location of the functional groups and the full structure of **1**. The key correlation peaks observed in the HMBC spectrum between H-1' of the xylosyl at δ = 4.84 (1H, d, J = 7.2 Hz) and C-3 (δ 88.4) of the aglycon, between H-1" of the rhamnosyl at δ = 6.58 (s) and C-2' (δ 78.2) of the xylose residue allowed the disaccharide chain at C-3 to be determined as α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranoside. The HMBC spectrum of **1** also showed long-range correlations between H-17 and C-16 (δ 73.5), C-20 (δ 87.1); between H-16 and C-20; between H-15 and C-30 (δ 14.5); between H-19 and C-9 and C-10; between H-18 and C-12, C-13, C-14, and C-17; and between C-24 and H-26 and H-27. The stereochemistry of **1** was resolved by analysis of NOESY

spectrum (Figure S7) and ^{1}H–^{1}H coupling constant value (*J*). The coupling constant between H-16 and H-17 was 7.8 Hz, suggesting a *cis*-relationship of the 16-OH group and the side chain, so a 16-*β*OH substituent was substantiated. In the NOESY spectrum, significant cross peaks between H-17/H-16, H-16/H_3-30 and H-24/Me-21 were observed, which enabled the establishment of OH-16*β*, and (20*S**, 24*R**) configurations. The NOESY correlation of H-3/H-5 showed *α*-orientation of H-3. The D-xylopyranose and L-rhamnopyranose were confirmed using GC-MS analysis after acidic hydrolysis of **1** following derivatisation with L-cysteine methyl ester and silylation [17,18]. Therefore, the structure of compound **1** was established as *α*-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranosyl]-20*S**,24*R**-epoxy-cycloartane-3*β*,7*β*,16*β*,25-quadriol and named soulieoside R as illustrated in Figure 1.

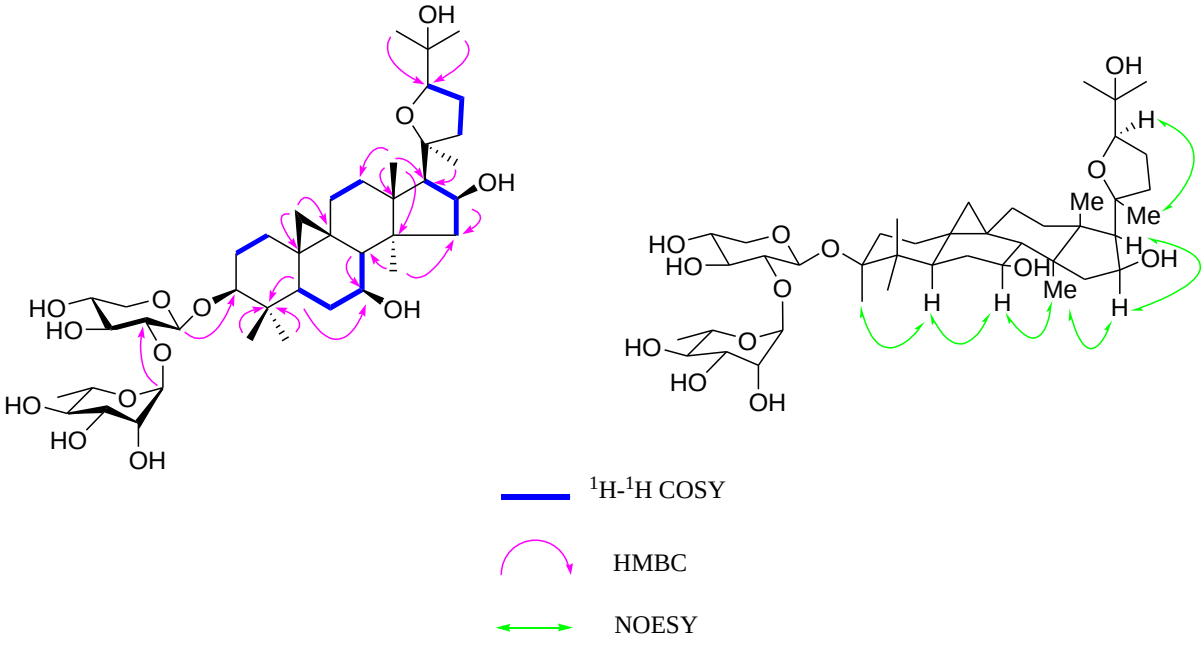

Figure 2. The key ^{1}H-^{1}H COSY, HMBC and NOESY correlations of compound **1**.

Acid hydrolysis of compound **1**

Compound **1** (2 mg) was heated in 2 mL of 2M trifluoroacetic acid at 95°C for 2 h. The reaction mixture was extracted three times with 2 mL of $CHCl_3$. The remaining aqueous layer was concentrated to dryness with EtOH to give a residue, and the residue was dissolved in anhydrous pyridine (2 mL). The sugar was derivatized with L-cysteine methyl ester hydrochloride (3 mg, 60°C, 1 h) and subsequently silylated with hexamethyldisilazane and chlorotrimethylsilane (Fluka) (2:1, 1.5 mL; 60°C, 30 min). Finally, the supernatant (0.5 mL) was analysed by GC-MS (Agilent 7890A/5975C, Agilent Technologies, Santa Clara, CA, USA) under the following conditions: capillary column HP-5 (30 m × 0.25 mm × 0.25 μm); temperature gradient: 150°C for 2 min, then 5°C/min to 210°C; carrier, helium gas (1.0 mL/min); and injection volume: 1.0 μL. The injection and detector temperature were set at 290°C, and the splitting ratio was 1/10. The presence of D-xylose in the acid hydrolysate of **1** was confirmed by comparison of their respective retention times with those of standard sample.

The cytotoxicity of compound **1** was assayed against three human cancer cell lines (HepG2, MCF-7, and HT-29) using the MTT method with 5-FU as the positive control. Compound **1** exhibited weak cytotoxic activity against all the tested cell lines with IC_{50} values > 40 μM.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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