






Two New Disaccharide Glycosides from the Root Cortex of *Paeonia ostii*

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Abstract: Two new glycosides 2-hydroxy-4-methoxy-acetophenone-3-*O*-[β -D-apiofuranosyl (1 \rightarrow 6)- β -D-glucoside (**1**) and 4-hydroxy-2-*O*- β -rutosyl acetophenone (**2**), along with two known compounds, paeonolide (**3**), involcranoside B (**4**) were isolated from the roots of *Paeonia ostii*. In addition, compound **4** was isolated from this genus for the first time. Their structures were established on the basis of spectral and chemical evidence. All the compounds showed inactive nitric oxide (NO) inhibitory effects.

Keywords: *Paeonia ostii*; disaccharide glycosides; water-soluble extraction; anti-inflammatory. © 2022 ACG Publications. All rights reserved.

1. Introduction

Paeonia ostii, a member of important Paeoniaceae family plant, is well-known for its genuine features and cultivated in areas of Fenghuang Mountain of Anhui Province for medicinal uses for more than 500 years. Modern researches have shown that monoterpenoids, paeonols along with their related glycosides in its root cortex [1,2]. Among them, paeonol exhibited pharmacological effects such as anti-inflammatory, neuroprotective, anti-cardiovascular, anti-tumor and others [3,4]. Researchers have prepared derivatives and dosage forms to enhance its stability, bioavailability and activity [5,6]. In a previous paper, we reported acetoisovanillone glycosides from the root cortex of *P. ostii* [7]. Ongoing our phytochemical studies, two new disaccharide glycosides (**1** and **2**) were obtained from the water soluble extraction of the medical part of this plant. This article described the isolates as well as their anti-inflammatory *in vitro*.

2. Experimental

2.1. Plant Material

The root-barks of *P. ostii* were collected at Fenghuang Mountain of Tongling City Anhui Province, in September 2018, and authenticated by Prof. Cheng-wu Fang, Anhui University of Chinese

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Medicine. The voucher specimen was preserved at the specimen center of School of Pharmacy with ACM20180911.

2.2. General Experimental Procedures

Optical rotations were obtained on a Jasco P-1020 automatic digital polarimeter (JASCO, Japan). UV spectra were acquired on a Shimadzu UV-2401PC spectrophotometer (Shimadzu, Japan). IR spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer (Bruker, Germany) with KBr pellets. ESI-MS and HR-ESI-MS were performed on an API QSTAR Pulsar i mass spectrometer (MDS Sciqaszex, Canada). NMR spectra were measured on JEOL JNM 400 and JEOL JNM 600 MHz spectrometers (JEOL, Tokyo, Japan). Sephadex LH-20 (Amersham Biosciences AB, Sweden), D101 macroporous adsorption resin (Solarbio Biotechnology Co., Ltd, China) and silica gel G (200-300 mesh, Qindao Marine Chemical Ltd, China) were used for column chromatography. Semi-preparative HPLC was performed on an Agilent 1260 (Agilent technologies Inc, US) with a YMC-Pack-ODS-A (10.0 mm × 250 mm, S-5 μm, 12 nm) column; Prep-HPLC was executed on Agilent 1260 Infinity II Preparative (Agilent Technologies Inc, US) with an Agilent Zorbax 300 SBC18 column (21.2 mm × 150 mm, 5 μm). MPLC was performed on Flash System/Cheetah (Agela Technologies) with a Spherical C18 column (20-45 μm, 100 Å).

2.3. Extraction and Isolation

The root-barks of *P. ostii* coarse powder (50.0 Kg) were percolated with 85% ethanol (70 L) and the excess of the solvent was evaporated in vacuum at 50°C resulting in a concentrated solution (density 1.10, 80°C), which was suspended in distilled-water and extracted successively with Pet (60-90°C), EtOAc and *n*-BuOH to give each organic fraction, respectively. And the water phase was removed the organic solvents under reduced pressure at 50°C. The filtrate was passed through a macroporous absorbent resin (D101) column and eluted with distilled water and homeopathic alcohol successively. The homeopathic alcohol partial gum (435.76 g) was submitted to a column chromatography (silica gel, 200-300 mesh, 3.5 Kg) and eluted with a CHCl₃-MeOH gradient system (9 : 1 → 1 : 1) to give five fractions. *Fr.3* (4.7) was further purified by CC (silica gel, 200-300 mesh) eluting with suitable solvents systems (CH₂Cl₂-MeOH-H₂O), some sub-Fractions were passed through Sephadex LH-20 (MeOH-H₂O, 90: 10), then subjected to RP-18 on MPLC separation with MeOH-H₂O (15: 85 → 85:15) to afford *Fr3.1- Fr3.4*, which was passed through a Sephadex LH-20 CC (90% MeOH) and then was subjected to further separation on semi-HPLC with a YMC-Pack-ODS-A column (10.0 mm × 250 mm, S-5 μm, 12 nm) to afford **1** (28 mg, *t_R* = 25 min, MeOH-H₂O = 20: 80, 3 mL·min⁻¹, λ = 254/280 nm) from *Fr.3-1*, and **2** (17 mg, *t_R* = 19 min, MeOH-H₂O = 30 : 70, 2 mL·min⁻¹, λ = 210/280 nm) from *Fr.3-2*. *Fr.4* (11.2 g) was further purified by silica gel CC (Ø 8.0 × 50 cm, 80 g) eluted with CH₂Cl₂-MeOH (85: 15) to give *Fr4.1- Fr4.3*, which was passed through Sephadex LH-20 (80% MeOH) and then was further separation via prep-HPLC with a Agilent Zorbax 300 SBC18 column (21.2 mm × 150 mm, 5 μm) to afford **3** (90 mg, *t_R* = 15 min, MeOH-H₂O = 15: 85, 8 mL·min⁻¹, λ = 210 nm) from *Fr4.3*, and **4** (4 mg, *t_R* = 8 min, AcCN-H₂O = 15: 85, 6 mL·min⁻¹, λ = 210 nm) from *Fr4.2*.

2.4. Spectroscopic Data

2-hydroxy-4-methoxy-acetophenone-3-O-[β-D-apiofuranosyl(1→6)]-β-D-glucopyranoside (1): White amorphous powder, $[\alpha]_{\text{D}}^{17.1} -51.56$ (*c* 1.04, MeOH); UV (MeOH): λ_{max} (log ε) = 213 (4.22) nm, 281 (3.95) nm; IR (KBr): ν_{max} 3380, 1641, 1511, 1442 cm⁻¹; ESI-MS *m/z* 499 [M + Na]⁺; HRESIMS *m/z* 499.1422 [M + Na]⁺ (calcd for C₂₀H₂₈O₁₃Na, 499.1422). ¹H NMR (600 MHz, MeOD) and ¹³C NMR (150 MHz, MeOD) data, see Table 1.

4-hydroxy-2-O-β-rutinosyl-acetophenone (2): White amorphous powder, $[\alpha]_D^{170} - 50.57$ (c 1.20, MeOH); UV (MeOH): λ_{\max} (log ϵ) = 212 (4.06) nm, 268 (3.91) nm, 314 (3.48) nm; IR (KBr): ν_{\max} 3425, 1637, 1513, 1438 cm^{-1} ; ESI-MS m/z 461 $[\text{M} + \text{H}]^+$, 943 $[\text{2M} + \text{Na}]^+$; HRESIMS m/z 461.1661 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{20}\text{H}_{29}\text{O}_{12}$, 461.1654). ^1H NMR (600 MHz, MeOD) and ^{13}C NMR (150 MHz, MeOD) data, see Table 1.

Paeonolide (3): White amorphous powder; ESI-MS m/z 483 $[\text{M} + \text{Na}]^+$; ^1H NMR (600 MHz, MeOD) δ : 7.74 (1H, d, $J = 8.8$ Hz, H-6), 6.84 (1H, d, $J = 2.2$ Hz, H-3), 6.66 (1H, dd, $J = 8.8, 2.2$ Hz, H-5), 3.87 (3H, s, OCH_3), 2.64 (3H, s, H-8), 5.07 (1H, d, $J = 7.6$ Hz, H-1'), 4.26 (1H, d, $J = 6.9$ Hz, H-1''); ^{13}C NMR (150 MHz, MeOD) δ : 122.4 (s, C-1), 160.1 (s, C-2), 102.8 (d, C-3), 166.0 (s, C-4), 109.1 (d, C-5), 132.8 (d, C-6), 200.4 (s, C-7), 31.9 (q, C-8), 56.3 (q, OCH_3), 101.1 (d, C-1'), 74.5 (d, C-2'), 77.8 (d, C-3'), 71.0 (d, C-4'), 77.0 (d, C-5'), 69.3 (t, C-6'), 104.9 (d, C-1''), 72.1 (d, C-2''), 73.9 (d, C-3''), 69.5 (d, C-4''), 66.5 (d, C-5'').

Involcranoside B (4): White amorphous powder; ESI-MS m/z 467 $[\text{M} + \text{Na}]^+$; ^1H NMR (600 MHz, MeOD) δ : 7.98 (2H, d, $J = 8.9$ Hz, H-2, -6), 7.14 (1H, d, $J = 8.9$ Hz, H-3, -5), 2.57 (3H, s, H-8), 4.98 (1H, d, $J = 7.6$ Hz, H-1'), 4.69 (1H, br s, H-1''); ^{13}C NMR (150 MHz, MeOD) δ : 132.5 (s, C-1), 131.5 (d $\times 2$, C-2, -6), 117.1 (d $\times 2$, C-3, -5), 162.8 (s, C-4), 199.4 (s, C-7), 26.33 (q, C-8), 102.0 (d, C-1'), 74.6 (d, C-2'), 77.8 (d, C-3'), 71.3 (d, C-4'), 77.0 (d, C-5'), 67.7 (t, C-6'), 101.3 (d, C-1''), 72.0 (d, C-2''), 72.2 (d, C-3''), 73.9 (d, C-4''), 69.7 (d, C-5''), 17.7 (q, C-6'').

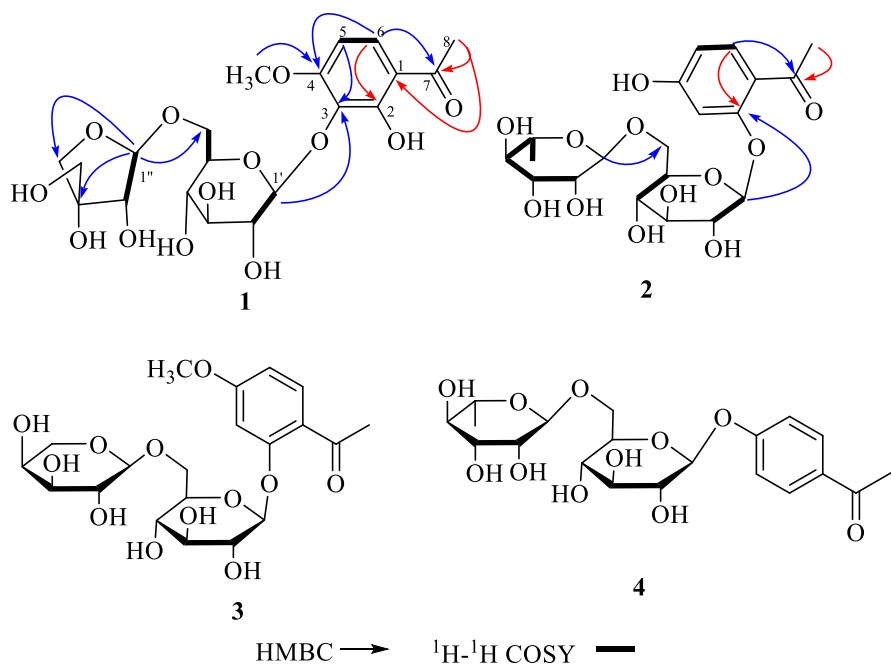


Figure 1. The structures of compounds 1-4 and the key correlations of compounds 1 and 2

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** was isolated as white amorphous powder. Its molecular formula was determined as $\text{C}_{20}\text{H}_{28}\text{O}_{13}$ m/z 499.1422 $[\text{M} + \text{Na}]^+$ (Calcd for $\text{C}_{20}\text{H}_{28}\text{O}_{13}\text{Na}$, 499.1422) from the HR-ESI-MS and ^{13}C -

NMR spectra. The ^1H -NMR spectrum (600 MHz, CD_3OD) (Table 1) presented an AB-system aromatic protons [δ 7.70, 6.67 (1H each, d, $J = 9.0$ Hz)], two anomeric characteristic protons at δ 4.89 (d, $J = 7.8$ Hz, H-1') and δ 4.92 (d, $J = 2.1$ Hz, H-1''), as well as two singlet methyl signals at δ 3.93 and 2.59. The ^{13}C -NMR spectrum presented 20 resonant carbon signals, including two methyls, three oxygenated methylenes, nine methanes and six quaternary carbons, which were assigned to an acetophenone aglycone, with two sugar units. Detailed comparison of the NMR spectroscopic data were in good agreement with 2-hydroxy-acetoisovanillone-3-*O*- β -D-glucopyranoside[7] except for one more pentose unit in **1**. Furthermore, it was found that the sugar signals of the **1** were consistent with those of apiopaeonoside[8]. The β -linkage of glucopyranosyl unit attached to C-3 was deduced from the HMBC correlations from H-5 (δ 6.67, d, $J = 9.0$ Hz) and H-1' (δ 4.89, d, $J = 7.8$ Hz) to C-3 (δ 132.6), and the D-apiose unit attached to C-6' was determined from H-1'' (δ 4.92) to (δ 66.7). The structure of the D-apiose unit was based on deduction from detailed analysis of 1D and 2D-NMR spectra. In the ^1H - ^1H COSY spectra, correlations of H-1''/H-2'', H-5''ax/H-5''eq were observed. Moreover, the small coupling constant $J_{1''2''} = 2.1$ Hz, indicated H-1'' and H-2'' located on the opposite of the furan ring, the C_{2-OH} and C_{3-OH} located on the ipsilateral of the sugar[8]. Others assignments depended on 2D-NMR correlations. Therefore, the structure of **1** was determined as 2-hydroxy-4-methoxy-acetophenone-3-*O*-[D-apio- β -D-furanosyl (1 \rightarrow 6)- β -D-glucopyranoside, named suffruticoside F (Figure 1).

Table 1. ^1H and ^{13}C NMR data of compounds **1-2** (at 600/150 MHz in CD_3OD , δ in ppm, J in Hz)

No.	1		2	
	δ_{C}	δ_{H}	δ_{C}	δ_{H}
1	115.3	-	116.2	-
2	155.9	-	164.9	-
3	132.6	-	104.9	6.58 (1H, d, 2.4)
4	158.6	-	165.5	
5	103.4	6.67 (1H, d, 9.0)	109.2	6.63 (1H, dd, 9.0, 2.4)
6	128.1	7.70 (1H, d, 9.0)	133.9	7.84 (1H, d, 9.0)
7	203.7	-	204.7	
8	25.6	2.59 (3H, s)	26.4	2.57 (3H, s)
OCH ₃	55.6	3.93 (3H, s)	-	-
1'	103.4	4.89 (1H, d, 7.8)	102.0	4.96 (1H, d, 7.2)
2'	74.2	3.49 (1H, dd, 9.2, 7.8)	74.5	3.46 (1H, m)
3'	76.5	3.40 (1H, t, 9.2)	77.8	3.47 (1H, m)
4'	70.3	3.32 (1H, t, 8.0)	71.3	3.37 (1H, m)
5'	76.2	3.34 (1H, ddd, 9.6, 6.0, 1.8)	77.0	3.71 (1H, dd, 9.0, 3.4)
6'	66.7	3.59 (1H, dd, 11.2, 6.0)	67.5	3.61 (1H, dd, 9.6, 6.4)
		3.86 (1H, dd, 11.2, 1.8)		4.01 (1H, dd, 9.4, 4.5)
1''	109.3	4.92 (1H, d, 2.1)	101.1	4.69 (1H, d, 1.8)
2''	76.6	3.76 (1H, d, 2.1)	71.9	3.87 (1H, dd, 3.6, 1.8)
3''	79.2	-	72.2	3.36 (1H, dd, 9.5, 3.6)
4''	64.3	3.50 (2H, s)	73.9	3.35 (1H, t, 9.5)
5''	73.6	3.79 (1H, d, 9.6)	69.7	3.63 (1H, m)
		3.66 (1H, d, 9.6)		
6''			17.7	1.19 (3H, d, $J = 6.0$)

Compound **2** was achieved as white amorphous powder. Its molecular formula was determined as $\text{C}_{20}\text{H}_{28}\text{O}_{12}$ based on the positive ion HR-ESI-MS at m/z 461.1661 [$\text{M}+\text{H}$]⁺ (Calcd for $\text{C}_{20}\text{H}_{29}\text{O}_{12}$ 461.1654) and ^{13}C NMR. The ^1H NMR spectrum (Table 1) appeared an ABX-system with aromatic protons [δ 7.84 (1H, d, $J = 9.0$ Hz), 6.58 (1H, d, $J = 2.4$ Hz), 6.63 (1H, dd, $J = 9.0, 2.4$ Hz)], two sets of anomeric protons appeared at δ 4.96 (d, $J = 7.4$ Hz) and δ 4.69 (d, $J = 1.8$ Hz), a singlet methyl signals at 2.57 (s), and a doublet methyl signals at 1.19 ($J = 6.0$ Hz). The ^{13}C -NMR spectrum exhibited 20 resonances signals, detailed analysis of them, which ascribable to a 2,4-di-oxysubstituted acetophenone with two hexose moieties. And the hexose moieties NMR spectroscopic properties were consistent with those of involcranoside B [9], the rutinoyl was deduced by the ^{13}C -NMR (Table 1) and the shift of C-6' was downfield to δ 67.5 ppm. And a small coupling of H-1'' ($J = 1.8$ Hz) hinted the

rhamnol was attached to C-6' with α -linkage. Furthermore, the expected HMBC (Figure 1) correlations of H-1' (δ 4.96, d, $J = 7.2$ Hz) and H-6 (δ 7.84, d, 9.0) to the carbon δ 164.9, indicated the disaccharide unit attached to C-2 via a β -linkage. Thus, the structure of compound **2** was identified as 4-hydroxyl-2-*O*- β -rutinosyl-acetophenone, named suffruticoside G.

The known isolates were identified as paeonolide (**3**)[10], involcranoside B (**4**)[9] based on MS and NMR data, and comparison with the references.

3.2. Anti-inflammatory Activity

The inhibitory effects of compounds **1–4** on nitric oxide production induced lipopolysaccharide were evaluated using a Griess assay[11]. All the isolates showed no obvious inhibitory effects, and positive quercetin IC₅₀ 15.23 \pm 0.65 μ M .

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Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

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