Xylariphilone: a new azaphilone derivative from the seagrass-derived fungus Xylariales sp. PSU-ES163

Jiraporn Arunpanichlert, Vatcharin Rukachaisirikul, Souwalak Phongpaichit, Orathai Supaphon and Jariya Sakayaroj

Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand; Natural Products Research Center of Excellence and Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand; National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand Science Park, Klong Luang, Pathumthani 12120, Thailand

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One new azaphilone derivative, named xylariphilone (1), along with 10 known compounds was isolated from the seagrass-derived fungus Xylariales sp. PSU-ES163. Their structures were elucidated on the basis of extensive spectroscopic analysis. The absolute and relative configurations of 1 were determined by circular dichroism spectroscopy and NOEDIFF data. The antimicrobial and cytotoxic activities of the isolated compounds were evaluated.

Keywords: Xylariales sp; seagrass-derived fungus; azaphilone derivative; antimicrobial activity; cytotoxic activity

1. Introduction

The order Xylariales has produced a wide range of bioactive compounds and novel secondary metabolites, including cytotoxic eutypellin A (Isaka et al. 2009), antibacterial multiformins A–D (Quang et al. 2005), antifungal sordaricin (Pongcharoen et al. 2008) and antimycobacterial 4-quinolinecarboxaldehyde oxime (Pongcharoen et al. 2007). During our ongoing search for bioactive substances from seagrass-derived fungi, we found that the broth ethyl acetate extract of the fungus Xylariales sp. PSU-ES163 exhibited cytotoxic activity against oral human carcinoma (KB) and human breast cancer (MCF-7) cell lines with IC₅₀ values of 20.25 and 46.67 μg/mL, respectively. According to the biological activities as well as previous chemical investigations of the order Xylariales, the broth and mycelial extracts from the fungus Xylariales sp. PSU-ES163 were subjected to chemical investigation. Herein, we report the isolation of one new azaphilone derivative, xylariphilone (1), together with 10 compounds, xylarenone (2) (Rukachaisirikul et al. 2015).
2007), isosclerone (3) (Rukachaisirikul et al. 2007), (3R,4R)-3,4-dihydro-3,4,8-trihydroxy-1(2H)-naphthalenone (4) (Iwasaki et al. 1972), (3R,4R)-3,4-dihydro-3,4,6,8-tetrahydroxy-1(2H)-naphthalenone (5) (Iwasaki et al. 1973), (−)-6-hydroxymellein (6) (Rukachaisirikul et al. 2007), de-O-methyldiaporthin (7) (Hallock et al. 1988), (3S,4R)-3-hydroxy-4-hydroxymethyl-4-butanolide (8) (Francisco et al. 2003; Miranda et al. 2004), (R)-6-methyl-5,6-dihydropyran-2-one (9) (Wolberg et al. 2001), (4R,6R)-4-hydroxy-6-methyltetrahydropyran-2-one (10) (Buchanan et al. 1996; Rukachaisirikul et al. 2007) and 8-methoxy-1-naphthol (11) (Rukachaisirikul et al. 2007). The antimicrobial and cytotoxic activities of the isolated compounds were examined.

2. Results and discussion
The broth and mycelial extracts of the fungus PSU-ES163 were purified using chromatographic techniques leading to the isolation of 1 new (1) and 10 known (2–11) compounds. All metabolites (Figure 1) were elucidated by analysis of spectroscopic data, including UV, IR, NMR and MS. The absolute and relative configurations of 1 were determined using the circular dichroism (CD) spectroscopy and selective NOEDIFF data.

Xylariphilone (1) was obtained as a colourless gum with [α]D24 = 25.1 (c 0.50, CHCl3). The molecular formula was deduced to be C11H16O4 on the basis of HREIMS with four degrees of unsaturation. The UV spectrum showed a maximum absorption band at 241 nm while the IR spectrum displayed absorption bands at 3394 and 1671 cm⁻¹ for hydroxy and conjugated carbonyl groups, respectively. The ¹H NMR spectroscopic data contained signals for two sets of nonequivalent methylene protons [δH 4.63 (d, J = 16.0 Hz, 1H) and 4.14 (br d, J = 16.0 Hz, 1H); 2.61 (ddd, J = 18.5, 6.0 and 0.5 Hz, 1H) and 2.36 (brdd, J = 18.5 and 10.5 Hz, 1H)], one oxymethine proton (δH 4.00, dd, J = 10.5 and 6.0 Hz), a 1-substituted 2-oxypropyl group [δH 3.64 (dd, J = 12.5 and 6.5 Hz, 1H), 2.19 (m, 1H), 2.17 (m, 1H) and 1.29 (d, J = 6.5 Hz, 3H)], two hydroxy protons (δH 3.66, br s and 2.47, s) and one methyl group (δH 1.26, s). The ¹³C NMR and DEPT spectra of 1 displayed 11 signals which were assigned to one ketone carbonyl (δC199.1), three quaternary (δC 153.2, 128.5 and 77.1), two oxymethine (δC 72.6 and 69.4), three methylene (δC 63.6, 38.2 and 36.1) and two methyl (δC 21.0 and 17.8) carbons. In the ¹H–¹H COSY spectrum, the nonequivalent methylene protons, H₉-H₁₀ (δH 2.61 and 2.36), were coupled with H-6 (δH 4.00), whereas the HMBC spectrum displayed the correlations of H₅-H₁₀ (δH 2.61)/C₄a (δC 153.2), C-6 (δC 72.6), C-7 (δC 77.1) and C-8a (δC 128.5) and those of H₅-10 (δH 1.26)/C-

Figure 1. Structures of compounds 1–11 from Xylariales sp. PSU-ES163.
6, C-7 and C-8 (δC 199.1). These data established a cyclohexenone with a methyl group at C-7 and a double bond at C-4a and C-8a. The chemical shifts of C-6 and C-7 as well as the HMBC correlations from 6-OH (δH 2.47) to C-5 (δC 36.1) and 7-OH (δH 3.66) to C-6, C-7 and C-8 indicated the attachment of the hydroxy groups at these two carbons. The oxymethine proton, H-3 (δH 3.64), showed a HMBC cross peak with C-1 (δC 63.63), which correlated in the HMQC spectrum with the nonequivalent oxymethylene protons (H-ab-1, δH 4.63 and 4.14). Accordingly, an ether unit (–CH2CH(CH3)OCH2–) was established. The linkage between C-1 and C-8a and that between C-4 (δC 38.2) and C-4a were indicated from the HMBC correlations of H-ab-1 with C-4a and C-8a and those of H-ab-4 (δH 2.19 and 2.17) with C-4a, C-5 and C-8a, thus indicating a tetrahydroisochromenone. The relative configuration of 1 was assigned from the coupling constants and NOEDIFF results (see Supplementary material). The coupling constant of 10.5 Hz between H-b-5 (δH 2.36) and H-6 indicated their location at pseudoaxial positions. Irradiation of H-6 enhanced the signal intensity of H-a-5, but did not affect that of H3-10, thus indicating that H-6 was cis to H-a-5 but trans to H3-10. According to the coupling constants and 12.5 and 6.5 Hz of H-3, H-3 was located at a pseudoaxial position. Irradiation of H-b-1 (δH 4.14) affected signal intensity of H-3 while irradiation of H-3 and H-a-5 enhanced the signal intensity of H-a-4 (δH 2.19). Consequently, H-3 had a cis relationship to H-6. The CD spectrum of 1 showed a positive first cotton effect (217 nm, Δε = +7.88) and a negative second cotton effect (234 nm, Δε = −2.61), the same sign as those of (+)-(R)-2-acetyl-3,6-dihydroxycyclohex-2-enone, CD (c = 1.00 × 10−3 mol/L, MeOH): λmax: 216 nm (Δε = +1.1) and 232 nm (Δε = −0.3) (Zaitsev & Mikhal’chuk 2001), indicating the absolute configuration at C-7 to be R. Therefore, the absolute configurations at C-3 and C-6 were assigned as R and S, respectively. It is worth to note that the absolute configuration at C-3 of 1 and the metabolites 6, 9 and 10 have identical R configuration. Consequently, xylariphilone (1) was identified as a new azaphilone derivative.

The isolated compounds 3–7 and 10 were evaluated for antimicrobial activity against *Staphylococcus aureus* ATCC25923, methicillin-resistant *S. aureus* SK1 (a clinical isolate), *Candida albicans* NCPF3153, *Cryptococcus neoformans* ATCC90113 and *Microsporum gypseum*. The remaining compounds were not tested due to their small quantities. None of them showed antimicrobial activity against the tested pathogenic bacteria and fungi at the concentration of 200 μg/mL. In addition, compounds 7 and 10 were tested for cytotoxic activity against KB, MCF-7 and noncancerous Vero (African green monkey kidney fibroblast) cells. Both were inactive against these cell lines.

3. Experimental

3.1. General experimental procedures

The IR spectra were recorded on a Perkin-Elmer 783 FTS 165 FT-IR spectrometer. Optical rotations were measured on a JASCO P-1020 polarimeter. Ultraviolet (UV) spectra were recorded on a Shimadzu UV-160A spectrophotometer. CD spectrum was recorded on a JASCO model J-810 polarimeter. EIMS mass spectrum of 1 was acquired using a MAT 95 XL mass spectrometer (Thermofinnigan). 1H and 13C NMR spectra were recorded on a 300 or 500 MHz Bruker FTNMR Ultra Shield spectrometer. Thin layer chromatography (TLC) and precoated TLC (PTLC) were performed on silica gel GF254 (Merck). Column chromatography (CC) was carried out on silica gel (Merck) type 60 (230–400 mesh ASTM), Sephadex LH-20 or on reverse phase silica gel C-18.

3.2. Fungal material

The endophytic fungus PSU-ES163 (BCC47786) was isolated from the leaves of the seagrass *Halophila ovalis* collected from Trang Province, Thailand. This isolate was identified based on
the nuclear ribosomal internal transcribed spacer (ITS) regions, the large (28S) subunit ribosomal RNA (LSU) and the small (18S) subunit ribosomal RNA (SSU). The sequences of this isolate were found to belong to Order Xylariales. The ITS sequence (accession number JN116682) showed the highest similarity with EP448415 Annulohypoxylon atroroseum (86.2%). The LSU sequence (accession number JQ419763) was closely related to Annulohypoxylon atroroseum (DQ840060) with 96.6% nucleotide identity. However, the SSU sequence (accession number JQ419764) gave the highest similarity (98.5%) with Xylaria acuta. Thus, this isolate could be identified to an order level as Xylariales sp.

3.3. Fermentation, extraction and isolation

The broth and mycelial ethyl acetate extracts of the fungus PSU-ES163 were prepared using the same procedure as described previously (Rukachaisirikul et al. 2007). For broth BuOH extract, the aqueous residue obtained after extraction of the filtrate from broth culture with ethyl acetate was divided into five portions. Each portion was extracted twice with an equal volume of BuOH (2 × 500 mL). The BuOH layer was then dried over anhydrous Na₂SO₄ and evaporated to dryness to obtain a dark brown gum (1.25 g). The broth EtOAc extract (829.5 mg) was subjected to CC over Sephadex LH-20 with 100% MeOH to afford six fractions (A–F). Fraction B (145.0 mg) was purified by CC over silica gel using a gradient of MeOH/CH₂Cl₂ to yield four subfractions (B1–B4). Subfraction B2 (7.7 mg) was purified by PTLC using EtOAc/CH₂Cl₂ (2:3) (3 runs) to yield 1 (1.4 mg). Fraction D (51.0 mg) was purified by CC over silica gel using a gradient of acetone/CH₂Cl₂ to yield five subfractions. The second subfraction (3.6 mg) was separated by PTLC with acetone/CH₂Cl₂ (1:99) (5 runs) to afford 3 (2.8 mg) while the fourth subfraction contained 4 (2.5 mg). Fraction E (55.7 mg) was subjected to CC over reverse phase silica gel using a gradient of MeOH/H₂O followed by CC over silica gel using a gradient of MeOH/CH₂Cl₂ and CC over Sephadex LH-20 with 100% MeOH/CH₂Cl₂ and CC over Sephadex LH-20 with MeOH/CH₂Cl₂ (1:1) to give 8 (1.2 mg). The mycelial EtOAc extract (1.70 g) was separated by CC over sephadex LH-20 with 100% MeOH to afford four fractions. The second fraction (990.5 mg) was fractionated by CC over silica gel using a gradient of MeOH/CH₂Cl₂ to obtain 9 (1.4 mg) and 10 (9.0 mg). Compound 11 (1.1 mg) was obtained from the third fraction (97.2 mg) after purification by CC over silica gel using a gradient of MeOH/CH₂Cl₂ and PTLC using CH₂Cl₂/hexane (1:1) (2 runs).

3.3.1. Xylariphilone (1)

Colourless gum [α]D24 25.1 (c 0.50, CHCl₃). UV (MeOH) λmax (log ε): 241 (3.86) nm. IR (neat) νmax: 3394, 1671 cm⁻¹. CD (MeOH) λmax (Δε): 217 (+7.88), 234 (−2.61), 251 (+3.93), 312 (−0.88) nm. HREIMS m/z: 212.1052 [M]+ (calcd for C₁₁H₁₆O₄, 212.1043). ¹H NMR (CDCl₃, 500 MHz): δH 4.63 (1H, d, J = 16.0 Hz, H₅-1), 4.14 (1H, br d, J = 16.0 Hz, H₅-1), 4.00 (1H, dd, J = 10.5, 6.0 Hz, H-6), 3.66 (1H, br s, 7-OH), 3.64 (1H, dd, J = 12.5, 6.5 Hz, H-3), 2.61 (1H, ddd, J = 18.5, 6.0 Hz, H-5), 2.47 (1H, s, 6-OH), 2.36 (1H, br dd, J = 18.5, 10.5 Hz, H₅-5), 2.19 (1H, m, H₆-4), 2.17 (1H, m, H₆-4), 1.29 (3H, d, J = 6.5 Hz, H-9), 1.26 (3H, s, H-10). ¹³C NMR (CDCl₃, 125 MHz): δC 199.1 (s, C-8), 153.2 (s, C-4a), 128.5 (s, C-8a), 77.1 (s, C-7), 72.6 (d, C-6), 69.4 (d, C-3), 63.6 (t, C-1), 38.2 (t, C-4), 36.1 (t, C-5), 21.0 (q, C-9), 17.8 (q, C-10).
3.4. Antimicrobial assay
Antimicrobial activity was determined as described by the Clinical and Laboratory Standards Institute (Drummond & Waigh 2000; Clinical and Laboratory Standards Institute (CLSI) 2002a, 2002b, 2002c). Vancomycin, amphotericin B and miconazole were used as positive controls for bacteria, yeasts and fungus with the MIC values of 1, 0.25 and 1 μg/mL, respectively.

3.5. Cytotoxicity assay
The activity assay against African green monkey kidney fibroblast (Vero) cells was performed in triplicate employing the method described by Hunt and co-workers (Hunt et al. 1999). The activities against KB and MCF-7 cell lines were evaluated using the resazurin microplate assay (O’Brien et al. 2000).

4. Conclusion
One new azaphilone derivative (1), along with seven known compounds, four naphthalenone (2–5), two isocoumarin (6–7) and one γ-lactone (8) derivatives, was obtained from the broth extract of Xylariales sp. PSU-ES163. Whereas three known compounds, two δ-lactone (9–10) and one naphthalene (11) derivatives, were isolated from the mycelial extract. Compound 1 was structurally related to pestafolide A (Ding et al. 2008), peneciraistin C (Ma et al. 2012), monascusone A and FK17-P2b2 (Jongrungruangchok et al. 2004) which have a tetrahydro-1Η-isochromen-8(5Η)-one core structure with the methyl group at C-7 and hydroxy groups at C-6 and C-7, identical to those in 1. The absolute and relative configurations of 1 were assigned by circular dichroism spectroscopy and NOEDIFF data which indicated that C-6 and C-7 in the tetrahydroisochromenone unit have the S and R configurations, respectively, identical to those of the above structurally related compounds (Jongrungruangchok et al. 2004; Ding et al. 2008; Ma et al. 2012).

Supplementary material
The 1H and 13C NMR spectra as well as the selected NOEDIFF correlations of 1 are available online.

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Disclosure statement
No potential conflict of interest was reported by the authors.

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