

A New Lignan Glycoside from the Roots of *Silene tatarinowii* Regel

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Abstract: A new lignan glycoside, siletatoside A (**1**), and three known lignans, (+)-isolariciresinol (**2**), balanophonin (**3**), and (+)-lariciresinol (**4**), were isolated from the roots of *Silene tatarinowii* Regel. Compounds **2–4** were isolated from the *Silene* genus for the first time. Their structures were determined based on physicochemical properties and spectroscopic methods. The structure of siletatoside A was elucidated for the first time. The cytotoxicity of **1–4** was evaluated *in vitro* in human HCT116, HT29, A549, and H1299 tumor cell lines. These compounds displayed weak cytotoxicity in the human cancer cell lines.

Keywords: *Silene tatarinowii*; lignan; siletatoside A; cytotoxicity. © 2020 ACG Publications. All rights reserved.

1. Plant Source

Silene tatarinowii Regel was collected in July 2018 from Shaanxi Province, China and authenticated by Prof. Jitao Wang (School of Pharmacy, Shaanxi University of Chinese Medicine, Xianyang, China). A voucher specimen (herbarium No.20180712) was stored in the Medicinal Plants Herbarium, Xianyang, China.

2. Previous Studies

Silene tatarinowii Regel, which belongs to the genus *Silene*, is used for the treatment of dropsy and rheumatism in China [1]. Characteristic components, such as ecdysteroids and triterpenoid saponins, have been isolated from plants of the *Silene* genus [2-3]. Ecdysteroids are present in 115 species of *Silene* and regarded as chemotaxonomic markers [4]. However, few phytochemical investigations on the roots of *S. tatarinowii* Regel have been reported. As part of an ongoing study on biological constituents from the roots of *S. tatarinowii* Regel, a new lignan glycoside and three known lignans, (+)-isolariciresinol (**2**) [5], balanophonin (**3**) [6], and (+)-lariciresinol (**4**) [7], were procured

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(Figure 1). In this study, structure elucidation of the isolated compounds and their cytotoxic activities are reported.

3. Present Study

The dry roots of *S. tatarinowii* Regel (9.8 kg) were ground into a crude powder and extracted three times with 70% EtOH under reflux. After the removal of the solvent under reduced pressure, the concentrated residue was continuously fractionated with petroleum ether and *n*-BuOH. The *n*-BuOH extract (180 g) was fractionated by column chromatography (CC) on silica gel and eluted with CHCl₃-MeOH (30:1 to 1:1) to produce four fractions (Fr. 1–4). A portion of Fr. 2 (26 g) with MeOH (100%) was purified over a Sephadex LH-20 column, eluted with (CHCl₃-MeOH, 1:1), and purified by HPLC (Ultimate XB-C₁₈, 10 mm × 250 mm) with MeOH-H₂O (28%, 3 mL/min) to produce compounds **1** (8.0 mg) and **2** (20.0 mg). Fr. 3 (31.8 g), using MeOH-H₂O (0:10 to 8:2) as the eluent, was also subjected to CC on octadecylsilyl gel to afford compounds **3** (11.2 mg) and **4** (12.6 mg).

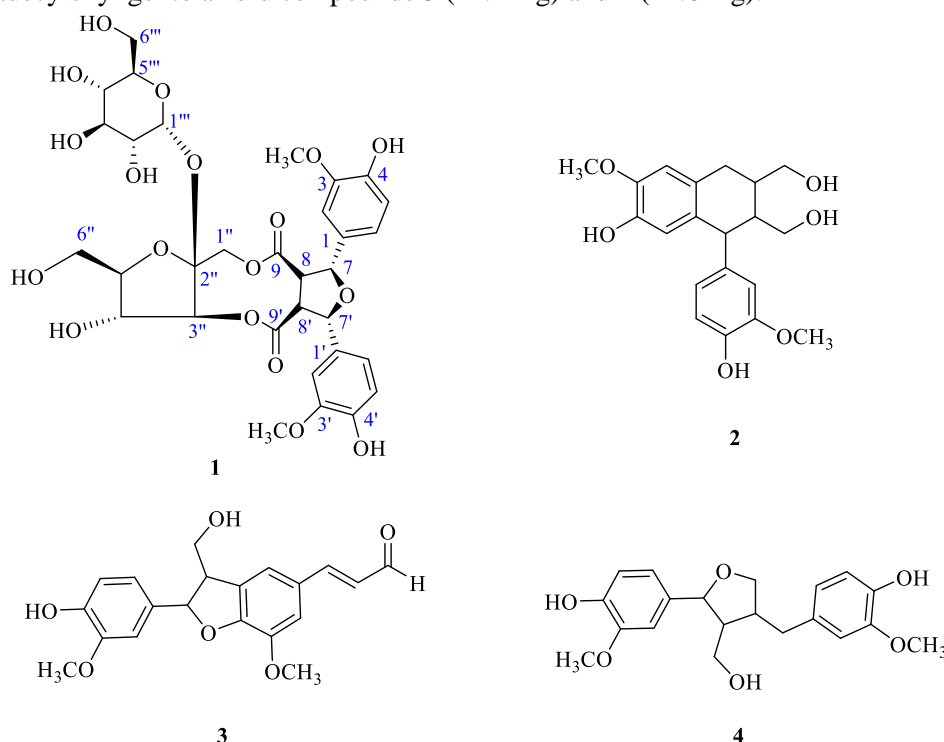


Figure 1. Structures of compounds **1-4**

Siletatoside A (1): yellow amorphous powder (MeOH); $[\alpha]_D^{25} +37.1$ (*c* 0.01, MeOH); IR ν_{\max} (in MeOH) cm^{-1} : 3377, 2940, 1735, 1600, 1450, 1377; UV λ_{\max} (MeOH): 232 nm; HR-ESI-MS at m/z 733.1950 $[\text{M} + \text{Na}]^+$; $^1\text{H-NMR}$ (pyridine-*d*₅, 400 MHz): δ_{H} 7.46 (1H, s, H-2), 7.38 (1H, d, *J* = 8.2 Hz, H-6), 7.31 (1H, d, *J* = 8.2 Hz, H-6'), 7.22 (1H, d, *J* = 7.6 Hz, H-5'), 7.20 (1H, d, *J* = 7.6 Hz, H-5), 6.18 (1H, d, *J* = 3.0 Hz, H-1'''), 5.78 (1H, d, *J* = 5.2 Hz, H-7), 5.72 (1H, d, *J* = 3.7 Hz, H-7'), 5.53 (1H, m, H-3'''), 5.22 (1H, d, *J* = 3.4 Hz, H-5'''), 5.12 (1H, d, *J* = 12.8 Hz, H-1'' α), 5.12 (1H, d, *J* = 12.8 Hz, H-1'' β), 4.92 (1H, m, H-2'''), 4.63 (1H, m, H-4'''), 4.63 (1H, m, H-3'''), 4.56 (1H, m, H-6'' α), 4.48 (1H, m, H-8), 4.48 (1H, m, H-8'), 4.45 (1H, m, H-6'' β), 4.28 (1H, m, H-4'''), 4.19 (1H, m, H-5'''), 3.78 (3H, s, H-10), 3.78 (3H, s, H-10'); $^{13}\text{C-NMR}$ (pyridine-*d*₅, 100 MHz): δ_{C} 174.6 (C-9), 170.7 (C-9'), 149.3 (C-3), 149.1 (C-3'), 149.1 (C-4), 148.8 (C-4'), 131.6 (C-1), 131.3 (C-1'), 121.2 (C-6), 121.1 (C-6'), 117.0 (C-5), 116.9 (C-5'), 112.4 (C-2), 111.9 (C-2'), 110.2 (C-2''), 94.9 (C-1'''), 89.1 (C-4'''), 84.0 (C-7), 82.3 (C-7'), 80.5 (C-3'''), 76.0 (C-3'''), 75.3 (C-2'''), 74.8 (C-5'''), 74.2 (C-5'''), 72.7 (C-4'''), 65.6 (C-6'''), 64.4 (C-1'''), 63.7 (C-6'''), 56.8 (C-8), 56.7 (C-8'), 56.4 (C-10), 56.3 (C-10').

Siletatoside A (**1**) was a yellow amorphous powder that showed maximum UV absorption at 232 nm. Its molecular formula was determined to be $C_{32}H_{38}O_{18}$ based on the high-resolution electrospray ionization mass spectrometry positive ion at m/z 733.1950 $[M + Na]^+$ (calcd for $C_{32}H_{38}O_{18}Na$, 733.1956). In the 1H -nuclear magnetic resonance (NMR) spectrum of **1**, six aromatic proton signals at δ_H 7.46 (s, H-2), 7.20 (d, $J = 7.6$ Hz, H-5), 7.38 (d, $J = 8.2$ Hz, H-6) and 7.48 (s, H-2'), 7.22 (d, $J = 7.6$ Hz, H-5'), and 7.31 (d, $J = 8.2$ Hz, H-6') were assigned to two ABX spin systems, along with two methoxy groups at δ_H 3.78 and 3.79 (each 3H, s). The anomeric proton doublet at δ 6.18 with a coupling constant (J) of 3.0 Hz indicated that **1** was an α -glucosyl [8]. Methine proton signals at δ_H 5.72 (H-7'), 5.78 (H-7), 4.48 (H-8), and 4.48 (H-8') were from the oxycyclopentane ring. The ^{13}C -NMR spectrum displayed 32 carbon signals. A distortionless enhancement of polarization transfer 135° experiment showed that there were eight quaternary carbons. There were two carbonyl signals at δ_C 170.7 and 174.6 in the low field region. There were two quaternary carbon signals at δ_C (131.3 and 131.6), six methine carbon signals at δ_C (112.4, 111.9, 116.9, 117.0, 121.1, and 121.2), and four oxygenated quaternary carbon signals at δ_C (148.8, 149.1, 149.1, and 149.4) owing to the dual sets of ABX systems. Twelve oxygenated carbon signals were ascribable to two glycosyl moieties (δ_C 94.9, 75.3, 76.0, 72.7, 74.2, and 63.7 and δ_C 64.4, 110.2, 80.5, 89.1, 74.8, and 65.6), which indicated the existence of a glucopyranose and a fructofuranose. Four methine carbon signals at δ_C (56.8, 56.7, 82.3, and 84.0) were assigned to an oxycyclopentane ring owing to the effect of electronic absorption of the oxygen atoms. Methine carbon signals C-7 and C-7' shifted to a lower field, as the C-7 and C-7' signals of the cyclobutane ring are usually perceived at δ_C 44.0 and 45.0, respectively [8]. When comparing the NMR data of **1** with a reference compound, 2,5-bis-(4-hydroxy-3-methoxyphenyl)-tetrahydrofuran-3,4-dicarboxylic acid [9], the aglycone of compound **1** exhibited similar spectroscopic features, based on heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond coherence (HMBC).

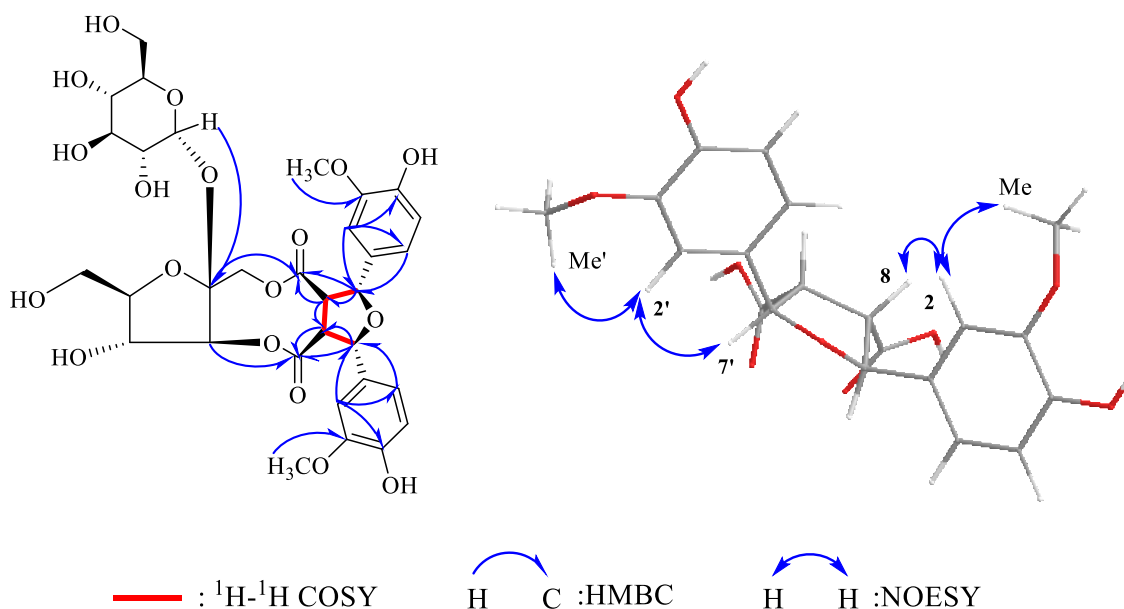


Figure 2. Key 1H - 1H COSY, HMBC and NOESY relevant of compound **1**

In addition, HMBC correlations (Figure 2) of H-2/C-1, C-4, C-6, and C-7; H-2'/C-1', C-4', C-6', and C-7'; H-6/C-4 and C-7; H-6'/C-4' and C-7'; H-7/C-2, C-6, C-8', and C-9; H-7'/C-2', C-6', C-8,

and C-9'; H-8/C-8' and C-9; and H-8'/C-8 and C-9' were observed. Furthermore, the HMBC spectrum correlation signal of H-1''' (δ_{H} 6.18) of the glucopyranosyl moiety and C-2'' (δ_{C} 110.2) of the fructofuranosyl moiety indicated that the sugar groups were connected as [β -D-fructofuranosyl-(2 \rightarrow 1)- α -D-glucopyranose] [10]. HMBC correlations of H-1''/C-9 and H-3''/C-9' suggested that the two carbonyl carbon were attached to the fructofuranosyl moiety. Moreover, the correlations of OMe-3/C-3 and OMe-3'/C-3' showed that the two methoxy groups were situated in C-3 and C-3'. In the nuclear Overhauser effect spectrum (Figure 2) of **1**, the nuclear Overhauser effect correlations observed of H-2/H-8 and OMe-3 and H-2'/H-7' and OMe-3' (Figure 2); thus, the relative configuration of **1** was determined. To determine the absolute configuration, experimental circular dichroism (CD) spectra were compared with predicted calculations from the time-dependent density functional theory, which is a quantum mechanical theory [11]. The calculated electronic CD spectrum for the 7*S*,8*R*,7'*R*,8'*S*-**1** stereoisomer matched and the experimental CD data of **1** are in good agreement (Figure 3) in the proposed structure.

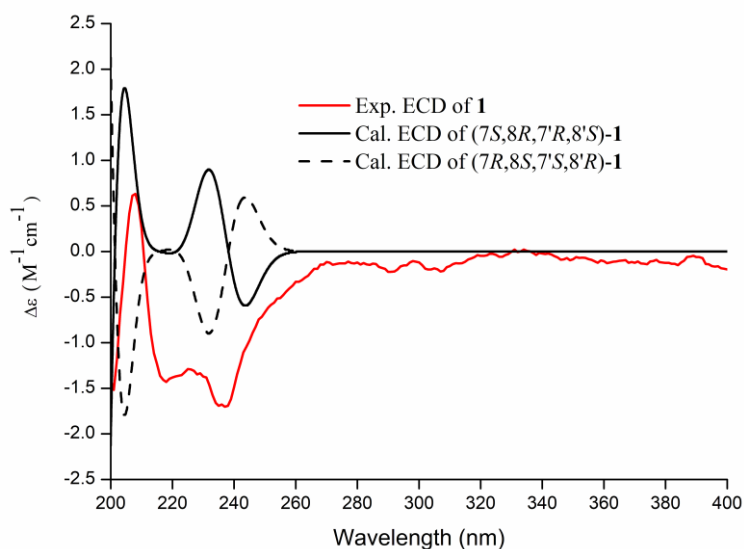


Figure 3. Experimental and calculated ECD spectra of **1**

The experimental ECD spectrum of **1** (red line) and the calculated ECD spectrum of (7*S*,8*R*,7'*R*,8'*S*)-**1** (black line) and (7*R*,8*S*,7'*S*,8'*R*)-**1** (black short dash)

Cytotoxicity data of compounds **1-4** against human HCT116, HT29, A549 and H1299 cell lines, the IC₅₀ values were shown in Table 1.

Table 1. Cytotoxicity of compounds **1-4** against four human cancer cell lines in vitro (IC₅₀, μM)^a

Compounds	Cell lines			
	HCT116	HT29	A549	H1299
5-Fu	2.4 ± 1.9	4.3 ± 2.1	4.0 ± 1.6	3.07 ± 0.52
1	87.3 ± 5.3	99.3 ± 2.4	>100	>100
2	89.3 ± 3.1	>100	>100	>100
3	69.3 ± 3.2	73.1 ± 2.0	>100	>100
4	>100	79.6 ± 6.8	>100	>100

^a IC₅₀ values are means from three independent experiments (average ± SD) in which each compound concentration was tested in three replicate wells; ^b5-fluorouracil (5-Fu) as positive control.

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Supporting Information

Supporting Information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

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