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AZAPHILONE DERIVATIVES ISOLATED FROM ENDOPHYTIC FUNGUS *PENICILLIUM SCLEROTIORUM*

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Summary

Endophytic fungi are one of the typical endophytic microbial groups that have recently attracted considerable research interest because of their roles in many aspects. The most special thing is the ability to biosynthesize secondary substances with biological activity, such as antioxidant, anti-microbial, anti-tumor, and anti-inflammatory effects. The endophytic fungal strains were first isolated from *Acorus gramineus* herbs and identified as *Penicillium sclerotiorum* by morphological, microscopic, and DNA sequencing based on the modified Sanger method. From the ethyl acetate fraction of the medium extract, six compounds were isolated and identified as peniazaphilones I (**1**), 11-*epi*-geumsanol F (**2**), sclerotioramine (**3**), *epi*-isochromophilone III (**4**), isochromophilone IV (**5**), and linoleic acid (**6**). Their structures were determined by means of spectroscopic methods (MS, 1D, and 2D NMR).

Keywords: *Penicillium sclerotiorum; Endophytic fungus; Acorus gramineus; Azaphilone; Derivative.*

1. Introduction

Endophytic fungi are the most popular microorganisms, present in the majority of plant species and existing in many parts of plants such as roots, stems, leaves, flowers, fruits, and seeds [1]. Endophytic fungi in plants are considered important sources of secondary metabolites and bioactive natural products such as alkaloids, terpenoids, steroids, quinones, coumarins, flavonoids, phenols, phenolic acids, and peptides [2]. Several endophytic fungi can produce bioactive compounds originating from plants that are similar or identical to those of their host plants, such as paclitaxel, podophyllotoxin, camptothecin, vinblastine, hypericin, and diosgenin [3]. In addition, the compounds isolated from endophytic fungi exhibited remarkable biological activities as antioxidant [4],[5], antibacterial [6], and anti-tumor activity [7],[8], which are potential sources for new drug research. Therefore, this study was undertaken to gain a deeper understanding of the mechanisms and chemical constituents of endophytic fungi involved in the production of bioactive secondary metabolites used in the treatment of several diseases. Our present study on chemical constituents from the ethyl acetate fraction of the endophytic fungus strains of *P. sclerotiorum* led to the isolation of six compounds. Their structures were identified as peniazaphilones I (**1**), 11-*epi*-geumsanol F (**2**), sclerotioramine (**3**), *epi*-isochromophilone III (**4**), isochromophilone IV (**5**), and linoleic acid (**6**) (Fig.1) by spectroscopic data and compared to those reported in the literature.

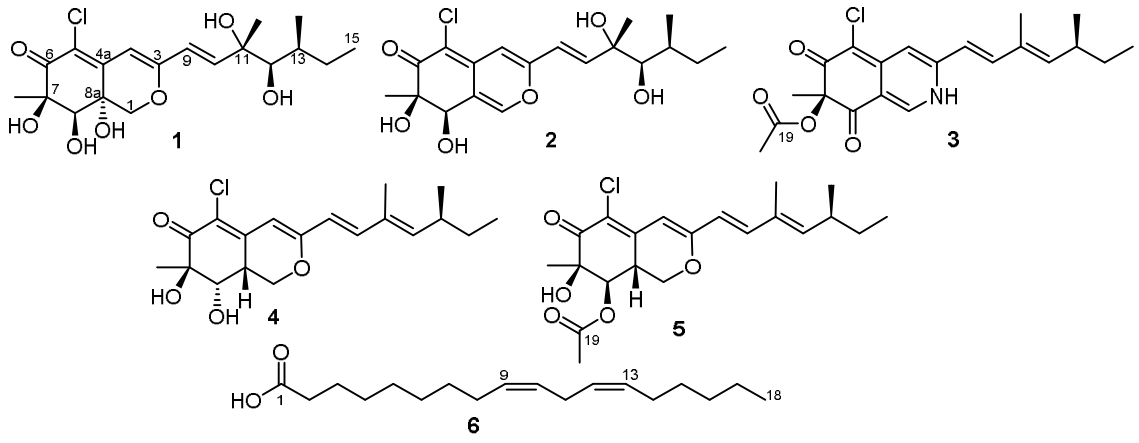

Fig. 1. Chemical structures of compounds **1-6**

2. Experimental

2.1. Materials and chemicals

The rhizomes of *Acorus gramineus* Aiton. (Acoraceae) were collected in Hoa Binh province in December 2024 for the isolation of the endophytic fungus strains. The voucher specimen of endophytic fungus strain (KN15.122024.HB) was deposited at the School of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City (UMP), Vietnam. The solvents were used for isolation and extraction, including ethyl acetate and methanol (Thermo Fisher Scientific, USA).

2.2. General experimental procedures

Silica gel (40-63 μm, Merck) and Sephadex LH-20 (GE Healthcare, Sweden) were used for column chromatography (CC). TLC was performed using normal phase *silica gel* 60 F_{254} plates (Merck). Spots were detected by UV and 5% vanillin-sulfuric acid/absolute ethanol. ESI-MS spectra were obtained from a Xevo G2-XS QTOF mass spectrometer (Waters, USA). NMR spectra were measured on a JEOL 400 MHz spectrometer (Japan), using TMS as an internal standard. NMR solvents were purchased from Sigma-Aldrich (USA). Microorganisms were manipulated under sterile conditions in an Esco AVC-4A1 class III biological safety cabinet.

Fungal Isolation and Scale-Up Cultivation

The rhizome of *Acorus gramineus* was washed with reverse osmosis water, then treated with 7% Javel (3 min), 70% ethanol (3 min) before being used to isolate the endophytic fungal strains. The fungal strain isolation procedure was followed as our previous protocol [9]. The potato dextrose agar-PDA (with chloramphenicol 25 mg/L) was used to isolate and investigate the growth ability of endogenous fungi. The fungial strain was identified based on the microscopic morphology characteristics with lactophenol cotton blue (LPCB) and DNA sequencing based on the modified Sanger method [10],[11].

The fungus was cultivated on 22 L of PDA agar at room temperature for 30 days. The agar and fungal mycelia were then harvested and extracted with EtOAc (30 L). The combined organic phase and water layer were filtered and concentrated *in vacuo* at 40 °C to yield the EtOAc extract (PSEA, 35 g).

2.3. Extraction and isolation

The EtOAc extract (30 g) was separated into 11 fractions (PSEA1 to PSEA11) by reversed phase *silica gel* C_{18} VLC using a linear gradient elution of MeOH – H_2O (0:100, 25:75, 50:50, 75:25, and 100:0). Fraction PSEA5 (2.48 g) was further separated by Sephadex LH-20 column chromatography (CC) and eluted with MeOH (100%) to give 3 sub-fractions (PSEA5.1 to PSEA5.3). Sub-fraction PSEA5.2 (167.6 mg) was continued to separate by preparative RP-HPLC (Phenomenex Luna C_{18}, 250 × 10 mm, 5 mm, 2 mL/min) with CH_3CN-H_2O (32:68, *v*/*v*) to obtain compounds 1 (7.0 mg, t_R=23.4 min) and 2 (6.8 mg, t_R=22.2 min). Fraction PSEA8 (787 mg) was separated into 4 fractions (PSEA8.1 to PSEA8.4) by Sephadex LH-20 CC (MeOH 100%). Sub-fraction PSEA8.3 (108.5 mg) was continued to be separated by semi-preparative HPLC (CH_3CN-H_2O, 50:50, *v*/*v*, 2 mL/min) to yield compound **3** (96.9 mg, t_R = 40.5 min).

Fraction PSEA10 (1.67 g) was separated into 4 fractions (PSEA10.1 to PSEA10.4) by Sephadex LH-20 CC (MeOH 100%). Sub-fraction PSEA10.3 (258 mg) was continued to separate by semi-preparative HPLC (CH_3CN-H_2O, 64:36, *v*/*v*, 2 mL/min) to yield compounds **4** (9.1 mg, t_R = 24.8 min), **5** (20.1 mg, t_R = 40.8 min), and **6** (61 mg, t_R = 62.3 min), respectively.

Peniazaphilones I (1): Yellowish oil. ESI-MS: *m/z* 403.1563 $[M+H]^+$, $C_{19}H_{27}O_7Cl$. ^1H NMR and ^{13}C NMR data are shown in Table 1 and Supplementary Information Table S1-S2.

11-Epi-geumsanol F (2)*:* Yellowish oil, ESI-MS: *m/z* 385.1516 $[M+H]^+$, $C_{19}H_{25}O_6Cl$. ^1H NMR and ^{13}C NMR data are shown in Table 1 and Supplementary Information Table S1-S2.

Sclerotioramine (3)*:* Orange-red amorphous powder. ESI-MS: *m/z* 390.1579 $[M+H]^+$, $C_{21}H_{24}O_4NCl$. ^1H NMR and ^{13}C NMR data are shown in Table 1 and Supplementary Information Table S1-S2.

Epi-isochromophilone III (4): Light yellowish-brown oil. ESI-MS: *m/z* 353.1637 $[M+H]^+$, $C_{19}H_{25}O_4Cl$. ^1H NMR and ^{13}C NMR data are shown in Table 1 and Supplementary Information Table S1-S2.

Isochromophilone IV (5): Yellowish oil. ESI-MS: *m/z* 395.1762 $[M+H]^+$, $C_{21}H_{27}O_5Cl$. ^1H

NMR and ^{13}C NMR data are shown in Table 1 and Supplementary Information Table S1-S2.

Linoleic acid (6): Colorless oil, ESI-MS: *m/z* 319.2104 $[M+K]^+$, $C_{18}H_{32}O_2$, ^1H NMR (400 MHz, $CDCl_3$) δ_H: 5.29 - 5.42 (4H, m, H-12, H-13, H-9, H-10), 2.77 (2H, t, *J*=7.6 Hz, H_2-11), 2.34 (2H, t, *J*=7.6 Hz, H_2-2), 2.05 (2H, q, *J*=7.2 Hz, H_2-8), 2.05 (2H, q, *J*=7.2 Hz, H_2-14), 1.63 (2H, m, H_2-3), 1.24 - 1.39 (14H, m, H_2-4, H_2-5, H_2-6, H_2-7, H_2-15, H_2-16, H_2-17), 0.90 (3H, t, *J*=6.8 Hz, H_3-18). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 178.9 (C-1), 130.2 (C-13), 130.0 (C-9), 128.1 (C-12), 127.9 (C-10), 34.0 (C-2), 31.5 (C-16), 29.6 (C-7), 29.4 (C-6), 29.2 (C-15), 29.1 (C-4), 29.1 (C-5), 27.2 (C-8), 27.2 (C-14), 25.6 (C-11), 24.7 (C-3), 22.6 (C-17), 14.1 (C-18).

3. Results and discussion

The endophytic fungi showed the ability to increase biomass rapidly and to produce secretions on the surface, which is one of the important factors in the isolation of fungi. The PDA medium showed suitability for fungi growth. Follow-up to the reference [12], PDA medium was selected for the scale-up cultivation.

In eight days, the endophytic fungal strain was preliminarily identified based on the microscopic morphology characteristics with LPCB color dyeing. Then, the colony of endophytic fungi was selected and identified by DNA sequencing based on the modified Sanger method. Genomic DNA was extracted from the mycelia of fungi, and the 18S rRNA genes were amplified by PCR, which were carried out by Phu Sa Genomics company, and the nucleotide sequence (5′-3′) was obtained as shown in the Supplementary Information data.

A BLAST analysis (NCBI database) on the resulting 18S rRNA gene partial sequence revealed 100.0% identity with the fungal strain *Penicillium sclerotiorum* MT071304.1.

The cultivation of the endophytic strain of *P. sclerotiorum* was further scaled up for the isolation of secondary metabolites at room temperature for 30 days. From the ethyl acetate extract, six compounds were isolated and structurally elucidated.

Compound **1** was obtained as a yellowish oil. The UV spectrum displayed a maximum absorption at 371 nm, suggesting the presence of an extended conjugated chromophore. The ESI-MS spectrum data showed the pseudomolecular ion peaks at *m/z* 403.1563 and 405.1648 $[M+H]^+$ with a ratio of 3:1, indicating the presence of one chlorine atom, which is calculated for the molecular formula $C_{19}H_{27}O_7Cl$ with 6 degrees of unsaturation. The ^{13}C NMR spectrum showed signals of 19 carbons including 4 methyl carbons (δ_C 26.5 (C-17), 26.4 (C-18), 14.4 (C-16), and 12.3 (C-15)), 2 methylene carbons (δ_C 74.9 (C-1) and 30.2 (C-14)), 6 methine carbons (δ_C 145.1 (C-10), 122.7 (C-9), 101.9 (C-4), 81.0 (C-12), 79.2 (C-8), and 36.4 (C-13)), and 7 non-protonated carbons (δ_C 195.3 (C-6), 162.3 (C-3), 146.2 (C-4a), 120.1 (C-5), 78.3 (C-7), 76.8 (C-11), and 69.0 (C-8a)). The ^1H NMR data showed the signals of two oxymethine protons (δ_H 3.81 (H-8) and 3.42 (H-12)), 4 methyl groups (δ_H 1.52 (H_3-18), 1.33 (H_3-17), 0.91 (H_3-15), and 0.87 (H_3-16), a pair of *trans*-coupled olefinic protons δ_H 6.29 (1H, d, *J* = 15.6 Hz, H-9) and 6.76 (1H, d, *J* = 15.6 Hz, H-10), two methine protons (δ_H 6.04 (H-4) and 1.72 (H-13)) and two methylene protons. The HMBC spectrum showed the correlations between H_2-1 and C-3/C-4a/C-8a, between H-4 and C-3 (δ_C 162.3)/C-5/C-8a, exhibiting the presence of a 3,4-dihydro-2*H*-pyran moiety. In addition, HMBC cross-peaks from H-8 to C-1 (δ_C 74.9)/C-4a/C-6 (δ_C 195.3)/C-7/C-8a/C-18, and from H_3-18 to C-6/C-7/C-8 verified a bicyclic core moiety, demonstrating the presence of an azaphilone skeleton. The COSY correlations were observed between the protons H-9/H-10 and H-12/H-13/H_2-14/H_3-15, together with the HMBC correlations from H-9 and C-3/C-4/C-10/C-11, H-10 and C-3/C-9/C-11/C-17, H-12 and C-10/C-11/C-13/C-14/C-16/C-17, H_3-13 and C-14/C-15/C-16 were used to construct the side chain of azaphilone with two hydroxy groups located at C-11 and C-12. Those NMR data suggested that compound **1** was a typical structure of the azaphilone framework, which has been reported in *P. sclerotiorum*. Based on the above NMR spectra data and comparison with the literature data [13], compound **1** was identified as peniazaphilones I, which was isolated from the mangrove endophytic fungi *P. sclerotiorum*.

Compound **2** was isolated as a yellowish oil. The positive ESI-MS spectrum exhibited the ion

peaks at *m/z* 385.1516 and 387.1551 $[M+H]^+$ with a ratio of 3:1, showing the presence of one chlorine atom, which was consistent with the molecular formula of $C_{19}H_{25}O_6Cl$ and calculated with 7 degrees of unsaturation. The ^{13}C NMR spectrum of compound **2** showed the presence of 19 carbons, including six methine carbons (δ_C 146.6 (C-1), 144.9 (C-10), 106.5 (C-4), 120.3 (C-9), 78.2 (C-12), and 35.4 (C-13)), one carbonyl carbon (δ_C 191.6, C-6), one methylene carbon (δ_C 28.6 (C-14)), seven non-protonated carbons (δ_C 156.7 (C-3), 140.4 (C-4a), 116.9 (C-8a), 107.8 (C-5), 76.3 (C-7), 71.7 (C-8), and 75.7 (C-11)), and four methyl carbons (δ_C 11.9 (C-15), 13.4 (C-16), 23.7 (C-17), and 23.8 (C-18)). The ^1H NMR spectrum data showed two singlet signals of aromatic protons at δ_H 7.35 (H-1) and 6.55 (H-4), two oxymethine protons at δ_H 4.35 (H-8) and 3.53 (H-12), a pair of *trans*-coupled olefinic protons (δ_H 6.64 (1H, d, J = 15.2 Hz, H-10) and 6.41 (1H, d, J = 15.2 Hz, H-9), one methylene group and four methyls (δ_H 1.35 (3H, s, H_3-17), 1.29 (3H, s, H_3-18), 0.98 (3H, d, J = 6.8 Hz, H_3-16), and 0.93 (3H, t, J = 7.6 Hz, H_3-15). The ^{13}C and ^1H NMR spectral data suggested that compound **2** was an azaphilone structure. The NMR data of compound **2** were similar to those of compound **1**, the main difference was the change of hydroxyl group at C-8a by an olefinic double bond between C-1 and C-8a ($D_{1,8a}$). The COSY and HMBC correlations of compound **2** were similar to those of **1** and are shown as in Fig. 2. The planar structure of **2** was closely related to those of geumsanol F and 11-epi-geumsanol F. The difference between geumsanol F and 11-epi-geumsanol F was the orientation of the hydroxyl group linked to carbon C-11, whereas the hydroxyl group in geumsanol F was β-orientation and *vice versa*. The NOESY spectrum of **2** showed the correlations between H-8/H_3-18, indicating that H-8 and CH_3-18 were on the same side of α-orientation. Correlations were observed between H-10 and H-12/H_3-16/H_3-17, between H_3-17 and H-12/H_3-16, suggesting that H_3-16 and H_3-17 were β-orientation as shown in Fig.3. On the basis of the spectroscopic data, compound **2** was identified as 11-*epi*-geumsanol F, which was isolated from the marine algae-derived fungus *Penicillium sclerotiorum* [12],[14].

Compound **3** was isolated as an orange-red amorphous powder. The ESI-MS spectral data exhibited the positive-ion peaks at *m/z* 390.1579 and 392.1541 $[M+H]^+$, corresponding to the molecular formula $C_{21}H_{24}ClNO_4$ and 9 degrees of unsaturation. Mass spectrometry data of 3 exhibited pseudomolecular ion peaks at *m/z* 390 and 392 with a ratio of 3:1, and an odd-numbered molecular ion peak indicates that compound **3** possessed one chlorine and one nitrogen atom in the structure. The ^{13}C NMR spectrum of **3** showed the presence of 21 carbon signals, including three carbonyl groups (δ_C 193.4 (C-8), 183.6 (C-6), and 171.5 (C-19)), six methine carbons (δ_C 138.0 (C-1), 110.0 (C-4), 116.1 (C-9), 142.5 (C-10), 148.8 (C-12), and 35.1 (C-13)), six non-protonated carbons (δ_C 146.1 (C-4a), 145.5 (C-3), 131.9 (C-11), 113.8 (C-8a), 102.1 (C-5), and 85.9 (C-7)), one methylene at δ_C 30.1, (C-14) and 5 methyls (δ_C 23.6 (C-18), 20.8 (C-20), 20.2 (C-16), 12.5 (C-17), and 12.1 (C-15)). ^1H NMR spectrum of **3** presented three methine at δ_H 7.76 (H-1), 6.76 (H-4), and 5.68 (1H, d, J = 9.6 Hz, H-12), a double bond of olefinic protons at δ_H 6.91 (1H, d, J = 16.0 Hz, H-10) and 6.05 (1H, d, J = 16.0 Hz, H-9), five methyls (δ_H 2.24 (H_3-20), 1.84 (H_3-17), 1.58 (H_3-18), 1.03 (H_3-16), and 0.86 (H_3-15) and a broad singlet signal at δ_H 10.5 accounted for -NH- group.

The COSY spectrum exhibited the correlations of *trans*-coupled olefinic protons H-9 (δ_H 6.05) and H-10 (δ_H 6.76), and five consecutive protons between H-12/H-13/(H_3-16)/H_2-14/H_3-15. The HMBC spectrum showed the correlations from H-1 to C-3/C-4a/C-8/C-8a, from H-4 to C-3/C-4/C-5/C-8a/C-9, from H-9 to C-3/C-4/C-10/C-11, from H-10 to C-3/C-12/C-17, from H-12 to C-10/C-14/C-16/C-17, from H_2-14 to C-12/C-13/C-15/C-16, from H_3-18 to C-6/C-7/C-8, and from H_3-20 to C-7/C-19. The above NMR spectral features showed that compound **3** was an azaphilone derivative with one oxygen group at C-2 replaced by an -NH- group and one acetyl moiety linked to a hydroxyl group at C-7. Based on the NMR spectral data and compared to those of the reference data [15], compound **3** was determined as sclerotioramine, a major compound of *P. sclerotiorum*.

Table 1. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data of compounds **1–5**

No.	**1**[a]		**2**[b]		**3**[b]		**4**[b]		**5**[b]	
	δ_C	δ_H, mult, *J* (Hz)	δ_C	δ_H, mult, *J* (Hz)	δ_C	δ_H, mult, *J* (Hz)	δ_C	δ_H, mult, *J* (Hz)	δ_C	δ_H, mult, *J* (Hz)
1	74.9	4.31, q, 12.0 Hz	146.6	7.35, s	138.0	7.76, s	68.2	4.26, dd, 12.0, 10.8, Hz	67.4	3.83, dd, 13.6; 10.8 Hz
								4.52, dd, 10.8; 4.8 Hz		4.37, dd, 10.8; 5.2 Hz
3	162.3	-	156.7	-	145.5	-	163.0	-	163.4	-
4	101.9	6.04, s	106.5	6.55, s	110.0	6.76, s	102.2	6.10, s	101.6	6.12, s
4a	146.2	-	140.4	-	146.1	-	145.7	-	145.7	-
5	120.1	-	107.8	-	102.1	-	115.4	-	118.5	-
6	195.3	-	191.6	-	183.6	-	192.8	-	187.1	-
7	78.3	-	76.3	-	85.9	-	77.3	-	74.9	-
8	79.2	3.81, s	71.7	4.35, s	193.4	-	73.6	4.13, d, 3.2 Hz	73.2	5.01, d, 10.4 Hz
8a	69.0	-	116.9	-	113.8	-	36.9	3.07, m	35.6	3.45, m
9	122.7	6.29, d, 15.6 Hz	120.3	6.41, d, 15.2 Hz	116.1	6.05, d, 16.0 Hz	118.9	6.00, d, 15.6 Hz	118.9	5.99, d, 15.2 Hz
10	145.1	6.76, d, 15.6 Hz	144.9	6.64, d, 15.2 Hz	142.5	6.91, d, 16.0 Hz	142.0	7.01, d, 15.6 Hz	142.0	6.97, d, 15.2 Hz
11	76.8	-	75.7	-	131.9	-	132.3	-	132.3	-
12	81.0	3.42, d, 2.4 Hz	78.2	3.53, d, 1.2 Hz	148.8	5.68, d, 9.6 Hz	147.3	5.63, d, 9.6 Hz	147.4	5.61, d, 10 Hz
13	36.4	1.72, m	35.4	1.71, m	35.1	2.47, m	35.0	2.46, m	35.0	2.45, m
14	30.2	1.42, m	28.6	1.43, m	30.1	1.43, m	30.1	1.42, m	30.1	1.40, m
		1.29, m		1.35, m		1.32, m		1.31, m		1.29, m
15	12.3	0.91, t, 7.2 Hz	11.9	0.93, t, 7.6 Hz	12.1	0.86, t, 7.2 Hz	12.0	0.85, t, 7.6 Hz	12.0	0.85, t, 7.6 Hz
16	14.4	0.87, d, 6.8 Hz	13.4	0.98, d, 6.8 Hz	20.2	1.03, d, 6.8 Hz	20.3	1.00, d, 6.8 Hz	20.3	0.99, d, 6.8 Hz
17	26.5	1.33, s	23.7	1.35, s	12.5	1.84, d, 1.2 Hz	12.4	1.82, d, 1.2 Hz	12.4	1.81, d, 0.8 Hz
18	26.4	1.52, s	23.8	1.29, s	23.6	1.58, s	23.5	1.38, s	20.7	1.43, s
19	-	-	-	-	171.5	-	-	-	170.4	-
20	-	-	-	-	20.8	2.24, s	-	-	20.7	2.23, s
						(-NH-) 10.5 brs				

[a]: *Measured in CD_3OD* [b]: *Measured in $CDCl_3$*

Compound **4** was isolated as a light yellowish-brown oil, whose molecular formula $C_{19}H_{25}O_4Cl$ was deduced by HR-ESIMS (*m/z* = 353.1637 $[M+H]^+$, implying 7 degrees of unsaturation. The ^{13}C NMR spectrum showed 19 carbon signals, including 2 methylene carbons (δ_C 68.2 (C-1), 30.1 (C-14)), 6 methine carbons (δ_C 142.0 (C-10), 102.2 (C-4), 118.9 (C-9), 73.6 (C-8), 36.9 (C-8a), and 35.0 (C-13)), 7 non-protonated carbons (δ_C 192.8 (C-6), 163.0 (C-3), 147.3 (C-12), 145.7 (C-4a), 132.3 (C-11), 115.4 (C-5), and 77.3 (C-7)), and 4 methyl carbons (δ_C 23.5 (C-18), 20.3 (C-16), 12.4 (C-17), and 12.0 (C-15)). The ^{1}H NMR data showed 4 methyl signals at δ_H 1.38 (s, CH_3-18), 1.82 (d, CH_3-17), 1.00 (s, CH_3-16), and 0.85 (t, CH_3-15), seven methine protons (including three olefinic, one aromatic, and three aliphatic protons), and two methylene protons. Comparing the ^{13}C and ^{1}H NMR data of **4** with that of compound **1**, it is found that their planar structures are very close. The differences between them were that the hydroxyl groups at C-8a, C-11, and C-12 in **1** were removed to form double bonds between C-1/ C-8a and C-11/ C-12 in **4**. The ^{1}H-^{1}H COSY spectrum showed an additional spin system H-8/H-8a/H_2-1 in comparison to those of compound **1**. The HMBC correlations of **4** were similar to those of **1**, as shown in Fig. 2. Based on the NMR data and compared to those of the reference data [16], compound **4** was identified as epi-isochromophilone III, which was isolated from soil fungus *P. multicolor*.

Compound **5** was isolated as a yellowish oil. The molecular formula of **5** was determined to be $C_{21}H_{27}O_5Cl$ by the HRESIMS peak at *m/z* 395.1762 $[M+H]^+$, with the 1:3 chlorine isotope peaks, corresponding to the molecular formula with 6 degrees of unsaturation. The ^{13}C NMR spectrum showed 21 carbon signals, including 2 methylene carbons (δ_C 67.4 (C-1), 30.1 (C-14)), 7 methine carbons (δ_C 147.4 (C-12), 142.0 (C-10), 101.6 (C-4), 118.9 (C-9), 73.2 (C-8), 35.6 (C-8a), and 35.0 (C-13)), 7 non-protonated carbons (δ_C 187.1 (C-6), 170.4 (C-19), 163.4 (C-3), 145.7 (C-4a), 132.3 (C-11), 118.5 (C-5), and 74.9 (C-7)), and 5 methyl carbons (δ_C 20.7 (C-20), 20.7 (C-18), 20.3 (C-16), 12.4 (C-17), and 12.0 (C-15)). The ^{1}H NMR data showed five methyl signals at δ_H 2.23 (3H, s, CH_3-20), 1.43 (3H, s, CH_3-18), 1.81 (3H, d, CH_3-17), 0.99 (3H, d, CH_3-16), and 0.85 (3H, t, CH_3-15), seven methine (including

four olefinic and three aliphatic protons), and two methylene protons. The ^{13}C and ^{1}H NMR data of **5** were similar to those of **4**. The differences between **5** and **4** were the addition of one acetyl group at carbon C-8 (δ_C 73.2), and the position of proton H-8 was changed to *b*-orientation in **5**. The same correlations as those of **4** were observed in the ^{1}H-^{1}H COSY and HMBC of **5**. A cross peak from the H-8 proton (δ_H 5.01, d, J = 10.4 Hz) to the acetyl carbonyl carbon (δ_C 170.4) in HMBC revealed that **5** confirmed an acetyl moiety located to carbon C-8. Therefore, the structure of compound **5** was identified as isochromophilone IV, which was isolated from *P. multicolor* [17].

Compound **6** was isolated as a colorless oil; its positive ESI-MS presented the ion peak at *m*/*z* 319.2039 $[M+K]^+$, corresponding to the molecular formula $C_{18}H_{32}O_2$ with 3 degrees of unsaturation. The ^{13}C NMR spectrum showed 18 carbon signals, including 12 methylene carbons (δ_C 34.0 (C-2), 31.5 (C-16), 29.6 (C-7), 29.4 (C-6), 29.2 (C-15), 29.1 (C-4), 29.1 (C-5), 27.2 (C-8), 27.2 (C-14), 25.6 (C-11), 24.7 (C-3), 22.6 (C-17)), four methine carbons (δ_C 130.0 (C-9), 127.9 (C-10), 128.1 (C-12), and 130.2 (C-13)), one carboxylic carbon (δ_C 178.9 (C-1), and one methyl (δ_C 14.1 (C-18)). The ^{1}H NMR data exhibited the signals of a methyl group at δ_H 0.90 (3H, t, CH_3-18), two pairs of double bond δ_H 5.29 - 5.42 (4H, m, H-12, H-13, H-9, H-10), and twelve methylene groups. In comparison with the published NMR data in the reference [18], compound **6** was identified as linoleic acid, which is reported for the first time in the fungus *Penicillium sclerotiorum*.

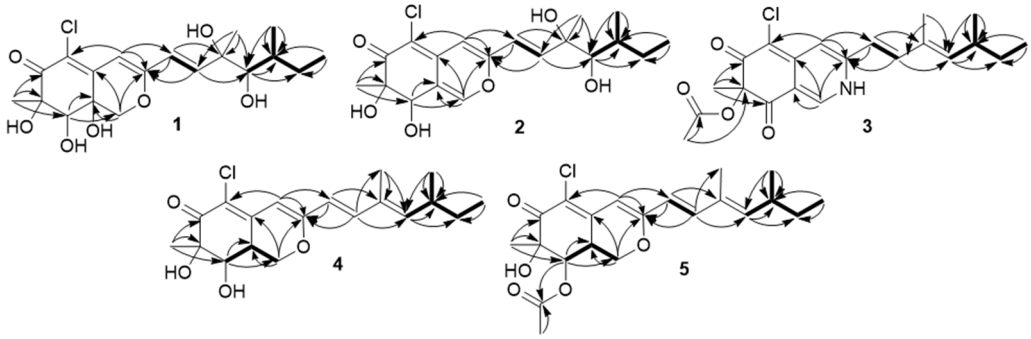

Fig. 2. The key HMBC (⟶) and COSY (▬) correlations of compounds **1-5**.

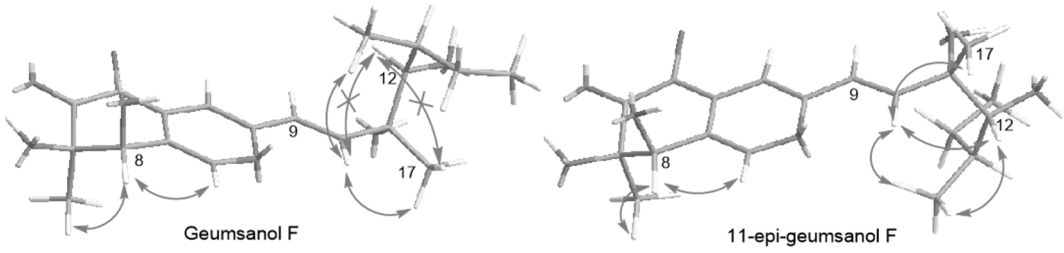

Fig. 3. The NOESY correlations of geumsanol F and 11-epi-geumsanol F.

Based on those references reported in the literature, peniazaphilone I was evaluated on anti-inflammatory effect against NO production on RAW 264.7 cell lines, cytotoxicity on MDA-MB-435 and A549 cancer cells, and antibacterial activities. The results showed that peniazaphilone I was found to be inactive in all tested conditions [13]. 11-*Epi*-geumsanol F and isochromophilone IV were evaluated on the anti-angiogenic effect in human endothelial progenitor cells. As a result, only isochromophilone IV displayed strong anti-angiogenic activities by blocking cell growth, migration, and tube formation without cytotoxicity on cell lines [14]. Furthermore, epi-isochromophilone III and isochromophilone IV exhibited antimalarial activity against *Plasmodium falciparum* (IC_{50} = 2.1–7.8 μg/mL), while epi-isochromophilone III showed

cytotoxicity against three cancer cell lines, KB, MCF-7, and NCI-H187 (IC_{50} 2.2–35.2 μg/mL) [16], and sclerotioramine showed strong antimycobacterial activity against *Mycobacterium tuberculosis* with MIC = 50.0 μg/mL and inhibitory effects on *Staphylococcus epidermidis*, *Candida albicans*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei* [15],[16].

4. Conclusion

In summary, the endophytic fungi from the rhizome of *A. gramineus* Aiton was isolated and identified as *Penicillium sclerotiorum* based on the microscopic morphological characteristics and DNA sequencing by the modified Sanger method. Six compounds were isolated from the ethyl acetate fraction of the endophytic fungi *P. sclerotiorum*. Their structures were identified as peniazaphilones (**1**), 11-*epi*-geumsanol F (**2**), sclerotioramine (**3**), *epi*-isochromophilone III (**4**), isochromophilone IV (**5**), and linoleic acid (**6**). The endophytic fungus strain *P. sclerotiorum* was first isolated and reported in the rhizome of *A. gramineus,* and the compounds **4** and **6** are first reported from *Penicillium sclerotiorum*.

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