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Anti-neuroinflammatory sesquiterpenes from Chinese eaglewood

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ABSTRACT

Nine new sesquiterpenes (**1**–**9**), together with seventeen known ones (**10**–**26**), were isolated from Chinese eaglewood. Their structures were established by extensive spectroscopic analysis, and the absolute configuration of **6** was determined by the modified Mosher's method. Compounds **7**, **10**, **14**, **15**, and **21** exhibited significant inhibition of nitric oxide production in lipopolysaccharide-stimulated BV-2 microglial cells with IC_{50} values in the range 7.1–53.8 μM.

1. Introduction

Chinese eaglewood (Chenxiang in Chinese) is the resinous wood of *Aquilaria sinensis* (Lour.) Gilg, which belongs to the Thymelaeaceae family [1]. It has been used as a sedative, analgesic, and digestive agent for centuries in traditional Chinese medical practices, as well as used as incense in Buddhist, Hindu, and Islamic ceremonies [2,3]. A wide array of pharmacological properties, such as sedative [4], laxative [5], neuroprotective [6], anti-inflammatory [7], cytotoxic [8], antibacterial and acetylcholinesterase inhibitory activities [9], have been reported for crude extracts or pure compounds from Chinese eaglewood. Previous phytochemical investigations have revealed that sesquiterpenes and 2-(2-phenylethyl)chromone derivatives are two predominant constituents of Chinese eaglewood [2]. In the course of a search for anti-neuroinflammatory agents from medicinal plants [10–13], the petroleum ether-soluble fraction from a 95% EtOH extract of the resinous wood of *Aquilaria sinensis* (Lour.) Gilg was found to significantly inhibit nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated BV-2 microglial cells (96% inhibition at 4.0 μg/mL). The following phytochemical procedure led to the isolation of nine new sesquiterpenes (**1**–**9**) and seventeen known ones (**10**–**26**) (Fig. 1). Herein, the isolation and structural elucidation of the nine new sesquiterpenes (**1**–**9**) are described as well as their inhibitory effects on NO production in LPS-stimulated BV-2 microglial cells.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on a Rudolph Autopol IV automatic polarimeter (NJ, USA). IR spectra were obtained using a Thermo Nicolet Nexus 470 FT-IR spectrophotometer (MA, USA) with KBr pellets. HRESIMS spectra were acquired using an LCMS–IT-TOF system fitted with a Prominence UFLC system and an ESI interface (Shimadzu, Kyoto, Japan). The NMR spectra were measured with a Varian INOVA-500 spectrometer (CA, USA) operating at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR, respectively. Column chromatographies were performed using silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, People's Republic of China), Sephadex LH-20 (Pharmacia), and LiChroprep RP-C_{18} gel (40–63 μm, Merck, Germany). Semipreparative HPLC was performed on a Shimadzu LC-20AT pump system (Shimadzu Corporation, Tokyo, Japan), equipped with an SPD-M20A photodiode array detector monitoring at 254 nm and 202 nm. A semipreparative RP-HPLC column (YMC-Pack ODS-A, 250 × 10 mm, 5 μm) was employed for the isolation. TLC was carried out using precoated silica gel GF_{254} plates, visualized under UV lamp at 254 nm, and sprayed with anisaldehyde reagent and heated until optimal color development. All purified compounds submitted for bioassay were at least 95% pure as judged by HPLC and supported by ^{1}H NMR analysis.

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2.2. Plant material

Chinese eaglewood (resinous wood of *Aquilaria sinensis* (Lour.) Gilg) was collected in Zhongshan, Guangdong Province, People's Republic of China, in November 2012. The plant material was authenticated by one of the authors (P.-F. Tu), and a voucher specimen (CX2012029) is deposited at the Modern Research Center for Traditional Chinese Medicine, Beijing University of Chinese Medicine, Beijing, China.

2.3. Extraction and isolation

The dried Chinese eaglewood (6.9 kg) was extracted with 95% EtOH under reflux for three times (120 L × 3, each for 2.5 h). After removing the solvent under reduced pressure, the combined and concentrated EtOH extract was suspended in 80% aqueous MeOH (1 L), and successively partitioned with petroleum ether and EtOAc, to afford petroleum ether (36.4 g) and EtOAc (682.0 g) soluble extracts, respectively. The petroleum ether extract (35.2 g) was submitted to vacuum liquid chromatography (VLC) on silica gel (200–300 mesh) eluted with a gradient of petroleum ether–EtOAc (50:1 → 0:1) and MeOH to provide 13 fractions (A–M). Fraction J (4.4 g) was further fractionated on a Sephadex LH-20 column chromatography (CC) eluting with CH_2Cl_2–*n*-hexane–MeOH (3:3:1) to yield five subfractions (J1–J5). Subfraction J4 (1.3 g) was resolved by silica gel CC using a stepwise gradient of *n*-hexane–EtOAc (1:0 → 0:1) as the mobile phase to give seven portions (J4a–J4g). J4c (170 mg) was purified by semipreparative RP-HPLC eluted with an isocratic 42% aqueous CH_3CN to produce **3** (1.1 mg, t_R = 18.5 min), **14** (13.0 mg, t_R = 35.4 min), **1** (2.3 mg, t_R = 41.5 min), **7** (10.3 mg, t_R = 49.6 min), and **13** (1.3 mg, t_R = 61.1 min), respectively. J4e (300 mg) was chromatographed over RP-C_{18} CC eluted by a stepwise gradient of aqueous MeOH (40% to 100% MeOH) and further purified by semipreparative RP-HPLC using an isocratic 40% aqueous CH_3CN to give **4** (1.1 mg, t_R = 35.9 min), **18** (8.0 mg, t_R = 40.9 min), and **11** (1.2 mg, t_R = 46.9 min), respectively. Compounds **5** (1.2 mg, t_R = 32.0 min), **2** (1.2 mg, t_R = 38.5 min), and **9** (16.0 mg, t_R = 43.3 min) were purified from J4d (35 mg) by semipreparative RP-HPLC with isocratic 42% aqueous CH_3CN as the mobile phase. Subfraction J5 (140 mg) was separated via silica gel CC eluting with a stepwise gradient of *n*-hexane–EtOAc (60:1 → 2:1) followed by purification on semipreparative RP-HPLC (isocratic 48% aqueous CH_3CN) to obtain **6** (16.0 mg, t_R = 18.8 min) and **20** (1.2 mg, t_R = 38.7 min). Fraction F (2.3 g) was subjected to Sephadex LH-20 CC eluting with CH_2Cl_2–*n*-hexane–MeOH (3:3:1) and purified by semipreparative RP-HPLC with isocratic 78% aqueous MeOH to afford compounds **24** (1.3 mg, t_R = 42.8 min) and **16** (1.4 mg, t_R = 46.6 min). Fraction H (4.1 g) was separated into 10 subfractions (H1–H10) by silica gel CC eluted with a stepwise gradient of *n*-hexane–EtOAc (30:1 → 0:1). Separation of H8 (120 mg) was achieved on semipreparative RP-HPLC (isocratic 68% aqueous CH_3CN), and afforded **19** (2.0 mg, t_R = 8.3 min), **25** (6.2 mg, t_R = 10.3 min), and **10** (1.5 mg, t_R = 14.5 min), respectively. Compound **21** (7.2 mg, t_R = 31.7 min) was purified by semipreparative RP-HPLC (isocratic 48% aqueous CH_3CN). Fraction K (1.6 g) was introduced to silica gel CC eluting with a stepwise gradient of petroleum ether–EtOAc (20:1 → 0:1), and purified by semipreparative RP-HPLC (isocratic 42% aqueous MeOH) to yield **17** (2.5 mg, t_R = 45.6 min), **22** (2.5 mg, t_R = 62.0 min), **26** (1.5 mg, t_R = 66.5 min), **8** (1.1 mg, t_R = 72.1 min), and **23** (2.2 mg, t_R = 75.1 min), respectively. Fraction I (1.2 g) was chromatographed over Sephadex LH-20 CC eluting with CH_2Cl_2–*n*-hexane–MeOH (3:3:1) to afford six subfractions (I1–I6). Compounds **12** (1.0 mg, t_R = 17.9 min) and **15** (1.2 mg, t_R = 60.0 min) were obtained from I5 (70 mg) by semipreparative RP-HPLC eluting with isocratic 40% aqueous CH_3CN.

(+)-9β-Hydroxyeudesma-4,11(13)-dien-12-al (**1**): colorless oil; $[\alpha]_D^{21}$ +42 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3440, 2930, 2861, 1686, 1636, 1456, 1400, 1384, 1262, 1169, 1055 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; positive-ion HRESIMS *m/z* 235.1693 $[M + H]^+$ (calcd for $C_{15}H_{23}O_2$, 235.1693).

(+)-Eudesma-4,11(13)-dien-8α,9β-diol (**2**): colorless oil; $[\alpha]_D^{21}$ + 34 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3425, 2918, 2850, 1645, 1577, 1541, 1435, 1384, 1165, 1058, 877, 720 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; negative-ion HRESIMS *m/z* 235.1710 $[M - H]^-$ (calcd for $C_{15}H_{23}O_2$, 235.1704).

(+)-8α-Hydroxyeudesma-3,11(13)-dien-14-al (**3**): colorless oil; $[\alpha]_D^{21}$ + 10 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3430, 2919, 2851, 1684, 1638, 1456, 1384, 1160, 1062, 892 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; positive-ion HRESIMS *m/z* 257.1506 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_2Na$, 257.1512).

(+)-Eudesma-3,11(13)-dien-8α,9β-diol (**4**): colorless oil; $[\alpha]_D^{21}$ + 6 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3418, 2918, 2851, 1644, 1440, 1382, 1215, 1071, 998, 955, 890 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; positive-ion HRESIMS *m/z* 259.1658 $[M + Na]^+$ (calcd for $C_{15}H_{24}O_2Na$, 259.1669).

(+)-Eudesma-4(14),11(13)-dien-8α,9β-diol (**5**): colorless oil; $[\alpha]_D^{21}$ + 35 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3430, 2929, 2850, 1646, 1578, 1438, 1384, 1201, 1049, 885 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; positive-ion HRESIMS *m/z* 237.1856 $[M + H]^+$ (calcd for $C_{15}H_{25}O_2$, 237.1849).

(4*R*,5*R*,7*S*,9*S*,10*S*)-(−)-Eudesma-11(13)-en-4,9-diol (**6**): colorless oil; $[\alpha]_D^{21}$ −17 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3417, 2928, 2851, 1644, 1540, 1446, 1384, 1258, 1169, 1048, 1013, 885 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; negative-ion HRESIMS *m/z* 237.1864 $[M - H]^-$ (calcd for $C_{15}H_{25}O_2$, 237.1860).

(+)-9β,10β-Epoxyeremophila-11(13)-en (**7**): colorless oil; $[\alpha]_D^{21}$ + 100 (*c* 0.1, MeOH); IR (KBr) ν_{max} 2922, 2861, 1642, 1452, 1383, 1273, 1089, 1014, 955, 890 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; positive-ion HRESIMS *m/z* 221.1900 $[M + H]^+$ (calcd for $C_{15}H_{25}O$, 221.1900).

(+)-11-Hydroxyvalenc-1(10),8-dien-2-one (**8**): colorless oil; $[\alpha]_D^{21}$ + 70 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3425, 2967, 2818, 2850, 1649, 1618, 1580, 1541, 1464, 1384, 1291, 1198, 1074, 906, 876 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; positive-ion HRESIMS *m/z* 235.1682 $[M + H]^+$ (calcd for $C_{15}H_{23}O_2$, 235.1693).

(−)-Eremophila-9-en-8β,11-diol (**9**): colorless oil; $[\alpha]_D^{21}$ −50 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3404, 2969, 2933, 2858, 1631, 1461, 1406, 1364, 1282, 1150, 990, 955, 845 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; negative-ion HRESIMS *m/z* 237.1857 $[M - H]^-$ (calcd for $C_{15}H_{25}O_2$, 237.1860).

2.4. Preparation of the (*R*)- and (*S*)-MTPA ester derivatives of compound **6**

The (*R*)- and (*S*)-MTPA ester derivatives of compound **6** were prepared as previously described [14]. Briefly, a solution of **6** (1.1 mg) in pyridine-d_5 [400 μL, 99.5% deuterated] was treated with (*S*)-(+)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride (5 μL) under a nitrogen gas stream in a clean NMR tube. The NMR tube was shaken at room temperature for 5 h to give the (*R*)-MTPA ester (**6a**). The same procedure was used to prepare the (*S*)-MTPA ester (**6b**) with (*R*)-MTPA chloride. The ^1H NMR spectra were obtained directly from the NMR reaction tubes and were assigned on the basis of their respective ^1H-^1H COSY spectra.

(4*R*,5*R*,7*S*,9*S*,10*S*)-(−)-Eudesma-11-en-4,9-diol, (*R*)-MTPA ester (**6a**): ^1H NMR (pyridine-d_5): δ 5.082 (1H, dd, *J* = 12.0, 4.0 Hz, H-9), 4.843 (1H, s, H-13a), 4.807 (1H, s, H-13b), 2.411 (1H, d, *J* = 12.5 Hz, H-6a), 2.202 (1H, t, *J* = 12.5 Hz, H-7), 2.139 (1H, d, *J* = 12.0 Hz, H-8a), 1.968 (1H, d, *J* = 12.5 Hz, H-3a), 1.850 (1H, m, H-8b), 1.712 (3H, s, H_3-12), 1.683 (1H, m, H-3b), 1.661 (1H, m, H-5), 1.608 (1H, d, *J* = 12.5 Hz, H-1a), 1.513 (2H, m, H_2-2), 1.402 (1H, m, H-6b), 1.306 (3H, s, H_3-14), 1.178 (1H, dt, *J* = 13.0, 3.5 Hz, H-1b), 1.012 (3H, s, H_3-15).

(4*R*,5*R*,7*S*,9*S*,10*S*)-(−)-Eudesma-11-en-4,9-diol, (*S*)-MTPA ester (**6b**): ^1H NMR (pyridine-d_5): δ 5.058 (1H, dd, *J* = 11.5, 4.0 Hz, H-9), 4.803 (1H, s, H-13a), 4.785 (1H, s, H-13b), 2.410 (1H, d, *J* = 13.0 Hz,