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Pterocidin, a Cytotoxic Compound from the Endophytic *Streptomyces hygroscopicus*

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Abstract A new cytotoxic compound, pterocidin, was isolated from the endophytic *Streptomyces hygroscopicus* TP-A0451, and the structure was determined on the basis of spectroscopic data. Pterocidin showed cytotoxicity against some human cancer cell lines with IC_{50} values of 2.9~7.1 μM.

Keywords pterocidin, *Streptomyces*, cytotoxic, δ-lactone

Actinomycetes associated with plants are recognized as an emerging source of novel natural products [1~3]. We have previously reported the isolation of pteridic acids A and B with plant growth promoting activity from the endophytic *Streptomyces hygroscopicus* TP-A0451 [1]. This strain has been so far identified to produce structurally diverse secondary metabolites; an antifungal prenylated indole [2], a sulfonated linear polyene antibiotic with antifungal activity [3], galbonolides A and B [4, 5], elaiophylin [6] and its derivatives, and herbimycins [7] and their hydroquinone congeners. Our continuous search for bioactive compounds from the strain TP-A0451 led to the isolation of pterocidin (**1**), a new cytotoxic compound. We herein describe the isolation and structure elucidation of **1**.

Ten liters of the culture broth of *S. hygroscopicus* TP-A0451 were extracted with 1-butanol, and the extract was fractionated on a silica gel column and further subjected to C-18 column chromatography to yield pterocidin (**1**, 11 mg) as pale yellow oil.

The molecular formula of **1** was established to be $C_{23}H_{34}O_6$ based on ^{13}C NMR and HRFABMS [*m/z* 429.2260, $(M+Na)^+$, Δ +0.7 mmu]. IR absorption of **1** indicated the presence of hydroxyl (3450 cm^{-1}) and α,β-unsaturated lactone carbonyl (1720 cm^{-1}) groups. The UV absorption [λ_{max} (MeOH) 229 (ε 22,900) nm] of **1** was indicative of an α,β-unsaturated carbonyl chromophore. The ^1H and ^{13}C NMR, DEPT, and HMQC spectra of **1** in $CDCl_3$ (Table 1) revealed the presence of signals due to one carbonyl carbon, one oxygenated sp^2 quaternary carbon, nine sp^2 methines, four sp^3 methines adjacent to oxygen atoms, one sp^3 methine, two sp^3 methylenes, three methoxy groups, and two methyls. Since six out of 7 unsaturations were accounted for, **1** was inferred to possess one ring.

DQF-COSY spectrum revealed two ^1H-^1H connectivities from H-2 to H-13 branching H-22 at C-12 and from H-15 to H-19. The HMBC correlations for H-15/C-13 and H-15/C-14 suggested the connectivity between C-13 and C-

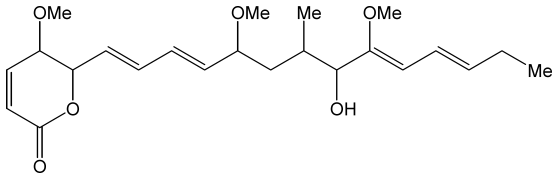

Fig. 1 Structure of pterocidin (**1**).

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Table 1 Physico-chemical properties of pterocidin

Appearance	Pale yellow oil
$[\alpha]_D^{20}$	−27.7° (*c* 0.46, $CHCl_3$)
HRFAB-MS	
Found	429.2260 $(M+Na)^+$
Calcd.	429.2253 (for $C_{23}H_{34}O_6Na$)
Molecular formula	$C_{23}H_{34}O_6$
UV (MeOH) λ_{max} nm (ε)	229 (22,900), 275 (2,500)
IR (neat) ν_{max} cm^{-1}	3450, 1720

Table 2 NMR assignment for pterocidin ($CDCl_3$)

Position	δ ^{13}C	δ ^{1}H (mult., *J* in Hz)
1	162.6	
2	123.3	6.16 (dd, 10.0, 0.7)
3	143.1	6.94 (dd, 10.0, 4.4)
4	71.2	4.02 (ddd, 4.4, 4.2, 0.7)
5	79.8	4.98 (ddd, 6.8, 4.2, 1.0)
6	125.8	5.89 (dd, 15.4, 6.8)
7	134.1	6.44 (ddd, 15.4, 10.5, 1.0)
8	131.4	6.24 (dd, 15.4, 10.5)
9	135.9	5.61 (dd, 15.4, 7.8)
10	79.7	3.73 (m)
11	39.6	1.60 (m), 1.66 (m)
12	33.1	1.96 (m)
13	74.5	4.06 (br d, 3.4)
14	155.3	
15	111.8	5.58 (d, 11.0)
16	122.4	6.34 (ddt, 15.4, 11.0, 1.5)
17	134.8	5.67 (dt, 15.4, 6.6)
18	26.0	2.10 (m)
19	13.7	1.02 (t, 6.8)
20	57.2	3.43 (s)
21	56.3	3.27 (s)
22	13.6	0.89 (d, 6.8)
23	60.0	3.70 (s)

14. The NOE between H-13 and H-15 suggested the *Z*-configuration at C-14/C-15. The coupling constants between H-6 and H-7 (J=15.4 Hz), H-8 and H-9 (J=15.4 Hz), and H-16 and H-17 (J=15.4 Hz) indicated that the configurations at C-6/C-7, C-8/C-9, and C-16/C-17 are *trans*. HMBC correlations for H-20/C-4, H-21/C-10, and H-23/C-14 confirmed the methoxy substituents at these carbons. The existence of α,β-unsaturated δ-lactone ring was indicated by the chemical shift of H-5 (δ 4.98) implying the connectivity between O-5 and the carbonyl C-1 and the coupling constant for H-2/H-3 (J=10 Hz)

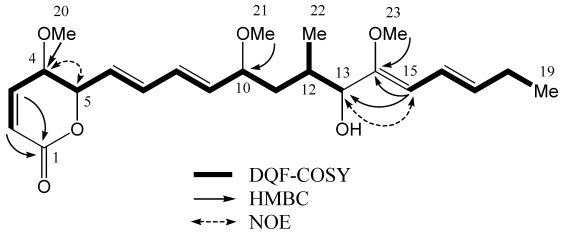

Fig. 2 Selected 2D NMR correlations and NOE observed for pterocidin (**1**).

that supports the *cis*-configuration for C-2/C-3. Thus, the structure of pterocidin was elucidated to be **1**. As for the stereochemistry, the relative configuration at C-4/C-5 was suggested to be *cis* by the strong NOESY correlation between H-4 and H-5. Absolute configuration of **1** is currently under investigation.

Pterocidin (**1**) is a new compound possessing an α,β-unsaturated γ-oxygenated δ-lactone at one end of the molecule with five chiral centers. Similar polyketide-derived δ-lactones have been isolated from actinomycetes [8~12] and marine sponges [13, 14] and many of them show antitumor activity. The structure of **1** is closely related to sultriecin [8], PD 113271 [9], and pironetin [10], antitumor compounds produced by *Streptomyces* species. Pterocidin (**1**) showed cytotoxicity against the cancer cell lines NCI-H522, OVCAR-3, SF539, and LOX-IMVI with IC_{50} values of 2.9, 3.9, 5.0, and 7.1 μM, respectively, but no significant activity was exhibited against microorganisms.

Experimental

General Experimental Procedures

Optical rotation was recorded on a JASCO DIP-1030 polarimeter. The IR and UV spectra were taken on Shimadzu FT-IR300 and Hitachi U-3210 spectrophotometers, respectively. NMR experiments were performed on a JEOL JNM-LA400 NMR spectrometer with TMS as an internal standard. FAB mass spectra were obtained on a JEOL HX-110 spectrometer.

Fermentation and Isolation

Isolation and fermentation of the producing strain *S. hygroscopicus* TP-A0451 was previously described [1, 15]. In brief, strain TP-A0451 was cultivated in a liquid medium, and the cultured whole broth (10 liters) was extracted with 1-butanol (10 liters). The organic layer was concentrated *in vacuo* to yield crude solid (33.7 g). The solid was suspended in CH_3CN and the soluble part was collected by filtration and evaporated to give dark brown oil

(6.6 g). This was chromatographed over silica gel column ($CHCl_3$ - MeOH = 100 : 1 ~ 1 : 1) and the fraction eluted with $CHCl_3$ - MeOH = 20 : 1 was collected and evaporated to give crude material (648 mg). It was further purified on C-18 column (200×40 mm, i.d., ODS-AM 120-S50, YMC Co., Ltd.) with a stepwise gradient of 20~80% CH_3CN in 0.15% K_2HPO_4 buffer (pH 3.5). Fractions containing pterocidin (eluted with 60% CH_3CN in buffer) was evaporated *in vacuo* and the remaining aqueous solution was extracted with EtOAc. The organic layer was evaporated to dryness to yield pterocidin (11 mg).

Cytotoxic Assay

IC_{50} values of pterocidin against human cancer cell lines were determined in the same manner as described [16].

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