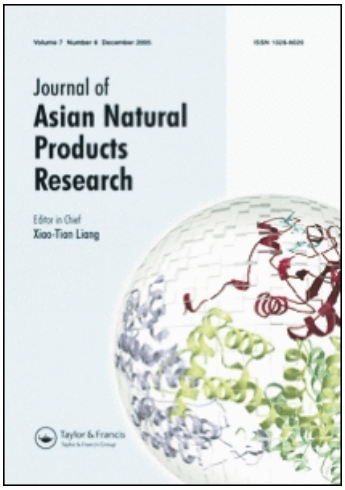

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Two new monoterpene glucosides, 4′-*O*-benzoylpaeoniflorin (**1**) and 4-*O*-galloylalbiflorin (**2**), were isolated from the 60% ethanol extract of the dried roots of *Paeonia lactiflora* Pall. Their structures were established on the basis of spectroscopic data.

Keywords: *Paeonia lactiflora* Pall.; monoterpene glucosides; 4′-*O*-benzoylpaeoniflorin; 4-*O*-galloylalbiflorin

1. Introduction

Paeonia lactiflora Pall. is one of the most important traditional Chinese medicines, which has been used as an anti-inflammatory, analgesic, sedative, antispasmodic, and astringent agents for a long time [1]. Intensive chemical investigations have been conducted and a series of monoterpene glycosides with a cage-like pinane skeleton were isolated from *P. lactiflora* [2,3]. In this paper, we report the isolation and structural elucidation of two new monoterpene glucosides, 4′-*O*-benzoylpaeoniflorin (**1**) and 4-*O*-galloylalbiflorin (**2**), from the 60% ethanol extract of *P. lactiflora.*

2. Results and discussion

Compound **1** was isolated as a colorless amorphous solid. The molecular formula was established to be $C_{30}H_{32}O_{12}$ based on HR-TOF-MS at *m*/*z* 607.1767 $[M + Na]^+$. The ^1H NMR spectrum of **1** showed two groups of monosubstituted phenyl signals at δ 8.13 (2H, m), 7.48 (1H, m), 7.31 (2H, t, $J = 8.0$ Hz) and δ 8.19 (2H, m), 7.48 (1H, m), 7.37 (2H, t, $J = 8.0$ Hz), an anomeric proton signal at δ 5.20 (1H, d, $J = 7.6$ Hz), an acetal signal at δ 5.94 (1H, s), and a methyl signal at δ 1.65 (3H, s). The ^{13}C NMR spectrum showed two groups of benzoyl signals at δ 130.7 (C-1″), 130.0 (C-2″, 6″), 128.8 (C-3″, 5″), 133.3 (C-4″), 166.7 (C-7″) and 131.0 (C-1‴), 130.1 (C-2‴, 6‴), 128.9 (C-3‴, 5‴), 133.3 (C-4‴), 166.3 (C-7‴), a group of sugar moiety signals at δ 100.5 (C-1′), 75.1 (C-2′), 76.2 (C-3′), 73.4 (C-4′), 75.9 (C-5′), 62.3 (C-6′), and a monoterpene aglycone moiety. The HMBC correlations (Figure 1) of H-7/C-1, C-2, C-4, C-5, H-9/C-2, C-4, C-6, C-8, and H-10/C-1, C-2, C-3 established the structure of the monoterpene aglycone, which was the same as that in paeoniflorin [2,4]. The location of the two benzoyl groups was established to be at C-8 and C-4′, respectively, according to the HMBC correlations of H-8/C-7″ and

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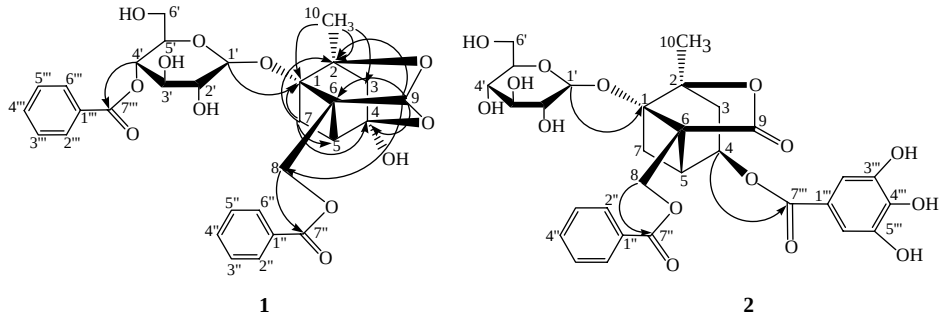
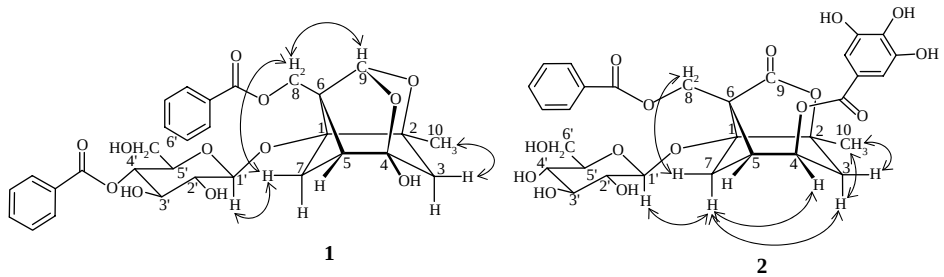


1 **2**

Figure 1. Key HMBC correlations of compounds **1** and **2**.

H-4′/C-7‴. Acid hydrolysis of **1** yielded D-glucose (D-Glc) by GC analysis with authentics as the reference [5]. The β-linkage of the glucosyl unit was determined by the coupling constant value of anomeric proton ($J = 7.6$ Hz). The HMBC spectrum showed the correlation between H-1′ and C-1, indicating that the glucose was connected to C-1. Thus, **1** was determined as 4′-*O*-benzoylpaeoniflorin. Its relative configuration was determined on the basis of NOESY correlations (Figure 2) between the signals of the following proton pairs (H-8/H-9; H-8/H-7; H-7/H-1′; H-10/H-3). Compound **1** represents the first example of the monoterpene glucosides in the genus *Paeonia* possessing a benzoyl substituent at C-4′.

Compound **2** was isolated as a white powder. The molecular formula was established as $C_{30}H_{32}O_{15}$ by HR-TOF-MS at *m*/*z* 631.1684 $[M-H]^-$. The ^{1}H NMR spectrum of **2** showed a group of signals assigned to a monosubstituted benzene ring at δ 8.20 (2H, d, $J = 7.2$ Hz), 7.34 (1H, m), 7.28 (2H, m), one singlet at δ 7.97 (2H, s) arising from a 1,3,4,5-tetrasubstituted benzene ring, and another singlet signal at δ 1.65 (3H, s) due to a methyl group which was directly linked to a quaternary carbon. The ^{13}C NMR spectrum showed a group of signals at δ 130.5 (C-1″), 130.0 (C-2″, 6″), 128.7 (C-3″, 5″), 133.2 (C-4″), 166.6 (C-7″), which was ascribable to a benzoyl group, a group of galloyl signals at δ 120.6 (C-1‴), 110.7 (C-2‴, 6‴), 147.4 (C-3‴, 5‴), 141.4 (C-4‴), and 166.5 (C-7‴), a monoterpene aglycone moiety, and a group of sugar moiety signals at δ 100.3 (C-1′), 74.8 (C-2′), 78.4 (C-3′), 71.6 (C-4′), 78.6 (C-5′), and 62.7 (C-6′). The β-D-Glc in compound **2** was confirmed by the NMR spectral data and acid hydrolysis followed by GC analysis [5]. According to the above analysis, the ^{1}H and ^{13}C NMR spectra of **2** were similar to those of albiflorin in showing the signals ascribable to a benzoyl group, a glucose moiety, and the same monoterpene aglycone, which



1 **2**

Figure 2. Important NOESY correlations of compounds **1** and **2**.

suggested that **2** was a derivative of albiflorin [2,6]. The difference was found in the presence of the galloyl signals (δ_H 7.97 (2H, s), δ_C 120.6, 110.7, 147.4, 141.4, and 166.5) in the NMR spectra of **2**. The HMBC correlation (Figure 1) of H-4 with C-7‴ indicated that the galloyl was connected to C-4, so the structure of **2** was elucidated as 4-*O*-galloylalbiflorin. The relative configuration of **2** was determined on the basis of NOESY correlations (Figure 2) between the signals of the following proton pairs (H-10/H-3; H-3α/H-7α; H-4/H-7α; H-7α/H-1′; H-8/H-7β). Compound **2** represents the first example of the monoterpene glucosides in the genus *Paeonia* possessing a galloyl substituent at C-4.

3. Experimental

3.1 General experimental procedures

The optical rotations were measured on a JASCOP-1020 digital polarimeter. The UV spectra were recorded on a SHIMADZU UV-2201 UV/vis recording spectrophotometer. The IR spectra were obtained using a Bruker IFS-55 plus spectrometer. The ESI-MS were taken on a Bruker Esquire 2000 mass spectrometer. The HR-TOF-MS were acquired using an Agilent 6210 mass spectrometer. The 1D and 2D NMR spectra were measured with a Bruker Avance-400 spectrometer and a Bruker Avance-600 spectrometer using C_5D_5N solvent. The silica gel (100–140 and 200–300 mesh) for column chromatography and silica gel GF_{254} for TLC were made by Qingdao Marine Chemical Factory of China. Sephadex LH-20 (Amershan Biosciences, Sunnyvale, CA, USA) and RP-18 silica gel (Merck, Darmstadt, Germany) were employed for column chromatography.

3.2 Plant material

The roots of *P. lactiflora* were collected in Pan'an city, Zhejiang Province, China, in May 2006, and were identified by Associate Prof. Qing He (Zhejiang University, Hangzhou, China). A voucher specimen (RMLPL-20060504) is deposited in the College of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University.

3.3 Extraction and isolation

The dry roots of *P. lactiflora* (10 kg) were refluxed thrice with 60% (v/v) EtOH. After concentration, the extract was suspended in H_2O, and then successively partitioned with $CHCl_3$, EtOAc, and *n*-BuOH. The EtOAc layer (63 g) was subjected to silica gel column chromatography and eluted with $CHCl_3$–MeOH of increasing polarity (100:0 → 0:100) to afford six fractions (fractions 1–6). Fraction 3 (10.3 g) was separated by Sephadex LH-20 column chromatography using $CHCl_3$–MeOH (1:1) as an eluent to give three fractions (fractions 31–33). Fraction 31 (6.5 g) was subjected to RP-18 silica gel column chromatography and eluted with MeOH–H_2O in gradient yielding eight fractions (fractions 311–318). Further separation of fraction 316 (1.3 g) was performed on preparative reversed-phase HPLC (YMC, 20 mm × 250 mm, MeOH–H_2O–CF_3COOH, 42:58:0.05, flow rate 10 ml/min) to give compound **1** (22.0 mg). Fraction 5 (25.1 g) was separated by Sephadex LH-20 column chromatography using $CHCl_3$–MeOH (1:1) as an eluent to afford three fractions (fractions 51–53). Fraction 51 (12.8 g) was subjected to an RP-18 silica gel column chromatography eluted with MeOH–H_2O in gradient yielding five fractions (fractions 511–515). Further separation of fraction 513 (660 mg) was performed on preparative reversed-phase HPLC (YMC, 20 mm × 250 mm, MeOH–H_2O, 35:65, flow rate 10 ml/min) to give compound **2** (162.0 mg).

3.3.1 4′-*O*-Benzoylpaeoniflorin (**1**)

A colorless amorphous solid; $[\alpha]_D^{25}$ −42.0 (c = 0.67, MeOH); UV λ_{max} (MeOH) nm

Table 1. ^{1}H NMR (400 MHz, C_5D_5N) and ^{13}C NMR (100 MHz, C_5D_5N) spectral data of compounds **1** and **2**.

	1		**2**	
Position	δ_H (J, Hz)	δ_C	δ_H (J, Hz)	δ_C
1		89.1		85.4
2		86.0		91.1
3	2.30 (1H, dd, 1.6, 12.0 Hz), 2.47 (1H, d, 12.0 Hz)	44.8	(α)2.51 (1H, dd, 7.0, 15.8 Hz), (β)2.24 (1H, d, 15.8 Hz)	39.3
4		106.0	5.59 (1H, m)	70.5
5	3.08 (1H, d, 6.4 Hz)	44.0	3.36 (1H, m)	38.5
6		71.8		56.4
7	2.34 (1H, d, 10.8 Hz), 2.92 (1H, dd, 6.4, 10.8 Hz)	23.6	(α)2.32 (1H, d, 11.2 Hz), (β)3.20 (1H, dd, 7.8, 11.2 Hz)	28.2
8	5.10 (1H, d, 12.0 Hz), 5.28 (1H, d, 12.0 Hz)	61.6	5.14 (1H, d, 12.0 Hz), 5.20 (1H, d, 12.0 Hz)	61.5
9	5.94 (1H, s)	101.8		175.2
10	1.65 (3H, s)	19.8	1.65 (3H, s)	20.1
1′	5.20 (1H, d, 7.6 Hz)	100.5	5.13 (1H, d, 7.6 Hz)	100.3
2′	4.06 (1H, m)	75.1	4.00 (1H, t, 8.0 Hz)	74.8
3′	4.03 (1H, m)	76.2	4.15 (1H, m)	78.4
4′	5.83 (1H, t, 9.2 Hz)	73.4	4.13 (1H, m)	71.6
5′	4.36 (1H, t, 9.2 Hz)	75.9	3.89 (1H, m)	78.6
6′	4.10 (1H, m), 4.19 (1H, dd, 2.0, 12.0 Hz)	62.3	4.29 (1H, m), 4.53 (1H, m)	62.7
1″		130.7		130.5
2″	8.13 (1H, m)	130.0	8.20 (1H, d, 7.2 Hz)	130.0
3″	7.31 (1H, t, 8.0 Hz)	128.8	7.28 (1H, m)	128.7
4″	7.48 (1H, m)	133.3	7.34 (1H, m)	133.2
5″	7.31 (1H, t, 8.0 Hz)	128.8	7.28 (1H, m)	128.7
6″	8.13 (1H, m)	130.0	8.20 (1H, d, 7.2 Hz)	130.0
7″		166.7		166.6
1‴		131.0		120.6
2‴	8.19 (1H, m)	130.1	7.97 (1H, s)	110.7
3‴	7.37 (1H, t, 8.0 Hz)	128.9		147.4
4‴	7.48 (1H, m)	133.3		141.4
5‴	7.37 (1H, t, 8.0 Hz)	128.9		147.4
6‴	8.19 (1H, m)	130.1	7.97 (1H, s)	110.7
7‴		166.3		166.5

(log ε): 229 (4.42); IR (KBr) ν_{max}: 3427, 1720, 1615, 1552, 1274, 713 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; ESI-MS (positive and negative): *m/z* 607 $[M+Na]^+$ and 583 $[M-H]^-$. HR-TOF-MS: *m/z* 607.1767 $[M+Na]^+$ (calcd for $C_{30}H_{32}O_{12}Na$, 607.1792).

*3.3.2 4-O-Galloylalbiflorin (**2**)*

A white powder; $[\alpha]_D^{25}$ −68.1 ($c = 0.67$, MeOH); UV λ_{max} (MeOH) nm (log ε): 221 (4.38), 279 (4.83); IR (KBr) ν_{max}: 3421, 1706, 1612, 1282, 1075, 713 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; ESI-MS (positive and negative): *m/z* 655 $[M+Na]^+$ and 631 $[M-H]^-$. HR-TOF-MS: *m/z* 631.1684 $[M-H]^-$ (calcd for $C_{30}H_{31}O_{15}$, 631.1663).

3.4 Acid hydrolysis and GC analysis of compounds 1 and 2

Compound **1** (2 mg) was hydrolyzed with 2 M HCl for 2 h in a boiling water bath. The mixture was evaporated to dryness under vacuum, and then the residue was dissolved in H_2O and extracted with $CHCl_3$. The aqueous layer was concentrated *in vacuo* to give a residue, which was dissolved in dry pyridine, to which was added L-cysteine methyl ester hydrochloride (2 mg; Sigma, St Louis, MO, USA). The reaction mixture was heated for 2 h at 60°C and concentrated to dryness with N_2 gas. Trimethylsilyl imidazole (200 μl; Fluka, St Gallen, Switzerland) was added to the residue, followed by heating for 1 h at 60°C. The residue was extracted with hexane and H_2O, and the organic layer was analyzed by gas chromatography: column, DB-1701 (0.25 mm × 30 m, 0.25 μm); detector, FID; column temperature, 160°C → 5°C/min → 230°C (staying 22 min); detector temperature, 280°C; injector temperature, 270°C; and carrier gas, He. One peak of the derivative of **1** was observed at t_R 22.23 min (D-Glc). The standard monosaccharides, D-Glc (Zhongyuan, China) and L-glucose (L-Glc) (Sigma), were subjected to the same reaction and gas chromatographic analysis under the same conditions. The peaks of the standard monosaccharide derivatives were recorded at t_R 22.11 (D-Glc) and 23.04 (L-Glc). Compound **2** was also subjected to acid hydrolysis and gas chromatographic analysis following the same procedure. One peak of the derivative of **2** was observed at t_R 22.13 min (D-Glc).

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