

A New Labdane Diterpene from the Aerial Parts of *Chloranthus serratus*

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Abstract: A new labdane diterpene (**1**) and five known compounds (**2–6**) were isolated from the aerial parts of *Chloranthus serratus*. The gross structure of compound **1** was resolved by NMR and HRESIMS data. The absolute configuration of **1** was determined by comparing CD spectrum and specific rotations with those of the co-isolated known analogue **2**. The known compounds were identified to be agathic acid (**2**), altechromone A (**3**), chlorajapolide F (**4**), shizukanolide (**5**), and chloranthalactone E (**6**) by comparing the NMR data with those in the literature. All compounds were evaluated for their inhibitions against NO production in LPS-activated RAW 264.7 macrophages, compounds **2** and **6** showed moderate inhibitions with IC₅₀ values of 45.2 ± 1.2 and 35.3 ± 0.9 μM.

Keywords: *Chloranthus serratus*; labdane diterpene; inhibitions; NO production. © 2020 ACG Publications. All rights reserved.

1. Plant Source

The aerial part of *Chloranthus serratus* were collected in November 2014 in Guangdong Province, P. R. China. Identity of the species was identified by Prof. Lin Jiang of School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou. The voucher specimen (accession number: SYXS201411) was deposited at Jiangxi Provincial People's Hospital.

2. Previous Studies

Chloranthus serratus is a perennial herb widely distributed in Southern China, especially in Anhui, Zhejiang, Hunan, Fujian, and Guangdong Provinces [1]. It is used for the treatment of bruises, furuncles, snakebites, and rheumatoid arthritis in Traditional Chinese Medicine (TCM) [1]. Previous investigations of the constituents of this species resulted in the isolation of lindenane sesquiterpenoids [2,3], eudesmane sesquiterpenoids [4], phenylpropanoids [2], and labdane diterpenoids [5], several sesquiterpenoids showed antimicrobial and anti-inflammatory effects.

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3. Present Study

The air-dried powder of the aerial parts of *C. serratus* (1.5 kg) was extracted with 75% EtOH (3 × 5 L) at room temperature (rt) to give 105 g of crude extract. The extract was suspended in H₂O (1 L) and further extracted with petroleum ether (3 × 0.5 L) and EtOAc (3 × 0.5 L), respectively. The EtOAc extract (80 g) was separated over MCI gel chromatographic column (CC) using MeOH/H₂O (3:7 → 10:0) as eluent to give five fractions (I–VI). Fraction IV was subjected to silica gel CC (petroleum ether/acetone, 20:1 → 1:1) to give five fractions (IVa–IVe). IVa was subjected to Sephadex LH-20 (ethanol) to obtain two fractions (IVb1–IVb2), further purification of IVb1 by silica gel CC (petroleum ether/EtOAc, 20:1 → 5:1) yielded **5**. IVb was purified by silica gel CC (petroleum ether/acetone, 2:1 → 1:1) to give **3**, **4**, and **6**. IVe was applied to sephadex LH-20 to obtain three subfractions (IVe1–IVe3), fraction IVE3 was further purified by HPLC (YMC ODS column) using CH₃CN/H₂O (55:45) as eluent to obtain **1** and **2** (Figure 1).

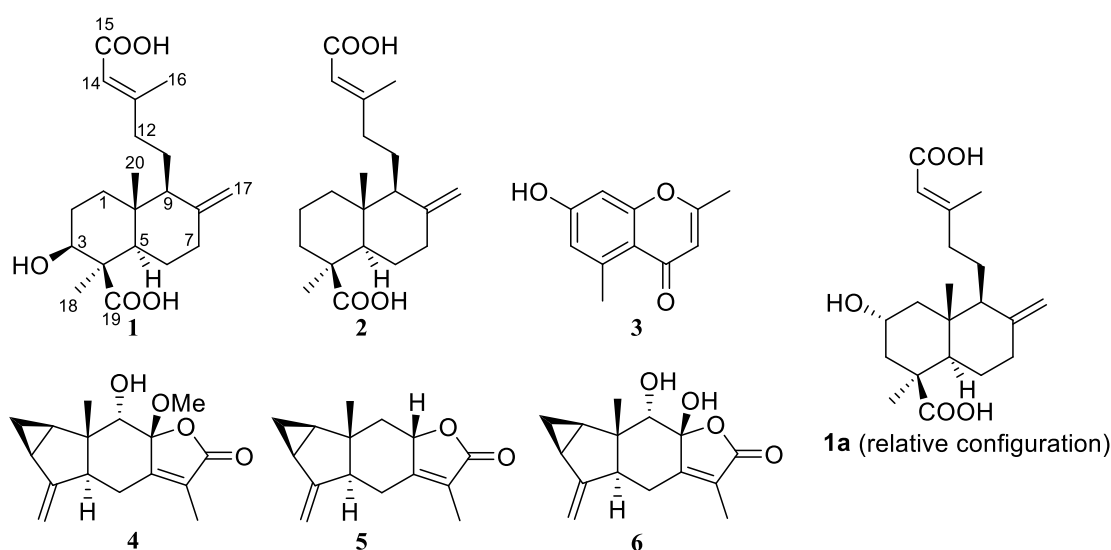


Figure 1. Structures of compounds **1–6** and **1a**

Compound **1**: colorless oil; $[\alpha]_D^{25} +61$ (c 0.1, MeOH); UV (MeOH) λ_{\max} ($\log \epsilon$) 216 (3.67); HRESIMS m/z 373.1986 $[M + Na]^+$ (calcd for C₂₀H₃₀O₅Na⁺, 373.1991).

Compound **1** was obtained as a colorless oil. Its molecular formula, C₂₀H₃₀O₅, was determined on the basis of its high-resolution ESIMS (HRESIMS) spectrum and the ¹³C NMR data, indicating 6 degrees of unsaturation. The ¹H NMR spectrum of **1** displayed resonances attributable to three methyl groups [δ_H 2.09 (s), 1.34 (s), and 0.66 (s)] including an olefinic methyl (δ_H 2.09), two protons for a terminal olefinic methylene [δ_H 4.52 (s) and 4.87 (s)], a proton bonded to carbons bearing heteroatoms [δ_H 3.15 (dd, $J = 12.0, 4.2$ Hz, H-3)]. The ¹³C NMR and HSQC spectra exhibited 20 carbon resonances, including three methyls (δ_C 13.5, 18.9, 24.7), six sp³ methylenes (δ_C 39.6, 38.7, 29.7, 27.2, 23.0, 40.7) and one sp² methylene (δ_C 107.2), three sp³ methines (δ_C 79.0, 56.4 × 2) and one sp² methine (δ_C 116.9), two sp³ quaternary carbon (δ_C 50.3, 41.0) and four sp² quaternary carbon (δ_C 149.0, 161.7, 170.4, 180.5). The low-field magnetic resonances above 100 ppm suggested the presences of two double bonds and two carbonyl carbon, which accounted for four degrees of unsaturations, the remaining two degrees of unsaturation required that compound **1** was bicyclic. The aforementioned structural features were very similar to those of a co-isolated known analogue, agathic acid (**2**). The obvious distinction between **1** and **2** was due to the presence of an additional oxygenated methine (δ_H 3.15, δ_C 79.0) in **1** instead of one methylene in **2**, indicating that compound **1** is a hydroxylated derivative of **2**, this was supported by the MS data. The hydroxy group was located at C-3 by the HMBC correlations of H₃-18 to C-3 and the ¹H-

^1H COSY correlations from H₂-1 (δ_{H} 1.20, 1.88) to H-3 (δ_{H} 3.15). The gross structure of **1** was confirmed by detailed analyses of the 2D NMR data (Figure 2).

Table 1. NMR data of **1**, **2**, and **1a** in CD₃OD^a (δ in ppm)

No.	1 ^a		2 ^a	1a ^b
	δ_{H} (mult., <i>J</i> in Hz)	δ_{C}	δ_{C}	δ_{C}
1	1.20, m	38.7, CH ₂	40.8, CH ₂	46.2, CH ₂
	1.88, m			
2	1.75, m	29.7, CH	21.1, CH	64.2, CH
	2.12, m			
3	3.15, dd (12.0, 4.2)	79.0, CH ₂	39.8, CH ₂	47.0, CH ₂
4		50.3, C	45.1, C	44.6, C
5	1.25, m	56.4, CH	57.4, CH	55.3, CH
6	1.90, m	27.2, CH ₂	27.5, CH ₂	25.7, CH ₂
	2.00, m			
7	1.90, m	39.6, CH ₂	40.4, CH ₂	38.2, CH ₂
	2.38, m			
8		149.0, C	149.4, C	147.4, C
9	1.58, m	56.6, CH	56.6, CH	55.2, CH
10		41.0, C	41.5, C	40.9, C
11	1.70, m	23.0, CH ₂	22.8, CH ₂	21.6, CH ₂
	1.54, m			
12	1.98, m	40.7, CH ₂	39.3, CH ₂	39.3, CH ₂
	2.27, m			
13		161.7, C	161.9, C	160.3, C
14	5.58, s	116.9, CH	116.8, CH	115.4, CH
15		170.4, C	170.3, C	168.9, C
16	2.09, d (1.2)	18.9, CH ₃	18.9, CH ₃	17.5, CH ₃
17	4.52, br s	107.2, CH ₂	106.9, CH ₂	106.2, CH ₂
	4.87, br s			
18	1.34, s	24.7, CH ₃	29.5, CH ₃	28.1, CH ₃
19		180.5, C	181.2, C	179.2, C
20	0.66, s	13.5, CH ₃	13.4, CH ₃	12.8, CH ₃

^a ^1H NMR (400 MHz), ^{13}C NMR (100 MHz). ^b ^1H NMR (600 MHz), ^{13}C NMR (150 MHz).

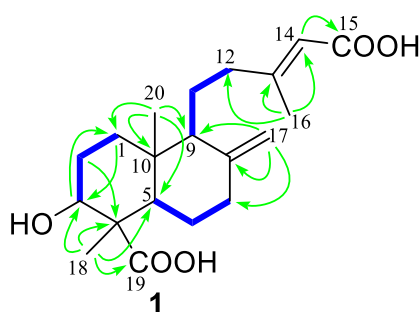


Figure 2. ^1H - ^1H COSY (—) and HMBC (→) correlations of **1**

The relative configuration of **1** was determined to be the same as that of **2** by comparing their ^{13}C NMR data, NOESY data, and coupling constants, (Figure 3). The NOE correlations from H₃-20 to H-6 β , H-2 β and between H-5 with H-9, H-3, H-1 α assigned H-3, H-5, and H-9 to be α -orientated, while

H₃-20 was in the opposite orientation. Besides, OH-3 was confirmed to be β -orientated by the coupling constants of $J_{H-3/H-2\beta}$ (12.0 Hz) and $J_{H-3/H-2\alpha}$ (4.2 Hz).

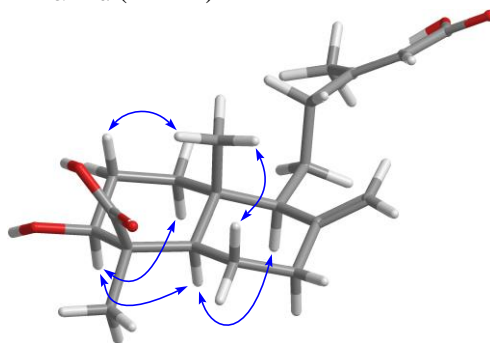


Figure 3. NOE correlations (\longleftrightarrow) of **1**

The absolute configuration of C-3, C-4, C-5, C-9, and C-10 was determined to be *S*, *R*, *R*, *S*, *R* based on their similar optical rotations (**1**: $[\alpha]_D^{25} +61$; **2**: $[\alpha]_D^{25} +48$) and CD spectra of **1** and **2** [6] (Figure 4). Compound **1** was given the trival name serralabdane F.

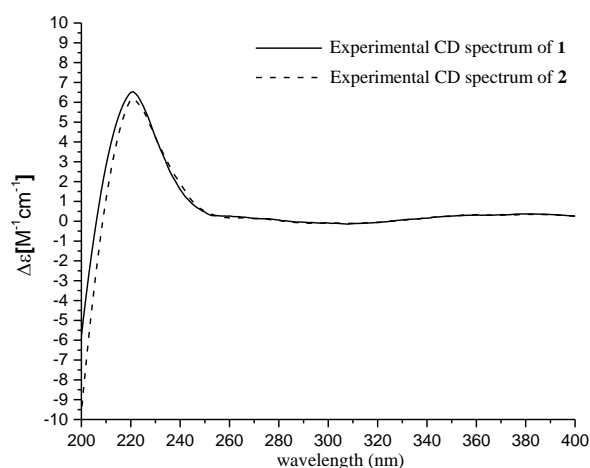


Figure 4. CD spectra (200–400 nm) of compounds **1** and **2**.

Compound **1** was an isomer of the known compound **1a** with the distinction owing to the location of the hydroxy group [6]. Totally, four labdane diterpenes were isolated from *Chloranthus serratus* in previous study [5], our study added new members to the labdane diterpenes from this plant. The known compounds were identified to be agathic acid (**2**) [7], altechromone A (**3**) [8], chlorajapolide F (**4**) [9], shizukanolide (**5**) [10], chloranthalactone E (**6**) [11] by comparison of their NMR data and specific rotations with those in the literature.

All compounds were tested for inhibitions against NO production in LPS-activated RAW 264.7 macrophages following the same procedures in the literature [3], as results, compounds **2** and **6** exhibited moderate inhibitory effects with IC₅₀ values of 45.2 ± 1.2 and 35.3 ± 0.9 μ M, while the positive control quercetin possessed an IC₅₀ of 15.2 ± 1.3 μ M.

Supporting Information

Supporting Information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

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