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A New Sesquiterpene from the Endophytic Fungus *Nigrospora sphaerica* #003

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Abstract: A new drimane sesquiterpene, 3*β*-OH-dendocarbin A (**1**), together with six known sesquiterpenes (**2–7**), were isolated from endophytic fungus *Nigrospora sphaerica* #003. The structure of **1** was elucidated by MS, 1D and 2D-NMR data analyses, and comparison data with those of known compounds. Compounds **1–7** were tested the cytotoxicity against five tumor cell lines (HL-60, A-549, SMMC-7721, MCF-7 and SW-480). However, none of them showed obvious activities, with $IC_{50} > 40$ μM.

Keywords: Endophytic fungus; *Nigrospora sphaerica*; sesquiterpene.

1. Introduction

Endophytic fungi are eukaryotic organisms that colonize and cause asymptomatic infections in healthy plant tissues for their entire life cycle or for, at least, a significant part of the latter [1]. They play important roles in the process of host plant growth and systematic evolution. Studies have shown that the endophytes produce bioactive substances to enhance the growth and competitiveness of the host in its natural habitat [2–3]. Thus, entophytes have been identified as an excellent source of bioactive natural products [4–5]. In our previous search for biologically active natural products of endophytic fungi, some bioactive and/or new compounds were isolated [6–7]. As a continuation of our studies, chemical investigation on fermentation broths of *Nigrospora sphaerica* #003 led to isolate a new drimane sesquiterpene, 3*β*-OH-dendocarbin A (**1**), together with six known sesquiterpenes (**2-7**) (figure 1). Compounds **2-7** were found for the first time from this species. The structures of the isolated metabolites were determined by extensive analysis of their spectroscopic data as well as by comparison with literature reports. Herein, we report the isolation and structure elucidation of these sesquiterpenes.

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2. Materials and Methods

*2.1. F*ungal material

The fungus *Nigrospora sphaerica* #003 was isolated from fresh leaves of *Eupatorium Adenophorum* collected in Kunming Institute of Botany, Kunming, Yunnan province, PR China in July 2012. The fungus was identified by observing the morphological characteristics and analysis of the internal transcribed spacer (ITS) regions (GenBank accession number: KT229615). The result from the BLAST search indicated that the sequence was the same (99%) to the sequence of *Nigrospora sphaerica* (compared to JN198501.1). The strain is preserved at the Kunming Institute of Botany, Chinese Academy of Sciences (No. F2012031).

2.2. Fermentation, Extraction and Isolation

This fungal strain was cultured on potato dextrose agar (PDA) medium at 25 ℃ for 5 days. The agar plugs were inoculated 500 mL Erlenmeyer flasks, each containing 200 mL of potato dextrose media. Flask cultures were incubated at 28 ℃ on a rotary shaker at 160 rpm for two days as seed culture. Sixty 500 mL Erlenmeyer flasks each containing 200 mL of potato dextrose broth (PDB) were individually inoculated with 10 mL of seed culture, and were incubated at 25 ℃ on a rotary shaker at 160 rpm for 15 days.

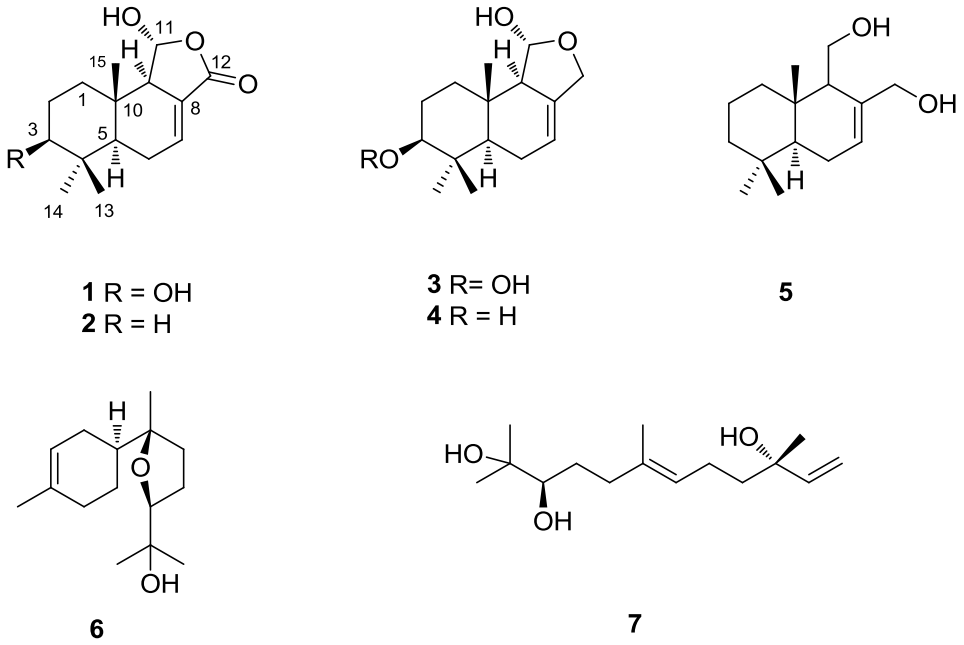

Figure 1. Structures of compounds **1**-**7**

The filtrate (12 L) of the fermented culture broth was extracted three times with EtOAc (12 L × 3) at room temperature, and the organic solvent was evaporated to dryness under reduced pressure to afford a brown crude extract (9.2 g), which was then fractionated by silica gel column chromatography (CC) eluted with a gradient of $CHCl_3$−MeOH (*v/v*, 100:0, 97.5:2.5, 95:5, 9:1, 8:2 and 1:1) to give six fractions. Fraction 2 was eluted with $CHCl_3$/MeOH (97.5:2.5), which was then purified by repeat silica gel CC and Sephadex LH-20 CC eluting with acetone to obtain **6** (4.1 mg). Fraction 3 was eluted with $CHCl_3$/MeOH (95:5). It was then separated by Sephadex LH-20 CC eluting with acetone to give four subfractions (3A−3D). Fraction 3B was then separated by preparative HPLC (MeCN/H_2O, from 30:70 to 60:40, *v/v*, 10 ml/min in 40 min) to give **2** (5.2 mg), **4** (11.0 mg) and **5** (2.0 mg). Fraction 3C was further purified by silica gel CC (petroleum ether–EtoAc, 1:1) to yield **1** (3.3 mg) and **3** (2.4 mg).

Fraction 4 eluted with $CHCl_3$/MeOH (9:1), was then purified into five subfractions (4A−4E) by MPLC using MeOH/H_2O as eluent. Fraction 4B was further purified by silica gel CC ($CHCl_3$/Me_2CO, from 20:1 to 3:1) to yield **7** (6.2mg)

3β-OH-dendocarbin A (**1**). White powder; $[\alpha]_D^{25}$ = – 42.91 (*c* 0.32, MeOH); UV (MeOH) λ_{max} (nm) (log ε): 207 (3.71); IR (KBr) ν_{max} (cm^{-1}): 3425, 2927, 1741, 1688, 1635, 1457, 1201, 1156. ESI-MS (−) *m/z* 265 $[M-H]^-$. HR-ESI-MS (–) *m/z* 266.1503 (calcd for $C_{15}H_{22}O_4$, 266.1518). ^{1}H NMR (Acetone-d_6, 600 MHz): 1.46 (1H, dt, *J* = 13.2, 3.8 Hz, H-1α), 1.83 (1H, d, *J* = 13.5 Hz, H-1β), 1.64 (2H, m, H-2), 3.26 (1H, m, H-3), 1.38 (1H, dd, *J* = 6.4, 4.0 Hz, H-5), 2.42 (1H, m, H-6α), 2.22(1H, m, H-6β), 6.73 (1H, s, H-7), 2.47 (1H, br s, H-9), 5.68 (1H, s, H-11), 0.90 (3H, s, H-13), 1.02 (3H, s, H-14), 0.86 (3H, s, H-15), 3.70 (1H, d, *J* = 4.8 Hz, 3-OH), 6.79 (1H, s, 11-OH).^{13}C NMR (Acetone-d_6, 150 MHz): 37.9 (C-1), 27.9 (C-2), 78.3 (C-3), 39.4 (C-4), 49.6 (C-5), 25.2 (C-6), 135.5 (C-7), 129.7 (C-8), 59.7 (C-9), 34.4 (C-10), 99.5 (C-11), 167.6 (C-12), 15.4 (C-13), 28.3 (C-14), 14.7 (C-15).

3. Results and Discussion

3.1. Structure elucidation

Compound **1** was obtained as a white powder. Its molecular formula was determined to be $C_{15}H_{22}O_4$ by HR-EI-MS at *m/z* 266.1503 (calcd for $C_{15}H_{22}O_4Na$, 266.1518), indicating five double-bond equivalents (DBEs). The IR absorptions at 3425, 1741, and 1635 cm^{-1} indicated the presence of hydroxyl, carbonyl and double bond groups. The ^{1}H NMR spectrum of compound **1** displayed three singlets for methyls at δ_H 0.86, 0.90, and 1.02 , two O-bearing methines at δ_H 5.68 (1H, s) and 3.26 (1H, m), and an olefinic proton at δ_H 6.73 (1H, s). The ^{13}C and DEPT spectra revealed 15 carbon signals, including three methyls, three methylenes, five methines (with one oxymethine and a hemiacetal methine) and four quarternary carbons (with a ester carbonyl). The above mentioned analysis accounted for 2 out of 5 degrees of unsaturation, indicating compound **1** should possess a tricyclic system.

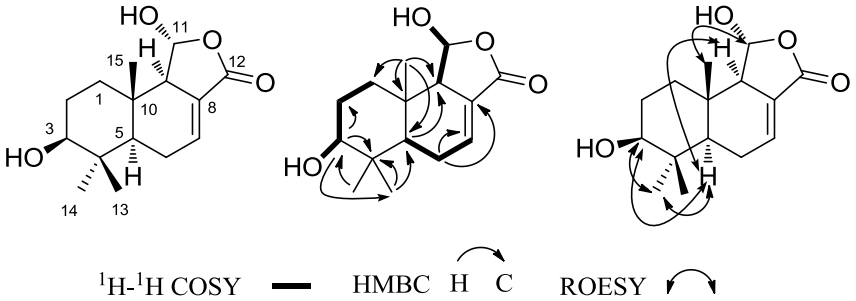

Figure 2. Key 2D NMR correlations of **1**

The 1D NMR spectra of **1** was resembled to the known sesquiterpene, dendocarbin A (**2**) [8], which was isolated from a japanese nudibranch *Dendrodoris carbunculosa*, except that the ^{13}C NMR signal of **2** at 44.1 ppm (C-3) was downshifted to 78.3 ppm in **1**, suggesting the presence of an O-bearing methine in **1** instead of a methylene in **2.** This assumption was further corroborated by the key HMBC correlation from H-3 to C-2, C-4 and C-13, and the crosspeak of 3-OH/H-3 in the COSY spectrum (figure 2). The relative structure of **1** was established by the ROESY experiment. In the ROESY spectrum, Me-15 showed a correlation with H-11, indicating Me-15 and H-11 in the same side, and they were arbitrarily assigned the β-orientation, while ROE correlation of H-5/H-3, H-9 and Me-14 revealed H-5, H-3, H-9 and Me-14 were in the opposite orientation (α). Thus, the structure of **1** was established and named 3β-OH-dendocarbin A.

The other known isolates, dendocarbin A (**2**) [8], danilol (**3**) [9], isodrimenilol (**4**) [8], 11,12-trihydrooxydrimene (**5**) [10], bisaboiol oxide B (**6**) [11], and (3R,6E,10S)-2,6,10-trimethyl-3-hydroxydodeca-6,11-diene-2,10-diol (**7**) [12] were readily identified by comparison of their spectral data (NMR and MS) with data from the literature.

3.2 Cytotoxicity activity

Five human cancer cell lines: breast cancer SK-BR-3, hepatocellular carcinoma SMMC-7721, human myeloid leukemia HL-60, pancreatic cancer PANC-1, and lung cancer A-549 cells were cultured in Dulbecco's modified Eagle's media (DMEM) supplemented with 10% fetal bovine serum and 2 mM L-glutamine. The cells were maintained at 37 ^{o}C in a humidified atmosphere at 95% air and 5% CO_2. Cell viability was measured by MTT assay [13]. However, No compounds showed significant activity, with IC_{50} values > 40 μM.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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