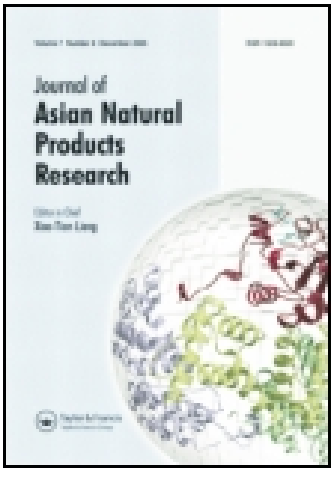

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Phenolic glycosides and other constituents from the bark of Magnolia officinalis

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Phenolic glycosides and other constituents from the bark of *Magnolia officinalis*

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A new phenolic glycoside, syringic acid 4-*O*-β-D-glucopyranosyl-(1 → 5)-α-L-rhamnopyranoside (**1**), together with 12 known compounds consisting of eight phenolic glycosides (**2**–**9**), two phenolic acids (**10** and **11**), and two norsesquiterpenoids (**12** and **13**), was isolated from the methanol extract of the bark of *Magnolia officinalis*. Their structures were elucidated on the basis of spectroscopic analysis and chemical methods. Compounds **1**–**11** were evaluated for their inhibitory activities against fructose-1,6-bisphosphatase, aldose reductase, lipase, dipeptidyl peptidase-IV, α-glucosidase, and three cancer cell lines. However, all the compounds showed weak or no activities in these tests.

Keywords: Magnoliaceae; *Magnolia officinalis*; phenolic glycoside; syringic acid 4-*O*-β-D-glucopyranosyl-(1 → 5)-α-L-rhamnopyranoside

1. Introduction

The bark of *Magnolia officinalis* (Magnoliaceae) has been used in traditional Chinese medicine for the treatment of abdominal distention and pains, dyspepsia, and asthmatic cough [1]. Some neolignans, lignans, alkaloids, and sesquiterpenes [2–5] were reported from this plant. In our previous investigation, 11 bioactive polar compounds were characterized from the methanol extract of the bark of *M. officinalis* [6]. Continuing examination of water-soluble portion of the same extract has resulted in the isolation of a new phenolic glycoside syringic acid 4-*O*-β-D-glucopyranosyl-(1 → 5)-α-L-rhamnopyranoside (**1**), along with 12 known compounds (**2**–**13**) (Figure 1). To our knowledge, all compounds were isolated from *M. officinalis* for the first time. Compounds **1**–**11** were evaluated for their inhibitory activities against fructose-1,6-bisphosphatase (FBPase), aldose reductase, lipase, dipeptidyl peptidase-IV (DPP-IV), α-glucosidase, and three cancer cell lines. However, all the compounds showed weak or no activities in these tests.

This report describes the isolation, structural elucidation, and bioassay results of these compounds.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder. The negative mode of ESI-MS of **1** gave a quasi-molecular ion peak at *m/z* 505 $[M - H]^-$, and HR-ESI-MS at *m/z* 529.1536 $[M + Na]^+$ indicated the molecular formula $C_{21}H_{30}O_{14}$. The IR spectrum displayed the presence of hydroxyl (3376 cm^{-1}), carbonyl (1695 cm^{-1}), and aromatic (1591 cm^{-1}) functionalities. The UV spectrum showed absorption maxima at 210 and 262 nm, which was characteristic of syringic acid [7]. The ^1H NMR data of **1** (Table 1) indicated two methoxys at δ_H 3.81 (6H, s) and two aromatic protons at δ_H 7.29

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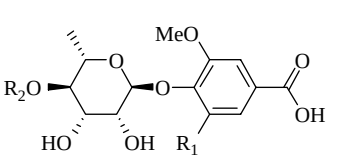
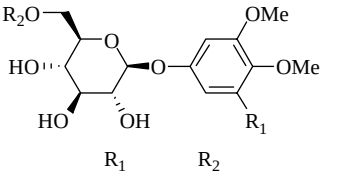
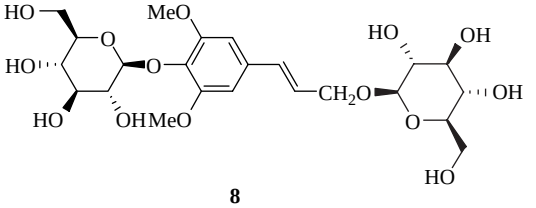


	R_1	R_2
1	OMe	β-D-Glc
2	OMe	H
3	H	H



	R_1	R_2
4	H	β-D-Api
5	OMe	β-D-Api
6	OMe	α-L-Rha
7	OMe	β-D-Xyl



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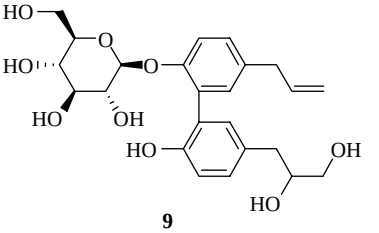

9

Glc: Glucopyranose; Api: Apiofuranose; Rha: Rhamnopyranose; Xyl: Xylopyranose

Figure 1. Structures of compounds **1**–**9.**

(2H, s), which are also identical to those of syringic acid [7]. In addition, it displayed signals attributable to two anomeric protons at δ_H 5.27 (br s) and 4.56 (d, $J = 8.0$ Hz), and other signals corresponding to sugar moieties at δ_H 4.30–3.20. One methyl at δ_H 1.22 (d, $J = 6.5$ Hz) indicated that it should be a rhamnosyl in compound **1**. These data suggested that **1** was a syringic acid 4-*O*-diglycoside, which was confirmed by ^{13}C NMR spectral data (Table 1). Particularly, the ^{13}C NMR spectrum showed 11 oxygen-bearing carbon signals and one methyl signal, indicating that two sugar units in **1** were hexosyl units. Acid hydrolysis of **1** liberated D-glucose and L-rhamnose, which were identified by gas chromatography (GC) analysis, comparison of retention times of sugars from hydrolysate with those of the authentic sugars. The 2D NMR data analysis led to unambiguous assignments of the NMR data of **1** (Table 1). Especially in the HMBC spectrum of **1**, long-range correlations of H-1′/C-4 and H-1″/C-4′ (Figure 2) demonstrated the linkage of sugar units. Therefore, compound **1** was concluded to be syringic

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data for **1** (in MeOH, δ in ppm, J in Hz).

No.	δ_H	δ_C
1		129.4
2, 6	7.29, 2H, s	108.1
3, 5		154.7
4		139.7
7		170.5
1′	5.27, 1H, br s	103.5
2′	4.10, 1H, dd ($J = 3.5$, 1.5)	72.2
3′	4.06, 1H, dd ($J = 8.5$, 3.5)	72.5
4′	3.61, 1H, t ($J = 8.5$)	83.6
5′	4.25, 1H, m	70.2
6′	1.22, 3H, d ($J = 6.5$)	18.3
1″	4.56, 1H, d ($J = 8.0$)	106.1
2″	3.23, 1H, m	76.4
3″	3.34, 1H, t ($J = 8.5$)	78.5
4″	3.27, 1H, t ($J = 8.5$)	71.8
5″	3.22, 1H, m	78.4
6″	3.78, 1H, dd ($J = 12.0$, 2.0) 3.63, 1H, dd ($J = 12.0$, 5.5)	63.0
3,5-OMe	3.81, 6H, s	56.9

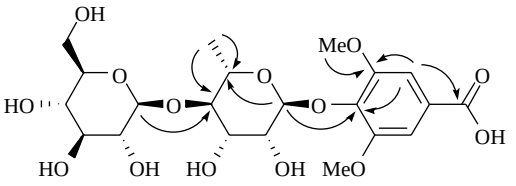

Figure 2. Key HMBC correlations of compound **1.**

acid 4-*O*-β-D-glucopyranosyl-(1 → 5)-α-L-rhamnopyranoside.

The known compounds were identified by comparison of spectroscopic data with those reported in the literature as syringic acid 4-*O*-α-L-rhamnopyranoside (**2**) [7], vannilic acid 4-*O*-α-L-rhamnoside (**3**) [8], 3,4-dimethoxyphenol β-D-apiofuranosyl (1 → 6)-β-D-glucopyranoside (**4**) [9], 3,4,5-trimethoxyphenol β-D-apiofuranosyl(1 → 6)-β-D-glucopyranoside (**5**) [10], 1-(α-L-rhamnosyl(1 → 6)-β-D-glucopyranosyloxy)-3,4,5-trimethoxybenzene (**6**) [11], 3,4,5-trimethoxyphenyl 1-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranoside (**7**) [12], isosyringinoside (**8**) [13], magnolignan A-2-*O*-β-D-glucopyranoside (**9**) [14], vannilic acid (**10**) [15], syringic acid (**11**) [16], blumenol A (**12**) [17], and blumenol B (**13**) [17].

Compounds **1**–**11** were evaluated for their inhibitory activities against FBPase (10 μM), aldose reductase (10 μM), lipase (10 μM), α-glucosidase (40 μM), and DPP-IV (10 μM), respectively. All tested compounds showed weak activities at the same concentration as the positive control drugs (CS-917, epalrestat, orlistat, acarbose, and INDP-2, see Table 2). The inhibiting rates of compounds **1**–**11** against α-glucosidase and DPP-IV are all $<10\%$. Compounds **1**–**11** were evaluated against three human cancer cell lines and were not active ($IC_{50} > 5$ μg/ml).

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a P-2000 automatic digital polarimeter (JASCO, Tokyo, Japan). UV spectra were obtained in MeOH on a JASCO V-650 spectrophotometer (JASCO). IR spectra were recorded on a Thermo Nicolet 5700 FT-IR microscope instrument (Thermo Electron Corp., Madison, WI, USA). HR-ESI-MS were obtained on an Agilent 6520 Accurate-Mass Q-TOF LC-MS (Agilent, Santa Clara, CA, USA). NMR spectra were taken on an Inova 500 spectrometer with solvent peak as references (Varian, Palo Alto, CA, USA). Macroporous resin D101 was a product of Chemical Plant of Nan Kai University (Tianjin, China). MCI CHP-20P (75–150 μm, Mitsubishi Chemical Corp., Tokyo, Japan) and RP-C18 (40–60 μm, YMC, Kyoto, Japan) were used for column chromatographic separation. Medium pressure liquid chromatography (MPLC) was performed on an EZ Purifier II flash

Table 2. Inhibitory activity (%) of compounds **1**–**11** (10 μM) against FBPase, aldose reductase, and lipase.

Compounds	FBPase	Aldose reductase	Lipase
1	35.6	–	–
2	42.5	–	–
3	39.7	–	–
4	28.8	33.1	–
5	30.1	–	–
6	35.6	–	–
7	35.6	16.3	–
8	20.5	19.0	–
9	39.7	–	–
10	23.1	–	–
11	32.9	–	13.6
CS-917	93.8	–	–
Epalrestat		98.3	
Orlistat			99.7

Note: –, Inhibiting rate $<10\%$.

chromatography system (Shanghai Li Sui E-Tech CO. Ltd, Shanghai, China). Analytical high performance liquid chromatography (HPLC) was conducted on a Waters 2695 pumping system equipped with a Waters 2996 photodiode array detector (Waters, Milford, MA, USA). The preparative HPLC was performed using a Waters 600 pump, a Waters 2487 detector, and a C18 column (250 mm × 20 mm, 5 μm; YMC). GC was carried out on an Agilent 7890 GC system (Agilent).

3.2 Plant material

M. officinalis was collected from Enshi City, Hubei Province of China, in May 2009, and identified by Prof. Bin Yang. A voucher specimen (No. 20090518) has been deposited at the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences.

3.3 Extraction and isolation

The dried and powdered *M. officinalis* (8 kg) was extracted with MeOH (3 × 32 liters) by ultrasonication. The MeOH solutions were combined and concentrated to yield a dried extract (1.5 kg). The MeOH extract (1.5 kg) was suspended in distilled water (3 liters) and then partitioned with $CHCl_3$ (3 × 3 liters). The water-soluble portion (650 g) was chromatographed on D101 macroporous resin, eluted with a gradient of EtOH:H_2O (0:100 to 95:5), to obtain five fractions (Fr. 1–Fr. 5). Fr. 1 (365 g, H_2O elute) was subjected to MPLC on an MCI CHP-20P column (75 ml/min, 265 nm) eluted with a gradient of EtOH:H_2O (0:100 to 100:0) to yield five fractions (Fr. 1.1–Fr. 1.5). Fr. 1.2 (21 g, 10% EtOH elute) was subjected to MPLC with an RP-C18 column (35 ml/min, MeOH–H_2O, 5–40%, 265 nm) to yield five major subfractions (Fr. 1.2.1–Fr. 1.2.5). Fr. 1.2.1 (0.9 g) was further separated by preparative HPLC (MeCN–H_2O, 7%, 10 ml/min, 265 nm) to give compound **8** (t_R 54 min, 8 mg). Compounds **5** (t_R 43 min, 13 mg), **6** (t_R 32 min, 17 mg), and **7** (t_R 55 min, 12 mg) were afforded from Fr. 1.2.2 (1.4 g) by preparative HPLC (MeCN–H_2O, 10%, 10 ml/min, 265 nm). Compounds **1** (t_R 56 min, 11 mg) and **4** (t_R 67 min, 7 mg) were afforded from Fr. 1.2.3 (1.8 g) by preparative HPLC (MeCN–H_2O, 10%, 10 ml/min, 265 nm) and compounds **9** (t_R 20 min, 50 mg), **12** (t_R 42 min, 37 mg), and **13** (t_R 36 min, 52 mg) were also obtained by preparative HPLC (MeCN–H_2O, 12%, 10 ml/min, 254 nm) from Fr. 1.2.4 (2.1 g). Fr. 1.3 (2.5 g, 20% EtOH elute) was subjected to MPLC with an RP-C18 column (15 ml/min, MeOH–H_2O, 10–50%, 265 nm) to yield five major subfractions (Fr. 1.3.1–Fr. 1.3.5). Compound **2** (t_R 53 min, 16 mg) was obtained by preparative HPLC (MeCN–H_2O, 12%, 10 ml/min, 265 nm) from Fr. 1.3.3 (340 mg). Fr. 1.4 (7.5 g, 20% EtOH elute) was subjected to MPLC with an RP-C18 column (35 ml/min, MeOH–H_2O, 10–50%, 254 nm) to yield six subfractions (Fr. 1.4.1–Fr. 1.4.6). Fr. 1.4.1 (1.3 g) was further separated by preparative HPLC (MeCN–H_2O, 12%, 10 ml/min, 265 nm) to give compound **3** (t_R 43 min, 17 mg). Fr. 1.4.4 (430 mg) was further separated by preparative HPLC (MeCN–H_2O, 10%, 10 ml/min, 254 nm) to give compounds **10** (t_R 41 min, 27 mg) and **11** (t_R 30 min, 30 mg).

3.3.1 *Syringic acid 4-O-β-D-glucopyranosyl-(1 → 5)-α-L-rhamnopyranoside (**1**)*

White amorphous powder; $[\alpha]_D^{20}$ − 42.3 ($c = 0.12$, MeOH); IR ν_{max} 3376, 2939, 1695, 1591, 1558, 1415, 1128, 1076, 1037, 973 cm^{-1}; UV λ_{max} (nm) (MeOH) 210, 262; for ^1H NMR (MeOH, 500 MHz) and ^{13}C NMR (MeOH, 125 MHz) spectral data, see Table 1; ESI-MS *m/z* 505 $[M-H]^-$; HR-ESI-MS *m/z* 529.1536

$[M + Na]^+$ (calcd for $C_{21}H_{30}O_{14}Na$, 529.1533).

3.4 Acid hydrolysis of 1

Compound **1** (3 mg) was hydrolyzed with 2 N HCl (1 ml) at 95°C for 4 h. After cooling to room temperature, the solution was extracted with $CHCl_3$ (3 × 1 ml). The aqueous layer was dried by blowing with N_2 to give a residue. The residue and standard D-glucose and L-rhamnose were treated with L-cysteine methyl ester hydrochloride (4 mg) in pyridine (1.0 ml) at 70°C for 2 h, respectively. The respective solutions were evaporated under a stream of N_2, and dried in vacuo. Next, 0.5 ml of *N*-trimethylsilylimidazole was added. The resultant reaction mixtures were maintained at 70°C for 1 h. The mixture was partitioned between H_2O (1.5 ml) and *n*-hexane (3 × 1 ml). The *n*-hexane layer was concentrated to 200 μl, and analyzed by GC under the following conditions: AB-5 (30 m × 0.32 mm × 0.25 μm); detector temperature, 280°C; injection temperature, 250°C; initial temperature, 100°C for 2 min and subsequent increase to 270°C at the rate of 10°C /min; final temperature, 270°C for 5 min; carrier, N_2 gas. D-glucose (t_R = 17.96 min) and L-rhamnose (t_R = 16.74 min) were detected from **1** by comparing their retention times with those of authentic samples (t_R = 17.99 and 16.76 min for D-glucose and L-rhamnose, respectively).

3.5 Bioactivity assays

Compounds **1**–**11** were tested *in vitro* for their inhibitory activity against α-glucosidase [18], DPP-IV [19], aldose reductase [20], and lipase [20] according to the procedure described in the literature. The cytotoxicity of compounds **1**–**11** against the human lung cancer cell line (A549), human liver cancer cell line (Bel-7402), and human colon cancer cell line (HCT-8) was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method, as described in the previous report [21].

Acknowledgements

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