

Penioctadecatrienoic A: A New Polyketide from Endophytic Fungus *Penicillium pinophilum* J70

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Abstract: Chemical examination of the rice solid fermented cultures of the fungus *Penicillium pinophilum* J70 isolated from the fresh leaves of *Hypericum japonicum* Thumb led to the isolation of one new polyketide, namely penioctadecatrienoic A (**1**), together with two known compounds (**2** and **3**). The structure of the new metabolite **1** was resolved on the basis of extensive spectroscopic analysis (NMR and HRESIMS data), in association with the modified Mosher's method for the absolute configurational assignment. All isolated metabolites were evaluated for their cytotoxic activity against ECA-109, the anti-inflammatory activity against NO production in lipopolysaccharides (LPS) stimulated RAW264.7 cells, and the antibacterial activity on *Staphylococcus aureus* ATCC 29213. As a result, compounds **1–3** showed moderately anti-inflammatory effects with the inhibitory rates ranging from 44.5% to 60.7% at the concentration of 20 μ M. In addition, compound **1** exhibited weakly antibacterial effect with an MIC value of 32 μ g/mL.

Keywords: Endophytic fungus; *Penicillium pinophilum*; polyketides; cytotoxic activities; anti-inflammatory activities. © 2023 ACG Publications. All rights reserved.

1. Fungal Source

The fungal strain J70 was isolated from the fresh leaves of *Hypericum japonicum* Thumb, which was sampled in June 2021 in Quanzhou, Fujian Province, China. The fungus was identified to be *Penicillium pinophilum* based on analyses of its internal transcribed spacer (ITS) gene sequences in association with microscopic observation. The ITS sequences was deposited in GenBank and was assigned the accession number OQ733174. The fungus was preserved in the Technology Innovation Center for Exploitation of Marine Biological Resources, TIO, MNR, Xiamen, China, and was assigned the registration number J70.

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2. Previous Studies

Endophytic fungi, which live internally in healthy hosts, have attracted considerable attentions because of their chemical and ecological potential. The complex relationships with host enables endophytic fungi to produce biologically active secondary metabolites with a wide array of structural diversities. Some of the metabolites are important source of pharmaceuticals [1-5]. The endophytic fungal strain *Penicillium pinophilum* have produced a number of biologically active secondary metabolites with various structural skeletons. For example, 5'-hydroxypenicillide, a new phenolic metabolite, was isolated from mangrove-associated fungus *P. pinophilum* (H608) [6]. Pinophilins D–F, three novel azaphilone derivatives, were obtained from gorgonian-derived fungus *P. pinophilum* XS-20090E18 [7]. In addition, 3-*O*-methylfunicone [8], (±)-asperlone A and (±)-asperlone B [9], pinodiketopiperazine A and 6,7-dihydroxy-3-methoxy-3-methylphthalide [10], dinapinones A1 and A2 [11], pinophilins A and B [12], dinapinone A [13], and 3-*O*-methyl-5, 6-epoxyfunicone [14] were also identified from *P. pinophilum* of endophytic origin. In our continuous study of secondary metabolites of fungi, the fungus *P. pinophilum* J70 attracted our attention due to its abundant secondary metabolites. Subsequently, chemical examination of the ethyl acetate (EtOAc) extract of the rice solid fermented cultures resulted in the isolation of three compounds (**1–3**), including one new metabolite **1** (Figure 1). Herein, the isolation, structure elucidations, and the biological activity of the compounds were reported.

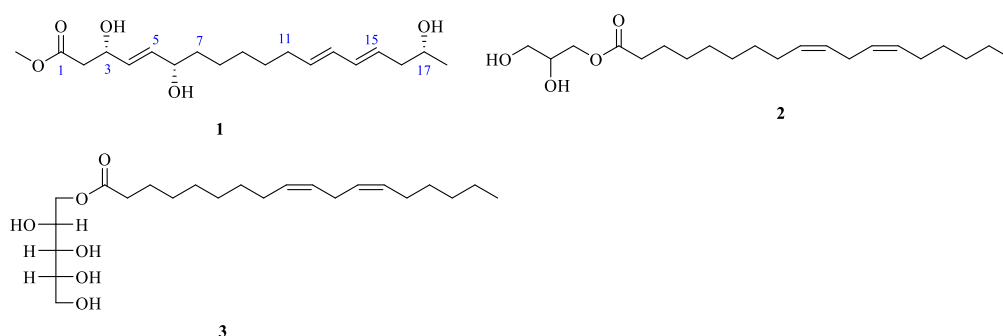


Figure 1. Structures of compounds **1–3** from *P. pinophilum* J70

3. Present Study

The producing fungus was inoculated in PDB medium for 4 days on a rotary shaker (180 rpm, 25 °C) to prepare seed cultures. Then, the cultures were inoculated in rice medium with 30 Fernbach flasks (1L), each containing 140 g of rice, 180 mg of peptone, 5.9 g of NaCl, and 180 mL of water, after autoclaving at 15 psi for 30 min. The inoculated rice medium was incubated in static conditions at 25 °C for 30 days. Then, the fermented materials were extracted with EtOAc for three times to get organic extract (35.0 g). The extract was subjected to silica gel column chromatography (CC) using CH₂Cl₂/MeOH gradient elution from 50:1 to 0:1 to obtain five fractions (Fr. A– Fr. E). Fraction Fr. B (1.90 g) was separated on the basis of CC on silica gel eluting with CH₂Cl₂/MeOH (7:1), and then further purified by silica gel CC (petroleum ether/EtOAc, 5:1) as well as by semipreparative HPLC with the mobile phase of MeCN/H₂O (22:3) to furnish compound **2** (6.5 mg). Fraction Fr. C (1.90 g) was chromatographed on the basis of the CC over ODS eluting with MeOH/H₂O gradient elution (3:17 to 1:0) to get a subfraction, which was further separated by silica gel CC with CH₂Cl₂/MeOH (50:1) elution, and then purified on the basis of semipreparative HPLC (MeCN/H₂O, 71:29) to obtain compound **3** (3.1 mg). Fraction Fr. E (3.09 g) was purified separately by the silica gel CC eluting with CH₂Cl₂/MeOH (5:1) and then with petroleum ether/EtOAc (1:1 to 0:1) elution, in association with semipreparative HPLC (MeCN/H₂O, 9:11) to furnish compound **1** (6.2 mg).

Compound 1: Colorless oil; $[\alpha]_D^{24} +10$ (c 0.7, MeOH); UV (MeOH) λ_{\max} (log ϵ) 246 (0.34) nm; ¹H and ¹³C NMR data, Table 1; HRESIMS m/z 361.2138 [M + Na]⁺ (calcd. for C₁₉H₃₂O₅Na⁺, 361.2142).

Compound **1** was isolated as a colorless oil. The molecular formula was assigned to be $C_{19}H_{32}O_5$ on the basis of a sodium adduct ion peak at m/z 363.2138 $[M + Na]^+$ (calcd for $C_{19}H_{32}O_5Na^+$, 363.2147) in the HRESIMS spectrum, revealing four degrees of unsaturation. Its 1H NMR spectrum exhibited signals for one methyl (δ_H 1.16), one methoxy (δ_H 3.69), six olefinic protons (δ_H 5.58, 5.59, 5.68, 5.71, 6.02, and 6.04) for three double bonds, three oxygenated methines (δ_H 3.76, 4.03, and 4.52), and a series of aliphatic methylenes (Table 1). The ^{13}C NMR spectrum in association with the HSQC data demonstrated the presence of 19 carbons, classifying one ester carbonyl carbon (δ_C 173.2), six olefinic carbons (δ_C 128.9, 131.8, 132.9, 133.7, 134.1, and 135.3) for three double bonds, three oxymethines (δ_C 68.6, 69.7, and 72.8), seven methylenes (δ_C 26.4, 30.2, 30.6, 33.5, 38.3, 43.3, and 43.4), one methyl (δ_C 22.9), and a methoxyl carbon (δ_C 52.1) (Table 1). The three double bonds and one carbonyl carbon accounted for all four indices of hydrogen deficiency, revealing **1** to be an acyclic molecule. The 1H and ^{13}C NMR data mentioned above are similar to those of methyl stearate [15], revealing **1** to be a structurally related derivative. The obvious differences were owing to the presence of three additional oxymethines and six olefinic carbons in **1** instead of nine methylenes in methyl stearate, revealing that **1** was an oxygenated derivative of methyl stearate. The structure of **1** was further established by detailed analyses of the 2D NMR spectra. The 1H - 1H COSY cross-peaks of H₂-2 (δ_H 2.53)/H-3 (δ_H 4.52)/H-4 (δ_H 5.68)/H-5 (δ_H 5.71)/H-6 (δ_H 4.03), and of H₃-18 (δ_H 1.16)/H-17 (δ_H 3.76)/H₂-16 (δ_H 2.23, 2.17)/H-15 (δ_H 5.58)/H-14 (δ_H 6.04)/H-13 (δ_H 6.02)/H-12 (δ_H 5.59), in association with the HMBC correlations from methoxyl proton (δ_H 3.69) to ester carbonyl carbon C-1 (δ_C 173.2), from H₂-2 to C-1, as well as from H₃-18 to C-16 (δ_C 43.4) and C-17 (δ_C 68.6) confirmed a methoxy residing at C-1, three hydroxys at C-3, C-6, and C-17, and three double bonds at C-4, C-12, and C-14, respectively, (Figure 2). To see the spectra of the compound please see supporting information of the article.

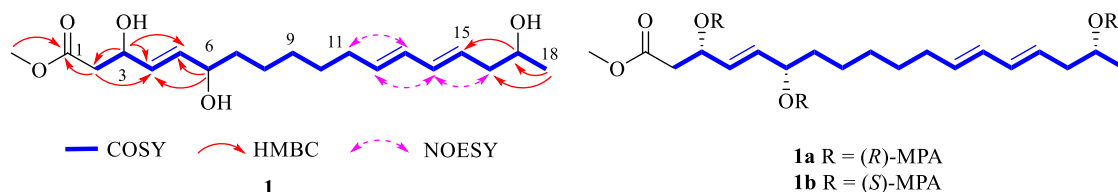


Figure 2. Selected 2D NMR correlations of **1**, **1a**, and **1b**

The geometries of the double bonds Δ^4 , Δ^{12} , and Δ^{14} were determined on the basis of coupling constants and the NOESY experiment. The coupling constant of H-4 and H-5 ($J_{H-4/H-5} = 15.5$ Hz) revealed the *E* configuration of Δ^4 . The NOESY correlations from H₂-11 (δ_H 2.08) to H-13, from H-12 to H-14, and from H₂-16 to H-14 demonstrated that both Δ^{12} and Δ^{14} had *E* configurations. In addition, the absolute configurations of C-3, C-6, and C-17 were determined to be *S*, *S*, and *R*, respectively, by the modified Mosher's method. The *tri*-(*R*)-MPA and *tri*-(*S*)-MPA esters (**1a** and **1b**) of **1** were synthesized. Analyses of the $\Delta\delta_H$ values ($\Delta\delta_H = \delta_R - \delta_S$) at H-3 (+0.1), H-4 (+0.59), H-5 (+0.47), and H-6 (+0.08) assigned the *S* configurations for C-3 and C-6 based on the acyclic 1,4-diol rules [16], while the C-17 was determined as *R* according to the $\Delta\delta_H$ values of H₃-18, H₂-16, H-12–H-15, and H₂-11 (Figure 3) [17]. Therefore, the structure of **1** was established as depicted and was given the trivial name penioctadecatrienoic A.

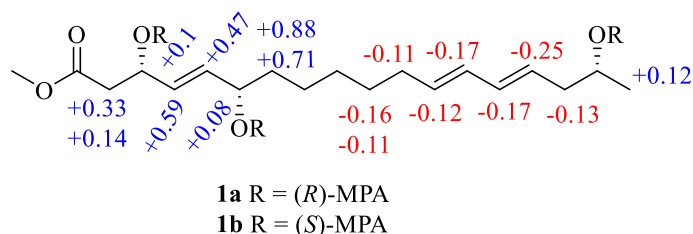


Figure 3. $\Delta\delta_H$ ($\delta_R - \delta_S$) values of the MPA esters for **1** in $CDCl_3$

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Table 1. ^1H NMR (600 MHz) data of **1**, **1a**, and **1b** and ^{13}C NMR (150 MHz) data of **1**

no.	1		1a	1b
	δ_{C} , type	δ_{H} , mult (<i>J</i> in Hz)	δ_{H} , mult (<i>J</i> in Hz)	δ_{H} , mult (<i>J</i> in Hz)
1	173.2, C			
2	43.3, CH ₂	2.53, m	2.56, dd (15.6, 4.5) 2.46, dd (15.6, 8.2)	2.42, dd (16.1, 5.0) 2.42, dd (16.1, 8.0)
3	69.7, CH	4.52, m	5.65, overlap	5.55, m
4	132.9, CH	5.68, dd (15.5, 5.0)	5.65, overlap	5.06, dd (15.1, 6.0)
5	135.3, CH	5.71, dd (15.5, 5.2)	5.65, overlap	5.18, dd (15.1, 6.0)
6	72.8, CH	4.03, m	5.23, m	5.15, m
7	38.3, CH ₂	1.52, m 1.47, m	2.21, m	1.41, m 1.33, m
8	26.4, CH ₂	1.40, m 1.34, m		
9	30.2, CH ₂	1.34, m		
10	30.6, CH ₂	1.41, m	1.18, m	1.34, m 1.29, m
11	33.5, CH ₂	2.08, m	1.93, q (6.8)	2.04, q (7.2)
12	133.7, CH	5.59, m	5.47, dt (15.3, 6.8)	5.59, dt (15.0, 7.2)
13	131.8, CH	6.02, m	5.81, dd (15.3, 10.1)	5.98, dd (15.0, 10.7)
14	134.1, CH	6.04, m	5.88, dd (14.6, 10.1)	6.05, dd (15.3, 10.7)
15	128.9, CH	5.58, m	5.21, dt (14.6, 7.5)	5.46, dt (15.3, 7.4)
16	43.4, CH ₂	2.23, m 2.17, m	2.21, m	2.34, m
17	68.6, CH	3.76, m	4.99, m	5.02, m
18	22.9, CH ₃	1.16, d (6.3)	1.25, d (6.3)	1.13, d (6.3)
	OCH ₃	52.1, CH ₃		

^a in CD₃OD, ^b in CDCl₃

In addition, the known compounds were assigned as 1-*O*-(9*Z*,12*Z*-octadecadienoyl)glycerol (**2**) [18] and 1-*D*-arabinitol-monolinoleate (**3**) [19] by comprehensive comparison of their NMR and specific rotations with those reported in the literature.

Compounds (**1–3**) were tested for their cytotoxicity against human cancer cell line of ECA-109, the inhibitory effects against NO production in LPS-stimulated RAW264.7 cells, and their antibacterial activity against *S. aureus* ATCC 29213 (Table 2). As a result, compounds **1–3** showed moderately anti-inflammatory activities with the inhibitory rates ranging from 44.5% to 60.67% at the concentration of 20 μM , while compound **2** showed weakly cytotoxicity against ECA-109 with the inhibitory rate of 31.3% (20 μM). In addition, compound **1** exhibited weakly antibacterial effect against *S. aureus* ATCC 29213 with an MIC value of 32 $\mu\text{g/mL}$.

Table 2. Effects of **1–3** against NO production in LPS-stimulated RAW264.7 and cytotoxicities against ECA-109 at 20 μM , and antibacterial activity against *S. aureus* ATCC 29213

Compounds	antiinflammatory	cytotoxic	antibacterial
	anti-NO (%)	inhibition (%)	MIC ($\mu\text{g/mL}$)
1	60.7 \pm 1.7	9.1 \pm 3.7	32
2	49.9 \pm 2.5	31.3 \pm 2.2	> 64
3	44.5 \pm 1.3	2.2 \pm 1.9	> 64

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Supporting Information

Supporting Information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

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