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New sesquiterpenes from the rhizomes of *homalomena occulta*

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ABSTRACT

Six new sesquiterpenes (**1**–**6**), along with eight known ones (**7**–**14**) were isolated from the rhizomes of *Homalomena occulta*. Structure elucidation of the new compounds was achieved through 1D NMR, 2D NMR spectroscopic techniques and HRESIMS, while the absolute configurations of compounds **1**, **2** and **5** were confirmed by X-ray crystallographic analysis. All of the isolates were evaluated for their activity against LPS-induced production of nitrogen oxide (NO) in macrophage cells, and compounds **1** and **5** showed inhibitory effect on NO production with the IC_{50} values of 21.2 and 15.4 μM, respectively.

1. Introduction

The genus *Homalomena* (Araceae) is widely distributed in America and the tropical areas of Asia and comprises over 140 species, but only four species belonging to this genus were found in China: *Homalomena aromatic*, *Homalomena occulta*, *Homalomena kelungensis* and *Homalomena hainanensis* [1]. *H. occulta* is widely distributed in Guangdong, Guangxi and Yunnan Provinces, the rhizomes of which have long been used as Chinese Traditional Medicine (TCM) for treatment of stomach aches, gastroenteritis, and rheumatic arthritis, and also for strengthening the tendons and bones [1,2]. Previous investigation revealed that *H. occulta* was a rich source of essential oil [3–5], and a few sesquiterpenes were also obtained from this herb, some of which exhibited antibacterial and stimulative effect on cellular processes of osteoblasts [6–9]. As part of our search for bioactive compounds from Chinese medicinal plants, the rhizomes of *H. occulta* have been investigated. Herein, we report the isolation and structure elucidation of six new sesquiterpenes (**1**–**6**) and eight known compounds (**7**–**14**) (Fig. 1), among them compound **1** was a rare example of a naturally occurring axane-type sesquiterpene, and compound **6** was a trihydroxyeudesmane semi-fumarate. These compounds were evaluated for their inhibitory effects on the production of NO through a macrophage-mediated bioassay.

2. Experimental details

2.1. General experimental procedures

Optical rotations were recorded on a Rudolph Research Autopol III automatic polarimeter. IR spectra were recorded on a JASCO FT-IR-6300 spectrophotometer or a PerkinElmer Frontier Mid-IR FTIR spectrophotometer with KBr disks. NMR spectra were obtained at 500 or 600 MHz for ^{1}H, and 125 or 150 MHz for ^{13}C, respectively, on Bruker Avance III 500 or Bruker Avance III HD 600 NMR spectrometer in acetone-d_6 or MeOH-d_4 using TMS as the internal standard. HRESIMS data were measured using a Micromass Autospec-Ultima ETOF spectrometer. Column chromatography was performed with silica gel (100–200 or 200–300 mesh, Qingdao Marine Chemical Inc. China) and Sephadex LH-20 (Pharmacia). HPLC separation was performed on a Waters 600 controller, a Waters 600 pump, and a Waters 2489 dual λ absorbance detector with an Ultimate XB-C_{18} (5 μm, 250 × 10 mm) column. TLC was carried out with glass precoated silica gel GF_{254} plates (Yantai Chemical Industry Research Institute, China). Spots were visualized by spraying with 10% H_2SO_4 in EtOH followed by heating.

2.2. Plant material

The rhizomes of *Homalomena occulta* were purchased at Anguo medicinal herb market of Hebei province, and identified by Professor Ying-Lin Wang from School of Combined Traditional Chinese and Western Medicine, Binzhou Medical University. A voucher specimen

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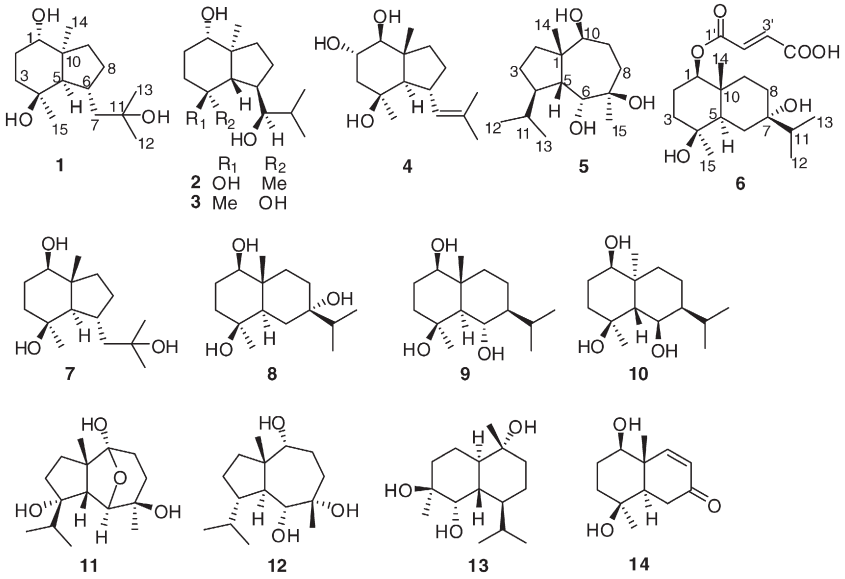

Fig. 1. Structures of compounds **1**–**14**.

(S-QNJ-2010) is deposited at Herbarium of the School of Pharmacy, Binzhou Medical University.

2.3. Extraction and isolation

The dried rhizomes of *Homalomena occulta* (9 kg) were powdered and extracted with 95% EtOH at room temperature and evaporated under reduced pressure to yield a residue (400 g), which was suspended in H_2O and then partitioned with ethyl acetate (EtOAc). The EtOAc extract (198 g) was subjected to CC over silica gel (100–200 mesh, petroleum ether-acetone, 10:0, 10:0.5, 10:1, 2:1, 1:1, 0:1) to give six fractions (Fr. 1-Fr. 6). Fr. 3 (45.5 g) was chromatographed over silica gel with a gradient of increasing ethyl acetate in petroleum ether (10:1, 10:2, 2:1, 1:1) to yield four subfractions, Fr. 3–1-Fr. 3–4. Fr. 3–2 was recrystallized in $CHCl_3$ to afford **12** (6.6 mg). Fr. 3–3 was recrystallized in acetone to afford **7** (2.1 g). Fr. 4 (23 g) was subjected to further silica gel CC with gradient petroleum ether-acetone (100:0–0:100) to give twenty subfractions, Fr. 4–1-Fr. 4–20. Fr. 4–3 (0.15 g) was loaded on CC over Sephadex LH-20 using petroleum ether-$CHCl_3$-CH_3OH (5:5:1) to give ten subfractions. Fr. 4–3-7 (50 mg) was purified by preparative TLC ($CHCl_3$-acetone, 3:1) to afford **10** (20 mg). Fr. 4–4 (0.2 g) was chromatographed over Sephadex LH-20 using petroleum ether-$CHCl_3$-CH_3OH (5:5:1) to give ten subfractions. Fr. 4–4-5 was recrystallized in acetone to afford **9** (9.1 mg). Fr. 4–4-6 was separated by preparative TLC ($CHCl_3$-acetone, 3:1) and semi-preparative HPLC with MeOH-H_2O (75:25, 2 ml/min) to afford **1** (2.2 mg). Fr. 4–4-7 was purified by semi-preparative HPLC with MeOH-H_2O (80:20, 2 ml/min) to afford **5** (9.7 mg). Fr. 4–6 (1.2 g) was chromatographed over Sephadex LH-20 using petroleum ether-$CHCl_3$-CH_3OH (5:5:1) to give five subfractions. Fr. 4–6-2 (15 mg) was purified by HPLC with MeOH-H_2O (70:30, 2 ml/min) to afford **11** (9.7 mg). Fr. 4–6-5 (44 mg) was purified by preparative TLC ($CHCl_3$-acetone, 3:1) to afford **2** (9 mg) and **3** (12 mg). Fr. 4–9 (2 g) was chromatographed over Sephadex LH-20 using petroleum ether-$CHCl_3$-CH_3OH (5:5:1) to give nine subfractions. Fr. 4–9-4 was purified by HPLC with MeOH-H_2O (70:30, 2 ml/min) to afford **4** (4.7 mg). Fr. 4–9-5 was purified by HPLC with MeOH-H_2O (70:30, 2 ml/min) to afford **13** (8 mg). Fr. 4–9-6 was purified by HPLC with MeOH-H_2O (65:35, 2 ml/min) to afford **14** (8 mg). Fr. 4–9-7 was purified by HPLC with MeOH-H_2O (70:30, 2 ml/min) to afford **6** (7 mg). Fr. 4–9-8 was recrystallized in acetone to afford **8** (1.1 g).

2.3.1. (1S,4S,5R,6R,10S)-ax-1,4,11-triol (**1**)

Colorless gum; $[\alpha]_D^{20} = +34.2$ ($c = 0.2$, MeOH); IR (KBr) ν_{max} 3325, 2969, 2947, 2860, 1464, 1365, 1049 cm^{-1}; ^1H NMR (MeOH-d_4, 600 MHz) data, see Table 1; ^{13}C NMR (MeOH-d_4, 150 MHz) data, see Table 2; (+)-HRESIMS m/z 279.1926 $[M + Na]^+$ (calcd for $C_{15}H_{28}NaO_3$, 279.1931).

2.3.2. (1S,4R,5S,6R,7R,10S)-opposit-1,4,7-triol (**2**)

Colorless needles; $[\alpha]_D^{20} = -24.1$ ($c = 0.1$, MeOH); IR (KBr) ν_{max} 3397, 2952, 2872, 1464, 1383, 1075, 1031, 916 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (acetone-d_6, 125 MHz) data, see Table 2; (+)-HRESIMS m/z 279.1926 $[M + Na]^+$ (calcd for $C_{15}H_{28}NaO_3$, 279.1931).

2.3.3. 7R-5βH,10α-opposit-1α,4α,7-triol (**3**)

Colorless gum; $[\alpha]_D^{20} = +35.2$ ($c = 0.2$, MeOH); IR (KBr) ν_{max} 3375, 2958, 2872, 1464, 1371, 1073, 1027, 948 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (acetone-d_6, 125 MHz) data, see Table 2; (+)-HRESIMS m/z 279.1926 $[M + Na]^+$ (calcd for $C_{15}H_{28}NaO_3$, 279.1931).

2.3.4. 2α-hydroxyhomalomenol A (**4**)

White amorphous powder; $[\alpha]_D^{20} = +31.6$ ($c = 0.15$, MeOH); IR (KBr) ν_{max} 3439, 2957, 2868, 1561, 1386, 1040, 1020, 836 cm^{-1}; ^1H NMR (MeOH-d_4, 500 MHz) data, see Table 1; ^{13}C NMR (MeOH-d_4, 125 MHz) data, see Table 2; (+)-HRESIMS m/z 277.1774 $[M + Na]^+$ (calcd for $C_{15}H_{26}NaO_3$, 277.1774).

2.3.5. (1S,4R,5R,6R,7R,10S)-isodauc-6,7,10-triol (**5**)

Colorless needles; $[\alpha]_D^{20} = +6.4$ ($c = 0.38$, MeOH); IR (KBr) ν_{max} 3412, 2935, 2872, 1453, 1372, 1010, 907 cm^{-1}; ^1H NMR (MeOH-d_4, 500 MHz) data, see Table 1; ^{13}C NMR (MeOH-d_4, 125 MHz) data, see Table 2; (+)-HRESIMS m/z 279.1927 $[M + Na]^+$ (calcd for $C_{15}H_{28}NaO_3$, 279.1931).

2.3.6. Eudesma-4β,7α-diol-1β-fumarate (**6**)

Colorless gum; $[\alpha]_D^{20} = -18.8$ ($c = 0.4$, MeOH); IR (KBr) ν_{max} 3406, 2935, 2865, 1699, 1642, 1262, 1174, 977, 905 cm^{-1}; ^1H NMR (MeOH-d_4, 600 MHz) data, see Table 1; ^{13}C NMR (MeOH-d_4, 150 MHz) data, see Table 2; (+)-HRESIMS m/z 377.1944 $[M + Na]^+$ (calcd for $C_{19}H_{30}NaO_6$, 377.1935).