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Diterpenes inhibiting NO production from *Euphorbia helioscopia*

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ABSTRACT

Three new jatrophane diterpenes (**1**–**3**), an unreported spectroscopic data jatrophane diterpenene (**4**), and nine known analogues (**5**–**13**) have been isolated from the whole plants of *Euphorbia helioscopia*. Their structures were established by detailed spectroscopic data analyses (IR, ESIMS, HR-ESIMS, and 1D and 2D NMR), and the structure of **1** was confirmed by X-ray crystallography. The diterpenes showed inhibitory activities on LPS-induced NO production in murine microglial BV-2 cells.

1. Introduction

Euphorbia helioscopia L., belonging to the family Euphorbiaceae, is widely distributed in mainland China [1]. The whole plants of *E. helioscopia*, a traditional Chinese medicine in China, have been used for the treatment of malaria, bacillary dysentery, and osteomyelitis [1,2]. Previous phytochemical investigations on *E. helioscopia* led to the isolation and identification of diterpenes, especially jatrophane-type diterpenes [3–8], which showed cytotoxic activities [4,5]. Though the chemistry and biological activities have been investigated, there have been no reports on the inhibitory effects of NO production of the chemical constituents or the extract from *E. helioscopia*. In our search for pharmacologically active substances in medicinal plants [9–11], much attention has been given to the occurrence of compounds with inhibitory effects of NO production, since NO plays an important role in the inflammatory process, and an inhibitor of NO production may be considered as a potential anti-inflammatory agent [12]. As a continuation of our search for inhibitors of NO production from plants, we investigated the chemical constituents of *E. helioscopia*. The phytochemical investigation led to the isolation of three new (**1**–**3**) and one unreported spectroscopic data (**4**) jatrophane diterpenenes, and nine known diterpenes (**5**–**13**) (Fig. 1). On the basis of detailed spectroscopic and spectrometric analyses (IR, ESIMS, HR-ESIMS, and 1D and 2D NMR) and X-ray crystallography, compounds **1**–**4** were elucidated and named euphorbiapenes A–D, and compounds **5**–**13** were characterized as euphoscopin F (**5**) [4], euphoscopin H (**6**) [8], euphoheliosnoid A (**7**) [7], euphoscopin C (**8**) [4], euphornin G (**9**) [8], 15-*O*-Acetyl-3-*O*-benzoylcharaciol (**10**) [13], ingenol monoacetate (**11**) [14], helioscopinolide A (**12**) [15], and helioscopinolide E (**13**) [15]. This paper herein describes the isolation and structure elucidation of these diterpenes and their inhibitory activities on lipopolysaccharide (LPS)-induced NO production in murine microglial BV-2 cells.

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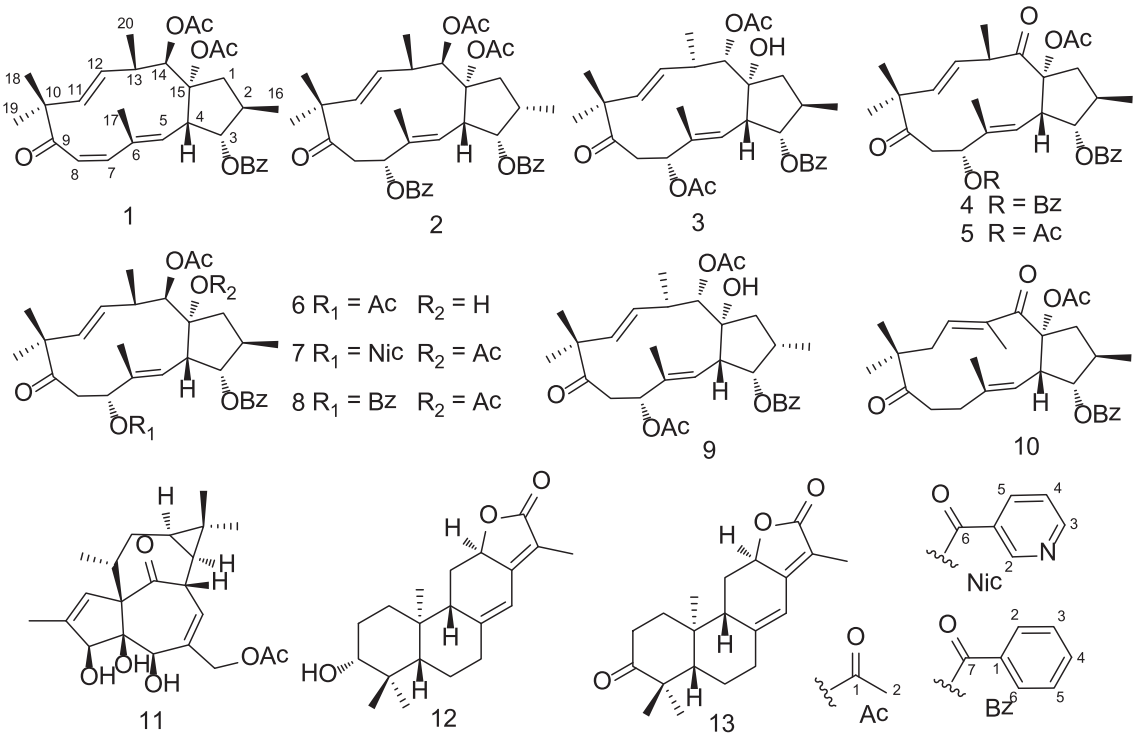

Fig. 1. Structures of **1–13** from *E. helioscopia*.

2. Experimental

2.1. General

Melting points were determined with an XT-4 microscopic thermometer. The optical rotations were measured in MeOH, using an Autopal IV automatic polarimeter made by Autopal Industries Limited Company. The IR spectra were taken on a Bio-Rad FTS 6000 Fourier transform infrared (FTIR) spectrometer with KBr disks. The ESIMS spectra were obtained on a LCQ-Advantage mass spectrometer made by Finnigan Company (America). HR-ESIMS spectra were recorded by IonSpec 7.0 T FTICR MS (IonSpec Co., Ltd., Lake Forest, CA) or Agilent 6520 Q-TOF LC/MS (Agilent, Santa Clara, CA). 1D and 2D NMR spectra were recorded on a Bruker AV 400 instrument (400 MHz for ^{1}H and 100 MHz for ^{13}C) with TMS as an internal standard. HPLC separations were performed on a CXTH system (Beijing Chuangxintongheng instrument Co. Ltd., P.R. China), equipped with a UV3000 detector at 210 nm, and a YMC-pack ODS-M80 column (20 × 250 mm, i.d.). Silica gel (200–300 mesh, Qingdao Marine Chemical Group Co. Ltd., P.R. China) was used for column chromatography. Chemical reagents for isolation were analytical grade and purchased from Tianjin Yuanli Chemical Co. Ltd. P.R. China. Biological reagents were from Sigma Company. The murine microglial BV-2 cell line was from Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (China).

2.2. Plant material

The whole plants of *E. helioscopia* L. were purchased in Aug. 2011 from Anguo Materia Medica Market, Hebei province, China. The botanical identification was made by Dr. Yuanqiang Guo (College of Pharmacy, Nankai University, China), and a voucher specimen (No. 20110817) was deposited at the laboratory of the Research Department of Natural Medicine, College of Pharmacy, Nankai University, China.

2.3. Extraction and isolation

The air-dried and powdered whole plants *E. helioscopia* (10 kg) were extracted with methanol three times (3 × 50 L) under reflux. The organic solvent was evaporated to afford a crude extract. The extract was suspended in H_2O (0.9 L) and then partitioned with ethyl acetate. The ethyl acetate soluble part (270 g) was subjected to silica gel column chromatography, using a gradient solvent system from 1 to 30% acetone in petroleum ether, to give 10 fractions (F_1–F_{10}) based on TLC analyses. F_3 was separated by MPLC over octadecylsilyl (ODS) eluting with a step gradient from 71% to 91% MeOH in H_2O to give four subfractions (F_{3-1}–F_{3-4}). The subfraction F_{3-2} was further purified by HPLC (YMC-pack J'Sphere ODS-M80, 20 × 250 mm, 85% MeOH in H_2O) to afford compounds **1** (11.6 mg) and **10** (6.0 mg). Fractions F_4, F_5, F_6, F_7, and F_8 were subjected to the same MPLC over ODS, eluting with a step gradient from 60% to 89% MeOH in H_2O, to give subfractions F_{4-1}–F_{4-5}, F_{5-1}–F_{5-12}, F_{6-1}–F_{6-7}, F_{7-1}–F_{7-16}, and F_{8-1}–F_{8-13}, respectively. Compound **2** (11.4 mg) was isolated from subfraction F_{5-5} (85% MeOH in H_2O), and the purification of subfraction F_{5-3} (77% MeOH in H_2O) to yield compounds **3** (4.2 mg) and **6** (7.2 mg), using the above HPLC system. With the same protocols for subfraction F_{4-3} (85% MeOH in H_2O), compounds **4** (17.4 mg), **5** (23.0 mg), and **8** (15.5 mg) were obtained. The purification of subfraction F_{7-4} (85% MeOH in