

Article

## Benzyl 2- $\beta$ -Glucopyranosyloxybenzoate, a New Phenolic Acid Glycoside from *Sarcandra glabra*

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**Abstract:** From the whole plant of *Sarcandra glabra*, a new phenolic acid glycoside, benzyl 2- $\beta$ -glucopyranosyloxybenzoate (**1**), together with seven known compounds including eleutheroside B<sub>1</sub> (**2**), 5-*O*-caffeoylshikimic acid (**3**), (–)-(7*S*, 8*R*)-dihydrodehydrodiconiferyl alcohol (**4**), (–)-(7*S*, 8*R*)-dihydrodehydrodiconiferyl alcohol 9-, 9'- and 4-*O*- $\beta$ -D-glucopyranoside (**5–7**), and (–)-(7*S*, 8*R*)-5-methoxydihydrodehydrodiconiferyl alcohol 4-*O*- $\beta$ -D-glucopyranoside (**8**) was isolated. Their structures were elucidated by spectral analysis including 1D-, 2D-NMR and HR-ESI-MS. Compound **2** was found to exhibit potent cytotoxic activity against BGC-823 and A2780 cancer cell lines using MTT method with IC<sub>50</sub> value of 2.53 and 1.85  $\mu$ M, respectively.

**Keywords:** *Sarcandra glabra*; chloranthaceae; benzyl 2- $\beta$ -D-glucopyranosyloxybenzoate; anticancer activity

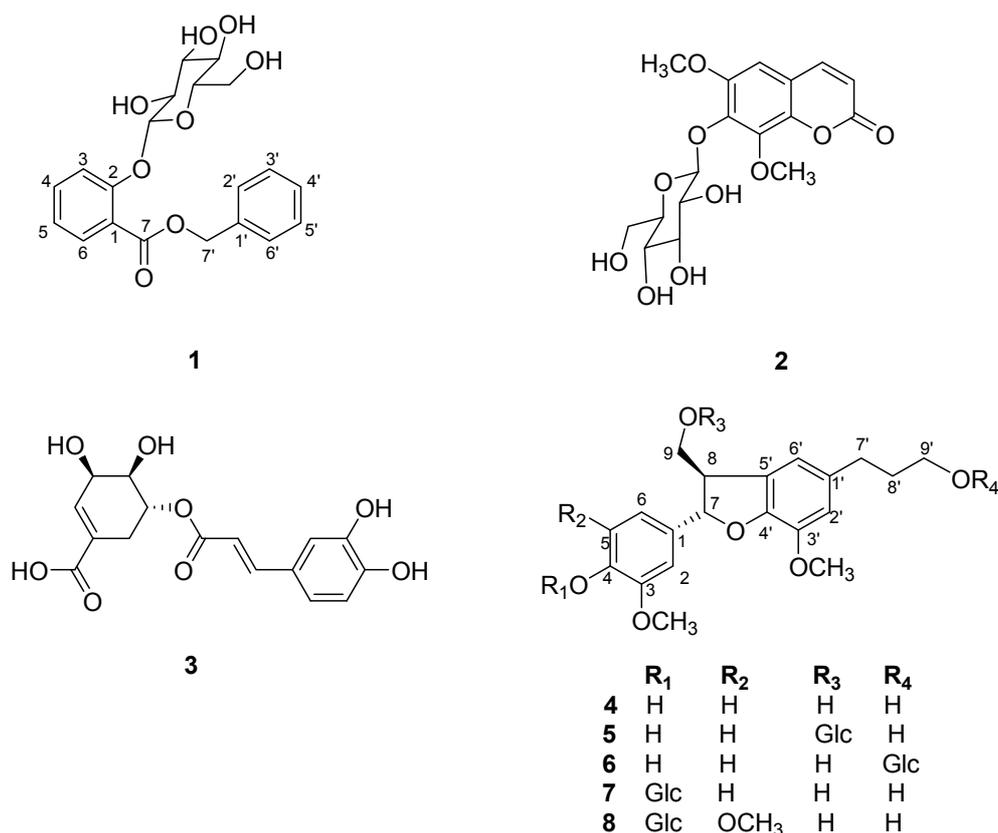
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### 1. Introduction

*Sarcandra glabra* (Thunb.) Makino (Chloranthaceae), an evergreen shrub growing in southern China, has been used in traditional medicine to treat bruises, bone fractures and arthritis [1]. The whole plant of *S. glabra* is specified in the 2010 edition of the Chinese Pharmacopoeia as a traditional

medicine used for its anticancer, antibacterial and antiviral activities [2]. So far, there have been many reports on the constituents in the whole plant of *S. glabra*, demonstrating the presence of sesquiterpenoids, coumarins, flavonoids, triterpenoids and phenolic acids [1,3–18]. Our previous phytochemical studies on the species resulted in the isolation of a new coumarin and three novel sesquiterpene glycosides [19,20]. In the continuation of our efforts to investigate antitumor agents from this plant, a new phenolic acid glycoside, benzyl 2- $\beta$ -glucopyranosyloxybenzoate (**1**) was isolated and determined using MS and NMR techniques. Also isolated from the 70% acetonic extract of the whole plants of *S. glabra* are eleutheroside B<sub>1</sub> (**2**) [21], 5-*O*-caffeoylshikimic acid (**3**) [17], (–)-(7*S*, 8*R*)-dihydrodehydrodiconiferyl alcohol (**4**) [22], (–)-(7*S*, 8*R*)-dihydrodehydrodiconiferyl alcohol 9-, 9'- and 4-*O*- $\beta$ -D-glucopyranoside (**5–7**) [22], and (–)-(7*S*, 8*R*)-5-methoxy-dihydrodehydrodiconiferyl alcohol 4-*O*- $\beta$ -D-glucopyranoside (**8**) [22] (Figure 1). In this paper, we report the isolation and structural elucidation of the new compound, as well as cytotoxicities of compound **2** against human cancer cell lines.

**Figure 1.** Structures of compounds **1–8**.



## 2. Results and Discussion

Compound **1** was obtained as a colorless amorphous powder and gave positive test with Molish reagent, thereby indicating its glycosidic nature. The HR-ESI-MS of **1** showed a quasi-molecular ion peak at  $m/z$  413.1192 [ $M + Na$ ]<sup>+</sup> (calcd. 413.1212 [ $M + Na$ ]<sup>+</sup>) in the positive-ion mode. In conjunction with the analysis of <sup>1</sup>H- and <sup>13</sup>C-NMR spectra, the molecular formula of **1** was deduced as C<sub>20</sub>H<sub>22</sub>O<sub>8</sub>, with 10 degrees of unsaturation. The IR spectrum showed hydroxyl groups (3,383 cm<sup>-1</sup>), an ester

group ( $1,706\text{ cm}^{-1}$ ) and an aromatic ring ( $1601$ ,  $1490$  and  $1074\text{ cm}^{-1}$ ). The  $^1\text{H-NMR}$  spectrum (Table 1) indicated the presence of 1,2-disubstituted benzene ring,  $\delta_{\text{H}}$  7.76 (1H, dd,  $J = 8.4$ ,  $1.8\text{ Hz}$ ), 7.54 (1H, m), 7.39 (1H, dd,  $J = 7.8$ ,  $1.8\text{ Hz}$ ) and 7.13 (1H, m), a monosubstituted benzene ring,  $\delta_{\text{H}}$  7.45 (2H, d,  $J = 7.2\text{ Hz}$ ), 7.39 (2H, t,  $J = 7.8\text{ Hz}$ ) and 7.33 (1H, m), an oxymethylene group ( $\delta_{\text{H}}$  5.33) and an anomeric proton ( $\delta_{\text{H}}$  4.92). The remaining sugar protons resonated from  $\delta_{\text{H}}$  3.91 to 3.39. The  $^{13}\text{C-NMR}$  spectrum (Table 1) displayed 20 signals for ester carbon at  $\delta_{\text{C}}$  168.2 (C-7), aromatic carbons from  $\delta_{\text{C}}$  158.8 to 119.0, anomeric carbon at  $\delta_{\text{C}}$  104.0 (C-10), other sugar carbons between  $\delta_{\text{C}}$  78.6 and 62.7 and oxygenated methylene carbon at  $\delta_{\text{C}}$  68.2. The HMBC spectrum of **1** showed correlations of C-7 with H-7' and H-6, C-2 with H-3, C-7' with H-2' and 6', C-1' with H-7', C-1 with H-3, C-3 with H-4, C-4 with H-5, and C-6 with H-5. The sugar group was presented at C-2, as indicated from the HMBC correlation between the anomeric proton ( $\delta_{\text{H}}$  4.92) and C-2 ( $\delta_{\text{C}}$  158.8). The  $^1\text{H-}$  and  $^{13}\text{C-NMR}$  data of **1** was completely assigned with the help of HMBC and HSQC, and presented in Table 1.

**Table 1.**  $^1\text{H-}$  and  $^{13}\text{C-NMR}$  data of compound **1** in  $\text{CD}_3\text{OD}$  ( $\delta$  in ppm,  $J$  in Hz).

Position	$\delta_{\text{C}}$	$\delta_{\text{H}}$	HMBC (H to C)
1	122.6		
2	158.8		
3	119.0	7.39 (dd, 7.8, 1.8)	C-1, C-2
4	135.4	7.54 (m)	C-3
5	123.9	7.13 (m)	C-4, C-6
6	132.3	7.76 (dd, 8.4, 1.8)	C-7
7	168.2		
1'	137.5		
2', 6'	129.4	7.45 (d, 7.2)	C-1'
3', 5'	129.8	7.39 (m)	
4'	129.5	7.33 (m)	
7'	68.2	5.34 (dd, 12, 12)	C-7, C-1'
Glc-1	104.0	4.92 (d, 7.2)	C-2
2	75.1	3.52 (m)	
3	77.7	3.48 (m)	
4	71.4	3.40 (m)	
5	78.6	3.46 (m)	
6	62.7	3.71 (dd, 12.0, 6.0) 3.89 (dd, 12.0, 1.8)	

The key HMBC correlations of **1** are shown in Figure 2. The absolute configuration of the glucose was not determined. On the basis of the above observations, compound **1** was assigned as benzyl 2- $\beta$ -glucopyranosyloxybenzoate. This is a new compound. The isolated compound **2** was evaluated using the MTT method *in vitro* for cytotoxic activities against five human cancer cell lines, HCT-8 (colon cancer), Bel-7402 (liver cancer), A549 (lung carcinoma), BGC-823 (gastric cancer) and A2780 (ovarian cancer). The results showed that the  $\text{IC}_{50}$  values of eleutheroside B<sub>1</sub> (**2**) against BGC-823 and A2780 were 2.53 and 1.85  $\mu\text{M}$ , respectively. Compound **2** showed no remarkable cytotoxic activity against HCT-8, Bel-7402 and A549 with  $\text{IC}_{50} > 10\ \mu\text{M}$ .



separated on a Sephadex LH-20 column eluted with MeOH and then preparative HPLC with MeOH–H<sub>2</sub>O to afford **1** (3 mg), **2** (30 mg) and **3** (5 mg). Fraction E was further chromatographed on silica gel column eluted with petroleum ether: ethyl acetate: methanol (20:1:0.1, v/v/v) and followed by LH-20 (MeOH) and preparative HPLC with MeOH–H<sub>2</sub>O to yield compounds **4** (4 mg), **5** (6 mg), **6** (4 mg), **7** (5 mg) and **8** (2 mg).

### 3.4. Spectral Data

Compound **1**: colorless amorphous powder.  $[\alpha]_D^{20}$   $-12.6$  ( $c = 0.13$ , MeOH). UV (MeOH)  $\lambda_{\max}$  ( $\log \epsilon$ ): 232 (3.49), 287 (2.92). IR (KBr)  $\nu_{\max}$  ( $\text{cm}^{-1}$ ): 3383, 2925, 2888, 1706, 1601, 1490, 1074. ESI-MS  $m/z$ : 413  $[\text{M} + \text{Na}]^+$ , 803  $[2\text{M} + \text{Na}]^+$ , 425  $[\text{M} + \text{Cl}]^-$ , 779  $[2\text{M} - \text{H}]^-$ . HR-ESI-MS:  $m/z$  413.1192  $[\text{M} + \text{Na}]^+$  (calcd for C<sub>20</sub>H<sub>22</sub>NaO<sub>8</sub>, 413.1212). For <sup>1</sup>H- and <sup>13</sup>C-NMR (CD<sub>3</sub>OD) spectra, see Table 1.

The structures of compounds **2–8** were identified by comparison of their spectral data with those reported in the literature.

### 3.5. Bioassays

The cytotoxic assays were measured on HCT-8 (human colon cancer), Bel-7402 (human liver cancer), A549 (human lung carcinoma), BGC-823 (human gastric tumor cells) and A2780 (human ovarian tumor cells) (obtained from the Institute of Materia Medica of Chinese Academy of Medical Sciences) using the MTT assay method. Cells were plated in the appropriate media on 96-well plates in a 100  $\mu\text{L}$  total volume at a density of  $1 \times 10^5$  cell/mL. Each tumor cell line was exposed to the test compound at various concentrations in triplicates and incubated at 37 °C and 5% CO<sub>2</sub> for 48 h. Cell viability was determined based on the mitochondrial conversion of MTT to formazan.

## 4. Conclusions

A new phenolic acid glycoside, benzyl 2- $\beta$ -glucopyranosyloxybenzoate (**1**), together with seven known compounds, including eleutheroside B<sub>1</sub> (**2**), 5-*O*-caffeoylshikimic acid (**3**), (–)-(7*S*, 8*R*)-dihydrodehydrodiconiferyl alcohol (**4**), (–)-(7*S*, 8*R*)-dihydrodehydrodiconiferyl alcohol 9-, 9'- and 4-*O*- $\beta$ -D-glucopyranoside (**5–7**), and (–)-(7*S*, 8*R*)-5-methoxydihydrodehydrodiconiferyl alcohol 4-*O*- $\beta$ -D-glucopyranoside (**8**) was isolated from the whole plant of *S. glabra*. The isolation of benzyl 2-*O*- $\beta$ -D-glucopyranosyl-2-hydroxybenzoate was a new addition to the molecular diversity of this species. Eleutheroside B<sub>1</sub> (**2**) exhibited potent activity against BGC-823 and A2780 cancer cell lines with IC<sub>50</sub> value of 2.53 and 1.85  $\mu\text{M}$ , respectively.

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*Sample Availability:* Samples of the compounds **1–8** are available from the authors.

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