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ISOCHROMOPHILOL A, A NEW AZAPHILONE ISOLATED FROM *PENICILLIUM* sp. RO369, A LEAF LITTER INHABITING FUNGUS FROM *TSUGA DIVERSIFOLIA*

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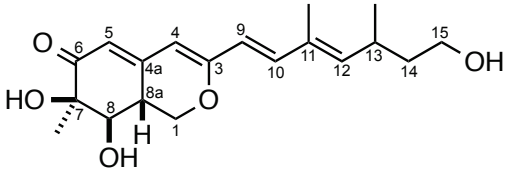
Abstract – A new sclerotiorin-type azaphilone, isochromophilol A (**1**), was isolated from *Penicillium* sp. RO369 associated with *Tsuga diversifolia*, along with eight known compounds (**2**–**9**). The structure of **1** was elucidated on the basis of spectroscopic data. Isochromophilol A (**1**) (16 μg) showed weak anti-methicillin-resistant *Staphylococcus aureus* (anti-MRSA) activity with an inhibition diameter of 8.8 ± 1.0 mm

Azaphilones[1] are a structurally diverse families of fungal polyketide metabolites possessing a highly oxygenated isochromene skeleton and are known as fungal pigments.[2] They are produced by numerous species of ascomyceteous and basidiomyceteous fungi, including *Aspergillus*, *Penicillium*, *Chaetomium*, *Talaromyces*, *Pestalotiopsis*, *Phomopsis*, *Emeriella*, *Epicoccum*, *Monascus,* and *Hypoxylon* genera.[1] (+)-Sclerotiorin (**2**) is one of the most representative azaphilones first isolated from *Penicillium sclerotiorum* by Curtin and Erilly in 1940.[3] Previous studies reported that five *Penicillium* spp. (i.e., *P. citreonigrum*,[1] *P. hirayamae*,[1] *P. japonica*,[1] *P. multicolor*,[1,4] and *P. sclerotiorum*[1,5]) and five unidentified *Penicillium* spp.,[1,6] along with *A. nidulans*,[1] *C. cpreum*,[1] *C. aureum*,[7] *T. helius*,[1] and *T. luteus,*[1] produce sclerotiorin-type azaphilones. Azaphilones exhibit a wide range of biological activities, including



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inhibition of gp120-CD4 binding, Grb2-SH2 interaction, MDM2-p53 interaction, heat shock protein 90 (Hsp90), and dihydrofolate reductase, as well as antimicrobial, antiviral, cytotoxic, anticancer, and anti-inflammatory activities. In our efforts to obtain new bioactive secondary metabolites from filamentous fungi,[8] we isolated *Penicillium* sp. RO369 from leaf litter of *Tsuga diversifolia*. Molecular phylogenetic analyses revealed that RO369 strain is closely related to *Penicillium daejeonium* which is the closest to *P. sclerotiorum* among the ten *Penicillium* spp. that produce sclerotiorin-type azaphilone. We investigated the chemical constitution of the RO369 strain, and isolated one new sclerotiorin-type azaphilone, isochromophilol A (**1**), along with eight known compounds (sclerotiorin (**2**),[3] ochrephilone (**3**),[9] isochromophilone IV (**4**),[10] isochromophilone VIII (**5**),[11] dechloroisochromophilone III (**6**),[12] 8-acetyldechloroisochromophilone III (**7**),[12] 2,4-dihydroxy-6-(5,7-dimethyl-2-oxo-*trans*-3,5-nonadinenyl-3-methylbenzaldehyde (**8**),[13] and PC-2 (**9**)[14]) (Figure S1). Here, we describe the isolation and structure elucidation of **1**.

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of Isochromophilol (**1**) in CD_3OD

Position	δ_H (ppm)	δ_C (ppm)
1a	4.77 (1H, dd 10.0, 5.0 Hz)	70.1
1b	3.78 (1H, dd 13.5, 10.0 Hz)	
3		163.0
4	5.78 (1H, s)	105.5
4a		154.8
5	5.67 (1H, d 2.0 Hz)	116.5
6		199,4
7		75.9
8	3.39 (1H, d 9.5 Hz)	75.7
8a	3.07 (1H, m)	37.4
9	6.04 (1H, d 15.5 Hz)	120.8
10	6.92 (1H, d 15.5 Hz)	140.9
11		134.0
12	5.62 (1H, d 9.0 Hz)	145.6
13	2.77 (1H, m)	30.8
14a	1.62 (1H, m)	41.0
14b	1.51 (1H, m)	
15a	3.53 (1H, m)	61.0
15b	3.48 (1H, m)	
7-Me	1.37 (3H, s)	19.6
11-Me	1.83 (3H, d 1.0 Hz)	12.5
13-Me	1.02 (3H, d 7.0 Hz)	21.0

Isochromophilol A {**1**, $[\alpha]_D^{20}$ -41 (*c* 0.47, MeOH)} was revealed to have the molecular formula, $C_{19}H_{26}O_5$, by HRESIMS data [*m/z* 357.1678, $[M+Na]^+$, Δ +0.0 mmu]. The ^{1}H-, ^{13}C-NMR (Table 1), and HMQC

spectra of **1** showed signals due to one carbonyl, three sp^2 quaternary carbon, five sp^2 methines, one sp^3 quaternary carbon, three sp^3 methines, three sp^3 methylenes, and three methyls. Among them, one sp^2 quaternary carbon (δ_C 163.0), one sp^3 quaternary carbon (δ_C 75.9), one sp^3 methines (δ_C 75.7), and two sp^3 methylenes (δ_C 70.1 and 61.0) were ascribed to those bearing oxygen atoms.

The planar structure of **1** was elucidated by analysis of 2D NMR data, including the ^1H–^1H COSY, HMQC, and HMBC spectra (Figure 1). The ^1H–^1H COSY spectra disclosed four structural units: **a** (C-1/C-8a and C-8a to C-8), **b** (C-9 to C-10), **c** (C-13 to C-13-Me), and **d** (C-14 to C-15). HMBC correlations for H_3-13Me (δ_H 1.02) to C-12 (δ_C 145.6) and C-14 (δ_C 41.0), and for H_3-11Me (δ_H 1.83) to C-10 (δ_C 140.9), C-11 (δ_C 134.0), and C-12 revealed the connectivity between C-12 and C-14 through C-13 (δ_C 30.8) and the connectivity of C-10, C-11Me (δ_C 12.5), and C-12 through C-11, respectively. HMBC cross-peaks of H-10 (δ_H 6.92) to C-3 (δ_C 163.0) and H-9 (δ_H 6.04) to C-4 (δ_H 105.5) indicated that C-9 connected to C-4 via C-3. The connectivity between C-1 (δ_C 70.1) and C-3 through an oxygen atom was revealed by an HMBC correlation for H-1a (δ_H 4.77) to C-3. HMBC cross-peaks of H-1a to C-4a (δ_C 154.8) and H-5 (δ_H 5.67) to C-4 suggested the connectivity of C-4, C-5 (δ_C 116.5), and C-8a (δ_C 37.4) through C-4a. HMBC correlations for H_3-7Me (δ_H 1.37) to C-6 (δ_C 199.4), C-7 (δ_C 75.9), and C-8 (δ_C 75.7) revealed that C-7Me (δ_C 19.6) connected to C-6 and C-8 through C-7. Finally, the connectivity between C-5 and C-6 was indicated by an HMBC cross-peak of H-5 to C-7. Thus, the planar structure of isochromophilol A was elucidated to be **1** (Figure 1).

The relative stereochemistry of **1** was elucidated from NOESY spectrum and $^3J_{H\text{-}H}$ coupling constants (Figure 2). The $^3J_{H\text{-}4/H\text{-}1a}$, $^3J_{H\text{-}4/H\text{-}1b}$ and $^3J_{H\text{-}4/H\text{-}8}$ values (5.0, 13.5, and 9.5 Hz, respectively) indicated an *anti*-relationship between H-8a/H-1b and H-8. The β- and α-orientations for hydroxy groups at C-7 and C-8, respectively, were revealed by NOE correlations for H-8/H-1b and H_3-7Me. The stereochemistry at C-13 was not elucidated. The geometries of C-3/C-4, C-4a/C-5, C-9/C-10, and C-11/C-12 were assigned as *Z*, *Z*, *E*, and *E*, respectively, by NOE correlations for H-4/H-5 and H-9, H-9/H_3-11Me, and H-10/H-12. Although we attempted to use the modified Mosher's method to explain the absolute configuration of **1** at C-8, the (*S*)- and (*R*)-MTPA esters of **1** could not be obtained.

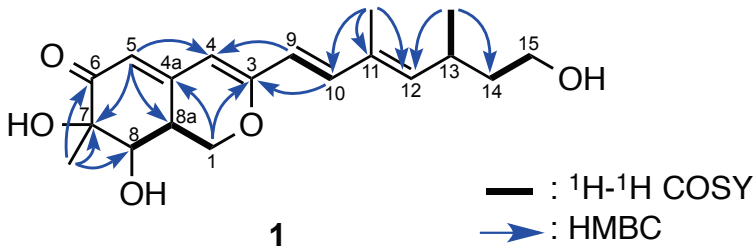

Figure 1. Selected 2D NMR correlations for isochromophilol A (**1**)

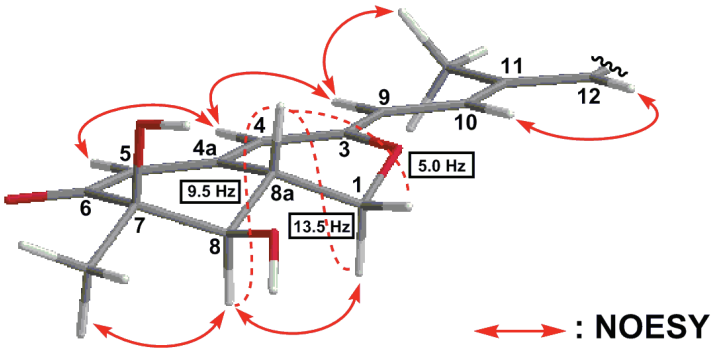

Figure 2. Selected NOESY correlations and relative stereochemistry for isochromophilol A (**1**)

Isochromophilol (**1**) was a new sclerotiorin-type azaphilone with a hydroxy group at C-15 of dechloroisochromophilone III (**6**).[12] Although approximately 50 sclerotiorin-type azaphilones have been reported so far,[1] they include few examples of compounds oxygenated at C-15.[15] Compounds **1**–**9** were evaluated for antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Isochromophilol A (**1**) showed weak anti-MRSA activity, with an inhibition diameter of 8.8 ± 1.0 mm. The diameter of the inhibition zone of the positive control was 20.3 ± 0.5 mm and that of the negative control was zero. Compounds **2**–**9** did not show the anti-MRSA activity.

EXPERIMENTAL

Optical rotation was recorded on a JASCO P-2100 polarimeter. UV spectra were recorded on a Shimadzu UV-1280 spectrophotometer. IR spectra were recorded on a Shimadzu IR Affinity-1 spectrometer. NMR spectra were recorded on a JEOL JNM-ECX500 spectrometer using 3.00 mm microcells (Shigemi Co., Ltd.). Chemical shifts (ppm) were referenced to the residual solvent peaks (δ_H 3.31 and δ_C 49.0 for CD_3OD). Positive-mode ESITOFMS was conducted on a Xevo G2-S QTof spectrometer (Waters Co. Ltd.) using a sample dissolved in MeOH.

Isolation and identification of *Penicillium* sp. RO369

Penicillium sp. RO369 was isolated from leaf litter of *Tsuga diversifolia* collected at the Tsukuba botanical garden of the National Museum of Nature and Science in Tsukuba, Ibaraki, Japan using the washing method.[16] The strain was identified using both phenotypic and molecular phylogenetical methods.[17] The fungal isolate was stored at Nihon University.

Fermentation and isolation

Penicillium sp. RO369 was cultured on a potato dextrose agar (PDA) medium at 25 °C for 7 days. The grown colonies were inoculated in potato dextrose broth (PDB) medium (100 mL × 20) and cultured at 25 °C at 180 rpm for 7 days. The mycelia and medium were separated by filtration, and the medium was

partitioned between EtOAc and H_2O. The EtOAc layer was subjected to a silica-gel column chromatography ($CHCl_3$/MeOH/H_2O, 1:0:0 → 4:1:0 → 6:4:1). A fraction eluted with $CHCl_3$/MeOH (10:1) was purified by C_{18} HPLC (COSMOSIL 5C_{18}-MS-II (Nacalai Tesque), 5 μm, 10 mm I.D. × 250 mm, solvent MeOH/H_2O, 50:50, flow rate 2.5 mL/min, detection 220 nm) to afford isochlomophilol A (**1**, 1.2 mg).

Isochromophilol A (1): colorless amorphous solid; $[\alpha]_D^{20}$ -41 (*c* 0.47, MeOH); UV (MeOH) λ_{max} 265 (ε 5500) and 374 (20700) nm; IR (ATR) ν_{max} 3340, 2950, 1650, and 1570 cm^{-1}; ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz), see Table 1; ESIMS *m/z* 357 $[M+Na]^+$; HRESIMS *m/z* 357.1678 $[M+Na]^+$ (calcd for $C_{19}H_{26}O_5Na$, 357.1678).

Antibacterial assay

The antibacterial activity of the compounds was evaluated via the disc diffusion method using MRSA strain N315 as pathogen. The culture was grown in Mueller-Hinton broth at 37 °C for 24 h. The turbidity of the bacterial suspension was adjusted to 0.5 McFarland standards. Sterile filter paper discs (6 mm) were impregnated with 20 μL (16 μg) of each compound. The discs were placed on the Mueller-Hinton agar spread with the bacterial suspension. After incubation at 37 °C for 24 h, the inhibition zones including the diameter of the disc were measured. The discs impregnated with 20 μL (16 μg) of Vancomycin were used as positive control whereas discs without the compounds (5% DMSO) were negative control. All assays were performed three times for each compound.

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SUPPORTING INFORMATION

Supplementary (^{1}H-, ^{13}C-NMR, MS, etc.) data and the structures of **2**-**9** associated with this article can be found in the online version at URL:
https://www.heterocycles.jp/newlibrary/libraries/previewprepress/26520044280

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