NOTES

Cytotoxic Metabolites Produced by a Fungal Strain from a *Sargassum* Alga

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We have focussed our attention on new antitumor metabolites from microorganisms which inhabit the marine environment.[1] As part of this program, we have found that a strain of *Penicillium waksmanii* Zaleski OUPS-N133 separated from the brown alga *Sargassum ringgoldianum* produces two novel compounds, pyrenocines D (**1**), and E (**2**), along with the known compounds, two pyrenocines A (**3**) and B (**4**), and three dioxopiperazine derivatives (**5**)~(**7**). We describe herein the isolation, structure elucidation and cytotoxicity of these metabolites.

The producing microorganism was cultured at 27°C for 3 weeks in a medium (23 liters) containing 1% malt extract, 1% glucose and 0.05% peptone in artificial seawater adjusted to pH 7.5. The mycelium and the culture filtrate were extracted with MeOH and AcOEt, respectively. The resulting MeOH and AcOEt extracts exhibited ED_{50} values of 38 and $<1\ \mu g/ml$ in the P388 lymphocytic leukemia test system in cell culture, respectively. The AcOEt extract was purified by bioassay-directed fractionation employing a combination of several column chromatographies. The extract (2.3 g) was passed through Sephadex LH-20 (3.0 × 33 cm), using CH_2Cl_2 - MeOH (1 : 1) as the eluent. The second fraction (2.0 g), in which cytotoxic activity was concentrated, was subjected to normal phase MPLC (silica gel, 2.0 × 23 cm) with a CH_2Cl_2 - MeOH gradient as the eluent to give the CH_2Cl_2 eluate (A, 112.8 mg), and two fractions B (705.5 mg) and C (109.8 mg) eluted with MeOH - CH_2Cl_2 (1 : 99). Fraction A was purified by reversed-phase HPLC (Shim-pack PREP-ODS, 2.0 × 25 cm) using MeOH - H_2O (4 : 1) as the eluent to afford **3** (67.8 mg) and **6** (5.4 mg). Fraction B was further chromatographed on a silica gel column (3.5 × 20 cm), followed by repeated HPLC (Shim-pack PREP-ODS, 2.0 × 25 cm) using MeOH - H_2O (4 : 1) and MeOH - H_2O (2 : 3) to afford **1** (5.2 mg), **3** (418.9 mg), **2** (23.7 mg) and **4** (111.0 mg). Fraction C yielded **4** (3.7 mg), **5** (3.8 mg) and **7** (3.8 mg) after purification by HPLC (Shim-pack PREP-ODS, 2.0 × 25 cm) using MeOH - H_2O (4 : 1) as the eluent.

The physico-chemical properties of compounds **1** and **2** are summarized in Table 1. Pyrenocine D (**1**) was assigned a molecular formula of $C_{11}H_{12}O_4$ as deduced from an M^+ peak in HREI-MS. A close inspection of the ^1H and ^{13}C NMR spectral data (Table 2) of **1** by DEPT and ^1H-^{13}C COSY experiments revealed the presence of two carbonyls for a lactone (C-2) and an aldehyde (C-12), a methoxyl group (C-8), two allylic methyls (C-7 and C-11), two sp^2-hybridized methines (C-3 and C-10), and four quaternary sp^2-carbons (C-4, C-5, C-6 and C-9) including two oxygen-bearing carbons (C-4 and C-6). The carbon signal for one (C-3) of two sp^2-methines appeared shifted more upfield than that of a general sp^2-methine, and the chemical shifts (δ_C 87.67 and 169.40) for C-3 and C-4 were found to be comparable to those (δ_C 91.4 and 165.8) of C-3 and C-4 in 3-hydroxycoumarin,[2] suggesting the presence of 3-methoxy-α-pyrone ring in **1**. The presence of the methoxyl group at C-4 was supported by long-range (LR) ^1H-^{13}C COSY correlations between C-4 and H-8. Assignments of C-5 and C-6 in the α-pyrone ring and the connection of C-6 and the methyl group (C-7) were demonstrated

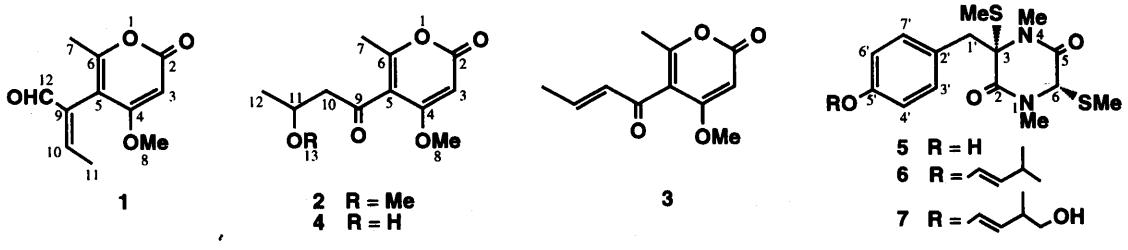

Table 1. Physico-chemical properties of pyrenocines D (**1**) and E (**2**).

	1	**2**
Appearance	Colorless needles	Colorless powder
Mp	112–114°C	82–84°C
$[\alpha]_D$		0° (12°, *c* 0.52, $CHCl_3$)
Molecular formula	$C_{11}H_{12}O_4$	$C_{12}H_{16}O_5$
HREI-MS	Calcd. for $C_{11}H_{12}O_4$: 208.0735 Obsd: 208.0731 $[M]^+$	Calcd. for $C_{12}H_{16}O_5$: 240.0996 Obsd: 240.0998 $[M]^+$
UV λ_{max}^{EtOH} nm (log e)	220 (4.14, sh), 282 (3.62)	215 (3.63, sh), 250 (3.29), 280 (3.06, sh)
IR υ_{max}^{KBr} cm^{-1}	2822, 2724, 1719, 1688, 1656, 1633, 1560	1742, 1694, 1628, 1563
Rf value on TLC	0.525 (CH_2Cl_2 - MeOH, 19:1, silica gel)	0.575 (CH_2Cl_2 - MeOH, 19:1, silica gel)
Solubility Soluble:	DMSO, MeOH, $CHCl_3$, acetone, AcOEt	DMSO, MeOH, $CHCl_3$, acetone, AcOEt
Insoluble:	H_2O	H_2O

Table 2. ^1H and ^{13}C NMR data of **1** and **2** in $CDCl_3$[a].

No.	**1**				**2**			
	δ_H	*J* Hz	δ_C	LR ^1H-^{13}C COSY(H)[b]	δ_H	*J* Hz	δ_C	LR ^1H-^{13}C COSY(H)
2			164.40 (q)[c]				163.50 (q)	
3	5.49 s		87.67 (t)		5.48 s		87.64 (t)	
4			169.40 (q)	8			168.40 (q)	8
5			104.99 (q)	3, 7, 12			115.91 (q)	3, 7
6			160.19 (q)	7			162.66 (q)	7
7	2.02 s		17.92 (p)		2.25 s		18.91 (p)	
8	3.73		56.24 (p)		3.86 s		56.39 (p)	
9			136.99 (q)	12			199.45 (q)	10A, 10B
10	7.05 q	7.0 (11)[d]	154.27 (t)	11	2.75 dd (A)	15.8 (10B), 4.7 (11)	51.59 (s)	12
					2.93 dd (B)	15.8 (10A), 8.4 (11)		
11	1.92 d	7.0 (10)	16.20 (p)		3.84 m		73.73 (t)	12, 13
12	9.53 s		191.91 (t)		1.19 d	6.0 (11)	18.34 (p)	
13					3.28 s		56.22 (p)	11

[a] Measured at 300 and 75.4 MHz for ^1H and ^{13}C, respectively.

[b] LR ^1H-^{13}C COSY correlations from C to H.

[c] Letters, p, s, t and q, in parentheses indicate, respectively, primary, secondary, tertiary and quaternary carbons, assigned by DEPT.

[d] Figures in parentheses indicate a proton coupling with that in question.

on the basis of LR ^1H-^{13}C COSY correlations from C-5 to H-3 and H-7, and from C-6 to H-7. In addition, the presence of a 2-butenal moiety (C-9～C-12) and the connection of C-5 and C-9 were deduced from the coupling relationship between H-10 and H-11, and LR ^1H-^{13}C COSY correlations from C-5 to H-12 and from

C-9 to H-12. The orientation of the Δ^9-double bond was based on the observation of an NOE between H-11 and H-8. The above evidence led to the structure **1** for pyrenocine D.

Pyrenocine E (**2**) had the molecular formula $C_{12}H_{16}O_5$ established by HREI-MS. The ^1H and ^{13}C NMR spectra (Table 2) of **2** exhibited signals for one secondary methyl (C-12), methylene (C-10), oxygen-bearing sp^3-methine (C-11), methoxy (C-13) and conjugated ketone (C-9) each in addition to signals corresponding to carbons anlogous to C-2 through C-8 of compound **1**. ^1H-^1H COSY and LR ^1H-^{13}C COSY correlations (C-9/H-10, C-10/H-12, and C-11/H-13) of **2** revealed that a side chain of the α-pyrone ring is a 3-methoxy-1-oxobutyl group. This finding allowed assignment of the structure **2** to pyrenocine E.

The known compounds, pyrenocines A (**3**)[3] and B (**4**),[3] and *cis*-bis(methylthio)silvatin (**6**)[4] and its derivatives **5**[4] and **7**[5] were identified by comparison of their spectral data with published values. Since compounds **2** and **4** showed no optical activity, they may be artifacts derived from **3**.

The cytotoxic activities of compounds **1**~**7** were examined in the P388 lymphocytic leukemia test system, according to the method reported previously.[6] Among them, compounds **2**~**4** exhibited significant cytotoxicity (ED_{50} values 1.30, 0.16 and 1.40 μg/ml, respectively).

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