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A New Isoflavonolignan Glycoside from the Roots of *Sophora tonkinensis*

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Abstract: A new isoflavonolignan glycoside, butesuperin B-7''-O-β-glucopyranoside (**1**) along with a known isoflavonolignan butesuperin A (**2**), were isolated from the roots of *Sophora tonkinensis*. The structure of the new compound was elucidated using spectroscopic methods, mainly 1D and 2D NMR. Compound **1** showed week cytotoxicity against BEL-7404 and NCI-H460 cells in MTT assay.

Keywords: *Sophora tonkinensis*; isoflavonolignan; butesuperin B-7''-O-β-glucopyranoside.

1. Introduction

The genus *Sophora* belongs to the family Leguminosae, containing about 21 species, 14 varieties and two forms that are widely distributed in Southwest China, South China and East China [1]. Among them, *Sophora tonkinensis* is an important traditional Chinese medicinal plant, known as Guang-Dou-Gen in Chinese, distributed chiefly in South China. Its roots are commonly used as a traditional Chinese drug to treat acute pharyngolaryngeal infections and sore throats [2]. Although previous phytochemical research on *S. tonkinensis* have revealed that the plant accumulated isoprenyl-substituted flavonoids and lupin alkaloids as its main constituents [3-7], there is no report on the isolation and identification of isoflavonolignans from *S. tonkinensis.*

Isoflavonolignans are a type of benzodioxane lignoids. As characteristic constituents of Leguminosae, all isoflavonolignans have been isolated from Leguminosae family *Butea superba* [8],

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Dipteryx odorata [9], *Xanthocercis zambesiaca* [10], *Pueraria alopecuroides Craib* [11] and *Alhagi pseudalhagi* [12].

In our chemical investigation, a new isoflavonolignan glycoside (**1**) and a known isoflavonolignan (**2**) (Figure 1), were isolated from the roots of *S. tonkinensis* by column chromatograph. Their structures were elucidated using 1D and 2D NMR spectroscopic data. To the best of our knowledge, this is the first report on the existence of an isoflavonolignan glycoside from natural source.

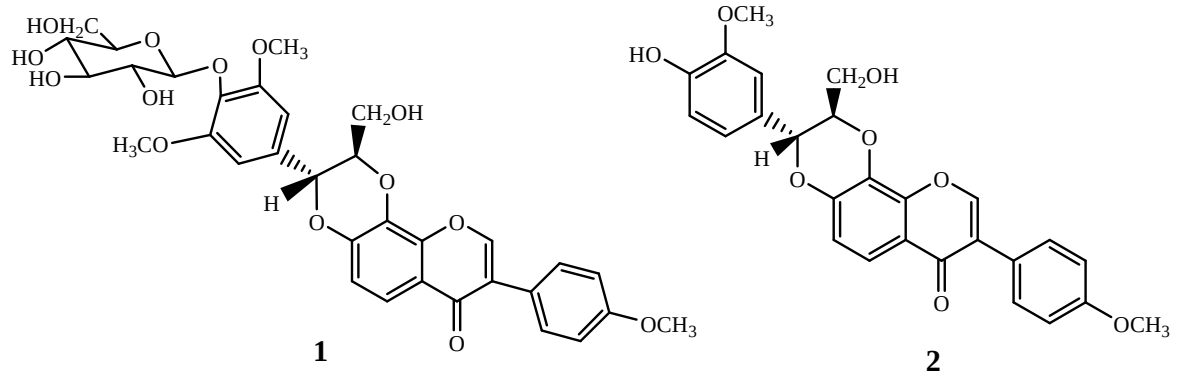

Figure 1. Structures of compounds **1** and **2**.

2. Materials and Methods

2.1. General

Optical rotations were measured using a PE 341LC plus digital polarimeter with a 0.5 dm length cell. HR-ESI-MS spectra were taken on a Bruker Daltonics Apex II mass spectrometer. All NMR spectra were recorded on a Bruker AV-500 spectrometer with tetramethylsilane (TMS) as an internal standard. Samples were dissolved in 0.6 ml DMSO-d_6 and transferred into a 5 mm NMR tube. All chemical shifts are expressed as δ(ppm), and scalar coupling constants are reported in Hz. Melting points were measured on an uncorrected X-4 digital melting point apparatus. Silica gel (100-200, 300-400 mesh, Qingdao Haiyang Chemical Co. Ltd., China), ODS (35-50 μm, Dasio) and MCI gel (CHP 20P, Mitsubishi Chemical) were used for column chromatography. Analytical grade solvents were produced by Shantou Xilong Chemical Factory.

2.2 Plant material

The roots of *S. tonkinensis* were collected in 2007 in Guangxi, China, and were identified by Prof. Ye-Cheng Deng, College of Life Science, Guangxi Normal University. A voucher specimen has been deposited in the Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources, College of Chemistry and Chemical Engineering, Guangxi Normal University.

2.3 Extraction and Isolation

The air-dried and powdered root materials (20.0 kg) were extracted with 95% EtOH at room temperature. Evaporation of EtOH under reduced pressure gave a brown residue (3.0 kg). The residue was later suspended in H_2O, and partitioned with petroleum ether, EtOAc and n-BuOH.

The EtOAc extract (1.0 kg) was applied to a silica gel (100-200 mesh) column with petroleum ether and EtOAc as binary mixtures of increasing polarity afforded 6 fractions (Fr. E1-E6). Fr. E2 (petroleum ether-EtOAc, 1:1) (50.0 g) was isolated on silica gel (300-400 mesh) eluted with petroleum ether-EtOAc step gradients (6:4→4:6) to give 5 subfractions (Fr. E2-1-E2-5). Further purification of Fr. E2-3 (5.2 g) was applied to column chromatography on silica gel with CH_2Cl_2-$(CH_3)_2CO$ (95:5→80:20) to obtain compound **2** (12.0 mg).

The n-BuOH extract (0.3 kg) was subjected to MCI gel column chromatography and eluted with MeOH-H_2O (3:7→5:5→7:3→10:0), successively. The 100% MeOH eluting extract (25.0 g) was separated by column chromatography on silica gel (100-200 mesh) using EtOAc-MeOH gradient (10:0→0:10) as mobile to yield 6 fractions (Fr. W1-W6). Fr. W2 (0.6 g) was rechromatographed on silica gel (300-400 mesh, MeOH-$CHCl_3$, 5:95→20:80) and compound **1** (16.5 mg) was obtained by ODS column chromatography (MeOH-H_2O, 7:3).

Compound **1** (Figure 1), butesuperin B-7''-O-β-glucopyranoside, pale yellow needle-like crystals (MeOH); mp 179-180 °C; $[\alpha]^{25}_D$ -15.0° (c 0.04, MeOH); ^{1}H and ^{13}C NMR data: see Table 1; HR-ESI-MS: *m/z* 655.2026 $[M+H]^+$ (calc. for $[C_{33}H_{34}O_{14}+H]^+$ 655.2021).

3. Results and Discussion

3.1. Structure elucidation

Compound **1** was obtained as pale yellow needle-like crystals with melting point of 179-180 °C. Its molecular formula was determined as $C_{33}H_{34}O_{14}$, on the basis of the HR-ESI-MS (positive) at *m/z* 655.2026 $[M+H]^+$ (calc. 655.2021), which indicated 17 degrees of unsaturation.

The ^{1}H NMR spectrum showed doublets signals due to two aromatic protons at δ_H 7.58 and 7.08 (each 1H and *J*=8.9 Hz each). The *J* values of 8.9 Hz indicated an *ortho*-coupling hence allowed the assignment of these two protons at positions C-5 and C-6 respectively. In the HMBC spectrum, the signal at δ_H 7.58 (H-5) indicated three key correlations with carbon signals at δ_C 175.1, 147.7 and 146.4 due to C-4, C-7 and C-8a respectively. The proton signal at δ_H 7.08 (H-6) correlated to quaternary carbon signal at δ_C 119.0 (C-4a) and two oxygenated carbon signals at δ_C 147.7 (C-7), 132.3 (C-8). In addition, H-5 and H-6 proton signals were observed in the COSY spectra to couple to each other. Besides, the ^{1}H NMR spectrum of **1** exhibited 1,4-disubstituted benzene signals at δ_H 7.49 (2H, d, 8.6, H-2′, 6′) and 6.96 (2H, d, 8.6, H-3′, 5′), which had been proved by the COSY spectra. The singlet proton signal at δ_H 3.75 corresponding to the carbon signal at δ_C 55.6 in the HMQC spectrum was assigned to the methoxy group. A correlation in the HMBC spectrum between δ_H 3.75 (OMe) and δ_C 159.6 confirmed the methoxy group attached at C-4′. Additionally, a diagnostic vinylic singlet at δ_H 8.47 (H-2), which was a resonance for isoflavone moiety [13], was observed in the HMBC spectrum to correlate to the carbon signals at δ_C 175.1 (C-4), 146.4 (C-8a) and 123.8 (C-1′). All above revealed a common isoflavone unit, retusin (7,8-dihydroxy-4′-methoxyisoflavone) structure [14] in compound **1**.

Further examination of NMR spectra (Table 1) indicated that compound **1** contains a phenylpropanoid unit. The deshielded doublet at δ_H 5.09 (H-1"), typical of a benzylic methine substituted by an oxygen, and the multiplet at δ_H 4.39 (H-2"), which were coupled each other in the COSY spectra, implied the presence of a 1,4-dioxane ring between an isoflavonoid moiety and a phenyl ring. On the basis of this observation, it could be proposed that compound **1** is composed of a 3,4,5-trioxygenated phenylpropanoid unit coupled via the dihydroxy functionality of a trioxygenated isoflavonoid moiety. The linkage of the isoflavonoid and phenylpropanoid units through a 1,4-dioxane bridge was deduced by HMBC experiments (Figure 2). The long-rang correlations between δ_H 5.09 (H-1") and δ_C 131.6 (C-4"), 106.8 (C-5", 9"), δ_H 4.39 (H-2") and δ_C 132.3 (C-8) in the HMBC spectrum indicated the linkage of the isoflavonol and phenylpropanol units at C-1" and C-2". And the correlation between δ_H 3.76 (6H, s, 2×OMe) and δ_C 153.2 (2×C) confirmed that two methoxy groups attached at C-6" and C-8" respectively. The *trans* stereochemistry between the methine hydrogens (H-

1" and H-2") was reasonably deduced by the coupling constant (J=7.8 Hz), and it was further confirmed in the NOE spectrum that was no correlation between δ_H 5.09 (H-1") and δ_H 4.39 (H-2") but a correlation between δ_H 4.39 (H-2") and δ_H 6.80 (H-5") [8-10].

Searching the literature indicated that compound **1** was closely related to butesuperin B [8]. Comparing the NMR spectral data of these two compounds, the differences were the presence of a sugar signals in the ^{1}H NMR and ^{13}C NMR spectrum of compound **1**. An upfield shift of 12.5 ppm (C-7") and a downfield shift of 4.7 ppm (C-6", 8") suggested that the sugar moiety was linked to C-7" of the aglycone. The signals of an anomeric proton and an anomeric carbon were observed at δ_H 4.94 and δ_C 102.9 respectively, which indicated the presence of a sugar moiety. Furthermore, the anomeric proton coupling constant (1H, d, 6.9 Hz) of the sugar moiety suggested it to be the β-anomer. In the ^{13}C NMR spectrum, the carbon signals at δ_C 102.9, 74.6, 77.7, 70.4, 76.9 and 60.3 and in the ^{1}H NMR spectrum, the proton signals at δ_H 4.94 (1H, d, 6.9) and 3.01-3.40 (m) also confirmed the sugar was β-glucopyranose. The sugar moiety of compound **1** was identified as D-glucose by TLC analysis after hydrolysis. Besides, the ESIMS fragment ions at 493 $[M+H-162]^+$ also indicated the presence of a sugar moiety. Thus, the structure of **1** was elucidated as butesuperin B-7"-O-β-glucopyranoside.

Additionally, butesuperin A (**2**) was identified by comparing its ^{1}H NMR and ^{13}C NMR data with literature [8].

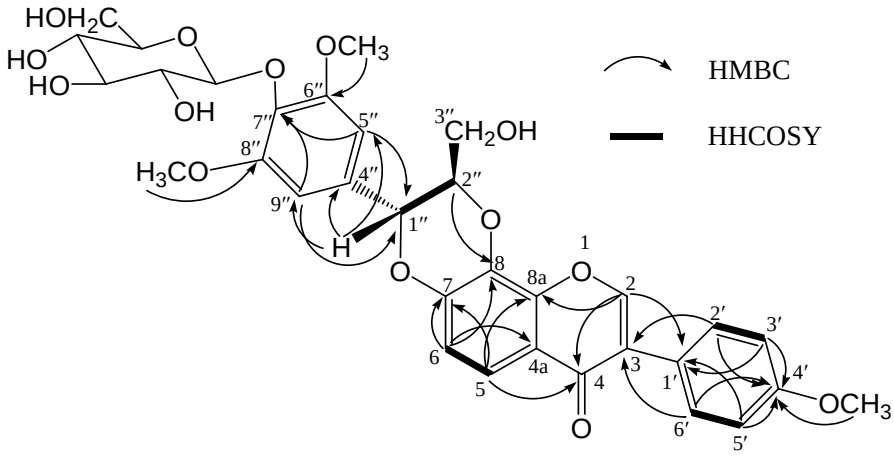

Figure 2. The key HMBC and HHCOSY correlations of compound **1**.

3.2 Cytotoxicity activity

Cytotoxic activity of compound **1** was evaluated against human hepatoma cells BEL-7404 and non-small-cell lung cancer cells NCI-H460 by the conventional MTT method. Compound **1** exhibited week cytotoxicity against these two selected cell lines (10 μg/mL, 28.35% and 20.36%, respectively) in MTT assay.

Table 1. ^{1}H and ^{13}C NMR data for compound **1** (500 MHz and 125 MHz, DMSO- d_6, J in Hz, δ ppm).[a]

Carbon	δ_H	δ_C
2	8.47(1H, *s*)	153.6
3		124.4
4		175.1
4a		119.0
5	7.58(1H, *d*, *J* = 8.9)	117.2
6	7.08(1H, *d*, *J* = 8.9)	115.4
7		147.7
8		132.3
8a		146.4
1′		123.8
2′,6′	7.49(2H, *d*, *J* = 8.6)	130.6
3′,5′	6.96(2H, *d*, *J* = 8.6)	114.1
4′		159.6
4′-OMe	3.75(3H, *s*)	55.6
1"	5.09(1H, *d*, *J* = 7.8)	78.4
2"	4.39(1H, m)	77.0
3"	3.67(1H, *dd*, *J* = 10.5, 5.0) 3.55(1H, *dd*, *J* = 10.5, 5.0)	61.4
4"		131.6
5", 9"	6.80(2H, *s*)	106.8
6", 8"		153.2
7"		135.4
6", 8" -OMe	3.76(6H, *s*)	57.0
Glc		
1'"	4.94(1H, *d*, *J* = 6.9)	102.9
2'"	3.21(*m*)	74.6
3'"	3.01(*m*)	77.7
4'"	3.11(*m*)	70.4
5'"	3.20(*m*)	76.9
6'"	3.40(2H, *m*)	60.3

[a] Assignments were confirmed by 2D experiments.

Acknowledgments

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Supporting Information

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

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