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NOTE

Penicillimide, an open-chain hemisuccinimide from Okinawan marine-derived *Penicillium copticola*

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Microbial products have contributed to human health care and the treatment of diseases.[1] Fungi isolated from marine environments are a rich source of bioactive natural products as well as terrestrial isolates, and a large number of metabolites with unique structures and bioactivities have been reported from marine-derived fungi.[1–4] During the course of an antifungal screening assay, we found that marine-derived *Penicillium copticola* strain TPU1270 isolated from Iriomote Island in Okinawa, Japan, exhibited growth inhibitory activity against *Mucor hiemalis* IAM6088 (20 mm at 200 μg per disc). Bioassay-guided isolation from the culture broth of strain TPU1270 yielded an open-chain hemisuccinimide, named penicillimide (**1**), together with five known eremophilane sesquiterpenes: sporogen-AO 1 (**2**),[5,6] 3-acetyl-13-deoxyphomenone (**3**),[7,8] 6-dehydropetasol (**4**),[9] 7-hydroxypetasol (**5**),[9] and petasol (**6**)[10] (Figure 1a). Compound **1** has been obtained by organic synthesis, and this is the first time to report this compound as a natural product. The isolation and antifungal activities of compounds **1**–**6** have been described herein.

The fungal strain TPU1270 was isolated from marine foam collected on the seashore in Iriomote Island, Okinawa Prefecture, Japan, in September 2012. Namely, ~1 ml of sterilized natural sea water was added to the marine foam collected in a sterilized plastic bag, the solution was mixed with 25 ml of sterilized natural sea water with 1.0% SDS and 50 μl of the resulting mixture was spread on an agar plate consisting of glycerol (Wako, Osaka, Japan) 0.6%, arginine (Wako) 0.1%, K_2HPO_4 (Wako) 0.1%, $MgSO_4$ (Wako) 0.05%, agar (Wako) 1.5%, cycloheximide (Wako) 100 μg ml^{-1} and rifampin (Wako) 5 μg ml^{-1} in natural sea water. The fungus that grew on the plate was isolated and maintained on a potato dextrose (PD) agar plate (BD, Franklin Lakes, NJ, USA), and was identified as *P. copticola* by a comparison of 228 bp ITS1 ribosomal DNA sequences (100% match). The mycelia that grew on the agar plate were inoculated into a 100-ml Erlenmeyer flask containing 50 ml of PD broth (BD), and the flask was shaken (150 r.p.m.) at 25 °C for 3 days (seed culture). Aliquots (2 ml) of the seed culture were inoculated into 15 Erlenmeyer flasks of 500 ml with each containing 200 ml of the main culture medium (sucrose (Wako) 3.0%, soluble starch (Wako) 3.0%, malt extract (BD) 1.0%, Ebios (Asahi Food & Healthcare, Tokyo, Japan) 0.3%, KH_2PO_4 0.5% and $MgSO_4{\cdot}7H_2O$ (Wako) 0.05%; adjusted to pH 6.0 before sterilization), and the flasks were cultured statically at room temperature for 21 days.

Acetone (1.5 l) was added to the culture broth (3 l) and filtered. The filtrate was evaporated to remove acetone, and the aqueous residue was applied on an ODS column. The column was eluted stepwise with H_2O-MeOH (100:0 to 0:100) to afford 10 fractions. The 40% MeOH fraction (309.2 mg) was separated by preparative HPLC on an ODS column (Pegasil ODS SP100, 10 mm × 250 mm, Senshu Scientific, Tokyo, Japan) with MeOH-H_2O = 44:56 (2.0 ml min^{-1}, UV 210 nm) to give 30.0 mg of **1** as solid. Preparative ODS HPLC (MeOH-H_2O = 55:45, 2.0 ml min^{-1}, UV 210 nm) of the 50% MeOH fraction (217.9 mg) afforded 15.0 mg of **2**. Compound **3** (4.0 mg) was isolated from the 70% MeOH fraction (30.4 mg) by preparative HPLC (ODS, MeOH-H_2O = 75:25), and **4** (15.1 mg) and **6** (12.0 mg) were obtained from the 60% MeOH fraction (181.2 mg) by preparative ODS HPLC (MeOH-H_2O = 66:34). Preparative HPLC (ODS, MeOH-H_2O = 33:67) of the 30% MeOH fraction (174.6 mg) yielded 5.5 mg of **5**.

The structures of compounds **2**–**6** were identified by comparing their spectroscopic data with the reported values for sporogen-AO 1 (**2**), 3-acetyl-13-deoxyphomenone (**3**), 6-dehydropetasol (**4**), 7-hydroxypetasol (**5**) and petasol (**6**)[6–12] (Figure 1a).

Compound **1** was obtained as pale yellow solid. The molecular formula of **1**, $C_{13}H_{14}NO_5Cl$, was deduced from high-resolution electron ionization mass spectrometry (HREIMS; *m/z* 299.0555 $(M)^+$, Δ –0.6 mmu) and NMR data. Compound **1** showed UV absorptions at 202 nm (log ε 3.69) and 280 nm (log ε 2.80). IR absorptions at 1635–1739 and 3419 cm^{-1} suggested the presence of carbonyl and hydroxy groups in the molecule. The ^1H and ^{13}C NMR signals were assigned by analyzing ^1H-^1H COSY, heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC) spectra (Table 1). The ^1H-^1H COSY spectrum of **1** revealed a 1,3,4-trisubstituted benzene ring (partial structure I) and partial structure II as shown by bold lines in Figure 1b. The ^{13}C NMR data of **1** showed the presence of three amide/ester carbonyl carbons at δ 173.8, 174.9 and 175.2. Partial structures I and II, three carbonyls and an OMe group were connected from the HMBC data (Figure 1b). NMR data measured in acetone-d_6 (Table 1) due to the 2-(3-chloro-4-hydroxyphenyl)acetylamide moiety

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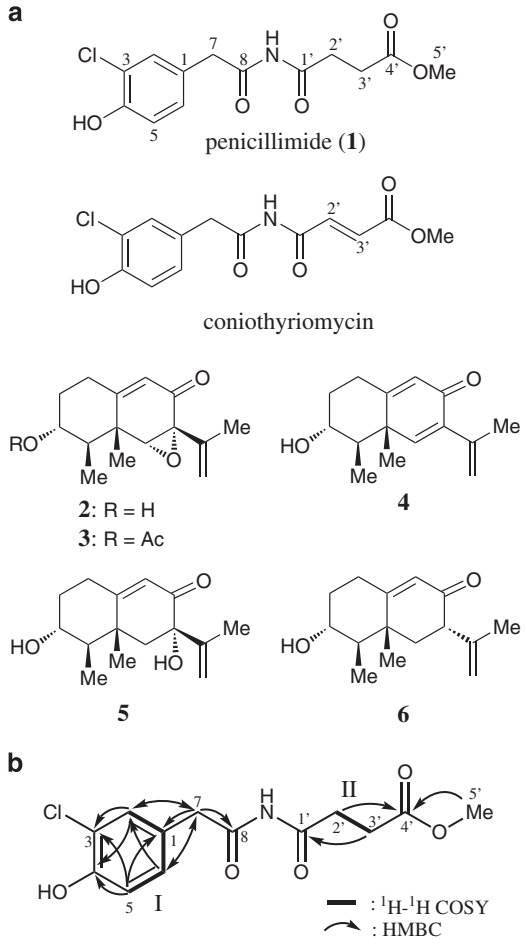

Figure 1 (**a**) Structures of compounds **1**–**6** isolated from *Penicillium copticola* TPU1270 and coniothyriomycin. (**b**) ^{1}H-^{1}H COSY and heteronuclear multiple bond correlation (HMBC) correlations for compound **1**.

in **1** were very similar to those of the same moiety in coniothyriomycin, previously identified as an antifungal metabolite from the fungus *Coniothryrium* sp.[13] A difference in the structures of compound **1** and coniothyriomycin was detected at the dicarboxylic acid units (Figure 1a). Compound **1** had the succinic acid unit, whereas coniothyriomycin had the fumaric acid unit ($\Delta^{2'}$-succnic acid unit). Thus, the structure of compound **1** was assigned as shown in Figure 1. Compound **1** was found in the SciFinder as a commercially available reagent for drug discovery, and therefore this study is the first to report compound **1** as a fungal fermentation product. Compound **1** might be named as dihydro-coniothyrionmycin, but a suffix 'mycin' will not be suitable as compound **1** was a fungal metabolite. Accordingly, compound **1** was named as penicillimide.

The antifungal activities of compounds **1**–**6** were evaluated against *M. hiemalis* IAM 6088 and *Aspergillus fumigatus* IAM 13869 using the paper disc method (Supplementary Table S1). The culture broth of strain TPU1270 inhibited the growth of *M. hiemalis*, and this activity was reproduced by compounds **2**–**4** and **6**. Compounds **2**, **4** and **6** displayed inhibition zones of 7, 11 and 8 mm, respectively, at 40 μg per disc, whereas compound **3** showed modest activity (9 mm at 80 μg per disc). These compounds also inhibited the growth of *A. fumigatus*. Penicillimide (**1**) and compound **5** were not active against either test fungi at 80 μg per disc. Amphotericin B, a positive control, showed

Table 1 ^{13}C (100 MHz) and ^{1}H (400 MHz) NMR data for compound **1** in CD_3OD and acetone-d_6

C	δ_C[a]	δ_H, mult. (*J* in Hz)[a]	*HMBC*[a]	δ_C[b]	δ_H, mult. (*J* in Hz)[b]
1	127.9			128.3	
2	132.1	7.22, d (2.1)	3, 4, 6, 7	132.1	7.31, d (2.2)
3	121.7			121.1	
4	153.6			153.1	
4-OH	—			—	8.67, s
5	117.8	6.86, d (8.3)	1, 3, 4, 6	117.7	6.95, d (8.3)
6	130.3	7.03, dd (2.1, 8.3)	2, 4, 7	130.4	7.10, dd (2.2, 8.3)
7	43.3	3.67, s	1, 2, 6, 8	43.1	3.81, s
8	173.8			172.4	
8-NH	—			—	9.78, s
1′	174.9			173.7	
2′	33.1	2.84, dd (5.7, 7.2)	1′, 3′, 4′	33.1	2.94, dd (6.5, 6.5)
3′	29.1	2.61, dd (5.7, 7.2)	1′, 2′, 4′	28.8	2.61, dd (6.5, 6.5)
4′	175.2			174.1	
5′′	52.4	3.66, s	4′	52.0	3.61, s

Abbreviation: HMBC, heteronuclear multiple bond correlation.
[a]NMR spectra were measured in CD_3OD.
[b]NMR spectra were measured in acetone-d_6.

inhibition zones of 12 and 13 mm at 10 μg per disc against *M. hiemalis* and *A. fumigatus*, respectively.

Although penicillimide (**1**) did not exhibit antifungal activity, coniothyriomycin ($\Delta^{2'}$-analog of **1**) was previously shown to be active against several fungal strains.[13,14] Therefore, a double bond at the side chain will be necessary for the antifungal activities of these imides.[14]

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Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)