Chemical Constituents of the Aerial Part of Valeriana officinalis var. latifolia Miq. With COX-2 Inhibitory Activity

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Abstract
Twelve compounds, including a new iridoid (1) and 11 known compounds, were obtained from the aerial part of Valeriana officinalis var. latifolia Miq. Their structures were assigned by spectroscopic and mass spectrometric methods. All isolates were screened in vitro for cyclooxygenase-2 (COX-2) inhibitory activity. Two compounds (9 and 10) exhibited significant COX-2 inhibitory activity, with IC50 values ranging from 0.67 to 6.77 μM. The possible recognition mechanism between compound 9 and COX-2 was predicted by molecular docking analysis.

Keywords
Valeriana officinalis var. latifolia Miq., valerianaceae, iridoids, lignans, COX-2 inhibitors

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Introduction
Valeriana officinalis var. latifolia Miq., a perennial herb, is widely distributed throughout China, such as Guizhou, Qinghai, Sichuan, Hebei, and Henan.1 As a traditional folk medicine, the roots of this plant have been used for the treatment of insomnia for centuries.2 Previous chemical studies on V. officinalis revealed the presence of a variety of sesquiterpenes,3 iridoids,3 lignans,4 and alkaloids.2 However, its aerial parts have seldom been investigated. Thus, this study on the chemical constituents of the aerial part led to the discovery of a new iridoid (1) and the identification of 11 known compounds (Figure 1). Herein, we report the isolation, structural elucidation, and cyclooxygenase-2 (COX-2) inhibitory activities of these isolates.

Results and Discussion
6α-Hydroxy-7α-(hydroxymethyl)-4-methylenhexahydrocyclopenta[c]pyran-1(3H)-one acetonide (I) has the molecular formula C13H18O4 according to HR-ESI-MS (m/z 261.1093, [M + Na]+, calcd for C13H22O4 261.1097). Its infrared (IR) absorptions implied the presence of an ester carbonyl group (1730, 1658 cm⁻¹) and a terminal double bond (890, 829, 767 cm⁻¹). The 1H-NMR spectrum indicated the presence of 2 singlet methyl groups (δH 1.37, s, 3H; 1.48, s, 3H) and 2 protons of terminal olefins (δH 5.02, d, J = 2.0 Hz; 5.16, d, J = 2.0 Hz). The 13C NMR and DEPT spectra (Table 1) of 1 exhibited 13 carbon signals, including an ester carbonyl (δC 174.8), a characteristic terminal double bond (δC 113.4 and 142.0), a hemiketal carbon (δC 97.7), 2 methyl groups (δC 19.1 and 29.5), 3 methylenes (δC 38.9, 60.6, and 71.9), and 4 methines (δC 39.9, 42.2, 43.9, and 44.3).
Further comparison of the 1D NMR data of compound 1 with those of 2 implied that both compounds shared a similar skeleton. The only differences were the presence of 2 methyl and a hemiketal carbon. In the heteronuclear multiple bond correlation (HMBC) spectrum, correlations from Me-13 (δ_H 1.37) and Me-14 (δ_H 1.48) to C-12 (δ_C 97.7) and from H2-10 (δ_H 3.95 and 4.21) to C-12 (δ_C 97.7) confirmed this deduction. Moreover, a cyclopentane ring was identified from the 1H-1H COSY cross-peaks of H2-5 (δ_H 1.62 and 2.19)/H-6 (δ_C 4.36), H-6 (δ_C 4.36)/H-7 (δ_H 2.25), H-7 (δ_C 2.25)/H-8 (δ_H 3.36), H-8 (δ_C 3.36)/H-9 (δ_H 3.51), H-9 (δ_C 3.51)/H2-5 (δ_H 1.62 and 2.19), and H-7 (δ_H 2.25)/H2-10 (δ_C 3.95 and 4.21) (Figure 2). The HMBC correlations of H2-11 (δ_H 5.02 and 5.16) to C-3 (δ_C 71.9), C-4 (δ_C 142.0), and C-9 (δ_C 39.9), H2-3 (δ_C 4.61 and 4.73) to C-1 (δ_C 174.8), and H-8 (δ_C 3.36) to C-1 (δ_C 174.8) enabled identification of the structure. Thus, the planar structure was established as shown in Figure 2.

**Table 1.** 1H (600 MHz) and 13C (150 MHz) NMR Data of 1 in CDCl3.

| Pos. | δ_H (J in Hz) | δ_C |
|------|--------------|-----|
| 1    | 174.8        |     |
| 3a   | 4.73, d, (11.6) | 71.9 |
| 3b   | 4.61, d, (11.6) |     |
| 4    |               | 142.0 |
| 5a   | 2.19, m      | 38.9 |
| 5b   | 1.62, m      |     |
| 6    | 4.36, m      | 71.4 |
| 7    | 2.25, m      | 42.2 |
| 8    | 3.36, dd, (12.0, 8.6) | 43.9 |
| 9    | 3.51, m      | 39.9 |
| 10a  | 3.95, dd, (12.3, 1.2) | 60.6 |
| 10b  | 4.21, dd, (12.3, 3.5) |     |
| 11a  | 5.16, d, (2.0) | 113.4 |
| 11b  | 5.02, d, (2.0) |     |
| 12   |               | 97.7 |
| 13   | 1.37, s      | 29.5 |
| 14   | 1.48, s      | 19.1 |

**Figure 2.** Key HMBC (→) and 1H-1H COSY (→) correlations of 1 and key NOESY (↔) correlations of 1.
The relative configuration was assigned by a NOESY experiment (Figure 2). The NOESY correlations from H-9 (δH 4.36) to H-6 (δH 4.36) and H-7 (δH 2.25) and from H-8 (δH 3.36) to H-6 (δH 4.36) implied that those protons were on the same side of the molecule and were assigned arbitrarily as β-oriented. Thus, the relative configuration of compound 1, different from that of compound 2, was assigned. The absolute configuration was established from the calculated electronic circular dichroism (ECD).b Compound 1 had 4 stereocenters at C-6, C-7, C-8, and C-9. The absolute configuration for compound 1 had 16 different molecules, and the ECD spectra for all possible compounds were calculated in Supporting Information. The results indicated that the absolute configuration was determined as (6S, 7S, 8R, and 9R)-1, which is also in good agreement with its relative configuration (Figure 3). Therefore, the structure of this compound was established.

Eleven known compounds were elucidated by extensive nuclear magnetic resonance spectroscopy (NMR) methods and compared with the reported data in the literatures. Their structures were assigned as 6β-hydroxy-7β-(hydroxymethyl)-4-methylenehexahydrocyclopenta[c]pyran-1(3H)-one (1),5 isobicyclogermacral (2),5 isobicyclo[4.3.0]non-7-en-6-one (3),5 4-methyl-2-butenyl-4-ene-2-carboxylic acid (4),5 friedelin (5),9 24-methylenecholesterol (6),10 trans-phytol (7),11 2-hydroxy-4,6-dimethoxyacetophenone (8),12 (-)-stigmasterol (9),13 1-hydroxy-5,6-dimethoxy-6-methylhept-2-en-1-one (10),14 4′,5,7-trimethoxyflavone (11),15 and β-sitosterol (12).16 (Figure 1). This is the first report to identify 9 compounds (1 and 4-11) in this plant, of which 8 (1, 4-9, and 11) were isolated from the family Valerianaceae for the first time.

6α-Hydroxy-7α-(hydroxymethyl)-4-methylenehexahydrocyclopenta[c]pyran-1(3H)-one acetone (1), with a 6-membered acetal, might be derived from a diol of this compound and acetone. Liquid chromatography–mass spectrometry experiments were used to determine whether the crude extracts of the plant contained compound 1. The results indicated that compound 1 was not included in the aerial part of the plant (Figure S33 in Supporting Information). Thus, this compound might be an artificial compound that formed during the separation. Moreover, the relative configuration of 2 was assigned by comparing the 1D NMR data with those in the literature.5 In addition, an Overhauser effect difference (NOEdiff) experiment was used for compound 2 to confirm its relative configuration. Overhauser effect (NOE) correlations from H-9 (δH 3.46) to H-8 (δH 3.00), H-5β (δH 2.15), and H-11β (δH 5.07) and the absence of correlations from H-9 to H-6 and H-7 assigned the relative configuration of compound 2 (Figure S32 in Supporting Information). Compounds 1 and 2 had different relative configurations.

Cyclooxygenase-2 is a crucial target for the development of agents to treat inflammation.17 All compounds were evaluated for COX-2 inhibitory activity at a concentration of 100 μM. Four active compounds (5, 8, 9, and 10) were further tested for their IC50 values (IC50: 0.67–93.5 μM) (Table 2). Two lignans (9 and 10) exhibited significant activity with IC50 values lower than 10 μM.

To investigate the molecular recognition mechanism between compound 9 and COX-2, compound 9 was applied to in silico analysis with a human COX-2 protein model (PDB code: 3LN1).18 As shown in Figure 4, the structure of 9 was packed into the catalytic site of the protein formed by TYR-341, GLN-178, PRO-500, ALA-502, ARG-499, and VAL-509. Moreover, 4 hydrogen bonds were observed to form between compound 9 and TYR-341, GLN-178, PRO-500, and ARG-499.

**Experimental Section**

**General Experimental Procedures**

1D and 2D NMR spectra were recorded on a Bruker Avance NEO 600 spectrometer using TMS as an internal standard. ESI-MS and HR-ESI-MS were recorded on Agilent 1100 and Thermo ultimate 3000/Q EXACTIVE FOCUS mass spectrometers, respectively, optical rotations on a JASCOP-1020 polarimeter, UV spectra on a Shimadzu UV-2401PC spectrometer, IR spectra on a Bruker FT-IR Tensor-27 and iCAN 9

![Diagram](image)

**Figure 3.** Calculated and experimental electronic circular dichroism (ECD) of (6S,7S,8R,9R)-1 and (6R,7R,8S,9R)-1.

**Table 2.** COX-2 Inhibitory Effects of the Isolates.\(^b\)

| Com. | IC50 (μM)   |
|------|-------------|
| 5    | 93.5 ± 1.5  |
| 8    | 68.8 ± 2.1  |
| 9    | 0.67 ± 0.05 |
| 10   | 6.77 ± 0.16 |
| Celecoxib\(^b\) | 0.02 ± 0.01 |

\(^b\)Celecoxib was used as a positive control.

Abbreviation: COX-2, cyclooxygenase-2.
infrared spectrophotometer with KBr disks, and CD spectra with an Applied Photophysics Chirascan spectrometer. Column chromatography was performed on silica gel (300-400 mesh; Qingdao Marine Chemical Co. Ltd), Sephadex LH-20 (40-70 µm, Amersham Pharmacia Biotech AB), and RP-C18 gel (40-63 µm, Merck). Semipreparative high-performance liquid chromatography (HPLC) was performed on an instrument consisting of a Hanbon NP7005c controller, a Hanbon NP7005 pump, and a Hanbon NU3000c UV detector with a YMC-Triart-C18 column (250 × 10.0 mm, 5 µm).

**Plant Material**

*Valeriana oficinalis* var. *latifolia* Miq., collected in Tongren, Guizhou Province of China, was identified by Dr Mingjin Huang. A voucher specimen (H20180913) was deposited in the Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Science.

**Extraction and Isolation**

The air-dried and powdered aerial parts of *V. oficinalis* (10 kg) were refluxed with methanol (3 × 40 L), 3 times (3 × 3 h). The combined solution was evaporated under vacuum to obtain 4.0 kg residue, which was suspended in water and then partitioned with ethyl acetate (EA) (3 × 15 L). The EA part (600 g) was separated on a silica gel column eluted with PE (light petroleum)/acetone gradient elution (50:1-1:0) to afford 7 fractions (Fr.1-Fr.7). Fr.2 (40.2 g) was applied to an MCI gel column, eluted with a gradient of CH₃OH/H₂O (60:40-95:5) to obtain 5 fractions (Fr.2A-Fr.2G). Fr.2D (5.2 g) was separated by column chromatography over silica gel to get 1 (20 mg), 2 (13 mg), 4 (9 mg), and 8 (12 mg). Fr. 2E (3.2 g) was separated by column chromatography over silica gel and further purified by semipreparative HPLC with X-bridge eluted with CH₃OH/H₂O (2.0 mL/min, CH₃OH:H₂O = 85:15, vol/vol) to yield 3 (12 mg, tₚ = 15 min), 5 (21 mg, tₚ = 23 min), and 12 (7 mg, tₚ = 38 min). Fr. 2F (1.5 g) was applied to Sephadex LH-20 and then further purified by semipreparative HPLC with X-bridge eluted with CH₃OH/H₂O (2.0 mL/min, CH₃OH:H₂O = 78:22, vol/vol) to obtain 10 (5.0 mg, tₚ = 27 min) and 11 (7.6 mg, tₚ = 35 min). Fr. 3 (20 g) was subjected to column chromatography over silica gel eluted with dichloromethane/acetic acid (100:1-1:1) and further purified on Sephadex LH-20 to obtain 6 (20 mg), 7 (9.5 mg), and 9 (38 mg).

**Bioassays**

The inhibitory activities of the isolated compounds toward COX-2 were evaluated using a COX-2 Inhibitor Screening

![Figure 4. Binding pose of compound 9 with cyclooxygenase-2 (COX-2).](image)
Kit (Beyotime). Stock solutions of test samples were prepared and diluted in DMSO. Celecoxib was used as a positive control. According to the manufacturer’s protocols, a recombinant human COX-2 enzyme in 96-well plates was incubated with varying concentrations of test samples for 10 min at 37 °C. Then, COX-2 probe and substrate were added to each well, respectively, and incubated for another 15 min at 37 °C in the dark. The intensity of fluorescence was measured using a microplate reader (BioTek) with an excitation wavelength of 560 nm and an emission wavelength of 590 nm.

Computational Section
A docking study was performed, as described previously. For enzyme preparation, a 3LN1 human COX-2 model was downloaded from the Protein Database (https://www.rcsb.org/structure/3LN1, code: 3LN1). The ligand was bound with 3LN1 at the molecular simulation level by utilizing Lmdock. Hydrogen atoms were added and the crystallographic water molecules in 3LN1 were removed. At neutral pH, all the dissociable residues in the system were set to their protonated states. Celcoxib, the inhibitor of COX-2 in 3LN1, was used as a reference compound to define the active site of 3LN1 for docking. All the parameters were default. Finally, the binding pose of compound 9 with 3LN1 was generated by MOE_2019.

Conclusions
A new iridoid (I) and 11 known compounds were isolated from the aerial part of Valeriana officinalis var. glutifolia Miq. This acetonide (I) is an artificial compound that formed during the separation. Their structures were assigned by spectroscopic methods. Nine compounds (I and 4-11) were identified in this plant for the first time. Two lignans (9 and 10) exhibited significant COX-2 inhibitory activity with IC_{50} values ranging from 0.67 to 6.77 μM. The possible recognition mechanism between compound 9 and COX-2 was predicted by molecular docking analysis.

Declaration of Conflicting Interests
The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material
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