A New Eudesmane Sesquiterpene from Nigrospora oryzae, an Endophytic Fungus of Aquilaria sinensis

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Abstract: A new eudesmane-type sesquiterpene, 11-hydroxycapitulatin B (1), along with a known related sesquiterpene, capitulatin B (2), was isolated from the endophytic fungus Nigrospora oryzae A8 from Aquilaria sinensis, the only plant resource for agarwood production in China. This research demonstrates that the endophytic fungi from A. sinensis might play a role in the formation of agarwood.

Keywords: Aquilaria sinensis; endophytic fungus; Nigrospora oryzae; eudesmane. © 2014 ACG Publications. All rights reserved.

1. Introduction

Agarwood (‘Chenxiang’ in Chinese) is a resinous wood formed in the heartwood of Aquilaria trees (Thymelaeaceae) in response to injury by cutting, drilling, burning, or incursion of moths, microorganisms, etc., which has been used for medicinal, religious, and ceremonial purposes for a long time [1–2]. Previous studies have revealed that the main active compounds in agarwood are sesquiterpenes and 2-(2-phenylethyl)chromone derivatives [3–5]. In order to improve the planting value of Aquilaria trees, great efforts have been made to induce healthy trees to produce these sesquiterpenes and 2-(2-phenylethyl)chromone derivatives [6–9], consequently forming agarwood. However, the production mechanism of those chemicals in agarwood is still unclear. Some fungi including endophytic ones have been demonstrated to trigger agarwood formation in A. sinensis (Lour.) Gilg [6,10–12], the only plant resource for agarwood production in China. Moreover, it is known that many endophytic fungi are capable of generating the same secondary metabolites as their host plants [13].

In order to examine if endophytic fungi from A. sinensis were capable of producing the same or similar bioactive chemicals as those in agarwood, phytochemical studies on cultures of several endophytic fungi from this plant were conducted [14–16]. A new humulane-type sesquiterpene (frabenol) was identified, which was considered to be biogenetically related to the agarwood fragrant chemicals [16]. During the ongoing investigation toward the chemical constituents of Nigrospora oryzae A8, another endophytic fungal strain from A. sinensis, a new eudesmane-type sesquiterpene,
11-hydroxycapitulatin B (1), along with a known related sesquiterpene, capitulatin B (2), was isolated and identified.

2. Materials and Methods

2.1. Microorganism Material

The endophytic fungal strain *N. oryzae* A8 was isolated from the root and the agarwood part of *A. sinensis*, which was collected at Xinyi, Guangdong province, P. R. China, in November, 2007. The strain was identified by sequence analysis of rDNA ITS (internal transcribed spacer) region. The sequence of the ITS region of A8 has been submitted to GenBank (Accession No. EU781665). By using BLAST (nucleotide sequence comparison program) to search the GenBank database, A8 has 99% similarity to *Nigrospora oryzae* (GenBank Accession No. JN211105). The strain is preserved at the Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, Guangdong Institute of Microbiology, with the ID number A8.

2.2 Fermentation and Isolation

For chemical investigations, the fungal strain was cultured on solid rice medium (100 mL of distilled water, 0.6 g of peptone, and 100 g commercially available rice were added into a 500 mL conical flask, then kept overnight prior to autoclaving) at room temperature under stationary conditions for 30 days. A total of 50 flasks of the fermented rice substrates were extracted for three times with EtOAc, and the organic solvent was evaporated under vacuum to afford the EtOAc extract. The extract was then dissolved in MeOH and extracted three times with petroleum ether, and the residue was evaporated under vacuum to afford the crude extract (66.3 g). The crude extract was submitted to a silica gel column and eluted with CHCl$_3$-MeOH gradient to yield eight fractions (Fr. 1–Fr. 8) on the basis of TLC analysis. Fr. 4 was further fractionated by a silica gel column (CH$_2$Cl$_2$-MeOH 10:1) to afford six subfractions (Fr. 4-1–Fr. 4-6). Fr. 4-5 was further subjected to reversed-phase silica gel C$_{18}$ (MeOH-H$_2$O 1:1) to afford compounds 1 (9.7 mg) and 2 (3.5 mg).

![Figure 1](image_url) Structures of compounds 1 and 2.

**11-Hydroxycapitulatin B** (1): Colorless oil. $[\alpha]_D^{20} = +6.4^\circ$ (c 0.14, MeOH); IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$: 3344, 2934, 2863, 1468, 1384, 1172, 1092, 984; positive HR-ESI-MS, $m/z$ [M + H$^+$]+ 255.2008, calcd. for [C$_{15}$H$_{27}$O$_3$] $^{+}$ 255.1960; $^1$H NMR (CD$_3$OD, 500 MHz) $\delta_H$ (ppm): 4.24 (1H, t, $J = 10.2$ Hz; H-6), 3.24 (1H, m, H-1), 1.88 (1H, m, H-9a), 1.71 (1H, m, H-9a), 1.66-1.53 (5H, m, H$_2$-2, H$_8$a, H-3b, H-7), 1.43 (1H, d, $J = 10.2$ Hz; H-5), 1.35 (3H, s, H$_3$-15), 1.28 (3H, s, H$_3$-12), 1.20 (3H, s, H$_3$-13), 1.19-1.10 (2H, m, H-8b, H-9b), 0.89 (3H, s, H$_3$-14); $^{13}$C NMR (CD$_3$OD, 125 MHz) $\delta_C$ (ppm): 79.2 (CH, C-1), 28.3 (CH$_2$, C-2), 40.7 (CH$_2$, C-3), 73.9 (C, C-4), 56.5 (CH, C-5), 72.8 (CH, C-6), 55.4 (CH, C-7), 23.1 (CH$_3$, C-8), 40.4 (CH$_3$, C-9), 40.9 (C, C-10), 75.4 (C, C-11), 23.3 (CH$_3$, C-12), 29.5 (CH$_3$, C-13), 13.9 (CH$_3$, C-14), 23.4 (CH$_3$, C-15).
3. Results and Discussion

The 1D NMR spectral data of 1 and 2 are typical of those of the eudesmane skeleton. By comparison of 1D NMR data with those reported in the literature [17], 2 was identified as capitulatin B.

Compound 1 was assigned a molecular formula of C_{15}H_{26}O_{3} based on the positive HR-ESI-MS (m/z [M + H]+ 255.2008, calcd. for [C_{15}H_{27}O_{3}]^{+} 255.1960), implying three degrees of unsaturation. The {\textsuperscript{13}}C NMR spectrum revealed the presence of 15 carbons, including four methyls, four methylenes, four methines, and three quaternary carbons. The {\textsuperscript{1}H} and {\textsuperscript{13}}C NMR spectra of 1 were very similar to those of 2. However, the two doublets for the methyls of the isopropyl group in 2 were replaced by two singlets (δ_{H} 1.20 and δ_{H} 1.28) in the 1H NMR spectrum of 1. Accordingly, a methine carbon (δ_{C} 26.2) in 2 was changed to a quaternary carbon (δ_{C} 75.4) in the 13C NMR spectrum of 1. This evidence suggested the presence of a 2-hydroxyisopropyl group in 1, which was further confirmed by the observed HMBC correlation from H-6 to C-11 (Figure 2). A triplet (1H, J = 10.2 Hz) of H-6 at δ_{H} 4.24 showed that H-5, H-6, and H-7 should be in an axial orientation, indicating the presence of a β-(2-hydroxyisopropyl) group and a 4α,6α-oxetane. Careful analyses of NOESY spectra (Figure 2) of 1 confirmed the stereochemistry of 1 as assigned. Thus the structure of 1 was established as 4α,6α-epoxy-1β,11-dihydroxy-4β-methyleudesmane, which was named 11-hydroxycapitulatin B.

There were very few reports on sesquiterpenes from the endophytic fungi of agarwood-producing plants [16,18]. This research led to the identification of a new and a known eudesmane-type sesquiterpene from the endophytic fungus N. oryzae A8 obtained from A. sinensis. The eudesmane skeleton was generally recognized as a biosynthetic precursor of eremophilanes [3–4], while 10-epi-eudesmane was regarded as a biosynthetic precursor of agarofurans, valencenes, and vetispiranes [3–4,19]. All of them were the common skeletons of sesquiterpenes from agarwood [3–4]. The current research demonstrated that the endophytic fungi from A. sinensis might play a role in the formation of agarwood. However, the relationship between endophytic fungi and agarwood formation should be further studied in detail.

Compounds 1 and 2 were assayed by the SRB method in vitro for inhibitory activity against human tumor cell lines SF-268, MCF-7, and NCI-H460, but both of them were shown to be inactive (IC_{50} >100 µg/mL).

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