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Monoterpene glycosides with anti-inflammatory activity from *Paeoniae Radix*

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ABSTRACT

Six new monoterpene glycosides, named 6′-*O*-nicotinoylalbiflorin (**1**), 4′-*O*-vanillylalbiflorin (**2**), paeonidanin L (**3**), paeoniflorigenin-1-*O*-*β*-D-xyloside (**4**), 6′-(2-hydroxypropanoyl)-paeoniflorin (**5**), oxylactiflorin (**6**), together with 16known ones (**7**–**22**) were isolated from the 70% ethanol extract of *Paeoniae Radix*. Their structures were elucidated based on spectroscopic analysis (1D and 2D NMR, HRESIMS, IR and UV), chemical evidences and comparison with literatures. The inhibitory effects of all the isolates were evaluated against lipopolysaccharide (LPS) stimulated PGE_2 production in RAW 264.7 macrophages.

1. Introduction

The peony is a perennial herb in *Paeonia,* the single genus of the family Paeoniaceae. Peony roots (“Shaoyao” in Chinese) are distinguished by their red or white color into two therapeutic herbal products, *Radix Paeoniae Rubra* (Chishao) and *Radix Paeoniae Alba* (Baishao). Chishao, red peony root, is brownish in appearance and officially originated from either *P. lactiflora* Pall. or *P. veitchii* Lynch with traditional usage for eliminating stasis and invigorating blood circulation. While baishao is white and peeled in appearance, only *P. lactiflora* Pall is officially considered, with claims of its nourishing blood, subduing hyperactivity and regulating menstruation [1–4]. There have been many researches on phytochemical constituents of peony roots, including monoterpenoids and their glycosides, flavonoids, hydrolysable tannins (polyphenols), and triterpenoids [5]. The “cage-like” monoterpene derivatives (paeoniflorin analogues) are considered as the main and characteristic bioactive components. These compounds have greater potentials for neuroprotective, anti-proliferative, anti-allergic, anti-coagulant, anti-inflammatory and anti-diabetic activities, etc. [6–13].

In our continuing efforts to seek the bioactive monoterpenes from genus *Paeonia*, a re-investigation on *Radix Paeoniae Alba* led to the discovery of twenty-two monoterpene derivatives (Fig. 1), including 6new ones, namely 6′-*O*-nicotinoylalbiflorin (**1**), 4′-*O*-vanillylalbiflorin (**2**), paeonidanin L (**3**), paeoniflorigenin-1-*O*-*β*-D-xyloside (**4**), 6′-(2-hydroxypropanoyl)-paeoniflorin (**5**), oxylactiflorin (**6**), together with 16 known analogues which were identified as albiflorin (**7**) [14], paeonivayin (**8**) [15], 4-*O*-galloylalbiflorin (**9**) [16], paeoniflorin (**10**) [14], oxypaeoniflorin (**11**) [14], mudanpioside E (**12**) [17], benzoylpaeoniflorin (**13**) [14], 6’-*O*-vanillylpaeoniflorin (**14**) [18], galloylpaeoniflorin (**15**) [19], 4′-*O*-galloylpaeoniflorin (**16**) [20], 4′-*O*-benzoylpaeoniflorin (**17**) [16], 4-*O*-galloylpaeoniflorin (**18**) [21], isomaltopaeoniflorin (**19**) [18], mudanpioside C (**20**) [17], lactiflorin (**21**) [22], 1-*O*-*β*-D-glucopyranosyl-8-*O*-benzoylpaeonisuffrone (**22**) [9].

Herein, we report the isolation and structure elucidation, and anti-inflammatory activity of these compounds.

2. Experimental

2.1. General experimental procedures

^1H and ^{13}C NMR spectra were measured on a Bruker AV 600 (Bruker Co. Ltd., Bremen, German) at 600 and 150 MHz with solvent signals (CD_3OD, δ_H 3.30/δ_C 49.0) as internal reference. IR spectra were taken on a JASCO FT/IRBCA plus spectrometer (JASCO International Co. Ltd., Tokyo, Japan) with KBr. UV spectra were acquired using a JASCO V-550 UV/Vis spectrometer (JASCO International Co. Ltd., Hachioji, Tokyo, Japan). Optical rotation was measured on JASCO P-1020 digital

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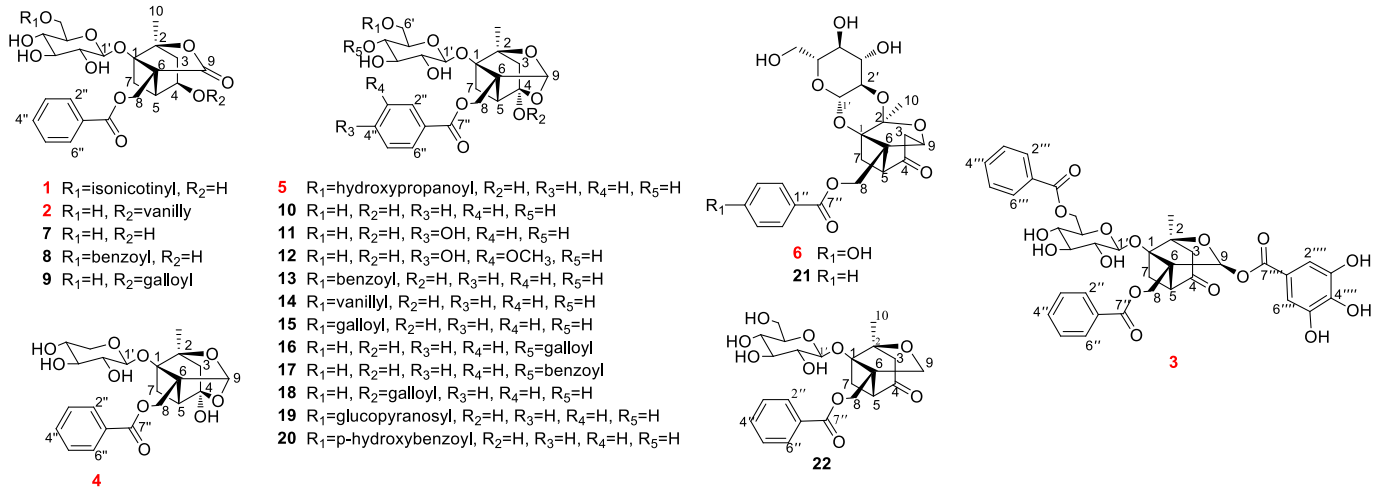


Fig. 1. Chemical Structures of Compounds **1–22** (red: new). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

polarimeter (JASCO International Co. Ltd., Hachioji, Tokyo, Japan). HR-ESI-MS spectra were measured on a Waters Synapt G_2 mass spectrometer (Waters, Manchester, U. K.) with a RP-18 column (1.7 μm, ϕ 3.0 × 150 mm; BEH). HPLC analyses were performed on a Waters 2695 separations module (Waters, Manchester, U.K.) equipped with a 2998 photodiode array (PDA) detector and an Alltech 3300 evaporative light scattering detector (ELSD; Alltech Inc., Deerfield, Illinois, U.S.A.) using a Phenomenex Gemini C18 column (5 μm, ϕ 4.6 × 250 mm; FLM Inc., Guangzhou, China). The semi-preparative HPLC analyses were performed on a Waters 1515 isocratic HPLC pump (Waters, Manchester, U.K.) coupled with a 2489 UV/Vis detector (Waters, Manchester, U.K.) and a Phenomenex Gemini C18 column (5 μm, ϕ 10 × 250 mm; FLM Inc., Guangzhou, China).

The methanol of HPLC grade was purchased from BCR International Co. Ltd. (Shanghai, China), while the acetonitrile was purchased from Merck (Darmstadt, Germany). Diaion HP-20 (Mitsubishi Chemical Co., Tokyo, Japan), silica gel (200–300 mesh, Qingdao Marine Chemical Ltd., Shandong, China), ODS-silica gel (12 nm, S-50 μm, YMC Ltd., Tokyo, Japan) and Sephadex LH-20 (Amersham Pharmacia Biotech, Sweden) were used for chromatography column (CC). TLC was performed on pre-coated silica gel plate (SGF_{254}, 0.2 mm, Yantai Chemical Industry Research Institute, Shandong, China).

2.2. Plant materials

Paeoniae Radix Alba were collected in Anhui, China, and purchased from Hubei tianji traditional Chinese medicine decoction piece Co. LTD. in Nov, 2016, and identified by Prof. G. X. Zhou of Jinan University. A sample (No. 20160601) is deposited in Jinan University, Guangzhou, China.

2.3. Extraction and isolation

Paeoniae Radix Alba (29.0 kg) were extracted with 70% EtOH-H_2O (120 L, *v*/v) by heat reflux for 2 times (2 h each) to afford a total extract (PRA, 4.0 kg), which was suspended in water and separated by HP-20 resin column chromatography (CC) eluted with EtOH-H_2O (15:85, 50:50, 95:5) gradient to afford 3 fractions (PRA-1 to PRA-3). Fr. PRA-2 (600.0 g) was separated over a silica gel column eluting with a CH_2Cl_2-CH_3OH gradient (from 95:5 to 0:100, *v*/v) to afford 14 fractions (Fr. 2A to 2 N). Fr. 2D (20.3 g) was chromatographed by ODS CC (CH_3OH-H_2O, from 25% to 100%, v/v) to afford 12 fractions (Fr. 2D1 to 2D12). Fr. 2D5, 8, 9 were subjected to preparative HPLC to yield compounds **7** [10.3 mg, t_R = 15.2 min, 15% CH_3CN (0.1% formic acid)], **22** [30.0 mg, t_R = 32.2 min, 22% CH_3CN (0.1% formic acid)] and **12** [30.0 mg, t_R = 29.0 min, 22% CH_3CN (0.1% formic acid)], respectively. Fr. 2D10 (9.7 g) was purified over ODS (CH_3OH-H_2O, 40% to 100%, *v*/v) to afford compounds **10** [1.6 g, 60% CH_3OH] and **19** [200.3 mg, 50% CH_3OH].

Fr.2E (8.5 g) was subjected to an ODS CC (CH_3OH-H_2O, 25% to 100%, *v*/v) to afford 14 fractions (Fr 2E1 to 2E14). Fr. 2E4 (1.6 g) was separated over a silica gel CC (EtOAc-CH_3OH, 9:1, v/v), followed by an ODS CC (CH_3OH-H_2O, 30%) , and further purified by a preparative HPLC to yield compounds **4** [15.9 mg, t_R = 12.1 min, 25% CH_3OH (0.1% formic acid)], **6** [25.3 mg, t_R = 16.7 min, 25% CH_3OH (0.1% formic acid)], **5** [30.9 mg, t_R = 20.7 min, 25% CH_3OH (0.1% formic acid)], **21** [102.1 mg, t_R = 25.2 min, 25% CH_3CN (0.1% formic acid)] and **1** [15.6 mg, t_R = 30.6 min, 25% CH_3CN (0.1% formic acid)]. Fr 2E7 (543.5 mg) and 2E10 (27.6 mg) were chromatographed by Sephadex LH-20 CC (CH_3OH-H_2O, 7:3, *v*/v), and then subjected to preparative HPLC to yield compounds **11** [12.0 mg, t_R = 27.9 min, 20% CH_3CN (0.1% formic acid)] and **3** [32.2 mg, t_R = 48.2 min, 22% CH_3CN (0.1% formic acid)], respectively. Compounds **2** [22.6 mg, t_R = 32.3 min, 22% CH_3CN (0.1% formic acid)] and **15** [16.5 mg, t_R = 27.9 min, 22% CH_3CN (0.1% formic acid)] were obtained from Fr 2E8 (192.1 mg) by ODS CC (CH_3OH-H_2O, 40% to 100%, *v*/v) followed by a preparative HPLC.

Fr·2H (161.0 g) was chromatographed by Sephadex LH-20 CC (CH_3OH-H_2O, 1:1, v/v) to give 8 subfractions (Fr. 2H1-2H8). Fr. 2H5 (53.2 g) precipitated crystal compound **18** (30.2 g). Fr. 2H6 (2.3 g) was chromatographed by ODS CC using 45% CH_3OH to afford 8 fractions (Fr 2H6A-2H6H). Fr.2H6A (0.8 g), 2H6C (43.9 mg) and 2H6F (46.2 mg) were further purified by preparative HPLC to obtain compounds **13** [200 mg, t_R = 27.2 min, 25% CH_3OH (0.1% formic acid)], **14** [2.1 mg, t_R = 36.7 min, 25% CH_3OH (0.1% formic acid)], **20** [37.6 mg, t_R = 39.6 min, 25% CH_3OH (0.1% formic acid)] and **16** [201.3 mg, t_R = 32.5 min, 25% CH_3OH (0.1% formic acid)]. Fr·2H8 (11.4 g) was chromatographed by MCI CC using a gradient elution (CH_3OH-H_2O, from 15% to 65%, *v*/v) to afford 7 fractions (Fr 2H8A to 2H8G). Fr. 2H8B (492.0 mg) was further purified by preparative HPLC to obtain compounds **8** [30.2 mg, t_R = 22.1 min, 25% CH_3OH (0.1% formic acid)] and **9** [62.1 mg, t_R = 28.0 min, 25% CH_3OH (0.1% formic acid)]. Fr. 2 K (2.3 g) was chromatographed by ODS CC using a 45% CH_3OH system to afford compound **17** (200.2 mg).

2.4. Data for structure elucidation of new compounds

6′-*O*-nicotinoylalbiflorin (**1**): White amorphous powder ; $[\alpha]_D^{28}$-

Table 1
1H and 13C NMR spectral data of **1–5** (^{1}H: 600 MHz; ^{13}C: 150 MHz, in CD_3OD) and **6** (^{1}H: 600 MHz; ^{13}C: 150 MHz, in DMSO-d_6).

Position	**1**		**2**		**3**		**4**		**5**		**6**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		86.9		86.3		88.2		89.4		89.3		84.6
2		93.2		92.9		89.4		87.1		87.1		102.3
3	2.10, dd (15.6, 6.6) 1.92, dd (15.6, 1.8)	41.6	2.69, dd (16.2, 7.2) 2.21, dd (16.2, 1.2)	39.8	2.73, dd (18.4, 1.3) 2.52, dd (18.4, 1.0)	50.0	2.13, d (12.5) 1.82, dd (12.5, 1.9)	44.5	2.15, d (12.6) 1.81, dd (12.6, 2.0)	44.5	2.79, dd (17.7, 4.5) Ha 2.42, d (17.7) Hb	47.1
4	4.09, dd (6.6, 4.8)	68.2	5.52, dd (6.6, 4.8)	71.6		209.6		106.3		106.3		216.6
5	2.86, t (7.8)	41.6	3.21, d (8.0)	39.5	3.10, d (7.4)	49.8	2.59, dd (6.8, 1.8)	43.9	2.59, dd (6.8, 1.7)	43.9	2.71, dd (13.2, 4.7)	36.9
6		56.6		57.4		64.4		72.3		72.1		55.0
7	2.78, dd (11.0, 7.8) Ha 1.80, d (11.0) Hb	28.3	2.95, dd (11.4, 8.0) 2.24, d (11.4)	28.0	3.04, dd (11.0, 7.7) 2.07, d (11.0)	27.9	2.51, dd (10.8, 6.9) Ha 1.86, d (10.8) Hb	23.2	2.51, dd (10.9, 7.0) 1.89, d (10.8)	23.2	2.36, t (13.9) Ha 2.06, dd (13.9, 4.7) Hb	30.1
8	4.77, d (12.2) Ha 4.66, d (12.2) Hb	61.9	4.89, d (12.2) Ha 4.66, d (12.2) Hb	61.8	4.83, d (11.8) 4.77, d (11.8)	64.6	4.76, d (12.2) 4.73, d (12.2)	61.7	4.75, d (12.2) 4.72, d (12.1)	61.6	4.62, s	61.5
9		177.8		177.4	6.54, s	100.7	5.42, s	102.3	5.42, s	102.3	4.81, d (4.3)	79.4
10	1.40, s	20.5	1.58, s	20.2	1.38, s	20.5	1.32, s	19.5	1.31, s	19.7	1.39, s	16.0
1′	4.59, d (7.7)	100.0	4.56, d (7.7)	100.1	4.69, d (7.7)	99.9	4.48, d (7.8)	100.9	4.55, d (7.7)	100.0	4.49, d (8.2)	94.6
2′	3.24, dd (9.0, 7.6)	74.8	3.24	74.9	3.34, dd (9.0, 1.5)	75.0	3.19, dd (9.2, 7.5)	75.0	3.19, dd (9.2, 7.5)	75.0	3.02	74.9
3′	3.33, d (7.1)	77.8	3.34, d (9.0)	78.0	3.47, t (9.0)	77.8	3.26, t (9.0)	77.9	3.26, t (9.0)	77.9	3.31	79.0
4′	3.35, d (2.0)	71.7	3.25	71.5	3.40, t (9.5)	72.0	3.46, ddd (10.0, 8.7, 5.4)	71.7	3.46, ddd (10.3, 8.7, 5.4)	71.7	3.17	70.1
5′	3.58, ddd (9.0, 6.7, 2.3)	75.3	3.26, d (2.0)	78.2	3.70, ddd (9.5, 7.0, 2.3)	75.4	3.79, dd (11.5, 5.4) 3.15, t (10.0)	78.0	3.79, dd (11.5, 5.4) 3.15, t (10.0)	78.0	3.12	72.9
6′	4.72, dd (11.8, 2.3) 4.53, dd (11.8, 6.7)	65.4	3.88, dd (12.0, 2.0) 3.63, dd (12.0, 5.8)	62.8	4.67, dd (11.8, 2.3) 4.56, dd (11.8, 7.1)	65.2				64.8	3.62, dd (12.1, 5.5) Ha 3.42, dd (11.5, 5.5) Hb	60.7
1″		131.2		131.1		131.2		131.2	4.51, dd (11.8, 2.1) 4.21, dd (11.8, 6.2)	131.2		119.6
2″, 6″	8.06, d (8.3)	130.7	7.93, d (8.2)	130.6	8.01, dd (8.3, 1.2);	130.5	8.06, d (8.4)	130.7		130.7	7.83, d (8.7)	131.6
3″, 5″	7.48, t (7.8)	129.6	7.32, d (8.0)	129.5	7.41, t (8.0);	129.7	7.49, t (7.8)	129.6	8.05, d (8.4)	129.6	6.86, d (8.7)	115.5
4″	7.61, d (6.2)	134.4	7.54	134.3	7.52, t (7.5)	134.4	7.62, t (6.7)	134.4	7.49, t (7.7)	134.4		162.2
7″		167.9		167.8		167.6		168.0	7.62, t (7.4)	168.0		165.3
1‴				122.1		131.2				176.0	5.30	3′ –OH
2‴	9.16, s	151.2	7.53, d (1.8)	114.0	7.72, dd (8.3, 1.2)	130.5			4.27, dd (7.0, 2.3)	67.8	5.19	4′ –OH
3‴		127.9		148.7	7.15, dd (8.3, 7.5)	129.3			1.38, d (6.6)	20.3	4.64	6′ –OH
4‴	8.43, d (8.0)	139.0		153.0	7.44, d (7.5)	134.3						
5‴	7.58, dd (8.0, 4.0)	125.4	6.82, d (8.0)	115.9	7.15, dd (8.3, 7.5)	130.5						
6‴	8.77, d (4.0)	154.3	7.55, dd (8.0, 1.8)	125.5	7.72, dd (8.3, 1.2)	130.5						
7‴		165.9		167.1		167.5						
1⁗						120.4						
2⁗,6 ⁗					6.92, s	110.6						
3⁗, 5⁗						146.5						
4⁗						140.4						
7⁗						167.0						
–OCH_3			3.80, s	56.5								

Multiplets and overlapped peaks were given without assigning multiplicity.

100.7 (*c* 0.5, CH_3OH); UV (CH_3OH) λ_{max} (logε): 202 (3.74), 227 (3.93), 264 (3.08), 271 (3.01) nm; IR (KBr) ν_{max}: 3443, 2920, 2555, 1772, 1281, 1067, 719 cm^{-1}; HR-ESI-MS: *m/z* 586.1929 $[M + H]^+$ (calcd. For $C_{29}H_{31}N_1O_{12}$ 586.1925); ^1H and ^{13}C NMR data see Table 1.

4′-*O*-vanillylalbiflorin (**2**): White amorphous powder; $[\alpha]_D^{28}$-34.9 (*c* 0.5, CH_3OH); UV (CH_3OH) λ_{max} (logε): 204 (3.99), 224 (3.94), 266 (3.54), 297 (3.32) nm; IR (KBr) ν_{max}: 3443, 2920, 1760, 1612, 1346, 710 cm^{-1}; HR-ESI-MS: *m/z* 631.2026 $[M + H]^+$ (calcd. For $C_{31}H_{34}O_{14}$ 631.2027); ^1H and ^{13}C NMR data see Table 1.

paeonidanin L (**3**): White amorphous powder; $[\alpha]_D^{25}$-87.1 (*c* 0.35, CH_3OH); UV (CH_3OH) λ_{max} (logε): 203 (4.46), 224 (4.64), 281 (4.11) nm; IR (KBr) ν_{max}: 3440, 2923, 1705, 1606, 1343, 716 cm^{-1}; HR-ESI-MS: *m/z* 759.1829 $[M + Na]^+$ (calcd. For $C_{37}H_{36}O_{16}$ 759.1901); ^1H and ^{13}C NMR data see Table 1.

paeoniflorigenin-1-*O*-*β*-D-xyloside (**4**): White amorphous powder; $[\alpha]_D^{28}$-32.0 (*c* 0.5, CH_3OH); UV (CH_3OH) λ_{max} (logε): 202 (3.82), 230 (4.01), 274 (2.89); IR (KBr) ν_{max}: 3426, 2978, 2929, 1711, 1444, 1269, 1073, 716 cm^{-1}; HR-ESI-MS: *m/z* 473.1422 $[M + Na]^+$ (calcd. For $C_{22}H_{26}O_{10}$ 473.1424); ^1H and ^{13}C NMR data see Table 1.

6′-(2-hydroxypropanoyl)-paeoniflorin (**5**): White amorphous powder; $[\alpha]_D^{28}$-31.7 (*c* 0.5, CH_3OH); UV (CH_3OH) λ_{max} (logε): 203 (4.14), 230 (4.36), 274 (3.22), 282 (3.12); IR (KBr) ν_{max}: 3426, 2929, 1711, 1444, 1269, 1073, 716 cm^{-1}; HR-ESI-MS: *m/z* 575.1743 $[M + Na]^+$ (calcd. For $C_{26}H_{32}O_{13}$ 575.1741); ^1H and ^{13}C NMR data see Table 1.

oxylactiflorin (**6**): White amorphous powder; $[\alpha]_D^{25}$-96.9 (*c* 0.5, CH_3OH), UV (CH_3OH) λ_{max} (logε): 203 (3.97), 210 (3.86), 260 (4.00) , IR (KBr) ν_{max}: 3538, 3294, 2932, 2891, 1769, 1453, 1267, 1125, 1021, 713 cm^{-1}。 HR-ESI-MS *m/z* 501.1368 $[M + H]^+$ (calcd. for $C_{23}H_{26}O_{11}$ 501.1373) , ^1H and ^{13}C NMR data see Table 1.

2.5. Acidic hydrolysis and sugar analysis

The absolute configurations of the sugar moieties in the structures were determined by the method of Tanaka et al. [23]. Each compound (1.0–2.0 mg) was refluxed with 2 mL of 2 M HCl for 2 h at 90 °C. The hydrolysates were extracted with equal volume of ethyl acetate twice. The aqueous layer was dried, and then reacted with 2.5 mg L-cysteine methyl ester hydrochloride in 1 mL of pyridine for 1 h at 60 °C. A total of 5 μL *o*-tolylisothiocyanate was added to the above mixture for 1 h at 60 °C. The reaction products were filtered by a 0.45 μm filter membrane for HPLC analysis at 35 °C with isocratic elution of 25% CH_3CN containing 0.1% formic acid for 30 min. Phenomenex Gemini C18 column (5 μm, ϕ 4.6 × 250 mm; FLM Inc., Guangzhou, China) was used to analysis at 0.8 mL/min flow rate. Peaks were detected by UV detector at 250 nm. The peaks of authentic samples of D-glucopyranose, L-glucopyranose, D-xylose and L-xylose after treatment in the same manner were detected at 19.8, 18.0, 23.0 and 21.2 min, respectively.

2.6. Anti-inflammatory activity assay

Cell culture and Viability: RAW 264.7 murine macrophage cell line was obtained from Chinese Academy of Sciences. The cells were grown in DMEM (Gibico, USA) containing 10% FBS (Gibico, USA), 100 U/mL penicillin and 100 μg/mL streptomycin. They were cultured at 37 °C in 5% CO_2. Cell viability was analyzed using MTT assay. Compounds were added to the cells and incubated for 1 h and then cells were treated with or without LPS for 18 h at 37 °C with 5% CO_2. MTT solution (5 g/L) was added to each well and incubated for 4 h at 37 °C. The formazan dyes in the cells were dissolved in 100 μL 10% SDS-HCl solution. The optical density was read at 570 nm (reference, 650 nm) using a microplate UV/vis spectrophotometer (Tecan, Mannedorf, Switzerland). The cell viability in the control group (cells were not treated by compounds and LPS) was set as 100%.

Effect of compounds **1–22** on the PGE_2 production: Cells were seeded in a 96-well plate at a density of 1×10^4 cells/well in DMEM and incubated for 24 h. The cells were pretreated with compounds for 30 min and treated with LPS (1 μg/mL) for 24 h in the presence or absence of different concentrations of the compounds. The concentration of PGE_2 in the culture medium was determined using commercial ELISA kits according to the instructions. The IC_{50} values were calculated from calibration curves of the absorbance inhibition by each compound at three concentrations against the increasing absorbance of macrophage cells stimulated only with LPS (without the compounds). Data were obtained from three independent experiments ($n = 3$).

3. Results and discussion

3.1. Structure elucidation of new compounds

Compound **1** was isolated as a white amorphous powder. The HR-ESI-MS of **1** displayed a quasi-molecular ion peak at *m/z* 586.1929 $[M + H]^+$ (calcd. For 586.1925), corresponding to the molecular formula $C_{29}H_{31}N_1O_{12}$. The analyses of NMR spectra revealed that **1** possessed 29 carbons. Among them, 23 carbon signals were assigned to a C_{10}-monoterpene aglycone [δ_C 86.9 (C-1), 93.2 (C-2), 41.6 (C-3), 68.2 (C-4), 41.6 (C-5), 56.6 (C-6), 28.3 (C-7), 61.9 (C-8), 177.8 (C-9), 20.5 (C-10)], a hexosyl [δ_C 100.0 (C-1′), 74.8 (C-2′), 77.8 (C-3′), 71.7 (C-4′), 75.3 (C-5′), 65.4 (C-6′)], and a benzoyl unit [δ_C 131.2 (C-1″), 130.7 (C-2″, 6″), 129.6 (C-3″, 5″), 134.4 (C-4″) and 167.9 (C-7″)], assembling into a main structure fragment as albiflorin (compound **7**). The remaining 6 carbon signals, including 5 sp^2 phenyl carbons and one carbonyl, in combination with unsaturation degrees and a nitrogen atom, suggested the presence of a nicotinoyl moiety in **1**. This deduction was further supported by carbon chemical shifts, ^1H-^1H COSY and HMBC spectral data. The ^1H-^1H COSY spectrum disclosed interactions of H-4‴/H-5‴/H-6‴, associated with a series of HMBC correlations of H-5‴/C-3‴, H-4‴/C-2‴, C-7‴ and H-2‴/C-6‴, C-4‴, C-7‴, undoubtedly allowed the assignment of a nicotinoyl group. Additionally, the nicotinoyl moiety (carbonyl carbon at δ_C 165.9) was determined to

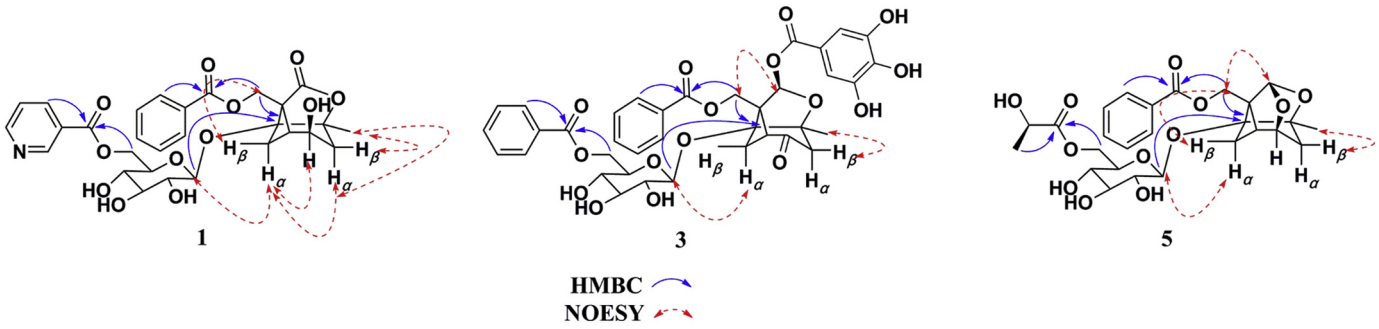
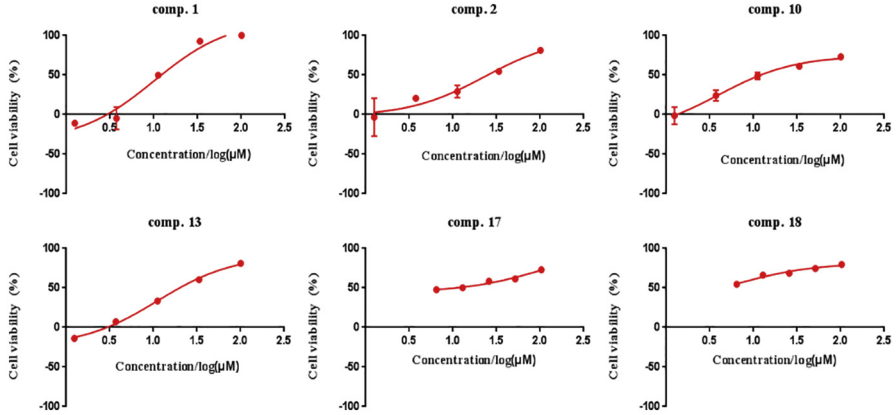


Fig. 2. Key HMBC and ROESY correlations of **1**, **3** and **5**.



Compounds	IC_{50} ± SD (μM)
1	11.21 ± 1.1
2	28.24 ± 0.9
10	15.12 ± 0.2
13	21.33 ± 2.1
17	12.95 ± 0.3
18	6.74 ± 0.7
Hydrocortisone[a]	10.5 ± 0.8

[a] Positive control

Fig. 3. Inhibitory effect of compounds **1**, **2**, **10**, **13**, **17** and **18** on PGE_2 production induced by LPS in macrophages (IC_{50} Values Expressed in μM).

connect to C-6 of glucosyl due to the obvious downfield shift of C-6 (δ_C 65.4) and HMBC correlations of H-6′/C-7‴. The configuration of the monoterpene aglycone in **1** was considered as the same as the well-known paeoniflorin-type monoterpene glucosides in ROESY experiment (H_3-10/H_2-3, H-3α/H-7α, H-4/H-7α, H-7α/H-1′ and H-7β/H-8b (see Fig. 2). Thus, the structure of **1** was elucidated as 6′-*O*-nicotinoylalbiflorin.

Compound **2** was obtained as a white amorphous powder with a molecular formula of $C_{31}H_{34}O_{14}$ based on HR-ESI-MS (*m/z* 631.2026 $[M + H]^+$, calcd. For 631.2027). Detailed inspection of the NMR spectroscopic data of **2** indicated it has the similar structure as compound **9** (4-*O*-galloylalbiflorin) [14]. The difference was found in the presence of the vanilly signals [δ_H 6.82 (1H, d, J = 8.0 Hz, H-5‴), 7.53 (1H, d, J = 1.8 Hz, H-5‴), 7.55 (1H, dd, J = 8.0, 1.8 Hz, H-6‴), δ_C 122.1 (C-1‴), 114.0 (C-2‴), 148.7 (C-3‴), 153.0 (C-4‴), 115.9 (C-5‴), 125.5 (C-6‴) and 167.1 (C-7‴)] in NMR spectra of **2**. Acid hydrolysis of **2** yielded D-glucose by HPLC analysis with authentic samples as the reference [23]. The HMBC correlation (see Fig. 2) of H-4 with C-7‴ indicated that the vanilly moiety was connected to C-4. On the basis of these data, the structure of **2** was settled down and named as 4′-*O*-vanillyalbiflorin.

Compound **3** was obtained as a white amorphous powder, and its molecular formula was determined as $C_{37}H_{36}O_{16}$ according to the HR-ESI-MS peak at *m/z* 759.1829 $[M + Na]^+$ (calcd. For $C_{37}H_{36}O_{16}$, 759.1901) with twenty degrees of unsaturation. The ^1H NMR spectrum of **3** displayed a methyl group at δ_H1.38 (3H, s), an acetal proton at δ_H 6.54 (1H, s), and an anomeric proton at δ_H 4.69 (1H, d, J = 7.7 Hz). The ^{13}C NMR spectrum displayed 37 carbons including two benzoate moieties [δ_C 131.2 (C-1″), 130.5 (C-2″, 6″), 129.7 (C-3″, 5″), 134.4 (C-4″), 167.6 (C-7″) and 131.2 (C-1‴), 130.5 (C-2‴, 6‴), 129.3 (C-3‴, 5‴), 134.3 (C-4‴), 167.5 (C-7‴)], a 1,2,4,5-tetrasubstituted benzene ring [δ_C 120.4 (C-1⁗), 110.6 (C-2⁗, 6⁗), 146.5 (C-3⁗, 5⁗), 140.4 (C-4⁗) and 167.0 (C-7⁗)], a set of glycosyl signals at δ_C 99.9 (C-1′), 75.0 (C-2′), 77.8 (C-3′), 72.0 (C-4′), 75.4 (C-5′) and 65.2 (C-6′), an acetal carbon at δ_C 100.7, and a carbonyl carbon at δ_C 209.6. The ^{13}C NMR spectrum was similar to that of paeonidanin A [24], except for the presence of a galloyl moiety instead of the methoxy group linked to the C-9. The HMBC correlations from H-3/C-1, C-4, C-5, H-5/C-1, C-4, C-8, C-9, H-7 to C-2, C-4, C-6, H-9/C-2, C-4, C-6, and H-10/C-1, C-2, C-3 were observed. In addition, HMBC correlations from H-1′ to C-1, H-8 to C-7″, H-6′ to C-7‴ and from H-9 to C-7⁗ confirmed that the sugar unit was connected to C-1, the two benzoyl moieties (carbonyl carbons at δ_C 167.6 and 167.5) were connected to C-8 and C-6′, respectively, and the galloyl moiety (carbonyl carbon at δ_C 167.0) was connected to C-9. Thus, the structure of **3** was elucidated and named as paeonidanin L.

The molecular formula of compound **4**, $C_{22}H_{26}O_{10}$, was established by HR-ESI-MS (*m/z* 473.1422 $[M + Na]^+$, calcd. For 473.1424). The analyses of NMR spectra revealed that **4** possessed 22 carbons, which were recognized as a benzoyl group, a monoterpene aglycone and a glycosidic moiety. Comparison of these NMR data with those of compound **10** (paeoniflorin) suggested that they had only different sugar unit, which was further confirmed by NMR and acid hydrolysis experiments. Accordingly, the ^{13}C NMR spectroscopic data of the sugar in **4** matched well with a xylose [25]. Acid hydrolysis of **4** [23] followed by HPLC analysis afforded a D-xylopyranosyl. A β-configuration at C-1″ in **4** was identified due to a large coupling constant (J = 7.8 Hz) of anomeric proton. Thus, compound **4** was elucidated as paeoniflorigenin-1-*O*-β-D-xyloside.

Compound **5** was obtained as a white amorphous powder, and its molecular formula of $C_{26}H_{32}O_{13}$ was elucidated from HR-ESI-MS data (*m/z* 575.1743 $[M + Na]^+$, calcd. For 575.1741). Detailed inspection of the NMR spectroscopic data of **5** indicated it was a derivative of compound **10** (paeoniflorin) [14]. The difference was found in the presence of the 2-hydroxypropanoyl signals at [δ_H 4.27 (2H, dd, J = 7.0, 2.3 Hz, H-2‴), 1.38 (3H, d, J = 6.6 Hz, H-3‴), δ_C 67.8 (C-2‴), 20.3 (C-3‴) and 176.0 (C-1‴)] in the NMR spectra of **5**. The HMBC correlation from H_2–6′/C-1‴ indicated that the 2-hydroxypropanoyl moiety was connected at C-6′ (see Fig. 2). Thus, the structure of **5** was assigned as 6′-(2-hydroxypropanoyl)-paeoniflorin.

Compound **6**, a white amorphous powder, showed the molecular formula $C_{23}H_{26}O_{11}$, as determined by HR-ESI-MS *m/z* 501.1368 $[M + Na]^+$ (calcd. For $C_{23}H_{26}O_{11}Na$, 501.1373) and NMR data. Comparing the ^1H and ^{13}C NMR spectroscopic data of **6** with those of a previously reported compound **21** (lactiflorin) [22] revealed the similarity of their chemical shifts, except for the presence of a *p*-hydroxybenzoyl moiety in the structure of **6** instead of the benzoyl moiety as in lactiflorin. Thus, compound **6** was determined as oxylactiflorin.

3.2. Anti-inflammatory effects of the compounds

All isolated compounds were evaluated for their inhibitory effects on PGE_2 production in macrophages induced by LPS. In addition, cytotoxicity in the present experiment was determined in parallel by the MTT method to find whether inhibition on PGE_2 production was due to the cytotoxicity of the test compounds. Compounds **1–22** exerted minimal cytotoxicity against Raw 264.7 cells (CC_{50} > 500 μM). As shown in Fig. 3, hydrocortisone (IC_{50} 10.5 ± 0.8 μM) was used as positive control. Compound **18** exhibited strong inhibitory activity on PGE_2 production, while compounds **1**, **2**, **10**, **13** and **17** showed medium anti-inflammatory activity.

4. Conclusion

In the present research, six new monoterpene glycosides (**1–6**) and sixteen known compounds (**7–22**) were obtained and identified from *Radix Paeoniae Alba*. Their inhibitory effects against PGE_2 production in

anti-inflammatory model were assessed. Although experiments showed some compounds have biological activities, further investigation of their therapeutic potential is needed. The diversity for botanical paeoniflorin compounds identified in this paper have provided a source for further pharmaceutical drug development.

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Declaration of Competing Interest

The authors declare no competing financial interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2019.104290.

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