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(±)-Paeoveitol, a Pair of New Norditerpene Enantiomers from *Paeonia veitchii*

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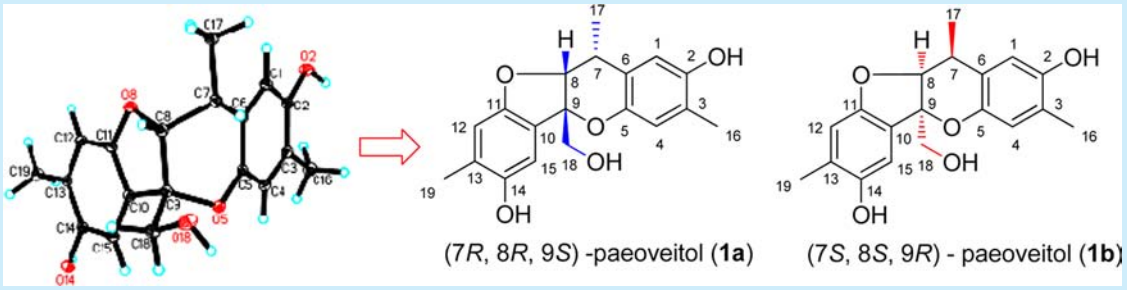
Supporting Information



ABSTRACT: (+)-Paeoveitol and (−)-paeoveitol, a pair of new norditerpene enantiomers, were isolated from the root of *Paeonia veitchii*. Their structures and absolute configurations were determined on the basis of extensive analysis of 1D and 2D NMR spectra, crystal X-ray diffraction, and electronic circular dichroism (ECD). A possible biogenesis involving two molecules of paeoniflorin was postulated.

Paeoniflorin-related monoterpene glycosides with a cage-like unit are rich in the genus *Paeonia* (Paeoniaceae) specifically[1] and generally recognized as the active constituents including activities in immunomodulation, anti-inflammation, hypoglycemic action, antidepression, and circadian regulation.[2] The root of *Paeonia veitchii*, the main source of traditional Chinese medicine "*Chuan-Chi-Shao*", is an important herb used as an analgesic, sedative, and cardiovascular agent. Previous studies of this plant resulted in the isolation of paeoniflorin-related monoterpenes, triterpenoids, and tannins.[3]

Our current investigation on the entitled plant led to a pair of new norditerpene paeoveitol, featuring an unexpected 6/5/6/6-fused tetracyclic ring system. Paeoveitol was resolved to be (+)-paeoveitol and (−)-paeoveitol by HPLC using a chiral column, and the absolute configurations were determined by computational methods via calculation of the electronic circular dichroism (ECD). Biosynthetically, paeoveitol might be derived from two molecules of paeoniflorin to fulfill the structure. Herein, we describe their isolation, structure elucidation, and possible biogenetic pathway.

The air-dried roots of *P. veitchii* (5.0 kg) were extracted with 90% EtOH−H_2O (20 L) at room temperature two times, for 2 h each time. The extract was concentrated *in vacuo* to give a residue, which was suspended in H_2O and partitioned between ethyl acetate (EtOAc) and *n*-BuOH, respectively. The EtOAc fraction (160 g) was further purified by silica gel column chromatography [acetone−petroleum ether (PE), 10:90, 20:80, 30:70, 50:50, 70:30, 80:20, 100:0, v/v] to give 10 fractions. Fr. 6 was purified by silica gel column chromatography (EtOAc−PE, 10:90, 20:80, 30:70, 50:50, 100:0) and Sephadex LH-20 (MeOH) to give a pair of enantiomers which were evidenced by a small amount of optical rotation values. Enantiomers were further separated by HPLC (chiralpak AS-H, isopropanol−*n*-hexane, 12:88, 4.6 mm × 250 mm) to yield (+)-paeoveitol (0.81 mg, t_R = 56.8 min) and (−)-paeoveitol (0.79 mg, t_R = 81.8 min).

Paeoveitol (**1**)[4] was obtained as a white prismatic crystal and had a molecular formula of $C_{19}H_{20}O_5$ by the positive HRESIMS (*m*/*z* 351.1203 [M + Na]$^+$, calcd: 351.1203), requiring 10 degrees of unsaturation. The presence of hydroxyl (3426 cm^{-1}), aromatic (1630, 1462 cm^{-1}), and ether (1179 cm^{-1}) groups was deduced from the IR spectrum.

In the ^{1}H NMR spectrum, four aromatic protons at δ_H 7.55 (1H, s, H-15), 7.19 (1H, s, H-1), 6.84 (1H, s, H-4), and 6.61 (1H, s, H-12); two methines at δ_H 5.56 (1H, overlapped, H-8) and 3.42 (1H, m, H-7); one methylene at δ_H 4.66 (1H, d, *J* = 12.0 Hz, H-18a), 4.44 (1H, d, *J* = 12.0 Hz, H-18b), together with three methyls at δ_H 2.23 (3H, s, H-19), 2.16 (3H, s, H-16), and 1.59 (3H, d, *J* = 6.6 Hz, H-17) were observed (Table 1), which were unambiguously designated by the HSQC experi-

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Table 1. NMR Spectroscopic Data of Compound 1[a] in Pyridine-d_5 (δ in ppm)

position	δ_H (multiplicity (m), J in Hz)	δ_C	HMBC
1	7.19 (1H, s)	113.7 (d)	3, 5, 7
2	–	152.6 (s)	–
3	–	123.8 (s)	–
4	6.84 (1H, s)	120.9 (d)	2, 6
5	–	148.1 (s)	–
6	–	128.6 (s)	–
7	3.42 (1H, m)	33.7 (d)	1, 5, 9
8	5.56 (1H, overlapped)	90.1 (d)	10, 11, 17, 18
9	–	90.4 (s)	–
10	–	126.6 (s)	–
11	–	155.0 (s)	–
12	6.61 (1H, s)	112.5 (d)	10, 14
13	–	128.7 (s)	–
14	–	151.5 (s)	–
15	7.55 (1H, s)	111.5 (d)	9, 11, 13
16	2.16 (3H, s)	17.0 (q)	2, 3, 4
17	1.59 (3H, d, 6.6)	14.3 (q)	6, 8
18	4.66 (1H, d, 12.0)	67.4 (t)	8, 10
	4.44 (1H, d, 12.0)		
19	2.23 (3H, s)	17.9 (q)	12, 13, 14

[a] ^{1}H NMR recorded at 600 MHz; ^{13}C NMR recorded at 150 MHz.

ment. The ^{13}C NMR (DEPT) spectrum exhibited 19 carbon resonances, assigned to nine quaternary carbons, six methines, one methylene, and three methyls. Taking the molecular formula and the ^{1}H NMR spectrum into consideration, two aromatic rings accounted for 8 of 10 degrees of unsaturation.

One aromatic ring was elucidated by the ^{1}H NMR spectrum and HMBC experiment (H-1 to C-3, C-5 and H-4 to C-2, C-6). In addition, the correlations of H-16 to C-2, C-3, and C-4 suggested that the methyl unit was located at C-3. From the above evidence, the structure of subunit A was obviously distinguished.

The existence of subunit B was confirmed by the correlations of H-12 to C-10, C-14; H-15 to C-11, C-13; H-19 to C-12, C-13, C-14 in the HMBC spectrum.

The sequential ^{1}H−^{1}H COSY correlations from H-7 to H-8 and H-17, together with the HMBC correlations of H-7 to C-9; H-8 to C-17, C-18; H-18 to C-8, led to the construction of subunit C.

Meanwhile, the cross-peaks of H-17 to C-6; H-7 to C-1, C-5; H-1 to C-7; H-8 to C-10 and C-11; H-18 to C-10; and H-15 to C-9 in the HMBC displayed the linkages of C-6/C-7 and C-9/C-10. Combined with the requirement accounted for the remaining 2 degrees of unsaturation, two ether bonds between C-5 and C-9, C-8 and C-11 were deduced, which was supported by the single-crystal X-ray diffraction.[5] Thus, the planar structure of compound **1** was elucidated as shown in Figure 1.

The single-crystal X-ray diffraction data showed the space group $C2/c$, indicating that paeoveitol (**1**) was a pair of enantiomers which was further confirmed by a small amount of the $[\alpha]_D$ value. The subsequent chiral resolution of **1** by HPLC afforded the anticipated enantiomers, (+)-paeoveitol and (−)-paeoveitol, which were opposite in terms of their CD curve and $[\alpha]_D$ spectra. ECD spectra derived from quantum-chemical calculations have been used successfully for the stereochemical assignment of small- and medium-sized molecules.[6] Starting from the relative stereochemistry evidenced from the single-crystal X-ray diffraction (Figure 2), the quantum chemical calculation on the ECD spectra was utilized to elucidate its absolute stereochemistry.

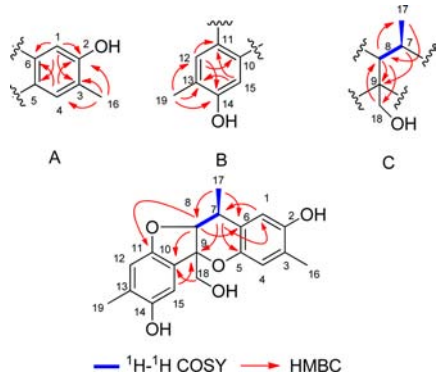

Figure 1. Fragment structures and selected 2D NMR correlations of **1**.

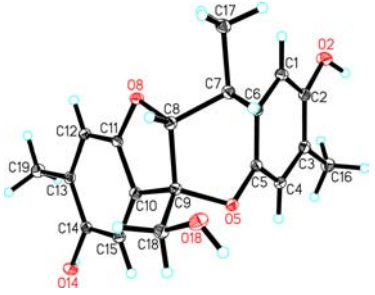

Figure 2. X-ray crystallographic structure of **1**.

To determine the absolute configuration of the enantiomers of **1**, the comparison between the experimental and calculated ECD spectra of (+)-paeoveitol and (−)-paeoveitol (Figure 3)

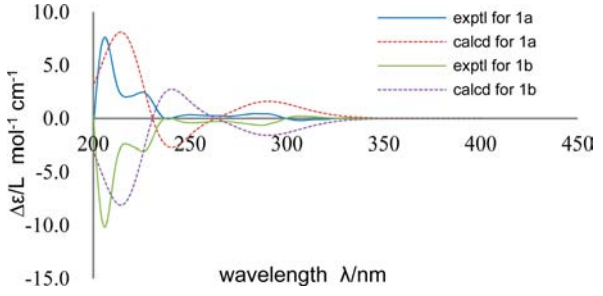

Figure 3. Calculated and experimental ECD spectra of **1a** and **1b**.

was performed using the time-dependent density functional theory (DFT) method at the B3LYP/6-311++G (2d, p) level. The calculated ECD spectrum for **1a** agreed well with that measured for (+)-**1a**, whereas the spectrum calculated for **1b** showed an opposite CD curve and was consistent with the experimental ECD spectrum for (−)-**1b**. Thus, the absolute stereochemistry of **1a** and **1b** was deduced to be 7*R*, 8*R*, 9*S* and 7*S*, 8*S*, 9*R* respectively (Figure 4).[7]

Paeoniflorin-related compounds were reported as the characteristic components of *P. veitchii*, which were the cage-like monoterpenes widely distributed in the *Paeonia* genus and might biosynthetically be generated from *p*-menthane.

Correspondingly, compound **1** might biosynthetically be derived from two molecules of paeoniflorin (Scheme 1). It was

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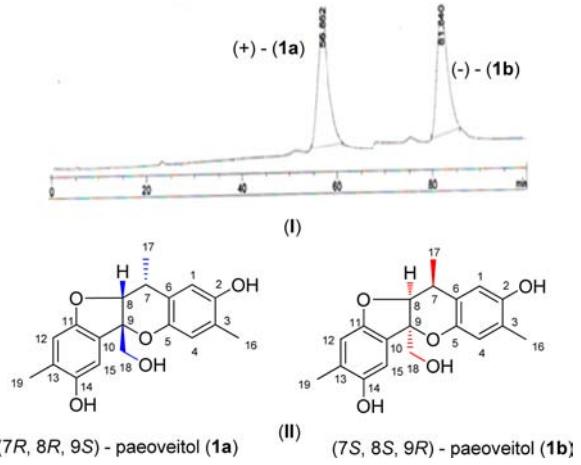
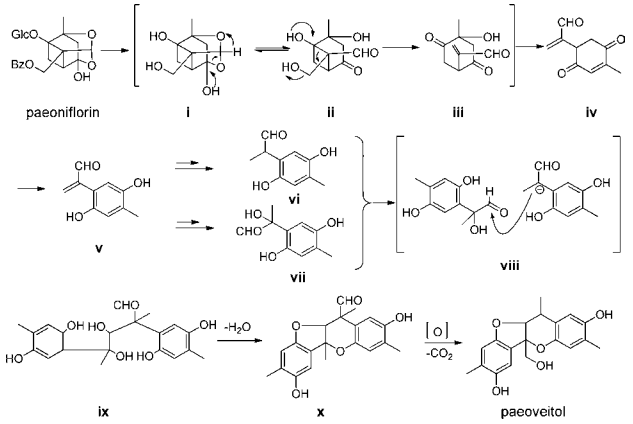


Figure 4. Chiral HPLC chromatogram (**I**) and structures of enantiomers (**II**): (+)-paeoveitol (**1a**) and (−)-paeoveitol (**1b**).

Scheme 1. Hypothetical Biogenetic Pathway of 1



assumed that intermediates (**i**, **ii**, **iii**) were obtained by hydrolysis of glucosyl, loss of benzoyl groups, cleavage of the hemiketal−acetal linkage, and the four-membered ring.[8] Subsequently, the structure of paeoveitol was established via a series of dehydration, cyclization, oxidation, and decarboxylation.

Previous reports demonstrated that paeoniflorin-related monoterpenes were the characteristic compounds of the *Paeonia* genus with antidepressant-like action,[2c,d] and antidepressants often involved the receptors of 5-hydrooxytryptamine 1A (5-HT_{1A}) and 5-hydrooxytryptamine 2C (5-HT_{2C}).[9] Thus the agitating activities of the 5-HT_{1A} and 5-HT_{2C} receptor assay of paeoveitols **1**, **1a**, and **1b** were studied on the HEK293 cell line *in vitro*, which showed no agitating activity on 5-HT_{1A} and 5-HT_{2C} receptors at the concentration of about 1 mM using serotonin hydrochloride (5-HT) as the positive control (bioassay tests in the Supporting Information).

In conclusion, paeoveitol (**1**) is the first report of a new norditerpene skeleton, possessing an unexpected 6/5/6/6-fused tetracyclic ring system. Biosynthetically, paeoveitol (**1**) might be derived from two molecules of paeoniflorin to form a norditerpene with a unique skeleton in the genus *Paeonia* and serve as another characteristic norditerpene for further investigation from natural sources.

■ ASSOCIATED CONTENT

Supporting Information

1D and 2D NMR, MS, IR, $[\alpha]_D$, UV spectra, the data for single-crystal X-ray diffraction of compound **1** (CIF). $[\alpha]_D$, ECD spectra for **1a** and **1b**, the agitating activities of the 5-HT_{1A} and 5-HT_{2C} receptor assay of paeoveitols **1**, **1a**, and **1b** together with experimental details. This material is available free of charge via Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(4) Paeoveitol (**1**): white prismatic crystal, mp: 151−152 °C, $[\alpha]_D^{12.6}$: −2.70 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ε): 203 (4.52), 297 (3.76) nm; IR (KBr) ν_{max}: 3426, 2966, 2926, 2857, 1630, 1501, 1462, 1420, 1179, 1063, 1004 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRESIMS: m/z = 351.1203 $[M + Na]^+$ ($C_{19}H_{20}O_5$, calcd for 351.1203).

(5) Crystal data for compound **1**: $C_{19}H_{20}O_5$, M = 328.35, monoclinic, a = 19.2190(18) Å, b = 8.9835(8) Å, c = 18.1799(16) Å, α = 90.00°, β = 95.0930(10)°, γ = 90.00°, V = 3126.4(5) $Å^3$, T = 100(2) K, space group $C2/c$, Z = 8, μ(MoKα) = 0.101 mm^{-1}, 16 133 reflections measured, 4377 independent reflections (R_{int} = 0.0347). The final R_1 value was 0.0456 ($I > 2\sigma(I)$). The final $wR(F^2)$ value was 0.1121 ($(I > 2\sigma(I))$). The final R_1 value was 0.0637 (all data). The final $wR(F^2)$ value was 0.1229 (all data). The goodness of fit on F^2 was 1.025. Crystallographic data of peoveitol (**1**) have been deposited at the Cambridge Crystallographic Data Centre (deposition no. CCDC970712).

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(7) (+)-Paeoveitol: $[\alpha]_D^{27.7}$: +30.08 (*c* 0.078, MeOH); (−)-paeoveitol: $[\alpha]_D^{28.0}$: −25.96 (*c* 0.066, MeOH).
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