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A New Isoflavonolignan Glycoside from *Abrus cantoniensis*

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Abstract: A new isoflavonolignan glycoside, namely cantoniensin A (**1**), along with three known compounds were isolated from *Abrus cantoniensis*. The structure of the new compound was elucidated on the basis of spectroscopic analyses, including 1D-, 2D-NMR and HRESIMS. Compound **1** is the second example of isoflavonolignan glycoside from nature and is also the first isoflavonolignan isolated from the genus of *Abrus*.

Keywords: Leguminosae; *Abrus cantoniensis*; cantoniensin A; isoflavonolignan; isoflavonolignan glycoside.

1. Introduction

Abrus cantoniensis Hance (Jigucao in Chinese), a traditional plant of Leguminosae family in Southern China and some countries of Southeast Asia, was extensively used as an edible vegetable and medicinal plant. The whole plant can be used to make beverages or herbal tea alone or with other plant materials. In China, it is used as a folk medicine for treating acute and chronic hepatitis, jaundice, rheumatism and removing the liver toxicants [1-2]. In previous studies, *A. cantoniensis* was reportedly showed potential biological activities including antioxidant [2-4], anti-tumor [5-6], immunoregulatory activity [5] and wound healing activity [7]. Some chemical constituents have been isolated from *A. cantoniensis*, such as triterpenes, thraquinones, flavonoids, and alkaloids [8-11], while isoflavones have never been reported in this plant.

To search for the bioactive constituents, an investigation of secondary metabolites of this plant was carried out and resulted a new isoflavonolignan glycoside, cantoniensin A (**1**), along with three known compounds (**2**-**4**) including two isoflavones were isolated from *A. cantoniensis*. To the best of our knowledge, isoflavonolignans were seldom isolated as nature sources and only found in Leguminosae family at present. Compound **1** represents the second example of isoflavonolignan

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glycoside from nature and also the first isoflavonolignan isolated from the genus of *Abrus*. Herein, we describe the isolation and structural elucidation of these compounds.

2. Materials and Methods

2.1. General

Optical rotation was recorded on a WZZ-2S polarimeter. NMR spectra were recorded on a Bruker AV-500 MHz spectrometer with TMS as the internal standard. HRESIMS was recorded on an Agilent Technologies liquid chromatograph connected to a Q-TOF mass spectrometer. Silica gel (200-300 mesh) and ODS-A (YMC-GEL) were used for open column chromatography (CC). TLC were conducted on silica gel GF254 plates to monitor the fractions. Sephadex LH-20 were used for column chromatography (CC). HPLC and Semi-preparative HPLC experiments were carried out using an Agilent 1100 HPLC system with a YMC ODS column (250×4.6 mm, 10 μm) and YiLiTe SinoChrom ODS-BP colunm (250×10 mm, 10 μm) respectively.

2.2. Plant Material

The air-dried whole herbs of *A. cantoniensis* were collected from Yulin of Guangxi Province, China in April 2017 and identified by Prof. Xi-Feng Sheng, Hunan normal university. A voucher specimen (No. JGC-2017) has been deposited in the Laboratory of Phytochemistry, School of Medicine, Hunan Normal University.

2.3. Extraction and Isolation

The air-dried plant of *A. cantoniensis* (20 kg) was extracted with 70% EtOH under reflux, and then concentrated under reduced pressure to give a crude extract (3 L). The extract was suspended in H_2O, partitioned successively with CH_2Cl_2, EtOAc and *n*-BuOH to afford CH_2Cl_2 (137.5 g), EtOAc (82 g), *n*-BuOH (190 g) soluble fractions. The EtOAc extract was subjected to a sephadex LH-20 column chromatography eluted with MeOH to provide 108 fractions which were analyzed by TLC and HPLC. Fr. 43~Fr.45 were further purified by preparative HPLC (30% ACN–H_2O) to obtain compounds **1** (13.0 mg). The CH_2Cl_2 portion was subjected to silica gel column eluted with CH_2Cl_2-MeOH (98:2, 96:4, 94:6, 92:8, 9:1, 1:1) afford fractions Fr. 1~ Fr. 92. Fr. 5 was chromatographed on ODS-A column eluted with MeOH-H_2O (70%, 80%, 90%, 100%) to yield Fr. 5.5, which further purified by preparative HPLC (38 % ACN- H_2O) to afford **2** (7 mg), **3** (11 mg) and **4** (6.5 mg).

2.4. Spectroscopic Data

*Cantoniensin A (**1**):* yellow amorphous powder. $[\alpha]_D^{20}$ = +12.0 (c 0.01, MeOH); ^{1}H-NMR and ^{13}C-NMR (DMSO-d_6, 500/125 MHz) see Table 1; HRESIMS calcd. for $C_{32}H_{32}O_{13}H$ $[M + H]^+$ *m/z* 625.1921, found 625.1925.

2.5. Acid Hydrolysis

Compound **1** (2 mg) was refluxed with 5% HCl in MeOH (5 mL) for 2 hours and then extracted with EtOAc (10 mL) for 3 times. The aqueous layer was neutralized with $NaHCO_3$ and concentrated in vacumn to give a residue. The sugar unit in the aqueous layer was identified as D-glucose on the basis of TLC (BuOH-AcOH-H_2O, 4:1:5 upper layer)[12].

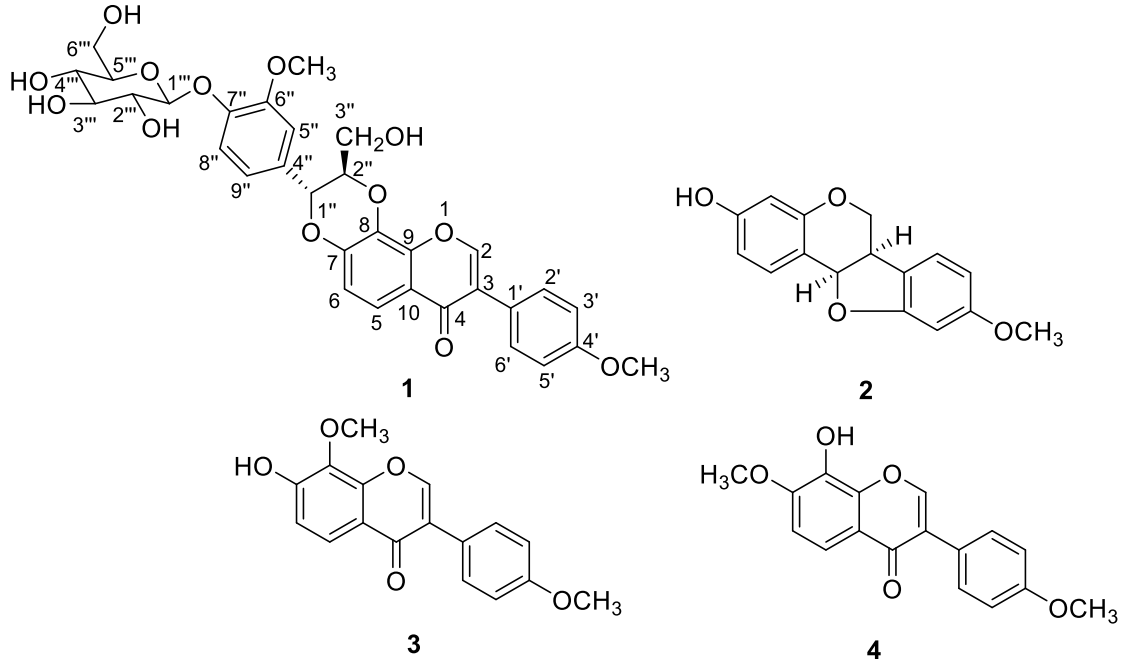

Figure 1. Structures of compounds **1**-**4**

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** was obtained as a pale yellow amorphous powder. Its molecular formula was determined to be $C_{32}H_{32}O_{13}$ with 17 degrees of unsaturation on the basis of the HRESIMS (*m/z* 625.1925 $[M + H]^+$, calcd. 625.1921) and the ^{13}C NMR data (Table 1).

The ^1H NMR spectrum of **1** displayed two ortho-coupling aromatic protons at δ_H 7.63, 7.11 (1H each, d, 8.8 Hz), which was also proved by COSY spectrum. The HMBC cross-peaks (Figure 2) from δ_H 7.63 (H-5) to C-4, C-7 and C-9 and from δ_H 7.11 (H-6) to C-7, C-8 and C-10 together with the COSY correlation of H-5/H-6 indicated the assignment of these two protons at C-5 and C-6 respectively. The ^1H NMR spectrum showed para-substituted benzene signals at δ_H 7.54 (2H, d, 8.7 Hz, H-2′, 6′) and 7.01 (2H, d, 8.7 Hz, H-3′, 5′), and a characteristic vinylic singlet at δ_H 8.51 (1H, s, H-2) for isoflavone moiety which was observed in the HMBC spectrum to correlate with C-4, C-9 and C-1′. Besides, a methoxy group was observed at δ_H 3.79 (3H, s, 4′-OMe) corresponding to the carbon signal at δ_C 55.6 in HMQC spectrum. The HMBC correlation from δ_H 3.79 (4′-OMe) to δ_C 159.5 demonstrated this methoxy group linked to C-4′. These above evidences revealed an isoflavone unit (7,8-dihydroxy-4′-methoxyisoflavone) in the structure of compound **1**.

Further examination of NMR spectra revealed a phenylpropanoid unit in the structure of **1**. The signals of δ_H 7.14 (1H, brs, H-5″), 7.15 (1H, d, 8.7 Hz, H-8″) and 7.01 (1H, d, 8.7 Hz, H-9″) along with the COSY spectrum proposed a 1,3,4-trisubstituted phenyl ring of the phenylpropanoid unit. The deshielded doublet at 5.17 (1H, d, 7.8 Hz, H-1″) is a typical oxygenated benzylic methine and this doublet was coupled with the multiplet at δ_H 4.42 (1H, m, H-2") in COSY spectrum. The signals at 3.68 (1H, *m*) and 3.45 (1H, *m*) was proved to be a hydroxymethyl at C-3″ by HSQC and COSY spectra. The HMBC correlations from H-1″ to C-4″, 5″ and C-9″, from H-2″ to C-4″ and form H-3″ to C-1″ and C-2″ confirmed that the phenylpropanoid unit is composed of a 1,2,3-tritrioxygenated side chain and a 1,3,4-trisubstituted phenyl ring. The HMBC correlations from δ_H 3.79 (6″-OMe), 7.15 (H-8″) to δ_C 149.4 (C-6″) confirmed the other methoxy group ws located at C-4″. Except the isoflavonol and phenylpropanoid units, the NMR spectra also showed an anomeric proton and an anomeric carbon at δ_H 4.95 and δ_C 100.4 respectively, which suggested the presence of a sugar moiety. The configuration of the glucosyl was confirmed as β-glucopyranose by the carbon signals at δ_c 100.4, 73.6, 77.5, 70.1, 77.3 and 60.3 and the the coupling constant (J = 8.7 Hz) of the anomeric proton. And the glucosyl was

identified as D-glucose by TLC analysis after hydrolysis [13]. The HMBC correlations from H-1‴ to C-7″ and NOESY correlation (Figure 3) between H-1‴ and H-8″ indicated the sugar moiety was connected with C-7″. Except for 16 degrees of unsaturation of the above fragments, the structure of **1** still remains one degree of unsaturation. Together with the molecular formula, a 1,4-dioxane ring between the isoflavonoid moiety and the phenylpropanoid unit was deduced. The linkage of the isoflavonol and phenylpropanol units at C-1" and C-2" was confirmed by the long-range correlation from H-2" to C-8. The *trans* stereochemistry was determined by the coupling constant (7.8 Hz) between H-1" and H-2" and the NOE correlations between H-2" and H-5", H-9", and NOE correlation between H-1" and H-3" [14-15].

Therefore, compound **1** was elucidated as butesuperin A-7"-O-*β*-glucopyranoside and named cantoniensin A (Figure 1). To best of our knowledge, there were very few isoflavonolignans reported as natural sources and only isolated in the family Leguminosae.

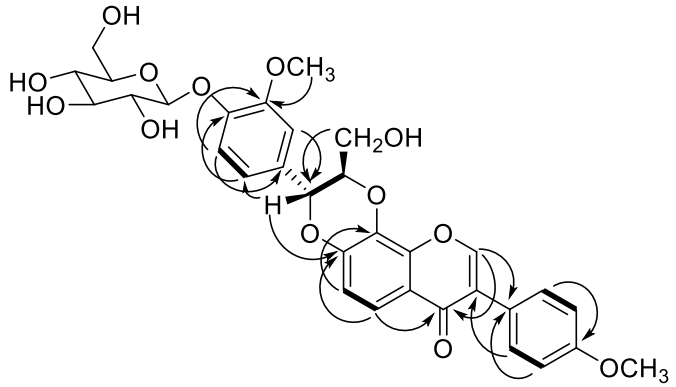

Figure 2. Key ^{1}H-^{1}H COSY and HMBC correlations of compound **1**

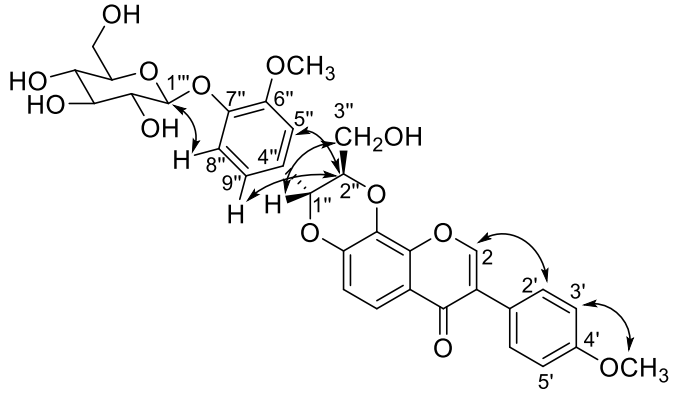

Figure 3. Key NOESY correlations of compound **1**

The three known compounds were identified as medicarpin (**2**) [16], 8-O-methylretusin (**3**) [17] and 8-hydroxy-4',7-dimethoxyisoflavone (**4**) [18] by comparing their spectroscopic data with those reported in the literatures. These three compounds were firstly isolated from the genus of *Abrus*.

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data for compound **1** in DMSO-d_6

Position	δ_H (ppm) J (Hz)	δ_C (ppm)
2	8.51 (1H, *s*)	153.6
3		124.4
4		175.1
5	7.63 (1H, *d*, *J* = 8.8)	117.2
6	7.11 (1H, *d*, *J* = 8.8)	115.5
7		147.7
8		132.3
9		146.4
10		118.9
1′		123.7
2′/6′	7.54 (2H, *d*, *J* = 8.7)	130.6
3′/5′	7.01 (2H, *d*, *J* = 8.7)	114.1
4′		159.5
4′-OCH_3	3.79 (3H, *s*)	55.6
1″	5.17 (1H, *d*, *J* = 7.8)	76.6
2″	4.42 (2H, *m*)	78.5
3″	3.45 (1H, *m*)	61.1
	3.68 (1H, *m*)	
4″		129.8
5″	7.14 (1H, *brs*)	112.6
6″		149.4
7″		147.5
8″	7.15 (1H, *d*, *J* = 8.7)	115.4
9″	7.01 (1H, *d*, *J* = 8.7)	120.8
6″-OCH_3	3.79 (3H, *s*)	56.3
Glc		
1‴	4.95 (1H, *d*, *J* = 8.7)	100.4
2‴	3.27 (1H, *m*)	73.6
3‴	3.30 (1H, *m*)	77.5
4‴	3.17 (1H, *m*)	70.1
5‴	3.28 (1H, *m*)	77.3
6‴	3.44 (1H, *m*)	60.3

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-ofnatural-products

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