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Labdane diterpenes from *Chloranthus serratus*

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ABSTRACT

Five new labdane diterpenes (**1**–**5**), serralabdanes A–E, were isolated from the whole plant of *Chloranthus serratus*. Their structures were elucidated by spectroscopic methods, and the absolute configuration of the 12,13-diol moiety in serralabdane C (**3**) was determined by observing the induced circular dichroism (ICD) after addition of dimolybdenum teracetate in DMSO solution. Serralabdanes A–E (**1**–**5**) showed inhibitory effects on lipopolysaccharide-induced nitric oxide production in RAW264.7 cells.

1. Introduction

Labdane diterpenes, possessing a variety of changeable skeletons, are mainly distributed widely in higher plants, such as Labiate, Asteraceae, Acanthaceae, Euhorbiaceae, Chloranthaceae, and Zingiberaceae, among others. Most of them have marked bioactivities, including anti-inflammatory, anti-bacterial, cardiotonic, and hypotensive activities [1]. *Chloranthus serratus* (Thnub.) Roem. et Schult., belonging in Chloranthaceae, is a perennial herbaceous plant usually distributed in eastern Asia. Its whole plant has been used for the treatment of bruises, bone fractures, rheumatoid arthritis, etc. in Chinese folk [2,3]. Previous investigations on the chemical constituents of this plant and their activities by our group [4] and other researchers [5–9] have discovered many terpenoids, mainly including sesquiterpene and sesquiterpenoid dimers, and some of which displayed significantly anti-inflammatory activities [4]. In our further study, five new labdane diterpenes (**1**–**5**), serralabdanes A–E, were isolated from the whole plant of *C. serratus*. Their structures and relative configurations were elucidated by spectroscopic methods (Fig. 1). And the absolute configuration of the 12,13-diol moiety in serralabdane C (**3**) was determined by observing the induced circular dichroism (ICD) after the addition of dimolybdenum teracetate in DMSO solution (Snatzke's method). Serralabdanes A–E (**1**–**5**) were evaluated for their inhibitory effects on lipopolysaccharide-induced nitric oxide production in RAW264.7 cells.

2. Experimental details

2.1. General

Optical rotations were obtained on a JASCO P-1020 polarimeter. CD spectra were measured with a JASCO 810 spectropolarimeter. UV spectra were recorded on a Shimadzu UV-2450 spectropolarimeter. NMR spectra were obtained on Bruker ACF-500 with TMS as the internal standard. High-resolution mass spectra were obtained on an Agilent UPLC-Q-TOF (6520B). Silica gel (200–400 mesh, Qingdao Haiyang Chem. Co.), MCI gel (75–150 μm, Mitsubishi) and RP-C_{18} (40–63 μm, Fuji) were used for column chromatography (CC). Recycling preparative HPLC was carried out using Agilent 1100 Series equipped with Shim-park RP-C_{18} column (5 μm, 20 × 200 mm) and 1100 Series Multiple Wavelength Detector.

2.2. Plant material

Whole plants of *C. serratus* were collected in May 2010 from Tiantang Village, Anhui Province of China, and authenticated

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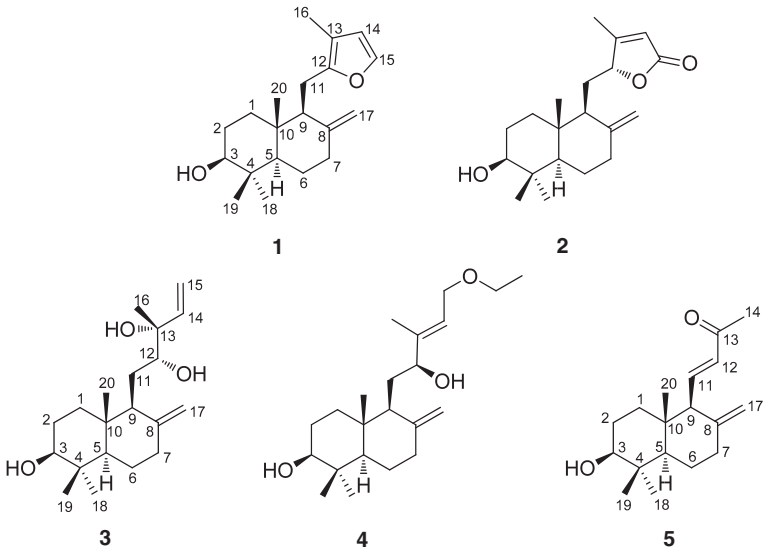

Fig. 1. Structures of compounds **1**–**5**.

by Prof. Gan Yao of the Institute of Botany, Jiangsu Province and Chinese Academy of Sciences. A voucher specimen (CS-2010005) has been deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

2.3. Extraction and isolation

The whole plants of *C. serratus* (1.8 kg) were extracted with 95% EtOH (4 × 6 L) to give 163 g of residue. The residue was suspended in water and partitioned with EtOAc to afford the EtOAc fraction (101 g). The fraction extract was chromatographed over a silica gel column eluted with petroleum ether–EtOAc in a gradient (1:0 to 0:1) to yield twenty fractions (1–20), monitored by TLC. Fraction 12 (2.0 g) was chromatographed on MCI gel, eluted successively with MeOH–H_2O (1:1 to 7:3) to give three subfractions (12a–12c). Fraction 12c (145.8 mg) was subjected to reversed-phase C_{18} silica gel, eluted with MeOH–H_2O (1:1 to 7:3), to give five subfractions (12c1–12c5). Fraction 12c2 (15.3 mg) was separated by recycling preparative HPLC using MeOH–H_2O (65:35) to give **1** (5.7 mg) and **2** (2.2 mg). Fraction 12c3 (25.6 mg) was separated by recycling preparative HPLC using MeOH–H_2O (70:30) to give **3** (2.7 mg), **4** (7.5 mg), and **5** (4.8 mg).

Serralabdane A (**1**): colorless oil; $[\alpha]^{25}_D$ − 3.8 (c = 0.22, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.71) nm; ^{13}C and ^1H NMR data, see Tables 1 and 2; HRESIMS m/z 303.2320 (calcd for $C_{20}H_{31}O_2$, 303.2319).

Serralabdane B (**2**): white powder; $[\alpha]^{25}_D$ + 5.2 (c = 0.26, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.83) nm; CD (1.7×10^{-4} MeOH) $\Delta\varepsilon_{216\ nm}$ −16.95; ^{13}C and ^1H NMR data, see Tables 1 and 2; HRESIMS m/z 341.2084 (calcd for $C_{20}H_{30}O_3Na$, 341.2087).

Serralabdane C (**3**): white powder; $[\alpha]^{25}_D$ + 28.8 (c =0.18, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.63) nm; ^{13}C and ^1H NMR data, see Tables 1 and 2; HRESIMS m/z 345.2402 (calcd for $C_{20}H_{34}O_3Na$, 345.2400).

Serralabdane D (**4**): white powder; $[\alpha]^{25}_D$ − 2.9 (c =0.33, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.71) nm; ^{13}C and ^1H NMR data, see Tables 1 and 2; HRESIMS m/z 373.2701 (calcd for $C_{22}H_{38}O_3Na$, 373.2701).

Serralabdane E (**5**): white powder; $[\alpha]^{25}_D$ + 2.6 (c = 0.21, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.18) nm, 205 (4.15); ^{13}C and ^1H NMR data, see Tables 1 and 2; HRESIMS m/z 277.1272 (calcd for $C_{18}H_{29}O_2$, 277.1262).

2.4. Anti-inflammatory bioassays

The protocol of the anti-inflammatory bioassays was provided in a previously published paper with dexamethasone as the reference [10].

Table 1
^{13}C NMR (125 MHz) spectral data of compounds **1**–**5** (in $CDCl_3$).

No.	1	2	3	4	5
1	37.2	36.7	37.1	37.0	38.6
2	28.2	28.0	27.9	28.6	27.6
3	79.0	78.5	78.8	78.8	78.8
4	39.4	39.1	39.0	39.2	39.0
5	54.8	52.2	54.6	54.7	53.7
6	24.1	24.0	24.0	24.0	22.9
7	38.1	37.9	38.2	38.1	36.4
8	148.4	148.1	148.4	148.4	147.9
9	54.4	54.3	52.1	52.9	60.5
10	39.5	39.1	39.2	39.2	39.3
11	21.9	27.8	26.3	28.0	145.8
12	150.9	83.4	75.9	77.2	133.7
13	113.6	169.2	75.8	140.3	197.9
14	113.1	116.6	141.0	124.8	27.3
15	139.6	173.1	114.5	66.7	
16	10.3	13.9	24.5	10.7	
17	107.4	106.5	106.9	107.1	109.0
18	28.6	28.3	28.3	28.3	28.3
19	15.7	15.4	15.4	15.4	15.1
20	14.5	14.5	14.7	14.6	15.6
–OEt				65.6	
				15.3	