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Highly oxygenated and structurally diverse diterpenoids from *Euphorbia helioscopia*

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ABSTRACT

A phytochemical investigation on the aerial part of *Euphorbia helioscopia* (Euphorbiaceae) led to the isolation of 22 highly oxygenated diterpenoids with structural types of *ent*-abietane, *ent*-kaurane, lathyrane, *ent*-atisane and ingenane. 17 of them, named euphelionolides A – N, 16-*epi*-18-hydroxy-abbeokutone, as well as eupheliotriols A and B, were identified to be previously undescribed compounds by extensive analysis of spectroscopic data. The stereostructures of euphelionolides A – K were determined by single crystal X-ray diffraction combined with analysis of substituent effects and comparison of optical characteristics. Eupheliotriol B is the first example of natural occurring lathyrol with 12*Z*-ene, while *ent*-atisanes are the first reported from the title plant. Furthermore, euphelionolides F and L exhibited significant cytotoxicity against MCF-7 and PANC-1 cell lines.

1. Introduction

Euphorbia helioscopia Linn. (Euphorbiaceae), known as “Zeqi” in Chinese, is widely distributed in most regions of China (Ma and Michael, 2008), and has been used as a traditional folk medicine for the treatment of chronic obstructive pulmonary diseases such as cough, phlegm-turbidity, asthma, and chronic bronchitis (Chen et al., 1979). Previous phytochemical studies on *Euphorbia* species disclosed the presence of terpenes (iridoids, sesquiterpenoids, diterpenoids, triterpenoids), steroids, flavonoids, acetophenones, tannins, phenylpropanoids, cerebrosides and glyceroglycolipids (Shi et al., 2008). Among these metabolites, diterpenoids are the majority of the genus. There over 700 diterpenoids with more than 20 skeletal types have been isolated from *Euphorbia* plants (Aljančić et al., 2011; Gao et al., 2016; Jadranin et al., 2013; Lu et al., 2008; Shi et al., 2008; Vasas and Hohmann, 2014; Yamamura et al., 1989; Zhou et al., 2016), which exhibited multiple biological activities, including antiproliferative, multi-drug-resistance-reversing, antimicrobial, anti-HIV, vascular-relaxing, immunomodulatory and anti-inflammatory effects (Barile et al., 2008; Lu et al., 2008; Pešić et al., 2011; Shi et al., 2008; Vasas and Hohmann, 2014). As a slightly poisonous plant, *E. helioscopia* was also clinically used to treat malignant tumors in China in view of poison-against-poison, and its ethyl acetate fraction played an important role on tumor cell proliferation, apoptosis, invasion, and metastasis *in vitro* and in the nude mouse xenograft model (Cheng et al., 2015). Based on the interest of anti-cancer ingredients and the structural diversity of *Euphorbia* diterpenoids, we investigated the EtOAc-soluble layer of the aerial part of the title plant, which led to the isolation of 17 previously undescribed and 5 known diterpenoids with multiple structural types. Herein, the isolation, structure elucidation, and *in vitro* cytotoxic activity of isolated compounds are reported.

2. Results and discussion

Diterpenoids **1**–**22** (Fig. 1) were isolated by repeated column chromatographies and semi-preparative HPLC from the EtOAc-soluble layer of 95% EtOH extract of *E. helioscopia*. Five known

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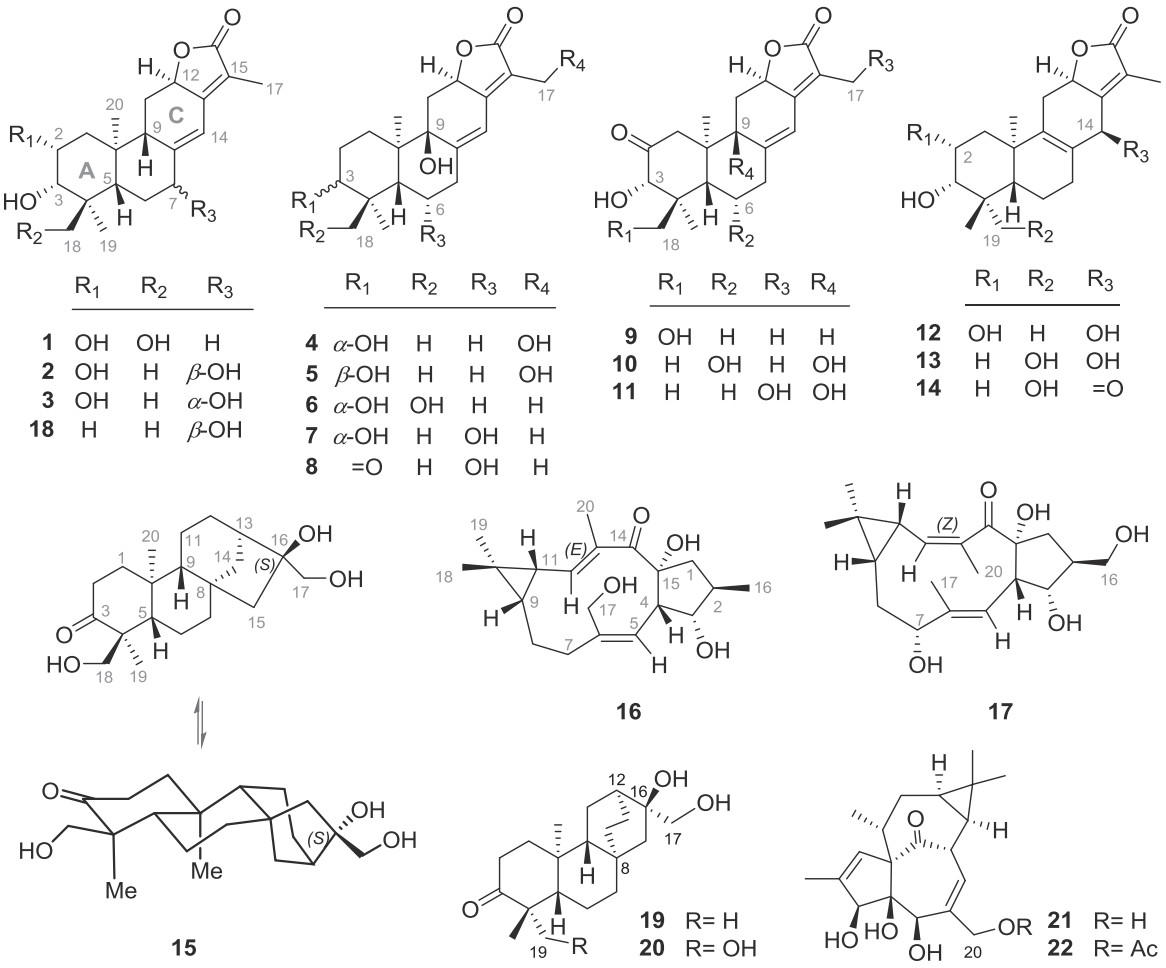

Fig. 1. Structures of diterpenoids **1**–**22**.

diterpenoids were identified as 3α,7β-dihydroxyjolkinolide E (**18**) (Vasas and Hohmann, 2014), *ent*-16β,17-dihydroxyatisan-3-one (**19**) (Vasas and Hohmann, 2014), eurifoloid Q (**20**) (Zhao et al., 2014), ingenol (**21**) (Wang et al., 2015), and 20-*O*-acetylingenol (**22**) (Wang et al., 2015) by comparison of the spectroscopic data with those reported in the literature.

Diterpenoid **1**, named euphelionolide A, was isolated as colorless crystals (EtOH), its molecular formula was deduced as $C_{20}H_{28}O_5$ by the HRESIMS $[M + Na]^+$ ion at *m/z* 371.1818 (calcd 371.1834), indicative of seven indices of hydrogen deficiency. The IR spectrum of **1** revealed the presence of hydroxyls (3308 cm^{-1}) and an α,β-unsaturated γ-lactone system (1751 and 1670 cm^{-1}) (Shizuri et al., 1983). The ^{13}C and ^{1}H NMR data (Tables 1 and 2) showed signals for 20 carbons and 25 carbon-bearing protons, which were resolved into three methyls (δ_H 0.92, 1.22 and 1.81), five methylenes (δ_C 24.1, 28.8, 37.6, 44.2 and 66.9), five methines (δ_H 1.77, 2.30, 3.61, 4.17 and 5.00), two double bonds (δ_H 6.41; δ_C 115.1, 116.8, 154.2 and 158.9), a conjugated ester carbonyl (δ_C 177.7), and two saturated quaternary carbons (δ_C 43.3 and 41.7). The above spectroscopic data were similar to those of jolkinolide E (**1a**, = 8(14),13(15)-abietadien-16,12β-olide) (Crespi-Perellino et al., 1996) except for the emerging of three oxygenated carbon signals (δ_C 66.9, 72.2 and 72.8) and the disappearance of two methylenes (C-2 and C-3) and one methyl (C-18 or C-19), indicating a trihydroxylated jolkinolide E. The hydroxymethyl was positioned to C-18 rather than C-19 by evidences of HMBC correlation of H_2-18/C-4 and NOESY cross-peak of Me-19/Me-20, and the two oxymethines were assigned to C-2 and C-3 as supported by the HMBC cross-peaks from H-2 and H-3 to C-4 (Fig. 2A and B, SI Figs. S6 and S7). Both hydroxyls on C-2 and C-3 were determined to be α-oriented on the basis of the coupling pattern of H-2 and its vicinal protons ($J_{1\alpha,2}$ = 3.4 Hz, $J_{1\beta,2}$ = 2.9 Hz, and $J_{2,3}$ = 3.8 Hz) and NOESY cross-peaks of H-3/H-5 and H-2/H-3 (Fig. 2B, SI Fig. S7). The absolute configuration of **1** was finally established to be (2*R*,3*S*,4*S*,5*S*,9*R*,10*S*,12*R*)-2,3,18-trihydroxy-8(14),13(15)-abietadien-16,12-olide by the Cu-Kα radiation X-ray diffraction analysis with the Flack parameter being 0.0 (2) (Fig. 2C).

Euphelionolides B – G (**2**–**7**) shared the same molecular formula of $C_{20}H_{28}O_5$ with **1** as extrapolated from their ^{13}C NMR data and HRESIMS $[M + Na]^+$ ions neighbor to *m/z* 371.1834. They were also trihydroxylated analogues of **1a** as judged from the comparability of IR bands, UV absorptions, positive and large specific optical rotations, and NMR data (Tables 1–3) with those of **1**, especially those characteristic spectral properties for the $\alpha\beta,\gamma\delta$-unsaturated 5-membered ring lactone (Shizuri et al., 1983).

The three hydroxyls of euphelionolide B (**2**) were assigned on C-2, C-3 and C-7 by the HMBC correlations from H-2 to C-1, C-3 and C-4, from H-3 to C-4, C-18 and C-19, and from H-7 to C-8, C-9 and C-14 (SI Fig. S12). The configurations of the three hydroxyls were elucidated to be 2α, 3α and 7β founded on the coupling pattern of their geminal protons ($J_{1\alpha,2}$ = 3.5 Hz, $J_{1\beta,2}$ = 3.6 Hz, $J_{2,3}$ = 3.6 Hz, $J_{6\alpha,7}$ = 2.1 Hz, and $J_{6\beta,7}$ = 2.8 Hz). This conclusion was further rationalized by analysis of substituent effects correlating with the