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TWO NEW GALLOYL GLUCOSIDES FROM THE BARK OF *CASTANOPSIS FARGESII*

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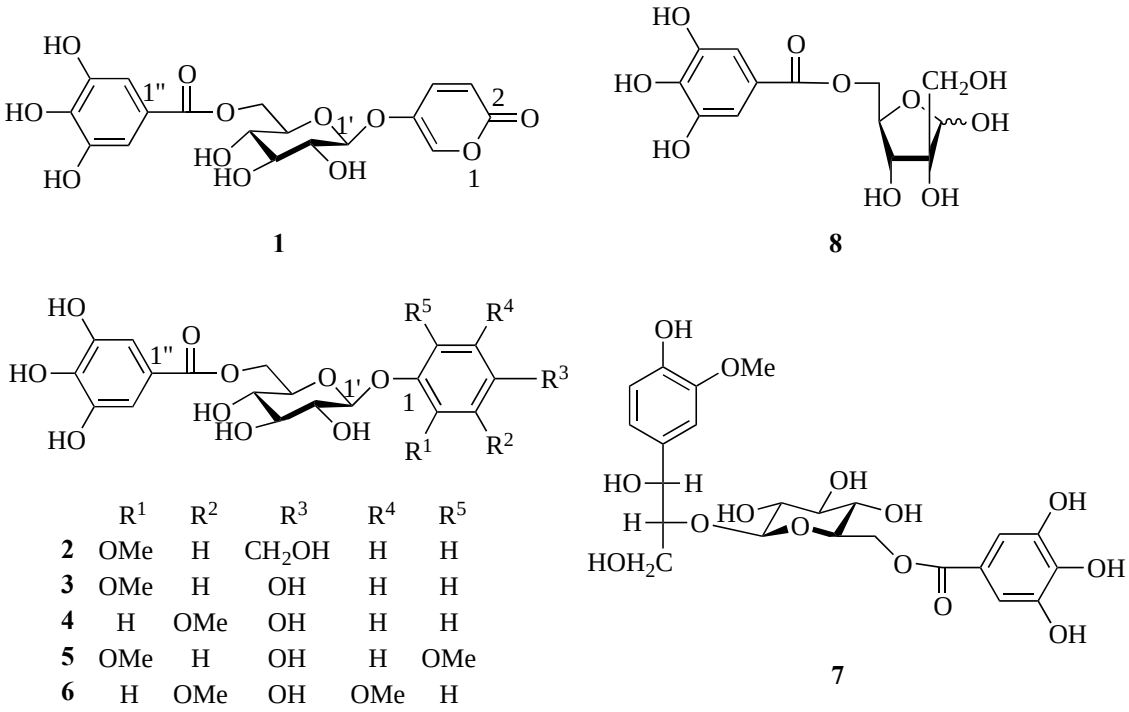
Abstract – In a continuing phytochemical investigation of the *Castanopsis* species (Fagaceae), two new galloyl glucosides, 5-*O*-β-D-(6'-*O*-galloyl)glucopyranosyl pyran-2-one (**1**) and 4-hydroxymethyl-2-methoxyphenol 1-*O*-β-D-(6'-*O*-galloyl)glucoside (**2**), were isolated from the ethanolic extract of the bark of *Castanopsis fargesii*, together with six known compounds. Their structures were elucidated on the basis of spectroscopic data analysis. The oxygen radical absorbance capacity (ORAC) values of the all compounds were measured and the known compound 4-hydroxy-2-methoxyphenol 1-*O*-β-D-(6'-*O*-galloyl)glucoside (**3**) showed the strongest antioxidant activity.

The genus *Castanopsis* belongs to the family Fagaceae, commonly found in the evergreen forests in East Asia, many of which are used for ornamental trees and construction materials. In previous papers, we have reported isolation and characterization of tannins and related compounds, including triterpene hexahydroxydiphenoyl (HHDP) esters, phenylpropanoid-substituted flavan-3-ols, ellagitannins, galloyl esters of quinic acid, phenolic glucosides, and flavonol glycosides from *C. fissa*,[1] *C. sclerophylla*[2,3] and *C. carlesii*.[4] In the course of a chemical study on tannins and related compounds from *Castanopsis* species, an ethanolic extract of the bark of *C. fargesii* was subjected to a chemical investigation leading to the isolation of eight galloyl glucosides including two new ones. Herein, the structural determination of the two new galloyl glucosides from the bark of *C. fargesii* was described, as well as the antioxidant activity of all compounds.

The fresh bark was extracted with 80% aqueous EtOH, and the extract was subjected to a combination of column chromatography using Sephadex LH-20, MCI gel CHP 20P, and Toyopearl Butyl-650**C** to afford eight compounds, including the two new galloyl glucosides **1** and **2** (Figure 1). The known compounds were elucidated on the basis of spectroscopic and comparison with literature data.



	R^1	R^2	R^3	R^4	R^5
2	OMe	H	CH_2OH	H	H
3	OMe	H	OH	H	H
4	H	OMe	OH	H	H
5	OMe	H	OH	H	OMe
6	H	OMe	OH	OMe	H

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

Compound **1** was isolated as an amorphous brownish powder, and the molecular formula $C_{18}H_{18}O_{12}$ was determined based on the liquid chromatography-mass spectrometry IT-TOF (LC-MS/IT-TOF), which showed the $[M-H]^-$ and $[M+Na]^+$ ion peaks at *m/z* 425.0724 (Calcd for $C_{18}H_{17}O_{12}$, 425.0725), and 449.0736 (Calcd for $C_{18}H_{18}O_{12}Na$, 449.0690), respectively. The dark blue coloration with ethanolic $FeCl_3$ reagent suggested presence of a pyrogallol ring in the molecule, and appearance of aromatic and carboxyl carbon signals in the ^{13}C NMR spectrum (δ_C 120.1, 109.1, 145.3, 138.5, and 166.6) along with a singlet aromatic signal at δ_H 7.09 in the ^1H NMR spectrum confirmed presence of a galloyl group (Table 1).[5] Chemical shifts of six aliphatic carbon signals at δ_C 101.4 (C-1'), 73.1 (C-2'), 75.5 (C-3'), 70.1 (C-4'), 74.2 (C-5'), 63.5 (C-6') and an anomeric proton signal with large coupling constant at δ_H 4.87 (1H, d, J = 7.1 Hz) indicating that this compound is a β-glucoside.[6] Large low field shift of glucose H-6 methylene protons at δ_H 4.34 and 4.61 showed that the galloyl group was attached to this position.[7] As for the aglycone, four *sp*2 carbon signals (δ_C 115.8, 156.7, 144.4, and 146.5) and a carboxy signal (δ_C 174.3) as well as unsaturation index (10) calculated from the molecular formula indicated presence of a pyran-2-one ring. This was supported by IR absorptions at 1740, 1648 and 1557 cm^{-1} belong to the

stretching vibrations of the three double bounds (C=O, C5–C6 and C3–C4).[8] Two doublet proton signals at δ_H 6.47 (d, J = 5.6 Hz, H-3) and 8.04 (d, J = 5.6 Hz, H-4) and one singlet signal at δ_H 8.19 (s, H-6) suggested that C-4 or C-5 of the pyran-2-one was substituted. The HMBC correlations of the glucose anomeric proton (δ_H 4.87) with C-5 (δ_C 146.5) of the pyran-2-one ring, indicated that the glucose was attached to the C-5 hydroxyl group through a glycosidic linkage (Figure 2). The HMBC correlations of the galloyl carboxyl group with glucose H-6 confirmed the location of the galloyl group. Based on these spectroscopic results, the structure of **1** was determined to be 5-*O*-β-D-(6'-*O*-galloyl)glucopyranosyl pyran-2-one.

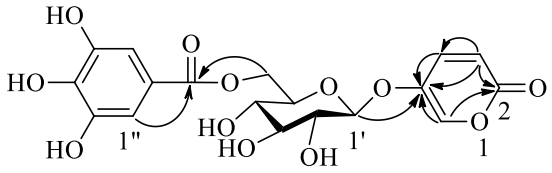


Figure 2. Selected HMBC correlations (H to C) **1**

Compound **2** was also positive to $FeCl_3$ (dark blue), and the molecular formula was deduced to be $C_{21}H_{24}O_{12}$ by LC-MS/IT-TOF (*m/z* 491.1174 $[M+Na]^+$, calcd for $C_{21}H_{24}O_{12}Na$, 491.1160). The ^1H and ^{13}C NMR spectrum of compound **2** (Table 1) were closely related to that of 4-hydroxy-2-methoxyphenol 1-*O*-β-D-(6'-*O*-galloyl)glucoside (**3**), showing signals assign to a galloyl group [(δ_H 7.14 (2H, s); δ_C 109.2 (2C), 120.4, 138.3, 145.2 (2C), 166.5], a sugar moiety [(δ_H 4.97 (1H, d, J = 7.5 Hz); δ_C 101.0, 76.2, 74.1, 73.4, 70.3, 63.8], an 1,3,4-trisubstituted benzene [(δ_H 6.80 (1H, dd, J = 1.6, 8.5 Hz), 7.00 (1H, d, J = 1.6 Hz), 7.08 (1H, d, J = 8.5 Hz); δ_C 111.2, 115.6, 119.6, 136.4, 145.4, 148.7], and a methoxyl group [δ_H 3.80 (3H, s); δ_C 55.5]. The large coupling constants of the remaining sugar proton signals ($J_{2,3}$, $J_{3,4}$, and $J_{4,5}$ = 9–10 Hz) indicated that this sugar was a β-glucopyranose in the $^4C_1$ conformation.[9] Differences included appearance of a signal assignable to a oxygenated methylene group [δ_H 4.50 (2H, s); δ_C 63.5] in the spectrum of **2**. Location of this methylene group at the C-4 position of the aglycone aromatic ring was indicated by appearance of HMBC correlations of this methylene proton with C-3 and C-5 (Figure 3). The HMBC experiment also determined the location of glucose unit, methoxyl group, and galloyl group at the C-1, C-2, and glc-C-6, respectively: the anomeric proton (δ_H 4.97) to aglycone C-1 (δ_C 145.4), the methoxyl group (δ_H 3.80) to the C-2 (δ_C 148.7), and the glucose H-6 [δ_H 4.33 (1H, dd, J = 7.2, 12.1 Hz), 4.62 (1H, dd, J = 1.9, 12.1 Hz)] to the galloyl carboxyl carbon (δ_C 166.5) (Figure 3). Based on these results, the structure of **2** was determined to be 4-hydroxymethyl-2-methoxyphenol 1-*O*-β-D-(6'-*O*-galloyl)glucoside.

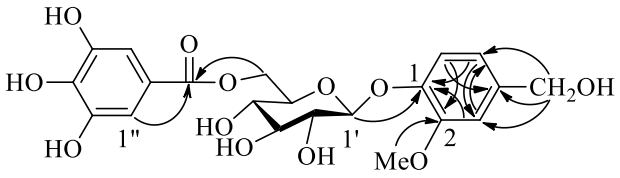


Figure 3. Selected HMBC correlations (H to C) of **2**

Table 1. ^{1}H-(500 MHz) and ^{13}C-(125 MHz) NMR data for **1** and **2** (in acetone-d_6)[a]

	1			**2**	
Position	^{1}H	^{13}C	Position	^{1}H	^{13}C
1			1		145.4
2		174.3	2		148.7
3	6.47 (d, 5.6)	115.8	3	7.00 (d, 1.6)	111.2
4	8.04 (d, 5.6)	156.7	4		136.4
5		146.5	5	6.80 (dd, 1.6, 8.5)	115.6
6	8.19 (s)	144.4	6	7.08 (d, 8.5)	119.6
7			7	4.50 (s)	63.5
1'	4.87 (d, 7.1)	101.4	1'	4.97 (d, 7.5)	101.0
2'	3.61 (dd, 7.1, 9.0)	73.1	2'	3.59 (dd, 7.5, 9.2)	73.4
3'	3.63 (t, 9.0)	75.5	3'	3.64 (t, 9.2)	76.2
4'	3.54 (t, 9.0)	70.1	4'	3.55 (t, 9.2)	70.3
5'	3.85 (m)	74.2	5'	3.86 (m)	74.1
6'	4.34 (dd, 6.5, 11.8)	63.5	6'	4.33 (dd, 7.2, 12.1)	63.8
	4.61 (dd, 2.1, 11.8)			4.62 (dd, 1.9, 12.1)	
1"		120.1	1"		120.4
2", 6"	7.09 (s)	109.1	2", 6"	7.14 (s)	109.2
3", 5"		145.3	3", 5"		145.2
4"		138.5	4"		138.3
7"		166.6	7"		166.5
			OMe	3.80 (s)	55.5

[a] Chemical shifts are given in δ values; multiplicities and coupling constants (*J* in Hz) in parentheses.

The known compounds were elucidated on the basis of spectroscopic data analysis as 4-hydroxy-2-methoxyphenol 1-*O*-β-D-(6'-*O*-galloyl) (**3**),[10] 4-hydroxy-3-methoxyphenol 1-*O*-β-D-(6'-*O*-galloyl) (**4**),[11] 4-hydroxy-2,6-dimethoxyphenol 1-*O*-β-D-(6'-*O*-galloyl) (**5**),[10] 3,5-dimethoxy-4-hydroxyphenol 1-*O*-β-D-(6'-*O*-galloyl)glucoside (**6**),[11] D-*threo*-guaiacylglycerol 8-*O*-β-D-glucopyranoside (**7**)[11] and 5-*O*-galloylhamamelose (**8**).[12] All the compounds were obtained from this plant for the first time.

Antioxidant activity was an important biological activity of phenolic compounds. Therefore, oxygen radical absorbance capacity (ORAC) values of the all compounds (**1**–**8**) were measured (Table 2) and compound **3** showed the strongest antioxidant activity.[13]

Table 2. ORAC values of compounds **1**–**8** obtained from *C. fargesii*

Compound	mmol Trolox equivalent/g
1	4.33
2	4.07
3	5.46
4	5.22
5	3.61
6	3.73
7	3.47
8	2.12

EXPERIMENTAL

Optical rotations were recorded on a Perkin-Elmer 341 digital polarimeter (Perkin-Elmer, Norwalk, CT, USA). Infrared (IR) spectra were obtained by a Nicolet 6700 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) with KBr pellets. The NMR spectra were measured in acetone at 27 °C, using a Bruker Avance 500 spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C) (Bruker Biospin AG, Fällanden, Switzerland). Coupling constants and chemical shifts were given in Hz and on a δ (ppm) scale, respectively. Lquid chromatography–mass spectrometry IT-TOF (LC-MS/IT-TOF) was obtained using a JEOL JMS-T100TD spectrometer (JEOL Ltd., Tokyo, Japan). The ORAC values were measured using ARVO X2 multilabel reader (PerkinElmer, MA). Column chromatography (CC) was obtained using Sephadex LH-20 (25–100 μm; GE Healthcare Bio-Science AB, Uppsala, Sweden), MCI gel CHP 20P (75–150 μm; Mitsubishi Chemical), and Toyopearl Butyl-650**C** (TOSOH Co., Tokyo, Japan) columns. TLC was performed on precoated Kieselgel 60 F_{254} plates (0.2 mm thick; Merck, Darmstadt, Germany) with toluene–HCO_2Et–HCO_2H (1:7:1, v/v) as the solvent, and spots were detected by praying with a 2% ethanolic $FeCl_3$.

Plant materials

The bark of *C. fargesii* was collected at Guangxi Institute of Botany, Guangxi Province, China, in August 2014, and identified by Prof. Shi-Hong Lu. The voucher specimen (2140821) as deposited in the Guangxi Key Laboratory of Functional Phytochemicals Research and Utilization, Guangxi Institute of Botany, China.

Extraction and separation

The fresh bark of *C. fargesii* (3.50 kg) was cut into small pieces and extracted with EtOH/H_2O (8:2, v/v, 20 L) by maceration at room temperature for 72 h. The extraction was repeated three times. After filtration, the filtrate was combined and concentrated under reduced pressure to give an aqueous solution. The solution was applied to a Sephadex LH-20 CC (8 cm i.d.×40 cm) with 0–100% MeOH (20% stepwise elution, each 1.5 L) to give seven fractions: frs 1 (2.42 g), 2 (12.45 g), 3 (11.74 g), 4 (22.02 g), 5 (28.59 g), 6 (19.55 g), 7 (23.30 g). The fraction 4 was subjected to MCI gel CHP 20P (4 cm i.d.×40 cm) with H_2O containing increasing amounts of MeOH (20–100%, 20% stepwise elution, each 1.0 L) to give nine fractions: frs 4-1 (2.40 g), 2 (0.82 g), 3 (0.83 g), 4 (1.07 g), 5 (0.95 g), 6 (1.87 g), 7 (2.57 g), 8 (2.00 g), and 9 (3.38 g). Fr. 4-2 was dissolved in EtOH and separated by Sephadex LH-20 (2.5 cm i.d.×25 cm) with EtOH-H_2O (100:0–50:50, 10% stepwise elution, each 0.2 L) and finally 100% MeOH to yield **8** (123 mg). Fraction 4-6 was purified using a Sephadex LH-20 column (2.5 cm i.d.×25 cm) with 0–100% MeOH in H_2O as the mobile phase (10% stepwise, each 0.2 L) and to yield compound **7** (22 mg). Fraction 4-7 further fractionated by Sephadex LH-20 (2.5 cm i.d.×25 cm) with EtOH-H_2O (100:0–50:50, 10% stepwise elution, each 0.2 L) and finally 100% MeOH, MCI gel CHP 20P with MeOH–H_2O (0–100%, 10% stepwise elution, each 0.2 L), and Toyopearl Butyl-650**C** with MeOH–H_2O (0–100%, 10% stepwise elution, each 0.1 L) to yield compounds **1** (12 mg), **2** (13 mg), **3** (6 mg), **4** (25 mg), **5** (16 mg), and **6** (40 mg).

Compound **1**, white amorphous powder, $[\alpha]_D^{28}$ +32.2 (*c* 0.6, MeOH); LC-MS/IT-TOF *m/z*: 425.0724 $[M-H]^-$ (Calcd for $C_{18}H_{17}O_{12}$, 425.0725) and 449.0736 $[M+Na]^+$ (Calcd for $C_{18}H_{18}O_{12}Na$, 449.0690); IR $\nu_{max}cm^{-1}$: 3359, 2351, 1740, 1648, 1557, 1186; UV λ_{max} (MeOH) nm (log ε): 262 (4.42), 211 (5.02); ^{1}H-NMR (125 MHz, acetone-d_6) and ^{13}C (500 MHz, acetone-d_6). See Table 1.

Compound **2**, white amorphous powder, $[\alpha]_D^{28}$ -16.3 (*c* 0.5, MeOH); LC-MS/IT-TOF *m/z*: 491.1174 $[M+Na]^+$ (Calcd for $C_{21}H_{24}O_{12}Na$, 491.1160); IR $\nu_{max}cm^{-1}$: 3351, 2356, 1692, 1613, 1195; UV λ_{max} (MeOH) nm (log ε): 259 (4.36), 208 (5.23); ^{1}H-NMR (125 MHz, acetone-d_6) and ^{13}C (500 MHz, acetone-d_6). See Table 1.

Antioxidant activity assays

Measurement of the ORAC values was performed using previously described methods.[1,14,15] The ORAC value was calculated on the basis of the standard curve for Trolox, and expressed as millimoles of Trolox equivalent to the samples (mmol Trolox equivalent/g) and averages were shown in Table 2.

ACKNOWLEDGEMENTS

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