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Atractylmacrols A-E, sesquiterpenes from the rhizomes of *Atractylodes macrocephala*

Shuang-Yan Wang[a,b,c,1], Lin-Fen Ding[a,1], Jia Su[b], Li-Yan Peng[b], Liu-Dong Song[a,*], Xing-De Wu[b,*]

[a] School of Pharmaceutical Science & Yunnan Key Laboratory of Pharmacology for Natural Products, Kunming Medical University, Kunming 650500, People's Republic of China

[b] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People's Republic of China

[c] School of Rehabilitation and Nursing, Yunnan Business Management College, Kunming 650106,Yunnan, People's Republic of China

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ABSTRACT

Phytochemical investigation of the rhizomes of *Atractylodes macrocephala* led to the isolation of five new sesquiterpenes, atractylmacrols A-E (**1**-**5**), as well as six known eudesmane sesquiterpenes (**6**-**11**). The structures of **1**-**5** were determined through interpretation of their 1D and 2D NMR spectroscopic data, as well as HREIMS values. Compounds **1**-**5** were evaluated for their inhibitory effects on LPS-induced nitric oxide (NO) production in RAW264.7 macrophages.

1. Introduction

Atractylodes macrocephala, belonging to the family Compositae, grows mainly in eastern China, especially in Zhejiang province (Shi, 1987). Its dried rhizoma 'Bai-Zhu' have long been used in traditional Chinese medicine for the treatment of stomach complaints, dyspepsia, and anorexia (Editorial Committee of Chinese Pharmacopoeia, 2010). Previous phytochemical investigations on *A. macrocephala* have revealed the presence of polyacetylenes (Chen, 1989) and sesquiterpenes (Chen et al., 1997; Huang et al., 1992; Li and Yang, 2014; Lin et al., 1997). Among the sesquiterpenes, atractylenolide III was found to induce apoptosis of lung carcinoma cells through mitochondria-mediated death pathway and maybe a potential candidate for treatment of human lung carcinoma (Kang et al., 2011a). In addition, atractylenolide III and atractylon were reported to exhibit anti-inflammatory activity (Chen et al., 2016; Kang et al., 2011b; Resch et al., 1998). To search further anti-inflammatory constituents from *A. macrocephala*, a 95% EtOH extract of rhizomas of this species was investigated. As a result, a rare cyperane sesquiterpene, atractylmacrol A (**1**), and four new eudesmane sesquiterpenes, atractylmacrols B-E (**2–5**), were isolated. In this paper, we report the isolation, structural elucidation, and the inhibition of LPS-induced nitric oxide (NO) production in RAW 264.7 macrophages of **1–5**.

2. Results and discussion

Powdered dried rhizome of *A. macrocephala* were extracted with 95% EtOH. The filtrate was concentrated and partitioned between H_2O and EtOAc. The EtOAc fraction was subjected to column chromatography over silica gel, Sephadex LH-20, and semi-preparative HPLC to obtain five new sesquiterpenes, atractylmacrols A-E (**1–5**), as well as six known analogues, including atractylenolide I (**6**) (Huang et al., 1992), atractylenolide II (**7**) (Huang et al., 1992), atractylenolide III (**8**) (Huang et al., 1992; Kim et al., 2007), 8β-methoxy-atractylenolide I (**9**) (Chen et al., 1997), eudesma-7(11)-en-4-ol (**10**) (Bohlmann et al., 1982; Zhao et al., 1997), eudesma-4(15),7(11)-dien-8-one (**11**) (Torii and Inokuchi, 1980; Chen et al., 2016) (Fig. 1). The structures of the known compounds were determined by comparison of their spectroscopic data with literature values.

Compound **1**, a colorless oil, exhibited a molecular formula of $C_{15}H_{24}O$, as deduced from the HREIMS (*m/z* 220.1834 $[M]^+$; calcd. for 220.1827) and ^{13}C NMR data, which indicated four degrees of unsaturation. The IR absorption bands at 1645 and 3442 cm^{-1} implied the presence of double bond and hydroxy groups, respectively. The ^1H NMR spectrum displayed three singlets at δ_H 0.84 (s, H_3-12), 1.08 (s, H_3-13), 1.63 (s, H_3-15) and a doublet at δ_H 1.03 (d, J = 6.3 Hz, H_3-14). The ^{13}C NMR spectrum (Table 1) showed signals for 15 carbons, including four methyl groups, four methylenes, three methines (including one oxygenated), and four quaternary carbons (including two sp^2 ones).

* Corresponding authors.
E-mail addresses: ynsld@126.com (L.-D. Song), wuxingde@mail.kib.ac.cn (X.-D. Wu).
[1] Contributed equally to this work.

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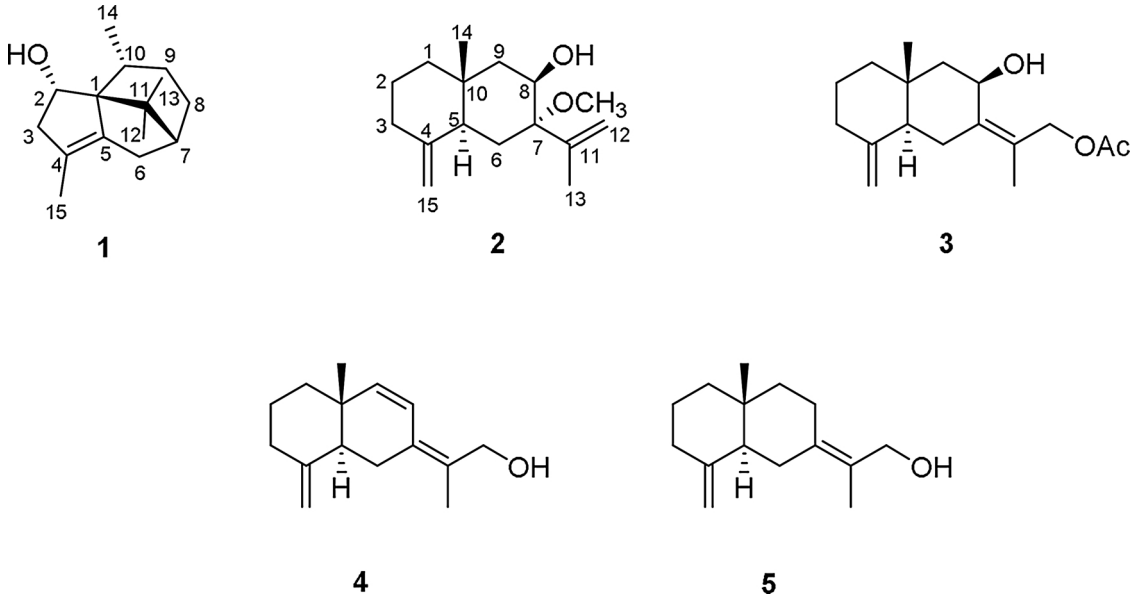

Fig. 1. Structures of compounds **1–5.**

Table 1
^{1}H and ^{13}C NMR spectroscopic data of compounds **1**-**3** in CD_3OD (δ ppm, J in Hz).

Position	**1**[a]		**2**[b]		**3**[a]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1α		66.2 s	1.30 (td, 13.0, 4.9)	41.6 t	1.15 (td, 12.9, 4.9)	42.0 t
1β			1.45 (br d, 13.0)		1.49 (br d, 12.9)	
2α	4.47 (t, 8.9)	73.8 d	1.59 (m)	22.7 t	1.57 (m)	23.0 t
2β			1.62 (m)		1.61 (m)	
3α	2.68 (m)	51.2 t	2.05 (td, 12.9, 6.1)	36.7 t	1.96 (td, 12.8, 5.2)	36.8 t
3β	2.42 (dd, 14.6, 8.9)		2.31 (br d, 12.9)		2.30 (br d, 12.8)	
4		127.4 s		150.8 s		150.8 s
5		143.3 s	2.10 (d, 13.0)	43.7 d	1.73 (dd, 13.8, 2.7)	49.9 d
6α	1.80 (m)	28.5 t	1.70 (d, 13.0)	22.8 t	2.41 (dd, 13.8, 2.7)	24.7 t
6β	2.22 (m)		1.83 (t, 13.0)		2.27 (t, 13.8)	
7	1.82 (m)	50.4 d		81.5 s		124.9 s
8α	1.31 (ddd, 13.4, 6.9, 3.7)	28.7 t	3.75 (br s)	70.3 d	4.96 (br s)	66.2 d
8β	1.88 (m)					
9α	1.13 (m)	30.0 t	1.74 (d, 12.9)	41.8 t	1.35 (dd, 14.4, 4.0)	47.3 t
9β	1.51 (m)		1.65 (d, 12.9)		1.90 (dd, 14.4, 1.9)	
10	2.19 (m)	33.4 d		35.4 s		35.7 s
11		42.6 s		146.1 s		140.6 s
12a	0.84 (s)	27.0 q	5.30 (br s)	117.4 t	5.11 (d, 11.7)	65.1 t
12b			5.15 (br s)		4.28 (d, 11.7)	
13	1.08 (s)	20.3 q	1.75 (s)	17.9 q	1.75 (s)	16.7 q
14	1.03 (d, 6.3)	20.7 q	0.93 (s)	18.4 q	1.03 (s)	18.1 q
15a	1.63 (s)	14.6 q	4.74 (br s)	105.0 t	4.77 (br s)	105.8 t
15b			4.53 (br s)		4.54 (br s)	
			2.99 (s)	49.3 q		
OAc						171.9 s
					2.05 (s)	21.4 q

The ^{1}H–^{1}H COSY correlations revealed the presence the two fragments: **a** $-CH_2-CH-CH_2-CH_2-CH(CH_3)-$ and **b** $-CH-CH_2-$ (Fig. 2). The HMBC correlations from H-2 (δ_H 4.47, t, J = 8.9 Hz) to C-1 (δ_C 66.2), C-3 (δ_C 51.2), C-4 (δ_C 127.4), and C-10 (δ_C 33.4) and from H-10 (δ_H 2.19, m) to C-1 (Fig. 2) suggested that the fragments **a** and **b** was connected through a sp^3 quaternary carbon at C-1. The above data revealed that **1** should be a cyperane sesquiterpene related to cyperene (Havlik et al., 2009; Joseph-Nathan et al., 1984), apart from the occurrence of an additional hydroxy group in **1**. The downfield chemical shifts of the proton and carbon resonances around C-2 (Table 1), as well as the HMBC correlations from H_2-3 (δ_H 2.68, m; 2.42, dd, J = 14.6, 8.9 Hz) to C-2 (δ_C 73.8) suggested that the hydroxy group was substituted at C-2.

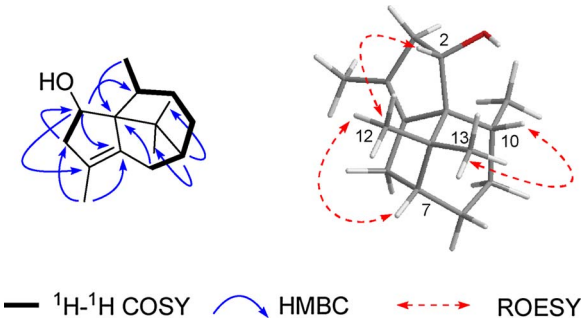

Fig. 2. Key 2D NMR correlations of **1**.

The relative configuration of **1** was determined by ROESY analysis (Fig. 2). The ROESY correlations of H-2/H_3-12, H-10/H_3-13, and H-7/H_3-12 indicated the β-orientation of H-2 and H-7, as well as the 1*S** and 7*R** configurations for **1**. Thus, the structure of **1** was deduced to be cypera-4-en-3α-ol and named as atractylmacrol A.

Compound **2** was obtained as a colorless oil, and its molecular formula was established as $C_{16}H_{26}O_2$ from HR-EI-MS (m/z 250.1959 $[M]^+$; calcd. for 250.1933) and ^{13}C NMR data, implying four degrees of unsaturation. The absorption band at 3476 cm^{-1} in the IR spectrum indicated the presence of hydroxy group. The ^{1}H NMR spectrum showed characteristic signals for two pairs of exocyclic olefinic protons at δ_H 5.30 (1H, br s, H-12a), 5.15 (1H, br s, H-12b), 4.74 (1H, br s, H-15a), and 4.53 (1H, br s, H-15b), and one methoxy group at δ_H 2.99 (3H, s). The ^{13}C and DEPT NMR spectra of **2** displayed 16 carbon resonances attributable to one methoxy, two methyls, seven methylenes (including two sp^2 ones), two methines (including one oxygenated), and four quaternary carbons (including one oxygenated and two sp^2 ones). The aforementioned data suggested that **2** was a eudesmane