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Excoecarins L and O from the mangrove plant *Excoecaria agallocha* L.

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ABSTRACT

Two new beyerene-type diterpenoids (**1** and **2**), along with six known metabolites (**3**-**8**) were isolated from the twigs and leaves of the mangrove plant *Excoecaria agallocha* L. collected from coastal areas in Vietnam. The structures and relative configurations of new compounds were established on the basis of HR-ESI-MS, 1D and 2D NMR experiments. Two new metabolites were tested for cytotoxicity against two human cancer cell lines (KB and LU-1), but both compounds did not show significant activity.

1. Introduction

Excoecaria agallocha L., a small mangrove tree belonging to the family Euphorbiaceae, is widely distributed in coastal areas of Vietnam. This plant is also found in India, East Asia and Australia. The latex of *E. agallocha* is poisonous and has been used as fish poison and arrow poison. The leaves of this species have been used in Vietnam for treatment of ulcers, rheumatism, leprosy, and paralysis (Chi, 2012). More than 140 metabolites, including diterpenoids, triterpenoids, flavonoids, alkaloids, sterols, tannins, and other compounds have been reported from *E. agallocha*. Among them, several compounds showed variety of bioactivities, such as antioxidant, antimicrobial, anti-inflammatory, analgesic, antiulcer, anticancer, antireverse transcriptase, antihistamine-release, antifilarial, DNA damage protective, antidiabetic, and antitumor effects (Li et al., 2009; Mondal et al., 2016; Yin et al., 2008). As a continuation of studies on bioactive compounds from Vietnam mangrove plants (Ba Vinh et al., 2017; Dat et al., 2015; Huong et al., 2014; Nguyen et al., 2015), we report herein the isolation and structure elucidation of two new diterpenoids, namely excoecarins L and O (**1** and **2**) along with six known compounds (**3–8**) from the MeOH extract of *E. agallocha*. To the best of our knowledge, excoecarin L (**1**) represents the first example of diterpene with 19-nor-beyerene skeleton isolated from *E. agallocha*.

2. Results and discussion

The concentrated methanol extract of *E. agallocha* was suspended in water and then successively partitioned with *n*-hexane and CH_2Cl_2. The CH_2Cl_2-soluble residue was subjected to various chromatographic separations to afford eight compounds (**1**-**8**), including two new diterpenoids (**1** and **2**) (Fig. 1). The known compounds were identified as aquillochin (**3**) (Tanaka et al., 1988), (+)-epipinoresinol (**4**) (Rahman et al., 1990), blumenol A (**5**) (Rahman et al., 1990), lup-20(29)-en-3-one (**6**) (Hisham et al., 1995), lupen-3-ol (**7**) (Khurshid Alam AHM et al., 2002), and *trans*-phytol (**8**) (Brown, 1994), by comparison of their NMR and MS spectral data with those reported in the literature.

Excoecarin L (**1**) was obtained as an amorphous white powder. Its molecular formula was determined by HR-ESI–MS as $C_{19}H_{28}O_4$ on the basis of the $[M+Na]^+$ sodiated-molecular ion peak observed at *m/z* 343.1897 (calcd. for $C_{19}H_{28}O_4Na^+$, 343.1880). The ^{13}C NMR and HSQC spectra revealed the presence of 19 carbon atoms corresponding to four quaternary carbons, six methines, eight methylenes, and one methyl. Among them, two olefinic methines (δ_C 135.20 and 135.58), four oxygenated carbons (two methylenes, one methine and one quaternary carbon resonating at δ_C 68.66, 69.44, 71.15 and 98.69, respectively) were evident. With six degrees of unsaturation established from the molecular formula, compound **1** was suggested to contain five rings and one double-bond. The ^1H NMR spectrum confirmed the presence of one *sec*-methyl group [δ_H 1.12 (3H, d, J = 7.0 Hz, H-18)], one oxymethine group [δ_H 3.75 (1H, ddd, J = 4.0, 11.0, 11.5 Hz, H-6)], two oxymethylene groups [δ_H 3.40 (1H, d, J = 11.0 Hz, H_a-17)/3.45 (1H, d, J = 11.0 Hz, H_b-17) and 3.80 (1H, d, J = 9.5 Hz, H_a-20)/3.89 (1H, dd, J = 3.5, 9.5 Hz, H_b-20)], and two olefinic protons of a disubstituted

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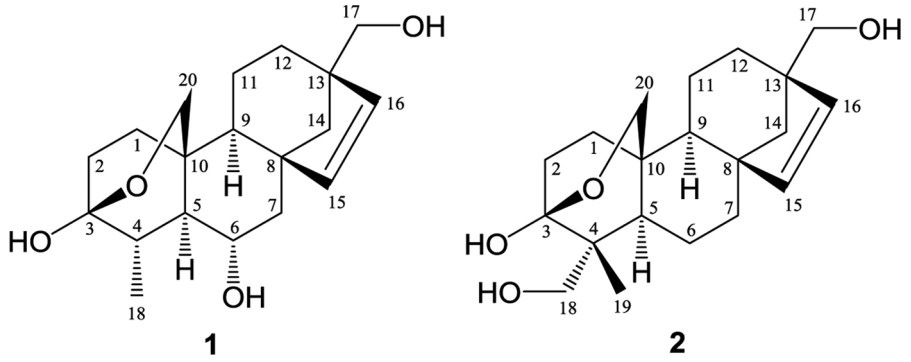

Fig. 1. Chemical structures of **1** and **2.**

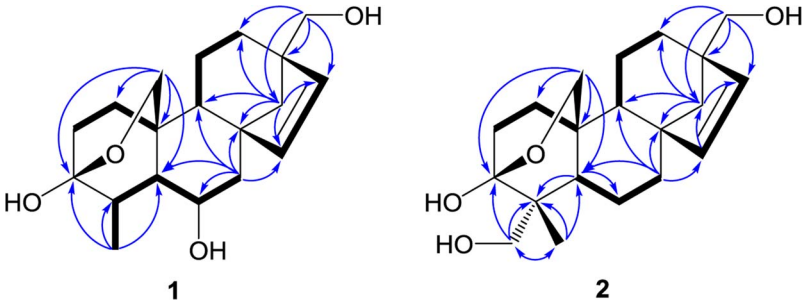

Fig. 2. COSY (⟨ ▬ ⟩) and key HMBC (⟨ ◠ ⟩) correlations of **1** and **2.**

double bond [δ_H 5.73 (1H, d, J = 6.0 Hz, H-15) and 5.66 (1H, d, J = 6.0 Hz, H-16)]. Detailed analysis of correlations provided by COSY and HMBC experiments (Fig. 2) revealed that the planar structure of **1** was similar to that of agallochin I, previously isolated from the same species (Anjaneyulu et al., 2002), except for the presence of an additional hydroxy group at C-17. In fact, the HMBC cross-peaks from H-17 to C-12, C-13, C-14, and C-16 placed the hydroxy group at C-17, whereas the other hydroxy group and the methyl group were placed at C-6 and C-4, respectively, due to the COSY correlations of H-18/H-4/H-5/H6/H-7. The downfield chemical shift of the quaternary carbon at δ_C 98.69 (C-3) in conjunction with the HMBC correlations from H-20 to C-1, C-3, C-5, and C-10 indicated that the ether bridge was positioned between C-20 and C-3, and the last hydroxy group was located at C-3.

The relative stereochemistry of **1** was obtained through analysis of ^{1}H NMR coupling constants and NOESY experiment. Specifically, the large J-values (J = 11.0 − 12.5 Hz) of H-5, H-6, H_a-7 and H-9 indicated the axial orientation of these protons. The NOE correlations between H-5/H-9, H_a-1, H_a-7; H_a-7/H_a-14, H-9; H_b-20/H-15; H-15/H-16 and H_a-20/H_a-11, H_b-1, H_a-2 confirmed the structure of beyer-15-ene diterpenoid skeleton. Finally, the configurations at C-4 and C-6 were determined on the basis of the NOE correlations between H-6/H_b-20, H-15, H-4 and between H_3-18/H_b-2 (Fig. 3). Therefore, compound **1** was elucidated as 3β,20-epoxy-3,6α,17-trihydroxy-19-nor-beyer-15-ene.

Excoecarin O (**2**) was also obtained as an amorphous white powder. Its molecular formula was established as $C_{20}H_{30}O_4$ based on HR-ESI–MS (m/z 357.2062 $[M+Na]^+$) and NMR data (see Table 1). The NMR spectroscopic features of **2** were similar to those of **1** and excoecarin D (Konishi et al., 2000). By comparison with **1**, compound **2** contains an additional methyl group and a hydroxyl group at C-4 and C-18, respectively, and lacks a hydroxy group at C-6. This assumption was confirmed by HMBC correlations (Fig. 2) from H-19 [δ_H 1.08 (3H, s)] to C-3 (δ_C 98.23), C-4 (δ_C 45.64), C-5 (δ_C 48.30), and C-18 (δ_C 70.85), and COSY correlations between H-5 [δ_H 1.35 (1H, m)]/H_b-6 [δ_H 1.69 (1H, m)] and between H_a-6 [δ_H 1.64 (1H, m)]/H_a-7 [δ_H 1.40 (1H, m)]. In the NOESY spectrum, the strong correlations between H-9/H-5, H_a-14; H-15/H_b-20, H-16; H_a-20/H_a-11, H_b-1 revealed that **2** possessed a beyer-15-ene type skeleton. Furthermore, methyl H_3-19 showed a correlation with H_b-20 whereas oxymethylene H_2-18 correlated with H-5 and H_b-2

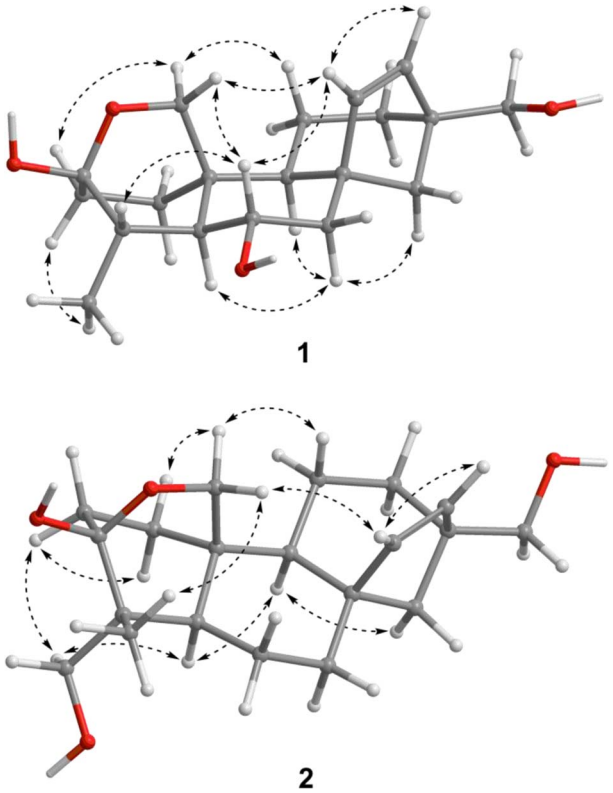

Fig. 3. Key NOESY correlations of **1** and **2.**

which established the relative configuration at C-4 (Fig. 3). Thus, compound **2** was elucidated as 3β,20-epoxy-3,17,18-trihydroxy-beyer-15-ene.

Compounds **1** and **2** were evaluated for their cytotoxic activity against two human cancer cell lines, as KB (epidermoid carcinoma) and LU-1 (lung adenocarcinoma), using the MTT colorimetric assay (Scudiero et al., 1988), but none of these compounds showed significant