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

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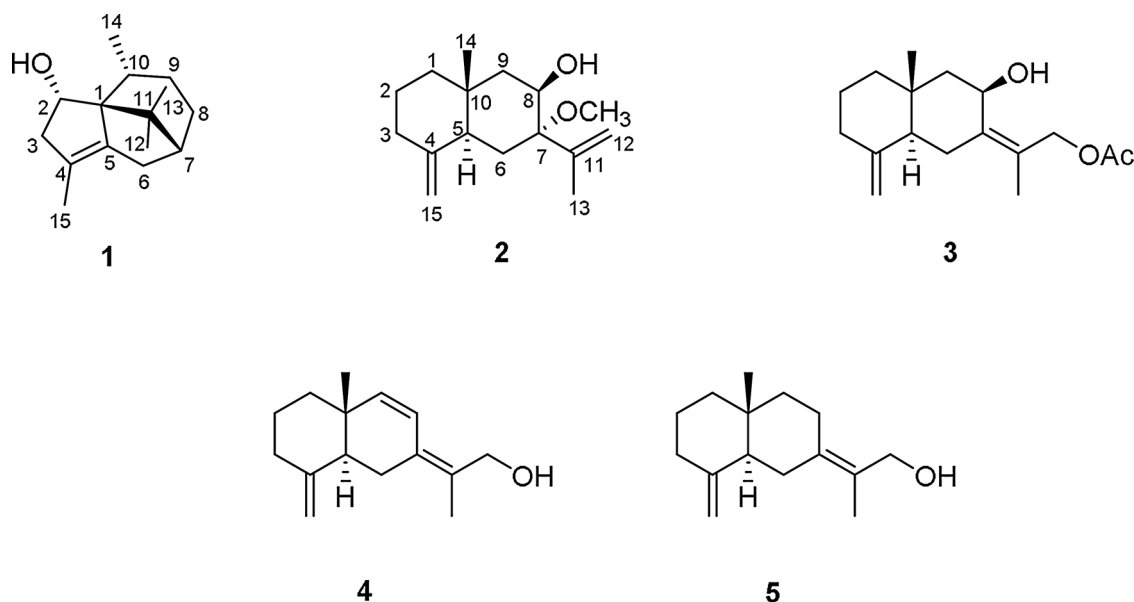
Fig. 1. Structures of compounds 1–5.

Table 1
^{1}H and ^{13}C NMR spectroscopic data of compounds 1-3 in CD$_3$OD (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-
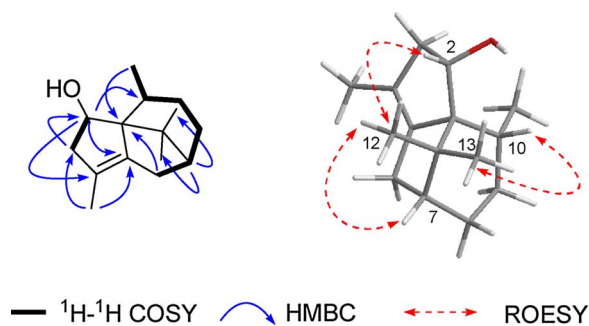

Fig. 2. Key 2D NMR correlations of 1.

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.

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 1S* and 7R* 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
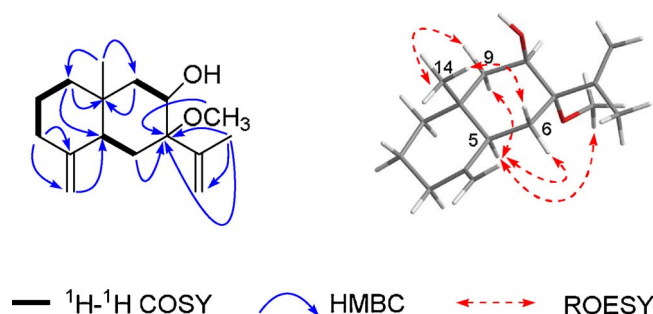
Fig. 3. Key 2D NMR correlations of **2**.

— ¹H-¹H COSY   HMBC   ROESY

sesquiterpene carrying a methoxy group. The ¹H and ¹³C NMR spectroscopic data of **2** (Table 1) was closely related to that of eudesma-4(15),11-dien-7α,8α,13-triol (Cheng et al., 1995). Important discrepancies, however, included the presence of a methoxy in **2** instead of a hydroxy at C-7 in latter, and the loss of a hydroxy at C-13 in **2**. This deduction was supported by HMBC correlations of OCH_3 with C-7 (δ_C 81.5) and of H_3-13 (δ_H 1.75, s), with C-7, C-11 (δ_C 146.1), and C-12 (δ_C 117.4) (Fig. 3). In the ROESY spectrum (Fig. 3), correlations of OCH_3 and H-6b with H-5 indicated that they were co-facial and assigned as α-oriented. Meanwhile, the broad singlet of H-8 in the ¹H NMR spectrum revealed H-8 was β-oriented. Furthermore, the ROESY correlation of between H-6a and H_3-14 was suggestive of the β-orientation of H_3-14. Consequently, the structure of **2** was assigned to be 7α-methoxyeudesma-4(15),11-dien-8β-ol and named as atractylmacrol B.

The HREIMS data of **3** showed a molecular ion at m/z 278.1876 [M]⁺ (calcd. 278.1882), corresponding to a molecular formula of $C_{17}H_{26}O_3$, consistent with five degrees of unsaturation. The IR spectrum exhibited absorption bands at 1741 and 3434 cm⁻¹, reminiscent of the presence of ester carbonyl and hydroxy functionalities, respectively. The ¹H NMR spectrum of **3** showed a terminal double bond at δ_H 4.77 (1H, br s, H-15a) and 4.54 (1H, br s, H-15b), which were correlated to the carbon resonated at δ_C 105.8 (C-15). Inspection of the ¹H and ¹³C NMR spectroscopic data of **3** revealed similarities to those of atractylenolide VII (Ding et al., 2005). Compared to atractylenolide VII, however, the NMR spectroscopic data of **3** lacked signals of a methylene moiety but exhibited resonances of an oxymethine group, suggesting that **3** was an oxygenated derivative of atractylenolide VII. HMBC correlations of H-8 (δ_H 4.96, br s) to C-7 (δ_C 124.9), C-9 (δ_C 47.3), C-10 (δ_C 35.7), as well as the ¹H-¹H COSY correlations between H-8 and H_2-9, revealed the additional hydroxy group was positioned at C-8. The relative configurations of C-5, C-8, and C-10 of **3** were assigned as being identical to those of **2** by analyzing coupling constants and the ROESY correlations. In addition, the Z-geometry of $\Delta^{7,11}$ double bond was deduced from the ROESY correlations of H-13 with H_2-6. Therefore, the structure of **3** was proposed to be (7Z)-13-acetoxyeudesma-4(15),7(11)–dien-8β-ol and named as atractylmacrol C.

The molecular of compound **4** was assigned as $C_{15}H_{22}O$ based on the HREIMS (m/z 218.1670 [M]⁺, calcd. for 218.1671) and ¹³C NMR data, requiring five degrees of unsaturation. The IR spectrum displayed absorption bands at 1646 and 3418 cm⁻¹, consistent with the occurrence of olefinic and hydroxy functionalities, respectively. The ¹H NMR spectrum (Table 2) of **4** exhibited typical signals assignable to two methyl groups at δ_H 1.87 (3H, s, H_3-13) and 0.83 (3H, s, H_3-14), and one terminal double bond at δ_H 4.85 (1H, br s, H-15a) and 4.63 (1H, br s, H-15b). The ¹H and ¹³C NMR spectroscopic data of **4** were almost identical to those of a known eudesmanolide, eudesma-4(15),7(11),8-triene (Bohlmann et al., 1980), excep for the absence of a methyl group of the latter. Instead, an oxygenated methylene group [δ_H 4.24 (1H, d, J = 11.8 Hz) and 4.14 (1H, d, J = 11.8 Hz)] was evident in 1D NMR spectra of **4**. These data indicated that **4** was a hydroxylated derivative of eudesma-4(15),7(11),8-triene. The hydroxy group was assign to C-12 via HMBC cross-peaks of H_3-13 with C-7 (δ_C 132.6), C-11 (δ_C 130.9),

Position	**4**[a]		**5**[b]	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1α	1.45 (td, 13.2, 5.6)	40.2 t	1.26 (td, 13.0, 4.9)	42.8 t
1β	1.59 (br d, 13.2)		1.45 (d, 13.0)	
2α	1.64 (m)	24.4 t	1.59 (m)	24. 5t
2β	1.68 (m)		1.63 (m)	
3α	2.04 (m)	37.7 t	1.99 (overlapped)	37.8 t
3β	2.34 (m)		2.30 (br d, 13.0)	
4		151.2 s		152.1 s
5	2.05 (dd, 14.0, 3.4)	48.5 d	1.76 (br d, 12.9)	51.6 d
6α	2.19 (dd, 14.0, 3.4)	27.5 t	2.51 (d, 12.9)	29.7 t
6β	2.49 (t, 14.0)		1.81 (t, 12.9)	
7		132.6 s		137.6 s
8α	6.44 (d, 10.0)	122.8 d	2.65 (br d, 13.0)	26.2 t
8β			1.98 (overlapped)	
9α	5.63 (d, 10.0)	141.1 d	1.53 (m)	43.4 t
9β			1.22 (m)	
10		38.3 s		37.2 s
11		130.9 s		126.1 s
12a	4.24 (d, 11.8)	62.5 t	4.08 (br s)	63.2 t
12b	4.14 (d, 11.8)		4.08 (br s)	
13	1.87 (s)	17.2 q	1.74 (s)	16.6 q
14	0.83 (s)	18.8 q	0.83 (s)	16.4
15a	4.85 (br s)	106.6 t	4.75 (br s)	106.0 t
15b	4.63 (br s)		4.49 (br s)	

and C-12 (δ_C 62.5). The ROESY correlations of H-5/H-6α and H_3-14/H-6β demonstrated the trans-linkage of the two six-membered rings and that H-5 was α-oriented, whereas H_3-14 had the β-orientation. The Z-geometry of the tetrasubstituted double bond between C-7 and C-11 was recognized by the ROESY correlations of H-8 with H_2-12. Accordingly, the structure of **4** was characterized as (7Z)-eudesma-4(15),7(11),8-trien-13-ol and named as atractylmacrol D.

Compound **5** was found to have the molecular formula of $C_{15}H_{24}O$ with four degrees of unsaturation, as determined by the molecular ion at m/z 220.1830 [M]⁺ (calcd. 220.1827) in the HREIMS and ¹³C NMR data. The similar ¹H and ¹³C NMR data of **5** and **4** revealed that they were structural congeners, with the major differences involving the absence of the Δ^8 double bond in **5** and the presence of two methylenes at C-8 and C-9 in **4**, which were supported by HMBC corss-peaks of H_2-8 (δ_H 1.98, m; 2.65, br d, J = 13.0 Hz) with C-6 (δ_C 29.7), C-7 (δ_C 137.6), and C-11 (δ_C 126.1), of H_2-9 (δ_H 1.22, m; 1.53, m) with C-1 (δ_C 42.8), C-5 (δ_C 51.6), and C-10 (δ_C 37.2). The relative configuration of **5** were elucidated as those of **4** by the analysis of the ROESY correlations. Furthermore, the E-geometry of $\Delta^{7,11}$ double bond was suspected from the ROESY correlations of H_2-12 with H-8α. Hence, the structure of **5** was established to be (7E)-eudesma-4(15),7(11),-dien-13-ol and named as atractylmacrol E.

The new compounds (**1**–**5**) were evaluated for their inhibitory effects against NO production in LPS-stimulated RAW 264.7 cells with MG 132 as a positive control (Qin et al., 2014). As a potent inhibitor of the proteasome, MG 132 decreases LPS-stimulated NO production in RAW 264.7 cells by inhibiting NF-κB signaling pathway (Trauzold et al., 2001). The results showed that none of them were active against NO production with IC_{50} values of more than 25 μM.

3. Experimental

3.1. General experimental procedures

UV spectra were obtained with the Shimadzu-UV-2401A spectrometer and IR spectra were measured on a Tenor-27 spectrometer. Optical rotations were recorded on a Horiba-SEPA-300 polarimeter. ESIMS were performed on an API QSTAR time-of-flight spectrometer. EIMS and HREIMS mass spectra were obtained on a Waters AutoSpec

Premier P776 spectrometer. The NMR spectra were determined on a Bruker AM-400 or DRX-600, and the chemical shifts were referenced to tetramethylsilane (TMS). Column chromatography (CC) was run on silica gel (200–300 mesh; Qingdao Marine Chemical Factory, Qingdao, China) and Sephadex LH-20 (Pharmacia Fine Chemical Co.Ltd.). TLC was performed on silica gel GF$_{254}$ (SiO$_2$; Qingdao Haiyang Chemical Factory, Qingdao, China.). Semipreparative HPLC was performed on an Agilent 1260 liquid chromatography system equipped with a Zorbax SB-C$_{18}$ column (9.4 mm × 150 mm), MPLC was performed on a Lisui EZ Purify III System (Shanghai Lisui Chemical Engineering Co., Ltd., Shanghai, China).

3.2. Plant material

The rhizomes of *A. macrocephala* were bought from Juhuacun Chinese Traditional Medicine Market, Kunming, Yunnan province, China, in April 2011 and identified by Prof. Xiao Cheng, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 20110506a) has been deposited in Kunming Institute of Botany, Chinese Academy of Sciences.

3.3. Extraction and isolation

The rhizome of *A. macrocephala* (10 kg) were extracted with 95% EtOH (v/v) at room temperature (3 × 30 L, 2 d each). After removal of the solvent in vacuo, the residue (600 g) was suspended in H$_2$O and successively extracted with EtOAc. The EtOAc extract (188 g) was subjected to MPLC over MCI eluting with MeOH–H$_2$O (60:40 to 90:10) to give eight fractions (A–H). Fraction D (10 g) was chromatographed on silica gel CC, eluted with a gradient of petroleum ether-acetone (20:1 to 2:1) to afford three fractions (D1-D3). Compound **8** (1.1 g) was crystallized using acetone as solvent from subfraction D2 (3 g). By the similar purification procedure, compound **6** (1.25 g) were obtained from fraction E (6 g). Fraction F (28 g) was separated by silica gel CC using petroleum–acetone (100: 1 to 1: 1) to afford six subfractions (F1–F6). Fraction F2 (120 mg) was purified by semipreparative HPLC eluting with MeOH–H$_2$O (75: 25, 3 mL/min) to yield compounds **4** (3 mg, t_R = 13.5 min) and **5** (3 mg, t_R = 16.2 min). Compound **3** (2 mg) was obtained from fraction F3 (80 mg) by silica gel CC (petroleum ether–EtOAc, 50: 1 to 1: 1). Fraction F4 (800 mg) was subjected to silica gel CC eluting with CHCl$_3$–acetone (50: 1 to 1: 1) and Sephadex LH-20 CC (CHCl$_3$–MeOH, 1:1) to provide **1** (1.5 mg), **2** (2.0 mg), **10** (8 mg), and **9** (552 mg). Fraction G (13 g) was subjected to silica gel CC eluted with CHCl$_3$–acetone (200: 1 to 1:1) to afford four fractions (G1–G3). Fraction G2 (2.5 g)was purified by Sephadex LH-20 (CHCl$_3$–MeOH, 1:1) to give **11** (880 mg). Compound **7** (1.1 g) was purified from fraction G3 (3.0 g) by crystallization from petroleum ether−acetone (1:1).

3.3.1. Atractylmacrol A (1)

Oiliness; $[\alpha]_D^{20}$ −10.3 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 204 (2.25) nm; IR (KBr) ν_{max} 3442, 2924, 1755, 1645, 1458, 1384, 1077, 1040 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; EI-MS *m/z* 220.0[M]$^+$ (18), 202 (5), 195 (7), 144 (100), 127 (27), 115 (66), 77 (22); HR-EI-MS, *m/z* 220.1834 [M]$^+$; (Cacld. for C$_{15}$H$_{24}$O 220.1827).

3.3.2. Atractylmacrol B (2)

Colorless oil; $[\alpha]_D^{20}$ −30.9 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ε) 223 (2.61), 202 (3.44) nm; IR (KBr) ν_{max} 3476, 2929, 1649, 1440, 1104, 1083, 1048, 1012, 897, 884 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; EI-MS *m/z* 250.0 [M]$^+$, 235 (10), 218 (100), 203 (78), 185 (31), 159 (46), 151 (49), 108 (51), 93 (68), 67 (66); HR-EI-MS, *m/z* 250.1959 [M]$^+$; (Cacld. for C$_{16}$H$_{26}$O$_2$ 250.1933).

3.3.3. Atractylmacrol C (3)

Colorless oil; $[\alpha]_D^{20}$ +9.1 (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ε)

202 (3.61) nm; IR (KBr) ν_{max} 3434, 2931, 1741, 1644, 1453, 1441, 1375, 1242, 1043 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 1; ESI–MS *m/z* 301.0 [M + Na]$^+$; HR-EI-MS, *m/z* 278.1876 [M]$^+$; (Cacld. for C$_{17}$H$_{26}$O$_3$ 278.1882).

3.3.4. Atractylmacrol D (4)

Colorless oil; $[\alpha]_D^{20}$ −180.6 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 295 (2.36), 243 (4.26) nm; IR (KBr) ν_{max} 3418, 2929, 1646, 1438, 1407, 1102, 1008, 886 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 2; EI-MS *m/z* 218.0 [M]$^+$ (39), 203 (24), 187 (30), 159 (39), 149 (61), 105 (58), 91 (100), 79 (58); HR-EI-MS, *m/z* 218.1670[M]$^+$; (Cacld. for C$_{15}$H$_{22}$O 218.1671).

3.3.5. Atractylmacrol E (5)

Colorless oil; $[\alpha]_D^{20}$ + 165.4 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 229 (3.26) 201 (3.59) nm; IR (KBr) ν_{max} 3443, 2930, 1645, 1443, 1383, 1165, 1045 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table 2; EI-MS *m/z* 220.0 [M + H]$^+$, HR-EI-MS, *m/z* 220.1830 [M]$^+$; (Cacld. for C$_{15}$H$_{24}$O 220.1827).

3.4. Determination of NO production

Murine macrophage cells RAW 264.7 were seeded into 96-well plates (2 × 10^5 cells/well) containing RPMI 1640 medium (Hyclone) with 10% FBS under a humidified atmosphere of 5% CO$_2$ at 37 °C. After 24 h incubation, cells were treated with the compounds with the maximum concentration of 25 μM in the presence of 1 μg/mL LPS for 18 h. Each compound was dissolved in DMSO and further diluted in cell culture media to obtain different concentrations. NO production was assessed by adding Griess reagent (100 μL of 1% sulfanilamide and 0.1% naphthylethylene diaminedihydrochloride in 5% H$_3$PO$_4$) to supernatant (100 μL) from LPS or the compound-treated cells in triplicate. After 5 min incubation, the absorbance was measured at 570 nm with a 2104 Envision Multilabel Plate Reader (Perkin-Elmer Life Sciences, Inc., Boston, MA, USA). MG132 (Sigma Aldrich, USA, purity 99%, IC$_{50}$ = 0.1 μM) was used as a positive control. Cytotoxicity was determined by the MTT assay as described (Mosmann, 1983).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.phytol.2017.11.021.

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