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Bioactive isopimarane diterpenoids from the stems of *Euonymus oblongifolius*

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ABSTRACT

Seven isopimarane diterpenes and one abietane diterpene, together with six known sesquiterpene derivatives, were isolated from the stems of *Euonymus oblongifolius*. Their structures were elucidated on the basis of spectroscopic analyses, and the absolute configuration of euonymusisopimaric acid A was confirmed by single-crystal X-ray crystallographic analysis using anomalous scattering of Cu Kα radiation. All of the isolated compounds were evaluated for their ability to inhibit LPS-induced nitric oxide production in the murine microglia BV2 cell line, and for their cytotoxic activity against five human cancer cell lines. Euonymusisopimaric acids A, E and F inhibited LPS-induced nitric oxide production in the murine microglia BV2 cell line, with IC_{50} values of 2.4, 4.8, and 1.6 μM, respectively. Euonymusisopimaric acid B exhibited moderate cytotoxicity against A549 cell line growth, with an IC_{50} value of 2.6 μM.

1. Introduction

Euonymus oblongifolius, a shrub or small tree, is distributed widely in the southern areas of China, such as Zhejiang, Fujian, Jiangxi, Hunan, and Guangdong (Editorial Committee of Flora of China, 1999). It belongs to the genus *Euonymus*, Celastraceae family. The plants of genus *Euonymus* (e.g., *Euonymus alatus* (Thunb.) *Sieb*) are commonly used in traditional medicines for regulating blood circulation, relieving pain, eliminating stagnant blood and treating dysmenorrhea (Zhao et al., 2011). However, previous research about *E. oblongifolius* has focused mainly on ecology and plant distribution (Dinan et al., 2001; Tokuoka and Tobe, 2006). In a previous study of a 95% ethanol extract of *E. oblongifolius* stems, two new neolignans were found (Li et al., 2012). In a continued search for bioactive metabolites from the same extract, a systematic phytochemical investigation was carried out, and 14 compounds were obtained including eight new diterpenes (**1**–**8**) and six known sesquiterpenes (**9**–**14**).

2. Results and discussion

A 95% ethanol extract of *E. oblongifolius* stems was divided by celite column into partitions of petroleum ether, chloroform, ethyl acetate, acetone, and 95% ethanol. Chromatographic purification of the soluble petroleum ether portion and the chloroform portion by silica gel column and preparative high performance liquid chromatography yielded eight new diterpenes (**1**–**8**) (Fig. 1) and six known sesquiterpenes (**9**–**14**).

Compound **1** was obtained as colourless needles. Its molecular formula was $C_{20}H_{30}O_3$, as determined by HRESIMS (*m/z* 319.2273 [M + H] $^+$, calcd for 319.2268). Its UV spectrum indicated an absorbance at λ_{max} 229 nm, and IR data indicated the presence of hydroxyl (3588 cm^{-1}), carboxyl (2949 cm^{-1}), and carbon-carbon double bonds moieties (1607 cm^{-1}). The ^{1}H NMR data indicated the presence of three tertiary methyl groups [δ_H 2.05, 1.25, and 0.92] and characteristic vinyl group hydrogen signals [δ_H 5.74 (1H, dd, J = 10.5, 17.5 Hz), 4.88 (1H, d, J = 17.5 Hz), 4.83 (1H, d, J = 10.5 Hz)]. The ^{13}C NMR and HSQC spectrum indicated signals for seven methylenes (δ_C 34.8, 24.1, 38.3, 17.5, 19.6, 43.1, 51.8) and two methyne groups (δ_C 51.4, 53.9), as well as one oxygenated quaternary group (δ_C 72.3), and an α, β-unsaturated carboxylic moiety (δ_C 174.9, 123.2, 149.9). The ^{1}H-^{1}HCOSY spectra data indicated the

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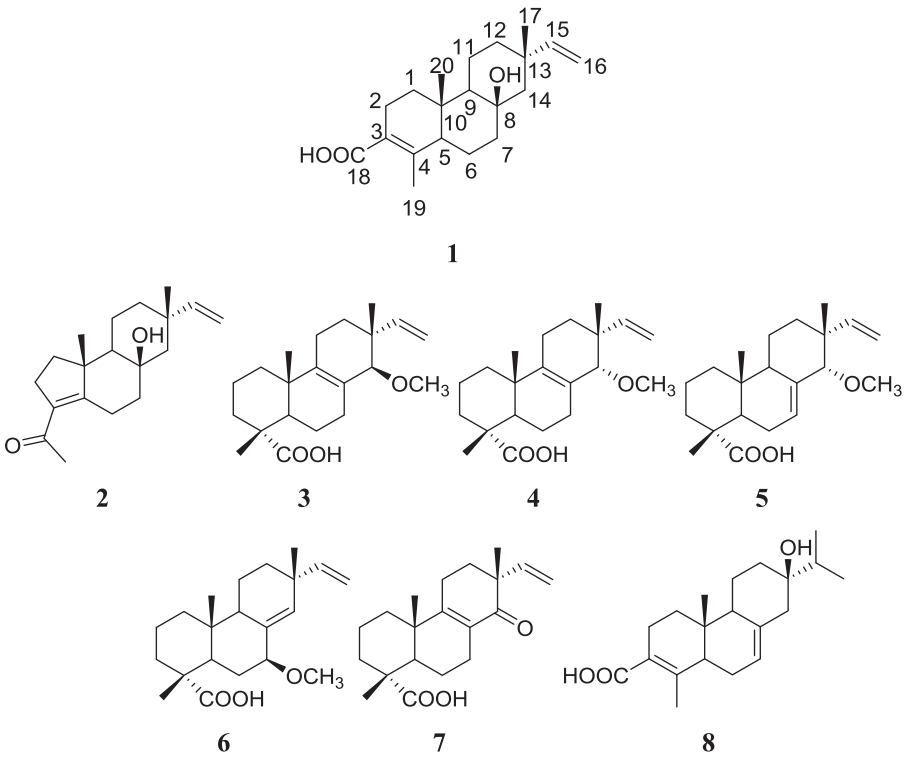

Fig. 1. The structures of **1**–**8** obtained from *E. oblongifolius*.

presence of an H-9—H-12 fragment. In the HMBC spectrum, correlations between CH_3-19 and C-3, C-4, and C-5 suggested that CH_3-19 was located at C-4. The correlation from CH_3-19 to 18-COOH suggested that CH_3-19 and 18-COOH were located at adjacent carbons. Thus, the structure of compound **1** was determined.

The relative configuration of **1** was established by NOESY correlations of H-9/H-1α and H-5/H-9. In the NOE experiment, while CH_3-17 was irradiated, the OH signal of δ_H 3.57 was enhanced, which suggested that OH and CH_3-17 were both β-oriented (Fig. 2). A single-crystal X-ray crystallographic analysis showed that there were four independent molecules in the unit, and the absolute structures were the same. Each of the two molecules formed a dimer through intermolecular hydrogen bonding between the carboxylic acid groups. The four molecules were numbered consistently as C1-C80 (Fig. 3). Thus, the chiral carbons of compound **1** were confirmed to be 8*R*, 10*S* and 13*S* (Fig. 4). Therefore, the structure of **1** was determined as (8*R*)-8-hydroxy-18(4 → 3) *abeo*-3, 15-isopimaradien-18-oic acid and was named euonymusisopimaric acid A.

The molecular formula of compound **2** was calculated to be $C_{19}H_{28}O_2$ according to its HRESIMS (*m/z* 289.2173 $[M + H]^+$, calcd for 289.2162). The ^{1}H NMR data showed vinyl group hydrogen signals [δ_H 5.73 (1H, dd, $J = 11.0, 17.5$ Hz), 4.88 (1H, br d, $J = 17.5$ Hz), 4.83 (1H, br d, $J = 11.0$ Hz)] and three sharp single tertiary methyl groups δ_H 1.12, 1.26, and 2.24. Comparisons between compounds **1** and **2** indicated that the methyl groups of compound **2** at δ_H 2.24 and 1.12 were at lower chemical shifts than 19-CH_3 and 20-CH_3 from a common isopimarane diterpene. The structure fragments of H-1—H-2, H-6—H-7, and H-9—H-11—H-12 were deduced from the ^{1}H-^{1}HCOSY spectra data. The HMBC data showed that H-1, H-2 and CH_3-20 were correlated with C-3, and that H-1, H-2, H-6, H-7 and CH_3-20 correlated with C-5; this key information verified that ring-A was a five-membered ring. In the HMBC spectrum, the correlations of H-6, H-7, and H-14 with C-8, and the chemical shift of C-8 (72.2) confirmed that OH was located at C-8. Thus, the structure of compound **2** was established.

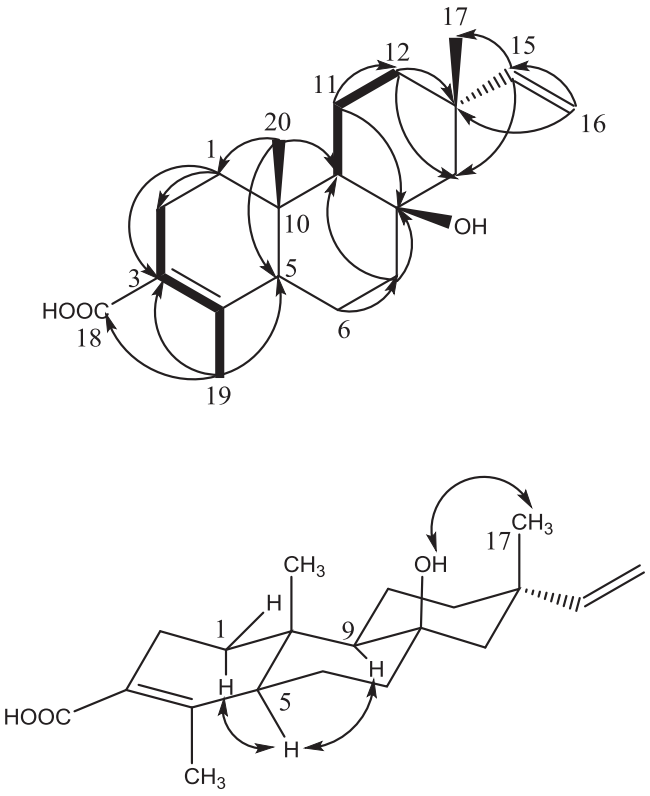

Fig. 2. Key ^{1}H-^{1}H COSY, HMBC, and NOE correlations of compound **1**.

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