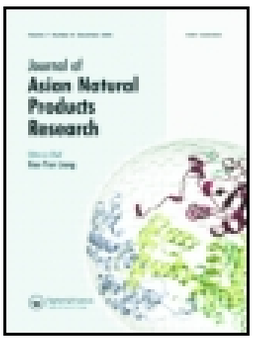

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Aromatic monoterpenoid glycosides from the seeds of *Paeonia ostii*

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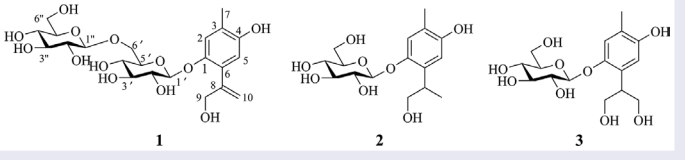
ABSTRACT

Phytochemical investigation into the seeds of *Paeonia ostii* T. Hong et J. X. Zhang (Paeoniaceae) led to the identification of three new aromatic monoterpenoid glycosides, named paeostisides A–C (**1–3**), along with one known compound. Their structures were identified by spectroscopic analysis and chemical method.



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Paeonia ostii; Paeoniaceae; monoterpenoid glycoside; paeostisides A–C

1. Introduction

Paeonia ostii T. Hong et J. X. Zhang (Paeoniaceae) is an ornamental and medicinal plant. The root barks of *P. ostii* together with *P. suffruticosa* Andr. have been used in traditional Chinese medicine as Cortex Moutan with heat-clearing, blood circulation promoting, and blood stasis dissipating effects [1]. The flowers of *P. ostii* are also used in aromatherapy with main constituents of (Z)-9-tricosene, ethyl myristate, and germacrone. [2]. The seeds of *P. ostii* were found to be rich in unsaturated fatty acids, and have been used as a new dietary oil source [3]. Previous phytochemical study on the seeds of *P. ostii* also revealed the existence of monoterpenoid glycosides, phenolic glycosides, flavonoid glycosides, and triterpenoids [4–6]. In order to know more about the polar components of the seeds of *P. ostii* for the purpose of finding potential application of the oil cake, systematically phytochemical investigation was undertaken. We herein report the isolation and structural elucidation of three new monoterpenoid glycosides, named paeostisides A–C, along with one known compound (Figure 1).

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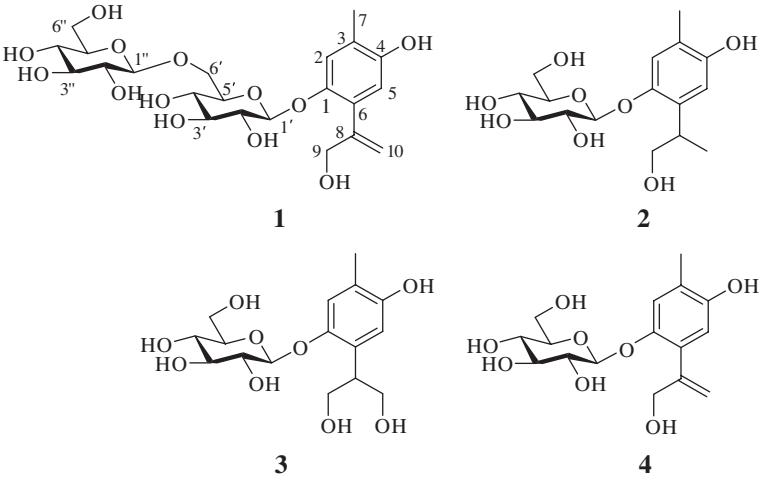

Figure 1. Structures of compounds **1**–**4**.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder. Its HRESIMS showed a quasi-molecular ion at *m/z* 527.1735 $[M + Na]^+$. In its ^1H NMR spectrum, two singlet aromatic proton signals at $\delta_{H\text{-}2}$ 6.98 (s, 1H) and $\delta_{H\text{-}5}$ 6.63 (s, 1H); two terminal olefinic proton signals at $\delta_{H\text{-}10a}$ 5.31 (br s, 1H) and $\delta_{H\text{-}10b}$ 5.11 (br s, 1H); two anomeric proton signals at $\delta_{H\text{-}1'}$ 4.86 (d, J = 7.4 Hz, 1H) and $\delta_{H\text{-}1'}$ 4.35 (d, J = 7.7 Hz, 1H); an oxygenated methylene at $\delta_{H\text{-}9}$ 4.44 (m, 2H); and a tertiary methyl at $\delta_{H\text{-}7}$ 2.20 (s, 3H) were recognized, as well as six aromatic carbon signals at δ_C 151.6, 148.9, 129.9, 126.0, 119.7, and 117.1; two olefinic carbons at δ_C 149.8 and 113.9; two anomeric carbon signals at δ_C 103.5 and 104.7 in its ^{13}C NMR spectrum, suggesting the existence of a 1,3,4,6-tetrasubstituted aromatic ring, a terminal olefinic function, and two saccharide moieties. HPLC-MS analysis of the hydrolytic product of compound **1** with appropriate derivatization [7], together with the large coupling constants of two anomeric proton signals, implied two sugar units as *β*-D-glucose. The HMBC correlations from H-7 to C-2, C-4; from H-9 to C-6, C-10; from H-10 to C-6, C-9; from H-1′ to C-1; and from H-1″ to C-6′ enabled deduction of the planar structure of **1** as shown in Figure 2. Its structure was further confirmed by NOE correlation signals between H-5 and H-9; between H-2 and H-1′ in the NOESY spectrum (Figure 3). Therefore, the structure of **1** was characterized as 4,9-dihydroxy-8(10)-ene-thymol-1-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside, and assigned the trivial name paeostiside A.

The ^{13}C NMR spectra of paeostiside B (**2**) and paeostiside C (**3**) revealed identical saccharide signals to those of the known compound 4,9-dihydroxy-8,10-dehydrothymol-1-*O*-*β*-D-glucoside (**4**) [4], and HPLC-MS analysis of their hydrolysates with appropriate derivatization confirmed the existence of D-glucose moiety in their structures. The molecular formula of **2** was determined to be $C_{16}H_{24}O_8$ based on the sodium adduct ion at *m/z* 367.1360 in its HRESIMS. Comparison of the ^1H and ^{13}C NMR data indicated that compound **2** differed from **4** only with the absence of the terminal olefinic function, and the presence of a secondary methyl (δ_H 1.18, d, J = 6.6 Hz, 3H; δ_C 17.4). In the HMBC spectrum of **2**, ^1H-^{13}C long-range correlation signals were observed between H-5 and C-8; between

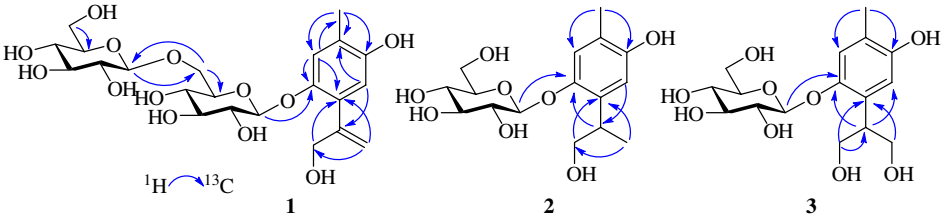

Figure 2. Key HMBC correlations of compounds **1**–**3**.

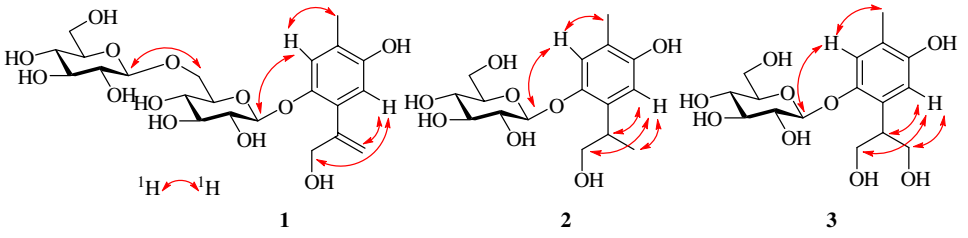

Figure 3. Key NOESY correlations of compounds **1**–**3**.

H-8 and C-1, C-5, C-9, C-10; between H-9 and C-6, C-10; and between H-10 and C-6, C-9 (Figure 2). Besides, NOE signals were found between H-5 and H-8, H-10 (Figure 3), which enabled characterization of the structure of **2** as 4,9-dihydroxy-thymol-1-*O*-*β*-D-glucopyranoside as shown in Figure 1. In comparison with ^{1}H and ^{13}C NMR spectra of **2**, the ^{1}H NMR spectrum of **3** showed another pair of methylene signals at δ_H 3.79 (dd, J = 10.8, 7.2 Hz, 1H) and δ_H 3.75 (dd, J = 10.8, 6.1 Hz, 1H), as well as the corresponding carbon signal at δ_C 64.5 in its ^{13}C NMR spectrum, with the loss of one methyl signal. The HMBC correlations from H-8 to C-1, C-5; from H-9 to C-6, C-8; and from H-10 to C-6, C-8 (Figure 2), and the NOE signals between H-2 and H-1′; and between H-5 and H-8 (Figure 3) revealed the structure of **3** as 4,9,10-trihydroxy-thymol-1-*O*-*β*-D-glucopyranoside as shown in Figure 1.

3. Experimental

3.1. General experimental procedures

Optical rotations were obtained on a PerkinElmer 341 polarimeter (PerkinElmer, Waltham, MA, USA). UV spectra were measured with a Cary 300-Bio UV-Visible spectrometer (Varian, Palo Alto, CA, USA). IR spectra were recorded on a Nicolet 6700 spectrometer (Thermo Scientific, Waltham, MA, USA). NMR data were obtained using a Varian-MERCURY Plus-400 (Varian, Palo Alto, CA, USA) or Bruker Advance III 500 (Bruker, Ettlingen, Germany) spectrometer with TMS as the internal reference. ESIMS analyses were carried out on a Shimadzu LC-MS-2020 (Shimadzu, Kyoto, Japan) with a Shimadzu SPD-M20 (Shimadzu, Kyoto, Japan) diode array detector using CNW C_{18} column (2.1 × 50 mm, 3.5 μm; Anpel Scientific Instrument Co., Ltd., Shanghai, China). HRESIMS analyses were performed on a Micromass Ultra Q-TOF mass spectrometer (Waters, Milford, MA, USA). Preparative HPLC was performed on a Unimicro EasySep TM-1010 binary pump system (Unimicro, Shanghai, China) with a Unimicro EasySepTM-1010 detector (Unimicro, Shanghai, China)

using a YMC-Pack ODS-A column (250 × 20 mm, 5 μm; YMC Co., Ltd., Kyoto, Japan) and a Diol-120-NP (250 × 20 mm, 5 μm; YMC Co., Ltd, Kyoto, Japan). Column chromatography (CC) was performed with Diol-gel (SMB100-20/45, Chromatorex, Fuji Silysia Chemical, Ltd., Kasugai, Japan), and C_{18} reversed-phase (RP-C_{18}) silica gel (150–200 mesh; Merck, Whitehouse Station, NJ, USA).

3.2. Plant material

The seeds of *Paeonia ostii* T. Hong et J. X. Zhang were collected in Bozhou, Anhui Province, China, in July 2014, and a voucher specimen has been identified by Prof. Jigang Han of Shanghai Chenshan Botanical Garden.

3.3. Extraction and isolation

Air-dried seeds of *P. ostii* (5 kg) were powdered and percolated with 95% EtOH (3 × 10 L) at room temperature to afford a crude extract (490 g). The extract was then suspended in 33% MeOH and filtrated, and the supernatant (300 g) was applied to RP-C_{18} gel CC eluted with MeOH/H_2O (from 3 to 100%, v/v) to yield eight fractions (Fr.1~8). Fr.3 (2.35 g) was further applied to Diol-120-NP CC with a petroleum ether/ethanol gradient (from 65 to 100%, v/v) as eluent to yield compound **3** (3.9 mg). Fr.4 (5 g) was subjected to Diol column and eluted with petroleum ether/ethanol (2:1→1:3, v/v) to give 13 subfractions (Fr.41~413). Fr.46 (450 mg) was purified by preparative HPLC with an eluent of 15% MeOH (MeOH/H_2O, v/v) to yield compounds **1** (5.2 mg) and **2** (3.5 mg). Fr.413 (560 mg) was subjected to preparative HPLC (MeOH/H_2O, 10:90→40:60, v/v) to yield compound **4** (21.6 mg).

3.3.1. Paeostiside A (1)

White amorphous powder; $[\alpha]_D^{20}$ − 28.6 (*c* 0.07, MeOH); UV (MeOH) λ_{max} nm (log ε) 203 (4.0), 286 (3.0); IR (KBr) ν_{max} 3358, 2922, 2852, 1659, 1632, 1412, 1040 cm^{-1}; for ^1H and ^{13}C NMR (CD_3OD) spectroscopic data, see Tables 1 and 2; HRESIMS: *m/z* 527.1735 $[M + Na]^+$ (calcd for $C_{22}H_{32}O_{13}Na$, 527.1741).

3.3.2. Paeostiside B (2)

White amorphous powder; $[\alpha]_D^{20}$ − 24.8 (*c* 0.11, MeOH); UV (MeOH) λ_{max} nm (log ε) 202 (3.9), 282 (2.8); IR (KBr) ν_{max} 3358, 2923, 2852, 1658, 1632, 1412, 1073, 1037 cm^{-1}; for ^1H and ^{13}C NMR (CD_3OD) spectroscopic data, see Tables 1 and 2; HRESIMS: *m/z* 367.1360 $[M + Na]^+$ (calcd for $C_{16}H_{24}O_8Na$, 367.1369).

3.3.3. Paeostiside C (3)

White amorphous powder; $[\alpha]_D^{20}$ − 50.0 (*c* 0.02, MeOH); UV (MeOH) λ_{max} nm (log ε) 203 (4.4), 283 (3.2); IR (KBr) ν_{max} 3358, 2923, 2852, 1658, 1632, 1410, 1066 cm^{-1}; for ^1H and ^{13}C NMR (CD_3OD) spectroscopic data, see Tables 1 and 2; HRESIMS: *m/z* 383.1320 $[M + Na]^+$ (calcd for $C_{16}H_{24}O_9Na$, 383.1313).

3.4. Hydrolyses of compounds 1–4

Several drops of 2N HCl were added to a solution of **1** (1 mg) in dioxane (1 ml) and the mixture was stirred at 90 °C for 3 h. The solvent was removed *in vacuo*, and the L-cysteine

Table 1. ^{1}H NMR spectral data of compounds **1**–**4** (500 MHz, δ in ppm, J in Hz, methanol-d_4).

Position	1	2	3	4
2	6.98, s	6.95, s	6.97, s	6.95, s
5	6.63, s	6.60, s	6.62, s	6.59, s
7	2.20, s	2.13, s	2.15, s	2.15, s
8		3.45–3.47, m	3.60–3.62, m	
9	4.43–4.45, m	3.59, dd (10.0, 6.6)	3.84, dd (10.7, 6.5)	4.39–4.41, m
	4.43–4.45, m	3.51, dd (10.0, 6.0)	3.59, dd (10.7, 6.0)	4.39–4.41, m
10	5.31, br s	1.18, d (6.6), 3H	3.79, dd (10.8, 7.2)	5.29, br s
	5.11, br s		3.75, dd (10.8, 6.1)	5.08, br s
1′	4.86, d (7.4)	4.73, d (7.3)	4.71, d (7.3)	4.76, d (7.3)
2′	3.43–3.45, m	3.44–3.46, m	3.42–3.44, m	3.22–3.24, m
3′	3.30–3.32, m	3.26–3.28, m	3.32–3.34, m	3.32–3.34, m
4′	3.41–3.43, m	3.28–3.30, m	3.35–3.37, m	3.33–3.35, m
5′	3.60–3.62, m	3.34–3.36, m	3.41–3.43, m	3.37–3.39, m
6′	4.17, dd (11.3, 2.0)	3.89, dd (12.3, 1.5)	3.87, dd (12.0, 2.2)	3.87, dd (12.0, 1.8)
	3.59, dd (11.3, 5.8)	3.69, dd (12.3, 5.1)	3.69, dd (12.0, 4.7)	3.66, dd (12.0, 5.1)
1″	4.35, d (7.7)			
2″	3.23–3.25, m			
3″	3.22–3.24, m			
4″	3.28–3.30, m			
5″	3.24–3.26, m			
6″	3.85, dd (11.8, 2.0)			
	3.66, dd (11.8, 5.5)			

Table 2. ^{13}C NMR spectral data of compounds **1**–**4** (125 MHz, methanol-d_4).

Position	1	2	3	4
1	148.9	145.0	150.4	149.0
2	119.7	120.2	121.2	120.0
3	126.0	124.0	124.6	126.0
4	151.6	151.9	152.1	151.6
5	117.1	113.8	114.7	117.0
6	129.9	133.6	130.5	130.0
7	16.4	16.1	16.1	16.2
8	149.8	35.2	43.8	149.8
9	65.8	68.9	64.8	65.7
10	113.9	17.4	64.5	113.8
1′	103.5	104.4	105.0	103.8
2′	75.1	75.3	75.2	75.2
3′	78.0	78.3	78.1	78.2
4′	71.4	71.5	71.5	71.5
5′	77.2	78.1	78.1	78.1
6′	70.0	62.7	62.6	62.6
1″	104.7			
2″	75.1			
3″	78.0			
4″	71.6			
5″	78.0			
6″	62.7			

methyl ester hydrochloride in pyridine was added. The reaction mixture was maintained at 60 °C for 1 h, and then *o*-tolyl isothiocyanate was added to the mixture and heated at 60 °C for another 1 h [7]. The reaction mixture was subjected to HPLC analysis with a UV detector (250 nm) at 40 °C characterized with a quasimolecular ion at *m/z* 447 $[M + H]^+$ using a CNW C_{18} column (2.1 × 50 mm, 3.5 µm) eluted with 23% CH_3CN-H_2O containing 0.2% formic acid at a flow rate of 0.3 ml/min. Authentic D- and L-glucose and compounds **2**–**4** were handled in a same manner. The retention times of the derivatives of sugar standards were

as follows: D-glucose (6.467 min), L-glucose (5.950 min), while the retention times of those of compounds **1–4** were at 6.450 min, 6.483 min, 6.500 min, and 6.517 min, respectively.

Supporting information

NMR spectra of compounds **1–4** together with the PDA chromatograms and mass spectra of the derivatives of hydrolysates of **1–4** are provided.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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