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A new sesquiterpenoidal glucoside from the roots of *Paeonia lactiflora*

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Highlights

A new sesquiterpenoidal glucoside, (+)-(1*R*,2*R*,4*S*,5*S*,10*R*)-2-*α*-D-glucopyranosyloxy-2-hydroxy-cadin-6,12-dien-15-oic acid (**1**), along with two known sesquiterpenoids (**2**-**3**) were isolated from the roots of *Paeonia lactiflora*. This report extended our knowledge about the diversity of compounds in *P. lactiflora* and lays the foundation for further research.

Traditionality

The dried roots of *Paeonia lactiflora* and *P. veitchii* are known as the important crude drug "*Chi-Shao*" in traditional Chinese medicine (TCM). With the function of clearing heat and cooling blood, dissipationg blood stasis and relieving pain, *Chi-Shao* has been widely used in clinics.

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Abstract

Objective: To study the chemical constituents of the roots of *Paeonia lactiflora*.

Materials and methods: The isolation and purification were carried out by column chromatography on macroporous adsorbent resin, MCI gel, silica gel, and Sephadex LH-20, as well as semi-preparative RP-HPLC. The structures were elucidated on the basis of physicochemical properties and spectroscopic analysis, as well as the ECD quantum chemical computation methods.

Results: A sesquiterpenoidal glucoside (**1**) along with two sesquiterpenoids (**2-3**) were isolated from the roots of *Paeonia lactiflora*, and their structures were identified as (+)-(1*R*,2*R*,4*S*,5*S*,10*R*)-2-*α*-D-glucopyranosyloxy-2-hydroxy-cadin-6,12-dien-15-oic acid (**1**), drim-7-en-3*β*,11,12-triol (**2**), and 3*β*-hydroxy-11,12-*O*-isopropylidenedrimene (**3**), respectively.

Conclusion: Compound **1** was identified as a new sesquiterpenoidal glucoside.

Keywords: *Paeonia lactiflora*, sesquiterpenoidal glucoside

Abbreviations: TCM, traditional Chinese medicine;

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Competing interests: The authors declare that there is no conflict of interests regarding the publication of this paper.

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Background

The dried root of *Paeonia lactiflora,* called *Chi-Shao*, is a famous herbal medicine used in Asia countries with a history of several thousand years. According to the *Chinese Pharmacopoeia*, *Chi-Shao* could eliminate pathogenic heat from blood and promote blood circulation by removing blood stasis [1]. Generally, peoniflorin is regarded as the indicative bioactive substance in *P. lactiflora* due to its high content and antihyperglycemic, anti-inflammatory, antioxidant, and other biological activities, and a number of monoterpenes, sesquiterpenoids and triterpenoids with a broad range of biological activities have been reported to be isolated from this species [2-7]. In our continuing efforts to search for biological constituents from *Chi-Shao* [6,7], this study has led to the discovery of a new sesquiterpenoidal glucoside (**1**) and two known sesquiterpenoids (**2-3**) (Figure 1). Detailed herein are the isolation, structural elucidation, and bioactivity evaluation of the isolates.

Materials and methods

Plant material and the extraction processes, see ref.7.

The instruments and equipments for testing optical rotations, UV, ECD, IR, NMR, and HRESIMS data, see ref. 7. Column chromatography (CC) was run using macroporous adsorbent resin (HPD-100), MCI gel (CHP 20P), silica gel (200-300 mesh, Qingdao Marine Chemical Inc., China), and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala Sweden). Analytical HPLC was performed with an Agilent HP 1260 using a Titank column (C_{18} 250 × 4.6 mm, 5 μm, Guangzhou FLM Scientific Instrument Co., Ltd). HPLC separation was conducted on Waters HPLC equipment, namely, using the following columns: Shiseido Capcell Pak MG Ⅲ C_{18} (250 × 4.6 mm, 5μm), Waters XBridgeTM Prep Shield RP18 (250 × 10 mm, 5 μm), Welch Ultimate® XB-Phenyl (250 × 10 mm, 5 μm) and Welch Ultimate® XB-C8 (250 × 10 mm, 5 μm). GC was carried out on an Agilent GC-series system and performed with an HP-5 column (30 m × 0.25 mm × 0.25 μm, Agilent, Santa Clara, CA).

The aqueous extracts were separated via a macroporous adsorbent resin (HPD-100, 30 kg) column (20 × 200 cm), eluting with 50 L H_2O, 150 L 50% EtOH, and 80 L 95% EtOH, successively. The 50% EtOH fraction was concentrated and subjected to chromatography over MCI gel (CHP 20P, 10 L) with successive elution using H_2O (30 L), 50% EtOH (80 L), 95% EtOH (30 L), and acetone (20 L), to afford fractions A−D. Fraction B was fractionated by Sephadex LH-20 column chromatography eluting with 50% MeOH to afford fractions B1−B8. Fraction B3 (42.8 g) was separated via MPLC over reversed-phase C_{18} silica gel using gradient elution (20−80% MeOH−H_2O) to give subfractions B3-1−B3-19 based on TLC analysis. Fraction B3-10 (1.8 g) was purified by RP C_{18} HPLC (C_{18} preparative column, 5 μm, 250 × 10 mm, 230 nm, MeCN−H_2O, 35:65, 2.0 mL/min) to give **1** (10.0 mg).

The 95% EtOH extracts were combined and concentrated, then the residue was suspended in H_2O and partitioned with EtOAc. The EtOAc extract (580 g) was subjected to a silica gel column (15 × 80 cm), eluting with petroleum ether-acetone (50:1 → 8:1) and then CH_2Cl_2-MeOH (20:1 → 1:1) to give 10 subfractions (E-N). Fraction L was subjected to a C_{18} silica gel column using gradient elution (MeOH−H_2O, from 80:20 to 100:0) to give nine fractions (L1−L9). L3 was separated by silica gel followed by semipreparative HPLC [C_{18}, 250 × 10 mm, 5 μm, MeOH:H_2O = 68:32, 2.0 mL/min, 230 nm] to yield **2** (14.7 mg) and **3** (14.7 mg). *In silico* prediction of ECD spectrum, see ref 6. The acid hydrolysis of compound **1** was performed by the reported protocol [8]. And the sugar analysis was performed as our previous reported protocol [7].

(+)-(1*R*,2*R*,4*S*,5*S*,10*R*)-2-α-D-glucopyranosyloxy-2-hydroxy-cadin-6,12-dien-15-oic acid (**1**). White powder. $[\alpha]^{20}_D$ +33.2 (c 0.5, MeOH); UV (MeOH) λ_{max} (log ε): 217 (5.00); CD (MeOH): 247 ($\Delta\varepsilon$ –0.81), 228 ($\Delta\varepsilon$ –0.45); IR (cm^{-1}): 3416, 2925, 2880, 2642, 2546, 1681, 1643, 1426, 1353, 1290, 1204, 1157, 1101, 1053, 1034, 1004, 968, 911, 894, 841, 772, 747, 714, 631, 577, 548; HRESIMS *m/z* 435.1994 $[M+Na]^+$ (calcd for $C_{21}H_{32}O_8Na$, 435.1989); ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data see Table 1.

Table 1 NMR data for Compound 1[a].

No.	δ_H (mult, *J*, Hz)	δ_C	No.	δ_H (mult, *J*, Hz)	δ_C
1	1.34 m	44.3	11	1.12 d (6.4)	15.1
2	3.22 td (4.2, 10.2)	85.9	12		147.9
3α	1.57 dd (11.9, 12.0)	41.0	13a	4.89 brs	113.1
3β	2.20 dt (3.4, 12.0)		13b	4.76 brs	

4	1.96 m	49.4	14	1.69 s	19.4
5	1.91 dd (10.5, 9.5)	44.0	15		171.1
6	6.80 brs	142.0	1′	4.90 d (4.0)	102.6
7		131.5	2′	3.36 dd (4.0, 9.6)	74.0
8*α*	2.40 m	26.2	3′	3.61 dd (9.6, 9.3)	74.9
8*β*	2.17 m		4′	3.28 t (9.3)	71.9
9*α*	1.19 m	26.9	5′	3.68 m	73.7
9*β*	2.14 m		6′a	3.73 dd (11.0, 1.4)	62.6
10	0.88 dd (10.5, 11.0)	44.5	6′b	3.66 dd (11.0, 5.0)	

[a] NMR data were measured in CD_3OD at 500 MHz for ^1H, and 150 MHz for ^{13}C.

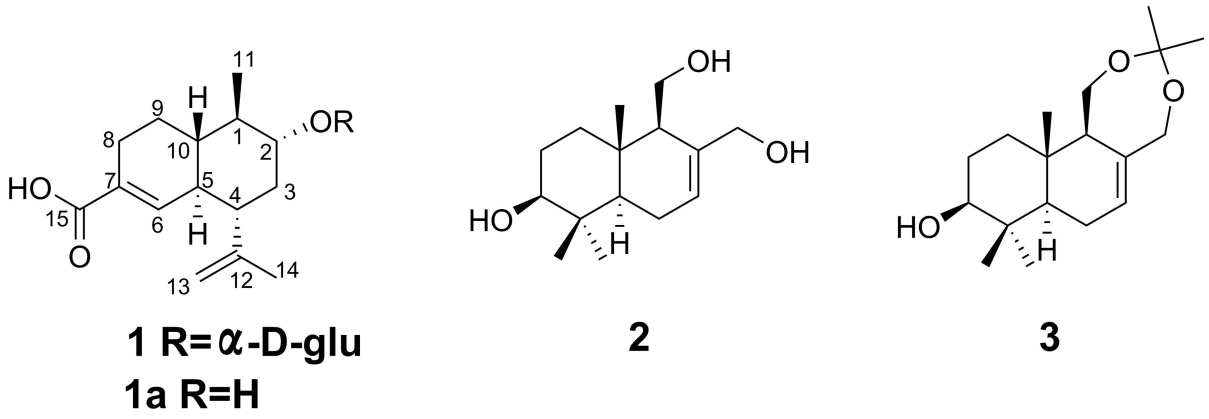

Figure 1. The structure of compounds 1-3.

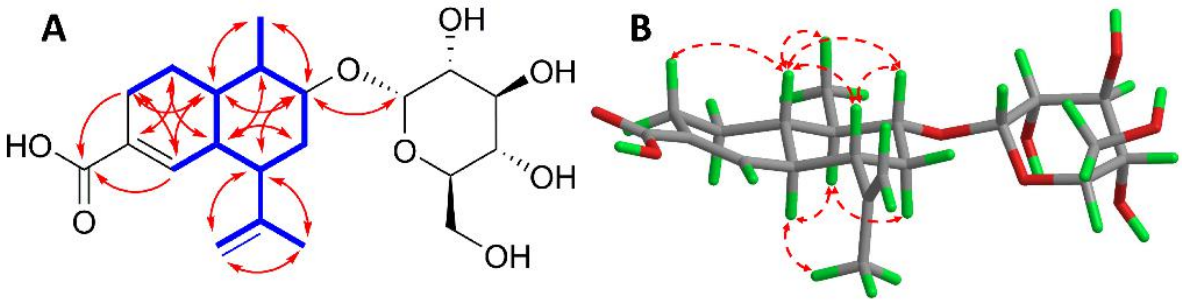

Figure 2. (A) The Key ^1H–^1H COSY (blue thick lines) and HMBC correlations (red arrows) of 1; (B) Key NOESY correlations for 1.

Results and Discussion

Compound **1** was purified as a white power with a molecular formula of $C_{21}H_{32}O_8$ as inferred from the HRESIMS ion (*m/z* 435.1994 [M + Na]$^+$, calcd 453.1989) along with the ^{13}C NMR data. The IR spectrum indicated the presence of hydroxyl (3486 cm^{-1}) and carboxyl (1681 cm^{-1}) groups. The ^{13}C NMR spectrum displayed 21 carbons (Table 1), consisting of characteristic signals for a glucose moiety (δ_C 102.6, 74.0, 74.9, 71.9, 73.7, 62.6). With the aid of HSQC experiment, the remaining 15 carbons were assigned as two methyls, four olefinic carbons, five methines (one oxygen-bearing), three methylenes, and one carbonyl carbon. Aglycone (**1a**) was obtained from an acid hydrolysis of **1** and the D-glucose was confirmed by using GC comparison of analysis of the hydrolysate according to the same protocol as earlier described [7]. The anomeric proton possessed a small *J* value of 4.0 Hz, indicating α configuration of the D-glucose residue. The NMR data of **1a** were proved to be identical to those of 2-hydroxy-cadin-6,12-dien-15-oic acid, suggesting that **1** was an *α*-D-glucosidic sesquiterpene. When compared the ^{13}C NMR spectrum with that of **1a,** the significant downfield shift of C-2 (δ_C 85.9) in **1** suggested that the *α*-D-glucose was connected to C-2,

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which was supported by the HMBC correlation from anomeric proton to C-2 (Figure 2A). All proton and carbon signals of **1** were assigned by $^{1}H-^{1}H$ COSY, HSQC, and HMBC data. The coupling constant of H-5 (dd, J = 10.5, 9.5 Hz) served to establish H-5, H-10 and H-4 as axial orientation. The NOESY cross-peaks of H-10/H-4, H-10/H-2β, H-10/H-8β, and H-10/H_3-11 indicated that the orientation of these protons are β, whereas the α orientation of H-1, H-5, and isopropenyl was deduced from the NOESY correlations of H-1/H-5, H-5/H-3α, and H-5/H_3-14 (Figure 2B). The CD curve of **1** was similar to that of (+)-(1*R*,2*R*,4*S*,5*S*,10*R*)-2-hydroxy-cadin-6,12-dien-15-oic acid (**1a**) [6], revealing a 1*R*,2*R*,4*S*,5*S*,10*R* configuration for **1**. This was confirmed by the result of comparison between the experimental and calculated ECD data of **1** (Figure 3). Therefore, the structure of **1** was defined as (+)-(1*R*,2*R*,4*S*,5*S*,10*R*)-2-α-D-glucopyranosyloxy-2-hydroxy-cadin-6,12-dien-15-oic acid.

By comparing with corresponding literature data, the known compounds were identified as drim-7-en-3β,11,12-triol (**2**) [9], and 3β-hydroxy-11,12-*O*-isopropylidenedrimene (**3**) [10].

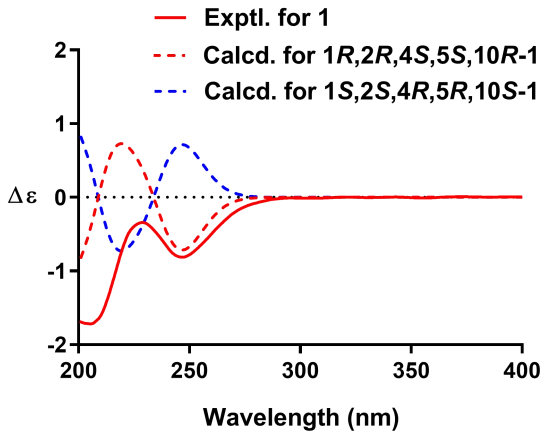

Figure 3. Experimental and computational ECD spectra for 1.

The isolates were tested for the inhibition of the NO production in lipopolysaccharide-induced RAW264.7 macrophage cells, TNF-α secretion in mouse peritoneal macrophages [11], protein tyrosine phosphatase 1B [12], and acetaminophen-induced HepG2 cell injury [13], as well as the cytotoxic properties toward HCT-116 colon, HepG2 liver, BGC-823 gastric and NCI-H1650 lung cancer cell lines [14], but they were all inactive at 10 μM.

Conclusion

In summary, the chemical constituent investigation of *Paeonia lactiflora* led to the discovery of a new sesquiterpenoidal glucoside (**1**) and two known sesquiterpenoids (**2-3**). This work extended our knowledge about the diversity of compounds in *P. lactiflora* and lays the foundation for further research.

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