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Diterpenoids from the roots of *Euphorbia ebracteolata* and their inhibitory effects on human carboxylesterase 2

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ABSTRACT

A chemical investigation of the roots of *Euphorbia ebracteolata* identified eighteen diterpenoids and glycosides. On the basis of spectroscopic data, they were determined to be *ent*-kauranes, *ent*-atisanes, tigliane derivatives, ingenane, and *ent*-abietanes, among which were eleven previously undescribed diterpenoids. The inhibitory effects of the isolated compounds against human carboxylesterase 2 (hCE-2) were evaluated *in vitro*, which revealed moderate inhibitory effects with IC_{50} values < 50 μM. Next, the inhibitory kinetics were evaluated for the putative hCE-2 inhibitor 4β,9α,16,20-tetrahydroxy-14(13 → 12)-abeo-12αH-1,6-tigliadiene-3,13-dione (IC_{50} 3.88 μM), and results indicated competitive inhibition with K_i 4.94 μM. Additionally, none of the diterpenoids showed cytotoxic effects against five human tumor cell lines as determined by MTT assays.

1. Introduction

Euphorbia ebracteolata Hayata, a perennial herbaceous plant in the Euphorbiaceae family, is distributed throughout Asia. Previous chemical studies of this plant have revealed the presence of diterpenoids (Liang et al., 2014; Liu et al., 2014a, b; Mu et al., 2013), acetophenones (Geng et al., 2015; Zhang et al., 2010), monoterpenes (Wang et al., 2015) and flavonol glycosides (Liu et al., 2004). Extracts of *E. ebracteolata* roots have been used to treat tuberculosis, ascites, and cancer in traditional Chinese medicine (Fu et al., 2006; Shi et al., 2005). The diterpenoids in particular have been shown to exert a range of biological effects, such as anti-tumor, anti-bacterial, anti-inflammatory and immunostimulatory activities (Xu et al., 1998, 2000; Zhang et al., 2009).

Human carboxylesterase 2 (hCE-2), which belongs to the serine protease superfamily (Takai et al., 1997; Wang et al., 2011), is widely distributed in the livers and intestines of mammals (Furihata et al., 2003; Kuykendall et al., 1993; Nishi et al., 2006) where it hydrolyzes a variety of endogenous and exogenous substances (Thomsen et al., 2014) such as drugs and environmental toxins. However, the metabolism of drugs by hCE-2 results in adverse clinical reactions (Laizure et al., 2014) such as the reduced biological availability of drugs. Thus, the development of an hCE-2 inhibitor is an active area of current medical research.

In the present investigation of the diterpenoids of *E. ebracteolata*, 11 previously undescribed diterpenoids were identified together with 7 known compounds (Fig. 1). The structures of the isolated diterpenoids were determined from various spectroscopic data. The inhibitory effects of the isolated compounds on hCE-2 were evaluated *in vitro* using a fluorescent bioassay. The inhibitory kinetics of the promising compound **9** were investigated. Finally, the cytotoxic activity of these compounds against five human cancer cell lines was evaluated *in vitro* using MTT assays.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder, with the molecular formula $C_{26}H_{42}O_9$ as established by the positive

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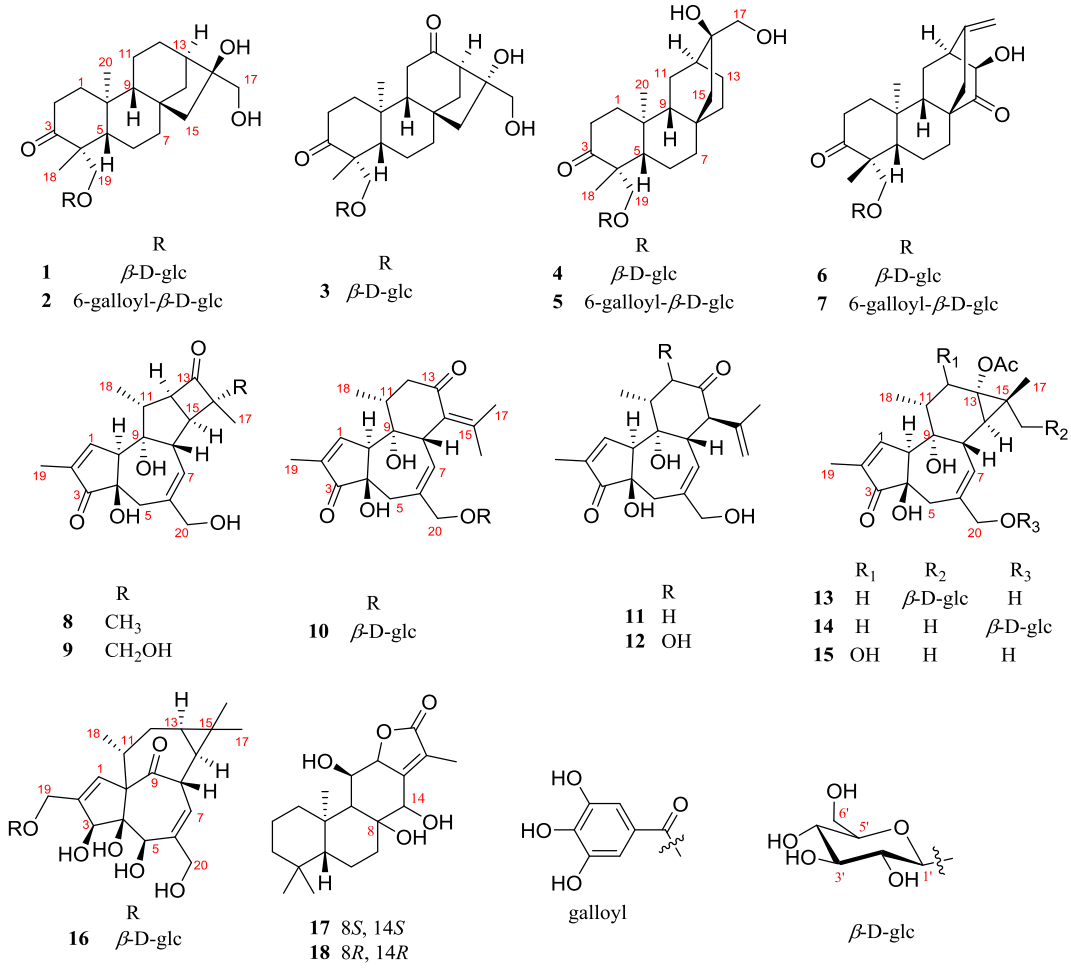

Fig. 1. Diterpenoids obtained from the roots of *Euphorbia ebracteolata*.

HRESIMS *m/z* 521.2738 $[M + Na]^+$ (calcd for $C_{26}H_{42}O_9Na$, 521.2729). The ^1H NMR spectrum suggested the presence of two methyl groups (δ_H 1.04, 1.20, each 3H, s), three oxygenated methylene groups (δ_H 4.18, 3.33; δ_H 3.24, 3.13; δ_H 3.66, 3.41), and five oxygenated methine groups (δ_H 4.02, 3.08 (2H), 3.00, 2.88) (Table 1). Twenty-six carbons were observed in the ^{13}C NMR spectrum, which indicated moieties such as a ketone (δ_C 213.3) and oxygenated carbons (δ_C 102.9, 78.4, 76.9, 76.7, 73.2, 70.7, 70.1, 69.1, 61.1) (Table 2). The NMR data suggested that compound **1** is an *ent*-kaurane type diterpenoid glycoside possessing a monosaccharide moiety. In the HMBC spectrum, the long-range correlations H-1 (δ_H 2.06, 1.19)/C-3, H-18 (δ_H 1.04)/C-3 (δ_C 213.3), H-19 (δ_H 4.18, 3.33)/C-3, H-5 (δ_H 1.21)/C-19 (δ_C 70.7), and H-18 (δ_H 1.04)/C-19 established the 3-ketone and 19-CH_2OH groups (Fig. 2). The observed HMBC correlations H-14 (δ_H 1.84)/C-16 (δ_C 78.4), H-15 (δ_H 1.34, 1.25)/C-16, H-15/C-17 (69.1), and H-17 (δ_H 3.24, 3.13)/C-13 (δ_C 40.5) determined the presence of the 16,17-diols. In the ^1H-^1H COSY spectrum, three different spin-spin systems (H-1/H-2, H5/H-6, H-9/H-11/H-12/H-13) were established by the correlations H-1 (δ_H 1.19)/H-2 (δ_H 2.77), H-5 (δ_H 1.21)/H-6 (δ_H 1.48), H-9 (δ_H 1.06)/H-11 (δ_H 2.01), and H-11 (δ_H 1.45)/H-12 (δ_H 1.75)/H-13 (δ_H 1.94)/H-14 (δ_H 0.99). The above-mentioned spectroscopic data indicated that the aglycon substructure of **1** was similar to *ent*-kaurane-16β,17,19-triol-3-one (Liu et al., 2017). The monosaccharide moiety was determined to be β-D-glucopyranose by the NMR data with the anomeric proton signal (δ_H 4.02, d, $J = 8.0$ Hz) and an acid hydrolysis experiment with D-glucose as a standard reference. Based on the long-range correlation of H-1′ (δ_H 4.02)/C-19 observed in the HMBC data, the glucose moiety was located at C-19 of the *ent*-kaurane diterpenoid. The relative configurations of diterpenoid skeleton were determined on the basis of the NOE correlations H-19/H-20 and H-13/H-17, which were same as those of *ent*-kaurane-16β,17,19-triol-3-one (Fig. 2). Data from ECD revealed a negative Cotton effect at 297 nm ($n \rightarrow \pi$), which suggested a 5*S* absolute configuration using the ketone octant rule (Ye, 1999) (Supplementary data, Fig. S9). Additionally, ^{13}C NMR calculations were performed to distinguish *ent*-kaurane diterpenoid **1** from *ent*-atisane diterpenoid **4** based on the linkages C-16/C-13 and C-16/C-12, respectively. The calculated ^{13}C NMR data of the aglycon substructure (without C-19) of compounds **1** and **4** is shown in Table 2, showing that the C-16 signal of compound **1** was downshifted by $\Delta\delta_C = 6$ ppm compared with that of compound **4**. Moreover, regression analysis of experimental versus calculated ^{13}C NMR chemical shifts of **1** at mPW1PW91/6-311 + G(d,p) level in gas phase showed a high degree of correlation ($R^2 = 0.9969$), which confirmed the *ent*-kaurane skeleton of **1** (Fig. S1, Supplementary Data). Therefore, the structure of compound **1** (*ent*-kaurane-16β,17-diol-3-one-19-*O*-β-D-glucopyranoside) was unequivocally characterized as shown.

The spectroscopic data of compound **2** indicated that it was similar to **1**, except that **2** contains an extra galloyl group (δ_H 6.96 s; δ_C 119.2, 108.6, 145.4, 138.5, 165.8). In the HMBC spectrum of **2**, a long-range correlation between H-6′ (δ_H 4.29) of the glucopyranose group and the carboxylic carbon (δ_C 165.8) of the galloyl moiety was