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Two new phenolic glycosides isolated from the fruits of *Citrus aurantium*

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[ABSTRACT] The present study was designed to investigate the chemical constituents of the fruit of *Citrus aurantium* L.. The compounds were isolated and purified by various chromatographic techniques, and their structures were elucidated on the basis of physicochemical properties and spectral data. Two new phenolic glycosides (compounds **1** and **2**) were obtained and identified as 1-*O*-3, 5-dihydroxyphenyl-(6-*O*-4-hydroxybenzoyl)-*β*-D-glucopyranoside (**1**) and 1-*O*-3, 5-dihydroxyphenyl-(6-*O*-3-methoxy-4-hydroxy benzoyl)-*β*-D-glucopyranoside (**2**), respectively.

[KEY WORDS] Phenolic glycoside; *Citrus aurantium* L.; Aurantii Fructus Immaturus; Phloroglucinol

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Introduction

Aurantii Fructus Immaturus (Zhishi in Chinese) is dried, immature fruit of *C. aurantium* L.. It has been used as an important traditional Chinese medicine (TCM) for relieving depression-like symptoms, such as pain, insomnia, sadness, and depression for more than 2 000 years [1]. Previous phytochemical investigations on Aurantii Fructus Immaturus have led to the isolation of volatile oils, flavonoids, phenolic glycosides, and alkaloids [2-3]. Some of these compounds have been shown to have various bioactivities [4], including antitumor [4], anti-hypertensive [5], anti-dyspepsia, anti-oxidative, anti-inflammatory [6], and anti-lipoxygenase activities [7]. In the present study, two new compounds named 1-*O*-3, 5-dihydroxyphenyl-(6-*O*-4-hydroxybenzoyl)-*β*-D-glucopyranoside (**1**) and 1-*O*-3, 5-dihydroxyphenyl-(6-*O*-3-methoxy-4-hydroxybenzoyl)-*β*-D-glucopyranoside (**2**) were isolated from the *n*-butyl alcohol fractions of 95% EtOH extract of the fruit of *C. aurantium* L.. This paper reports the isolation and structural elucidation of these two new phenolic glycosides (Fig. 1).

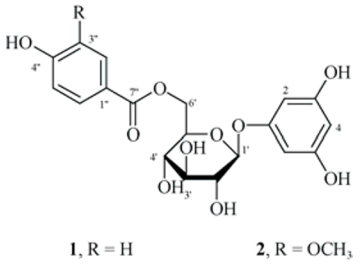

Fig. 1 Structures of compounds 1 and 2

Results and Discussion

Compound **1** was isolated as a white, amorphous powder. The IR spectrum of **1** indicated the presence of hydroxy (3 343 cm^{-1}), carbonyl (1 696 cm^{-1}), and aromatic ring signals (1 608 cm^{-1}, and 1 513 cm^{-1}). The molecular formula, $C_{19}H_{20}O_{10}$, was determined by HRESI-MS (*m/z* 431.094 4 $[M + Na]^+$, Calcd. for *m/z* 431.094 9). The ^1H NMR spectrum (Table 1) of compound **1** in DMSO-d_6 revealed three phenolic proton signals at δ_H 10.27 (1H, br s), 9.24 (2H, s), and signals at δ_H 5.95 (2H, d, *J* = 1.8Hz) and 5.91 (1H, d, *J* = 1.8 Hz) showed three substitutions in para [3]. Two additional doublets at δ_H 7.85 (2H, d, *J* = 8.7 Hz) and δ_H 6.84 (2H, d, *J* = 8.7 Hz), each integrating for two protons, revealed the presence of a symmetric phenolic derivative with two equivalent pairs of

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These authors have no conflict of interest to declare.

Table 1 The NMR data (400 MHz for ^{1}H, 100 MHz for ^{13}C, DMSO-d_6) of compounds 1 and 2 (*J* in Hz)

No.	1			2			
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC	NOESY
1		159.5, C			159.6, C		
2, 6	5.95 (2H, d, 1.8)	95.3, CH	C-1, C-3, C-4	5.94 (2H, s)	95.3, CH	C-1, C-3, C-4	
3, 5		159.3, C			159.3, C		
4	5.91 (1H, d, 1.8)	97.1, CH	C-1, C-2, C-3	5.91 (1H, s)	97.1, CH	C-2, C-3	
1′	4.80 (1H, d, 7.7)	100.5, CH	C-1, C-2′	4.80 (1H, d, 7.6)	100.5, CH	C-1, C-2′	
2′	3.23 (1H, m)	73.6, CH	C-1′	3.23 (1H, m)	73.5, CH	C-1′, C-3′	
3′	3.33 (1H, m)	76.8, CH	C-1′, C-4′, C-5′	3.32 (1H, m)	76.8, CH	C-1′, C-4′, C-5	
4′	3.27 (1H, m)	70.5, CH	C-1′, C-3′	3.25 (1H, m)	70.5, CH	C-1′, C-3′	
5′	3.65 (1H, t, 7.7)	74.3, CH	C-1′, C-4′	3.66 (1H, m)	74.3, CH	C-1′, C-4′	
6a	4.12 (1H, dd, 11.9, 7.1)	64.4, CH_2	C-5′, C-7"	4.13 (1H, dd, 11.8, 7.8)	64.5, CH_2	C-5′, C-7"	
6b	4.54 (1H, d, 11.7)			4.58 (1H, d, 11.8)			
1"		120.7, C			120.9, C		
2"	7.85 (1H, d, 8.7)	132.1, CH	C-3", C-4", C-7"	7.43 (1H, d, 1.8)	113.0,CH	C-1", C-3", C-4", C-6"	H-6", H-7
3",	6.84 (1H, d, 8.7)	115.8, CH	C-1", C-7"		151.9, C		
4"		162.4, C			147.8, C		
5"	6.84 (1H, d, 8.7)	115.8, CH	C-1", C-7"	6.86 (1H, d, 8.2)	115.7, CH	C-1",C-2", C-3", C-4", C-6", C-7"	H-6"
6"	7.85 (1H, d, 8.7)	132.1, CH	C-3", C-4", C-7"	7.53 (1H, dd, 8.2, 1.8)	124.2, CH	C-1",C-2", C-3", C-4", C-5", C-7"	H-2", H-5"
3-OH	9.24 (1H, s)		C-2", C-3", C-4"	9.23 (1H, s)		C-2",C-3", C-4"	
5-OH	9.24 (1H, s)		C-4", C-5", C-6"	9.23 (1H, s)		C-4",C-5", C-6"	
	3"-OCH_3			3.80 (3H, s)	56.0	C-2", C-4"	H-2"
4"-OH	10.27 (1H, s)			9.94 (1H, s)		C-3", C-4", C-5"	H-5"
7"		166.0			166.0		

ortho-coupled protons, suggesting a para substituted benzene [8]. The signal of a sugar unit at δ_H 4.80 (1H, d, *J* = 7.7 Hz) with the six carbonyl signals at δ_C 100.5, 76.8, 74.3, 73.6, 70.5, and 64.4 indicated the presence of glucose [9]. The ^{13}C NMR spectrum displayed a signal at δ_C 166.0, a chemical shift attributable to the carbonyl group of a benzophenone. Signals corresponding to 12 aromatic carbons were also detected (δ_C162.4, 159.5, 159.3, 132.1, 120.7, 115.8, 97.1, and 95.3); some of them being superimposed (δ_C 132.1, 2C; 115.8, 2C; and 95.3, 2C). These data were in good correlation with the hypothesis of a phloroglucinol and a benzophenone derivative with a second fully substituted aromatic ring, and a phenolic glycoside structure which has been reported previously [10]. An acid hydrolysis experiment proved the absolute configuration of the glucose was *D* by comparing its optical rotation value with that of authentic sample. Its anomeric proton at δ_H 4.80 (1H, d, *J* = 7.7 Hz) indicated *β*-configuration for the anomeric proton.

Meanwhile, the HMBC experiment exhibited correlations of **1** (Fig. 2) from 4.80 (H-1′) to 159.5 (C-1); 4.12, 4.54 (H-6a′, H-6b′) to 166.0 (C-7"); 7.85 (H-2") to 166.0 (C-7"); and 6.84 (H-3") to 166.0 (C-7"), which indicated that C-1 and C-7" were attached to the glucose C-1′ and C-6′, respectively. Thus,

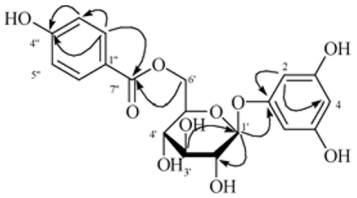

Fig. 2 The key HMBC (H→C) correlations of the moieties of compound 1

compound **1** was determined as 1-*O*-3, 5-dihydroxyphenyl-(6-*O*-4-hydroxybenzoyl)-*β*-D-glucopyranoside.

Compound **2** was isolated as a white, amorphous powder. The HRESI-MS peak at *m/z* 461.105 1 $[M + Na]^+$ (Calcd. for *m/z* 461.105 4) indicated its molecular formula to be $C_{20}H_{22}O_{11}$, with one methoxy more than that of compound **1** (431.094 4 $[M + Na]^+$). The IR and NMR spectra of compound **2** were almost identical to those of compound **1** (see Experimental section). However, the detailed NMR analysis showed an additional methoxy group at δ_H 3.80 (3H, s, -OCH_3) and δ_C 56.0 (3"-OCH_3). The ^{1}H NMR spectrum of Compound **2** in DMSO-d_6 revealed three phenolic proton signals at δ_H 9.94 (1H, s), 9.23 (2H, s). And δ_H 7.53 (1H, dd, *J* = 8.2, 1.8 Hz), 7.43 (1H, d, *J* = 1.8 Hz), and 6.86 (1H, d, *J* =

8.2 Hz) indicated a 1, 3, 4-trisubstituted pheny ring [11]. Signals at δ_H 5.94 (2H, s), 5.91 (1H, s), and 4.80 (1H, d, J = 7.6 Hz) were same as those of compound **1**. These data suggested that compound **2** was a 3"-methoxy derivative of compound **1**, which was further confirmed by NOESY experiments (Fig. 3) on compound **2**. The absence of correlations between δ_H 3.80 (3"-OCH_3) and δ_H 7.43 (H-2"); and δ_H 9.94 (4"-OH) and δ_H 6.86 (H-5") in the NOESY spectrum and the obvious cross-peak between δ_H 6.86 (H-5") and δ_H 7.43 (H-2") supported the existence of the 3-methoxy-4-hydroxy benzoyl fragment. The HMBC correlations from δ_H 4.80 (H-1′) to δ_C 159.6 (C-1), δ_H 4.13(H-6a′) to δ_C 166.0 (C-7"), δ_H 4.58 (H-6b′) to δ_C 166.0 (C-7"), and δ_H 7.43 (H-2") to δ_C 166.0 (C-7") indicated that C-1 and C-7" were attached to the glucose C-1′and C-6′, respectively. On the basis of the aforementioned results, the structure of compound **2** was determined to be 1-*O*-3, 5-dihydroxyphenyl-(6-*O*-3-methoxy-4- hydroxybenzoyl)-*β*-D-glucopyranoside.

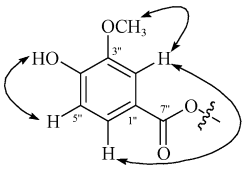

Fig. 3 The key data NOESY spectrum of compound 2

Experimental

Apparatus and Reagents

The optical rotations were determined on a Perkin-Elmer 241 polarimeter (Perkin-Elmer Co., Jena, Germany). The UV spectra were recorded on a Shimadzu-2201 (Shimadzu, Tokyo, Japan). The IR spectra were recorded by a Bruker IFS-55 infraredspectro photometer (Bruker Co., Zurich, Switzerland). The NMR spectral data were recorded on Bruker AV-600 (400 MHz for ^{1}H and 100 MHz for ^{13}C) with tetramethylsilane (TME) as the internal standard (Bruker Co., Zurich, Switzerland). The CD spectra were measured on Bio-Logic MOS 450 (Bio-Logic, Paris, France). Precise molecular weights were measured on a Waters Mass Spectrophotometer (Waters Co., Milford, MI, USA). Analytical HPLC was carried out on a Shimadzu LC-10AT liquid chromatography (Shimadzu, Kyoto, Japan). Preparative HPLC separation was performed on a YMC-Pack ODS-A column, in an HPLC system equipped with a Shimadzu LC-8A pump and a Shimadzu SPD-10A UV-vis detector. Silica gel (200-300 mesh, Qingdao Marine Chemical Factory, Qingdao, China) and Sephadex LH-20 (Pharmacia, Uppsala, Sweden) were employed in open column chromatography operation.

Plant materials

Aurantii Fructus Immaturus was purchased from Anguo Changda Chinese Herbal Pieces Ltd. (Production license number: Hebei province Y20050130, Batch number: 100701, Anguo, China), and authenticated by Prof. LU Jin-Cai (School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, China) A voucher specimen was deposited in the School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, China.

Extraction and isolation

The dried fruit of *C. aurantium* L. (8 kg) was extracted using conventional heating reflux method with 95% ethanol and the solvent was removed by vacuum rotary evaporator, to afford the crude extract (ca. 1.25 L). The crude extract was successively partitioned with petroleum ether, ethyl acetate, and *n*-butanol to yield three layers of extracts. The *n*-butanol layer extract (780 g) was subjected to silica gel column chromatography eluted with methylene chloride/methanol in a polarity increasing gradient manner (100 : 0 to 0 : 100) to give 6 fractions (Fr. 1-6). Fraction 5 (100 g) was further separated by another silica gel column (CH_2Cl_2-CH_3OH, 100 : 0 to 0 : 100) to afford 6 fractions (Fr. 5a-5f). Fraction 5f (2.5 g) was purified by Sephadex LH-20 column chromatography and preparative HPLC (MeOH/H_2O, 33 : 67, *V/V*; 4 mL·min^{-1}; 210 nm) to obtain compounds **1** (t_R = 14 min; 12.4 mg) and **2** (t_R = 18 min; 10.3 mg).

Hydrolysis of compounds 1 and 2

A solution of compounds **1** and **2** (each 7.5 mg) in MeOH (2.5 mL) was treated separately with 5% H_2SO_4 (2.5 mL) at 80 ºC for 4 h. The hydrolyzed mixture was neutralized to pH = 7 with $Ba(OH)_2$ and concondenseed to dryness under reduced pressure. The reaction mixture was diluted with H_2O and extracted with EtoAc. The H_2O layer was condensed to dryness. The monosaccharide was isolated and determined to be cymarose for compounds **1** and **2** by means of preparative TLC developed by EtoAc : Py : EtOH : H_2O (8 : 1 : 1 : 2, R_f = 0.48) and comparing with authentic sample. The absolute configuration was considered to be *D*-form for compound **1** by its optical value: cymarose $[\alpha]_D^{20}$ +48.39 (*c* 0.04, H_2O).

Identification

1-*O*-3, 5-dihydroxyphenyl-(6-*O*-4-hydroxybenzoyl)-*β*-D-glucopyranoside (1) White powder; HRESI-MS *m/z* 431.094 4 $[M + Na]^+$ (Calcd. for $C_{19}H_{20}NaO_{10}$, 431.094 9). $[\alpha]_D^{20}$ –32.41 (*c* 0.1, MeOH). UV (MeOH, λ_{max}, nm): 258.6, 214.0. IR (KBr) υ_{max} 3 342 (OH), 1 696 (C=O), 1 607, 1 513.6 (C=C), 1 488, 1 383, 1 283, 1 167, 1 073, 830, 770, 616.9 cm^{-1}. CD (CH_3OH, $\Delta\varepsilon$) λ_{max} 218 (–12.01), 235.5 (–13.07), 267.5 (–46.47), 285 (0.49) nm. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 3.23(1H, m, H-2′), 3.33 (1H, m, H-3′), 3.27 (1H, m, H-4′), 3.65 (1H, t, J = 7.7 Hz, H-5′), 4.12 (1H, dd, J = 11.9, 7.1 Hz, H_a-6′), 4.54 (1H, d, J = 11.7 Hz, H_b-6′), 4.80 (1H, d, J = 8.2 Hz, H-1′), 5.19 (1H, d, J = 4.3 Hz, sugar-OH), 5.32 (2H, d, J = 3.4 Hz, sugar-OH), 5.91 (1H, d, J = 1.8 Hz, H-4), 5.95 (2H, d, J = 1.8Hz, H-2, H-6), 6.84 (2H, d, J = 8.7 Hz, H-3", H-5"), 7.85 (2H, d, J = 8.7 Hz, H-2", H-6"), 9.24 (2H, s, 3,5-OH), 10.27 (1H, br s, 4"-OH). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 159.5 (C-1); 95.3 (C-2, C-6); 159.3 (C-3, C-5); 97.1 (C-4); 100.5 (C-1′); 73.6 (C-2′); 76.8 (C-3′); 70.5 (C-4′); 74.3 (C-5′); 64.4 (C-6′); 120.7 (C-1"); 132.1 (C-2", C-6"); 115.8 (C-3", C-5"); 162.4 (C-4"); and 166.0 (C-7").

1-*O*-3, 5-dihydroxyphenyl-(6-*O*-3-methoxy-4-hydroxybenzoyl)-*β*-D-glucopyranoside (2) White powder; HRESI-MS *m/z* 461.1051 $[M + Na]^+$ (Calcd. for $C_{20}H_{22}NaO_{11}$, 461.105 4). $[\alpha]_D^{20}$ -48.39 (*c* 0.06, MeOH). UV (MeOH, λ_{max}, nm): 261.6, 221.0, 212.0. IR (KBr) υ_{max} 3 358 (OH), 2 919, 1 697 (C=O), 1 606, 1 514.9 (C=C), 1 488, 1 429, 1 383, 1 286, 1 222, 1 074, 830, 763.8, 625.4 cm^{-1}. CD (CH_3OH, $\Delta\varepsilon$) λ_{max} 217.5 (–34.92), 236.5 (–14.73), 266.5 (–58.01), 285 (–25.56) nm. ^1H NMR (400 MHz, DMSO-d_6) δ_H: 3.80 (3H, s, 3"-OCH_3), 4.13 (1H, dd, *J* = 11.6, 7.1 Hz, H_a-6′), 4.58 (1H, d, *J* = 11.6 Hz, H_b-6′), 4.80 (1H, d, *J* = 7.5 Hz, H-1′), 5.20 (1H, s, sugar-OH), 5.35 (1H, d, *J* = 2.7 Hz, sugar-OH), 5.91 (1H, s, H-4), 5.94 (2H, s, H-2, H-6), 6.86 (1H, d, *J* = 8.2 Hz, H-5"), 7.43 (1H, d, *J* = 1.8 Hz, H-2"), 7.53 (1H, dd, *J* = 8.2, 1.8 Hz, H-6"), 9.23 (2H, s, 3, 5-OH), 9.94 (1H, s, 4"-OH). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 159.6 (C-1); 95.3 (C-2, C-6); 159.3 (C-3, C-5); 97.1 (C-4); 100.5 (C-1′); 76.8 (C-3′); 74.3 (C-5′); 73.5 (C-2′); 70.5 (C-4′); 64.5 (C-6′); 120.9 (C-1"); 113.0 (C-2"); 151.9 (C-3"); 147.8 (C-4"); 115.7 (C-5"); 124.2 (C-6"); 166.0 (C-7"); and 56.0 (3"-OCH_3).

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