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TWO NEW GALLOYL GLUCOSIDES FROM THE LEAVES OF *CASTANOPSIS FORDII*

Yong-Lin Huang,[a]* Zhang-Bin Liu,[a] Ya-Feng Wang,[a] Li Ge,[b] Rui-Jie He,[a] Jin-Lei Liu,[a] Ke-Di Yang, [b]* and Dian-Peng Li [a]

[a]Guangxi Key Laboratory of Functional Phytochemicals Research and Utilization, Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences, Guilin 541006, China. [b]Gaungxi university, Nanning 530004, China

Abstract – Two new galloyl glucosides, furan-2-carbonyl 6-*C*-β-D-(6'-*O*-galloyl)glucopyranoside (**1**), grasshopper ketone 5-*O*-β-D-(6'-*O*-galloyl)glucopyranoside (**2**), and one known compound 4-quinolone-2-carboxylic acid, were isolated from ethanol extract of the fresh leaves of *Castanopsis fordii* (Fagaceae). The chemical structures of these compounds were determined by one and two dimensional (1D and 2D)-NMR, liquid chromatography/time-of-flight/mass spectrometry (LC/TOF/MS), chemical evidence, and comparison with the literature.

Castanopsis is a genus belonging to the Fagaceae family. In previous studies, the main of the secondary metabolites from *Castanopsis* were found to be tannins and related compounds,[1-5] including polyphenols and flavonoids from *C. fordii*.[6,7] In the course of a chemical study on tannins and related compounds from *Castanopsis* species, the ethanol extract of the fresh leaves of *C. fordii* was subjected to a more careful investigation leading to the isolation of three compounds including two new ones. In this paper, we describe the isolation, structure determination of the two new galloyl glucosides from the fresh leaves of *C. fordii*.

Fresh leaves of *C. fordii* were extracted with 80% aqueous ethanol, and the extract was subjected to a combination of column chromatography using Sephadex LH-20, MCI gel CHP 20P, Toyopearl HW-40F, and Toyoperal Butyl-650C to afford three compounds, including the two new galloyl glucosides **1**, **2**, and one known compound **3** (Figure 1). The known compound **3** was elucidated on the basis of spectroscopic and comparison with literature data as 4-quinolone-2-carboxylic acid.[8]

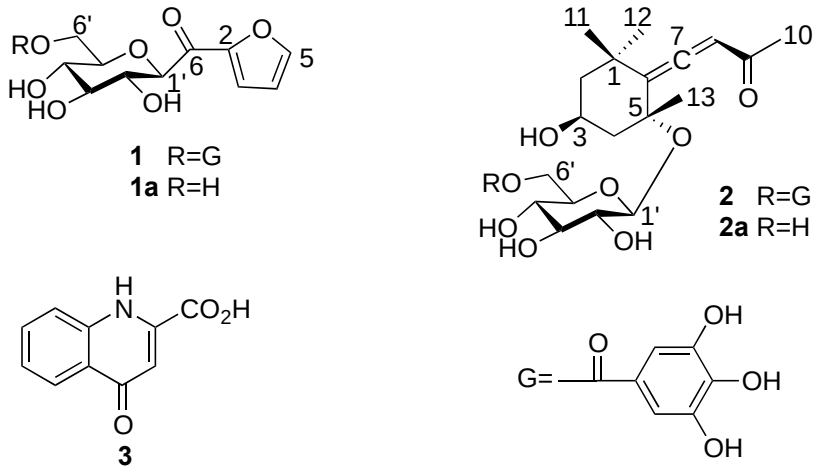

Figure 1. Structures of compounds **1**, **1a**, **2**, **2a** and **3**

Compound **1** was isolated as an amorphous powder and gave a dark blue coloration after reaction with ethanolic $FeCl_3$, suggesting the presence of a pyrogallol ring in the molecule. The molecular formula was determined to be $C_{18}H_{18}O_{11}$ by the liquid chromatography/time-of-flight/mass spectrometry (LC/TOF/MS), which showed the $[M-H]^-$ peak at *m/z* 409.0728 (Calcd for $C_{18}H_{17}O_{11}$, 409.0776). In the ^1H NMR spectra (Table 1) showed the presence of one 2-furanyl group from the proton signals at δ_H 7.73 (1H, d, J = 1.7 Hz), 6.50 (1H, dd, J = 3.7, 1.7 Hz), and 7.48 (1H, d, J = 3.7 Hz), and analysis of the ^{13}C NMR spectra data showed that this compound contained a furan-2-carbonyl moiety from signals at δ_C 150.9, 123.9, 113.4 and 150.1, and one carbonyl group at δ_C 185.5.[9] The ^1H NMR spectra showed one anomeric proton singlet appeared at δ_H 4.43 (1H, d, J = 9.6 Hz) and six proton signals appeared at δ_H 3.63 to 4.50 were suggested to be one sugar moiety, and this was supported by ^{13}C NMR signals at δ_C 80.1, 71.6, 70.2, 77.4, 78.3, and 64.3. In addition, this sugar moiety was confirmed as a C-β-glucopyranosyl unit by comparing the chemical shift and coupling constant of those compounds isoorientin and vitexin.[10,11] A two-proton singlet at δ_H 6.99 (2H, s) suggested that the presence one galloyl group, and supported by

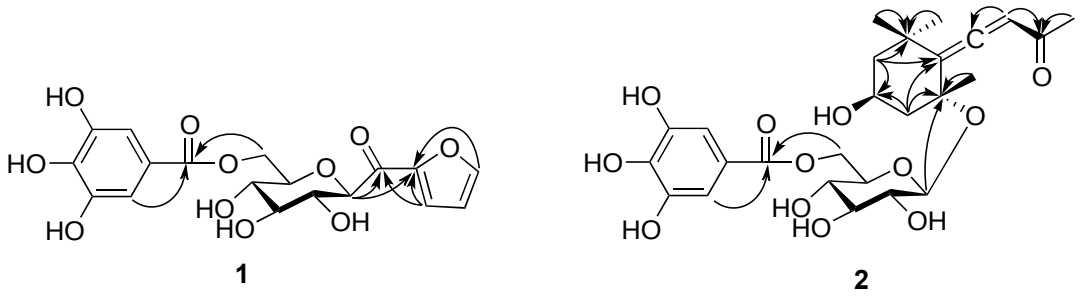

Figure 2. Selected HMBC correlations (H to C) **1** and **2**

^{13}C NMR signals at δ_C 120.5, 110.0 (2C), 145.2 (2C), 138.7, and 167.8. The ester of furan-2-carbonyl group is located at glc-C-1, and the galloyl group is located at glc-C-6, respectively. These were confirmed by the HMBC correlations of H-3 and H-1' with C-6 (δ_C 185.5), and H-6' with galloyl-C-7 (δ_C 167.8). Finally, enzymatic hydrolysis of **1** with tannase, which yielded gallic acid and **1a** (scleropentaside A).[9] Based on these spectroscopic and chemical results, the structure of **1** was concluded to be furan-2-carbonyl C-β-D-(6'-*O*-galloyl)glucopyranoside.

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

	1			**2**	
Position	^{1}H	^{13}C	Position	^{1}H	^{13}C
1			1		35.5
2		150.9	2	1.80 (ddd, 12.4, 3.6, 1.7)	19.6
				1.15 (dd, 12.4, 11.0)	
3	7.73 (d, 3.7)	123.9	3	4.39 (m)	61.2
4	6.50 (dd, 3.7, 1.7)	113.4	4	2.37 (m)	46.4
				1.19 (m)	
5	7.48 (d, 1.7)	150.1	5		77.1
6		185.5	6		117.4
			7		210.5
			8	5.78 (s)	99.8
			9		197.5
			10	2.08 (s)	26.3
			11	1.22 (s)	29.0
			12	1.29 (s)	26.4
			13	0.96 (s)	31.7
1'	4.43 (d, 9.6)	80.1	1'	4.43 (d, 8.2)	97.0
2'	3.63 (dd, 9.6, 9.0)	71.6	2'	3.97 (dd, 9.7, 8.2)	73.5
3'	3.48 (dd, 9.0, 8.5)	70.2	3'	4.14 (t, 9.7)	77.0
4'	3.56 (dd, 9.0, 8.5)	77.4	4'	3.25 (t, 9.7)	70.0
5'	3.78 (m)	78.3	5'	3.76 (m)	73.7
6'	4.30 (dd, 12.2, 6.2)	64.3	6'	4.12 (dd, 11.8, 6.3)	63.6
	4.50 (dd, 12.2, 2.2)			4.40 (dd, 11.8, 2.0)	
galloyl			galloyl		
1		120.5	1		119.5
2, 6	6.99 (s)	110.0	2, 6	6.93 (s)	108.7
3, 5		145.2	3, 5		145.5
4		138.7	4		138.4
7		167.8	7		165.8

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

Compound **2** also gave dark blue coloration with ethanolic $FeCl_3$ reagent, and the molecular formula $C_{26}H_{34}O_{12}$ was decided from the $[M-H]^-$ peak at *m/z* 537.1931 in the LC/TOF/MS (Calcd for $C_{26}H_{33}O_{12}$, 537.1978). The ^1H and ^{13}C NMR exhibited a two-proton singlet at δ_H 6.93 (2H, s), and δ_C 119.5, 108.7 (2C), 145.5 (2C), 138.4, and 165.8 indicated that **2** had a galloyl group. The ^1H NMR showed an anomeric proton signal at δ_H 4.43 (1H, d, J = 8.2 Hz) suggest that **2** had a β-glucopyranose moiety, and this was supported by ^{13}C NMR signals at δ_C 97.0, 73.5, 77.0, 70.0, 73.7, and 63.6. As the aglycone, the appearance of signals four tertiary methyl groups [δ_H 2.08, 1.22, 0.96, and 1.29; δ_C 26.3, 29.0, 31.7, and 26.4], one oxy-bearing methine [δ_H 4.39; δ_C 61.2], two sp^3 quaternary carbons [δ_C 35.5 and 77.1], two methylenes [δ_H 1.80 (1H, ddd, J = 12.4, 3.6, 1.7 Hz), 1.15 (1H, dd, J = 12.4, 11.0 Hz), 2.37 (1H, m), and 1.19 (1H, m), δ_C 19.6 and 46.4], an allenic unit [δ_H 5.78 (1H, s), δ_C 117.4, 210.5, and 99.8], and a carbonyl carbon (δ_C 197.5), suggested that the aglycone was grasshopper ketone.[12] Enzymatic hydrolysis with tannase **2** yielded gallic acid and **2a** (citroside A), confirming that **2** is a galloyl ester of citroside A.[12] The galloyl moiety was determined to be located at glc-C-6 by the HMBC correlations of H-6 (δ_H 4.12 and 4.40) with galloyl-C-7 (δ_C 165.8). Based on these spectroscopic and chemical results, the structure of **2** was concluded to be grasshopper ketone 5-*O*-β-D-(6'-*O*-galloyl)glucopyranoside.

The known compound of **3** was elucidated on the basis of spectroscopic data analysis as 4-quinolone-2-carboxylic acid.

EXPERIMENTAL

The NMR spectra were measured in acetone-d_6 or DMSO-d_6 at 27 °C, using a Bruker Avance 500 spectrometer (500 MHz for ^1H 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. Infrared (IR) spectra were obtained by a Nicolet 6700 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) with KBr pellets. Liquid chromatography/time-of-flight/mass spectrometry (LC/TOF/MS) was obtained using a JEOL JMS-T100TD spectrometer (JOL Ltd., Tokyo, Japan). Optical rotations were recorded on a Perkin-Elmer 341 digital polarimeter (Perkin-Elmer, Norwalk, CT, USA). Column chromatography (CC) was performed using Sephadex LH-20 (25–100 μm; GE Healthcare Bio-Science AB, Uppsala, Sweden), MCI gel CHP 20P (75–150 μm; Mitsubishi Chemical), Diaion HP20SS (Mitsubishi Chemical, Tokyo, Japan), and Toyopearl Butyl-650C (TOSH Co., Tokyo, Japan) columns with H_2O containing increasing proportion of MeOH (0-100%). The enzymatic hydrolysis used tannase (Shanghai Rui Qi Biology Technology Co., Ltd, China). 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 spraying with a 2% ethanolic $FeCl_3$.

Plant materials

The fresh leaves of *C. fordii* were collected at Yangshuo County, Guangxi Province, China, in August 2014, and identified by Prof. Shi-Hong Lu, Guangxi Institute of Botany. The voucher specimen (20140821) 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. fordii* (5.0 kg) was chipped into small pieces and extracted two times with EtOH/H_2O (8:2, v/v, 20 L) by maceration at room temperature. After filtration, the filtrate was combined and concentrated under reduced pressure to give an aqueous solution. The solution was subjected to Sephadex LH-20 (8 cm i.d.×40 cm, 0-100% MeOH in H_2O) column chromatography to give nine fractions. Fraction 2 (19.61 g) was separated by MCI gel CHP 20P (6.5 cm i.d.×35 cm, 0-100% MeOH in H_2O) column chromatography to give three fractions : frs 2-1 (8.91 g), 2 (9.04 g), and 3 (1.53 g). The fraction 2-1 was further separated by Diaion HP20SS (4.0 cm i.d.×32 cm, 0-100% MeOH in H_2O) and Toyopearl Butyl-650C (2.0 cm i.d.×27 cm, 0-100% MeOH in H_2O) column chromatography to give compound **1** (25 mg). Fraction 2-2 was purified using Diaion HP20SS (4.5 cm i.d.×27 cm, 0-100% MeOH in H_2O) column chromatography to yield five subfractions: frs 22-1 (2.75 g), 2 (1.69 g), 3 (1.72 g), 4 (0.88 g), 5 (1.28 g), and 6 (0.29 g). The fraction 22-2 was separated by Sephadex LH-20 (2.5 cm i.d.×32 cm, 0-100% MeOH in H_2O) and Diaion HP20SS (2.5 cm i.d.×27 cm, 0-100% MeOH in H_2O) column chromatography to afford compound **3** (31 mg). Fraction 22-4 was purified using Toyopearl Butyl-650C (2.0 cm i.d.×27 cm, 0-100% MeOH in H_2O) column chromatography to yield compound **2** (30 mg).

Compound 1, white amorphous powder, $[\alpha]_D^{28}$ -30.6 (*c* 0.6, MeOH); LC-MS/IT-TOF *m/z*: 409.0728 $[M-H]^-$ (Calcd for $C_{18}H_{17}O_{11}$, 409.0776); IR $\nu_{max}cm^{-1}$: 3321, 1806, 1769, 1618, 1557, 1498; UV λ_{max} (MeOH) nm (log ε): 282 (4.89), 218 (4.35); ^{1}H-NMR (125 MHz, acetone-d_6) and ^{13}C (500 MHz, acetone-d_6). See Table 1.

Compound 2, white amorphous powder, $[\alpha]_D^{28}$ -76.1 (*c* 0.4, MeOH); LC-MS/IT-TOF *m/z*: 537.1931 $[M-H]^-$ (Calcd for $C_{26}H_{33}O_{12}$, 537.1978); IR $\nu_{max}cm^{-1}$: 3389, 1900, 1712, 1318, 1186; UV λ_{max} (MeOH) nm (log ε): 268 (4.42), 225 (5.18); ^{1}H-NMR (125 MHz, DMSO-d_6) and ^{13}C (500 MHz, DMSO-d_6). See Table 1.

Tannase hydrolysis of 1 and 2

A solution of **1** (5.1 mg) in water (1.0 mL) was incubated with tannase at room temperature for 2 h. The reaction mixture was filtrated and the filtrate concentrated to dryness under reduced pressure. The residue was dissolved in EtOH and applied to a column of Sephadex LH-20 with EtOH to give gallic acid (1.7 mg) and **1a** (scleropentaside A, 2.3 mg); ^{1}H-NMR (125 MHz, DMSO-d_6) δ: 7.61 (1H, d, J = 3.7 Hz, H-3), 6.71 (1H, dd, J = 3.7, 1.7 Hz, H-4), 8.02 (1H, d, J = 1.7 Hz, H-5), 4.28 (1H, d, J = 9.6 Hz, H-1'), 3.45 (1H, dd, J = 9.6, 9.0 Hz, H-2'), 3.28 (1H, dd, J = 9.0, 8.5 Hz, H-3'), 3.13 (1H, dd, J = 9.0, 8.5 Hz, H-4'), 3.24 (1H, m, H-5'), 3.65 (1H, dd, J = 12.2, 2.2 Hz, H-6a'), 3.43 (1H, dd, J = 12.2, 6.2 Hz, H-6b'). Hydrolysis of **2** (6.0 mg) was also achieved in a similar manner to give gallic acid (1.6 mg) and **2a** (citroside A, 3.5 mg); ^{1}H-NMR (125 MHz, pyrdine-d_5) δ: 1.78 (1H, ddd, J = 12.4, 3.6, 1.7 Hz, H-2a), 1.13 (1H, dd, J = 12.4, 11.0 Hz, H-2b). 2.33 (1H, m, H-4a), 2.00 (1H, m, H-4b), 6.13 (3H, s, H-8), 2.25 (3H, s, H-10), 1.22 (3H, s, H-11), 1.65 (3H, s, H-12), 1.65 (3H, s, H-13), 4.43 (1H, d, J = 7.8 Hz, anomeric). The gallic acid was confirmed by silica gel TLC comparison with the standard [R_f 0.8, toluene:HCO_2Et:HCO_2H (1:7:1), characteristic blue coloration with $FeCl_3$ reagent].

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