LEPTOSINS I AND J, CYTOTOXIC SUBSTANCES PRODUCED BY A *Leptosphaeria* sp.

PHYSICO-CHEMICAL PROPERTIES AND STRUCTURES

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Leptosins I and J, belonging to a series of epipolythiodioxopiperazines, have been isolated from the mycelium of a strain of *Leptosphaeria* sp. OUPS-4 attached to the marine alga *Sargasssum tortile*. Their relative stereostructures have been elucidated by chemical and spectral evidence. These compounds exhibited significant cytotoxic activity against cultured P388 cells.

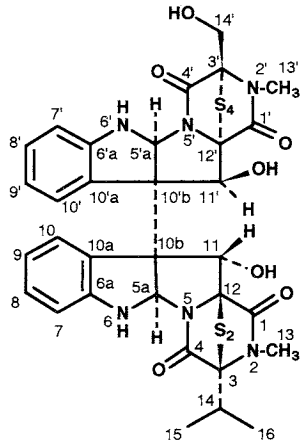
Some bioactive compounds isolated from marine animals have proven to be produced by bacteria[1~4]. These reports led us to search for new antineoplastic materials from microorganisms inhabiting the marine environment. As part of this program, we have already reported that the antitumour and cytotoxic compounds, leptosins A (**1**)~H, belonging to a series of epipolythiodioxopiperazines, were produced by a strain of *Leptosphaeria* sp. OUPS-4 isolated from the marine alga *Sargasssum tortile* C. Agaroh (Sargassaceae) (Fig. 1)[5,6]. Our continuing search for cytotoxic metabolites from this fungal strain led to the isolation of novel epipolythiodioxopiperazines designated leptosins I (**2**) and J (**3**) (Fig. 1). In this paper, we describe the physico-chemical properties, structure elucidation and cytotoxic activity of these compounds.

Results and Discussion

The fungal strain was cultured in a medium containing glucose, peptone and yeast extract in artifical seawater. The MeOH extract of the mycelium was purified by bioassay-directed fractionation employing a combination of Sephadex LH-20 and silica gel column chromatographies and HPLC to afford leptosins I (**2**) and J (**3**).

Leptosin I (**2**) is a pale yellow powder with the properties listed in Table 1. The molecular formula of **2** was established as $C_{32}H_{32}N_6O_7S_4$ by HRFAB-MS (m/z 741.1301 $(MH)^+$, $\Delta+0.8$). A close inspection of the ^1H and ^{13}C NMR spectra of **2** (Table 2) by DEPT and ^1H-^1H and ^1H-^{13}C COSY experiments revealed signals for the following functionalities as observed in leptosins A (**1**)~C reported previously[5]: two oxygen-bearing methine groups (C-11 and C-11') linked to two quaternary sp^3-hybridized carbons, two methines (C-5a and C-5'a) bearing two nitrogens and a quaternary sp^3-carbon, two quaternary sp^3-carbons (C-3 and C-12) each bearing a nitrogen and a sulfur, four amides (C-1, C-4, C-1' and C-4'),

Fig. 1. Structure of leptosins and derivatives.



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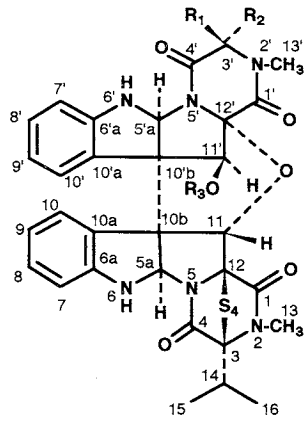
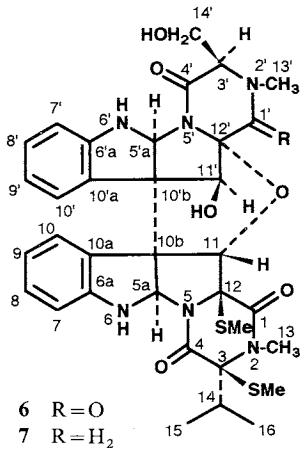


2 $R_1=CH_2OH$, $R_2=R_3=H$
3 $R_1=R_3=H$, $R_2=CH_2OH$
4 $R_1=CH_2OAc$, $R_2=H$, $R_3=Ac$
5 $R_1=H$, $R_2=CH_2OAc$, $R_3=Ac$



6 R=O
7 $R=H_2$

Table 1. Physico-chemical properties of leptosins.

	Leptosin I	Leptosin J
Appearance	Pale yellow powder	Pale yellow powder
MP	218~220°C	215~216°C
$[\alpha]_D$	+212° (24°C, *c* 0.13, $CHCl_3$)	+188° (24°C, *c* 0.21, $CHCl_3$)
Molecular formula	$C_{32}H_{32}N_6O_7S_4$	$C_{32}H_{32}N_6O_7S_4$
HRFAB-MS	Calcd. for $C_{32}H_{33}N_6O_7S_4$: 741.1293 Obsd: 741.1301 $(M+H)^+$	Calcd. for $C_{32}H_{33}N_6O_7S_4$: 741.1293 Obsd: 741.1305 $(M+H)^+$
UV λ^{EtOH} nm ($\log\varepsilon$)	209 (4.45), 240 (3.91), 305 (3.45)	209 (4.45), 240 (3.94), 298 (3.44)
IR ν_{max} (KBr) cm^{-1}	3510, 1667, 1610, 1591	3479, 1667, 1610, 1594
CD λ^{EtOH} nm ($\Delta\varepsilon$)	235 (+23.9), 249 (+1.7), 269 (+16.9), 298 (+3.9), 312 (+5.2), 339 (−5.2) (*c* 1.74×10^{-5} M in EtOH)	233 (+20.9), 248 (+3.1), 266 (+13.6), 295 (+5.5), 309 (+6.8), 337 (−4.3) (*c* 2.46×10^{-5} M in EtOH)
Rf value on TLC	0.477 ($CHCl_3$-MeOH, 9:1, silica gel)	0.452 ($CHCl_3$-MeOH, 9:1, silica gel)
Solubility Soluble:	DMSO, MeOH, $CHCl_3$	DMSO, MeOH, $CHCl_3$
Insoluble:	H_2O, Hexane	H_2O, Hexane

two *N*-methyl groups (C-13 and C-13′), isopropyl (C-14, C-15 and C-16) and hydroxymethyl (C-14′) groups, two 1,2-disubstituted benzenes (C-6a to 10a and C-6′a to C-10′a), each bonding to an amino group as one substituent, and two benzylic quaternary sp^3-carbons (10b and 10′b). Additionally, signals for one methine (C-3′) and one quaternary sp^3-carbon (C-12′) appeared at δ_C 66.8 and δ_C 92.2, respectively. The C-3′ proton resonated as a broad singlet signal at δ_H 3.97 in **2**, and was coupled to the proton signals of the hydroxymethyl group at δ_H 4.06 (dd, J=10.5, 9.0 Hz, 14′-H) and δ_H 4.52 (dd, J=10.5, 3.5 Hz, 14′-H) to appear as a doublet doublet signal at δ_H 4.28 (dd, J=9.0, 3.5 Hz, 3′-H) in the acetate (**4**) of **2** described below (Table 3), indicating the C-3′ methine to be adjacent to the C-14′ hydroxymethyl. The lowfield

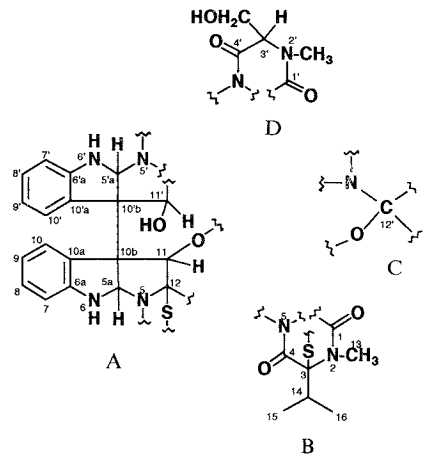
Table 2. ^{1}H and ^{13}C NMR spectral data of leptosin I (**2**)[a].

Position	δ_H[b]	NOESY	δ_C	HMBC[c]
1			165.65 (q)[d]	
3			82.30 (q)	
4			167.92 (q)	
5a	6.43 d (3.9)	5′a	79.44 (t)	6a, 11, 12, 10′b
6	4.28 br s			
6a			150.11 (q)	
7	6.25 d (7.8)		110.35 (t)	9, 10a
8	7.19 t (7.8)		131.17 (t)	6a, 10
9	6.91 t (7.8)		119.58 (t)	7, 10a
10	7.75 d (7.8)	11, 10′, 11′	124.84 (t)	6a, 8
10a			125.89 (q)	
10b			64.18 (q)	
11	5.14 s	10, 11′	87.02 (t)	5a, 10a
12			78.15 (q)	
13	2.98 s	14, 15, 16	29.92 (p)	1, 3
14	2.69 heptet (6.8)	13	36.17 (t)	3, 4, 15
15	1.16 d (6.8)	13	18.01 (p)	3, 14, 16
16	1.48 d (6.8)	13	18.67 (p)	3, 14, 15
1′			161.98 (q)	
3′	3.97 br s	13′	66.75 (t)	1′, 4′
4′			166.63 (q)	
5′a	5.87 d (5.5)	5a	79.19 (t)	10b, 11′
6′	5.36 br s			
6′a			152.39 (q)	
7′	6.62 d (7.8)		111.35 (t)	9′
8′	7.04 t (7.8)		130.36 (t)	6′a, 10′
9′	6.45 t (7.8)		120.21 (t)	7′, 10′a
10′	6.04 d (7.8)	10	126.28 (t)	6′a, 8′
10′a			120.21 (q)	
10′b			63.82 (q)	
11′	5.35 d (5.4)	10, 11	73.82 (t)	10b, 5′a
12′			92.22 (q)	
13′	2.99 s	3′, 14′	32.56 (p)	1′, 3′
14′	3.93 br s	13′	60.65 (s)	4′
11′-OH	3.07 br s			
14′-OH	3.24 br s			

[a] Signal assignments were based on ^{1}H-^{1}H and ^{1}H-^{13}C COSY and HMBC spectra.
[b] ^{1}H chemical shift values (δ ppm) followed by multiplicity and then the coupling constant (J/Hz) in parentheses.
[c] Long range ^{1}H-^{13}C correlations from H to C observed in the HMBC experiment.
[d] Letters, p, s, t and q , in parentheses indicate respectively primary, secondary, tertiary and quaternary carbons, assigned by DEPT.

resonance of the C-12′ carbon at δ_C 92.2 was ascribed to a carbon bearing both oxygen and nitrogen atoms because carbons bearing both sulfur and nitrogen atoms resonated at δ_C 75 to δ_C 83 in related compounds[5,6]. HMBC correlations (Table 2) for the functional groups thus established led to partial structures A, B, C and D (Fig. 2). Typical HMBC correlations are as follows; 5a-H to C-6a, C-11, C-12 and C-10′b, 11-H to C-5a and C-10a, 5′a-H to C-10b and C-11′, 13-H to C-1 and C-3, 14-H to C-4, 15-H to C-3, 13′-H to C-1′ and C-3′, and 14′-H to C-4′.

Fig. 2. Partial structures of **2**.



Treatment of **2** with $NaBH_4$ and CH_3I gave two bis (methylthio) derivatives **6** and **7** (Fig. 1), which had the molecular formulae $C_{34}H_{38}N_6O_7S_2$ and $C_{34}H_{40}N_6O_6S_2$ established by $(M+1)^+$ peaks in FAB-MS, respectively. The ^1H and ^{13}C NMR signals of **6** (Tables 3 and 4) were similar to those of **2** except for additional signals for two *S*-methyl groups. These findings indicated that **6** was produced by reduction followed by methylation of a tetrasulfide bridge. In the ^1H NMR spectrum of **7**, one (13′-H) of *N*-methyl signals and 3′-H was found shifted upfield by 0.6 and 1.1 ppm, respectively, relative to those of **6**, and one additional methylene signal appeared at δ_H 3.27 (d, $J=12.0$ Hz, 1′-H) and δ_H 3.42 (d, $J=12.0$ Hz, 1′-H) (Table 3), as deduced from the ^1H-^1H COSY spectrum. This evidence indicated that one (C-1′) of the amide carbonyl groups in **6** was replaced by a methylene in **7**. The reason why the C-1′ amide carbonyl group was reduced by $NaBH_4$ is not known. However, the formation of **6** and **7** from **2** demonstrated that **2** had a tetrasulfide bridge and that the sulfur-bearing carbons were C-3 and C-12, supporting that C-12′ is linked to an oxygen atom.

Standard acetylation of **2** with acetic anhydride-pyridine produced the diacetate derivative **4** in which the C-11′ and C-14′ proton signals appeared shifted downfield by approximately 1.23 and 0.15~0.61 ppm, respectively (Table 3), and the C-11 proton signal remained essentially unchanged. This confirmed the presence of a secondary hydroxy group at C-11′ in addition to a primary hydroxy group at C-14′, and established that C-11 is linked to an ether oxygen. From the molecular formula of **2**, it was deduced that the ether linkage is between C-11 and C-12′.

The presence of one tetrasulfide bridge and one ether linkage in **2** as described above led to the connection of partial structures B and C to both N-5 and C-12, and C-11 in partial structure A, respectively, and the remaining partial structure D was therefore connected to N-5′, C-11′ and C-12′ in the combined structure of A, B and C to lead to planar structure of **2** for leptosin I.

The relative chemistry for **2** was deduced from detailed analysis for the NOESY spectra of **2** and **6** (Tables 2 and 3), which exhibited similar cross peaks except for those for the *S*-methyls. NOE correlations for 12-SCH_3/11-H and 10-H/11-H in **6** implied that 12-SCH_3,11-H and the C-10a~C-10b bond are oriented *cis* to one another, and the C-10a~C-10b bond must be therefore *cis* to the N-6~C-5a bond. In other words, the C-10b~C-10′b bond and 5a-H are both *trans* to 11-H. In addition, NOEs for 11′-H/11-H and 11′-H/10-H showed 11′-H to be oriented *cis* to the C-10′b~C-10b bond and the C-12′ ether linkage, while an NOE between 5a-H and 5′a-H indicated that 5a-H and 5′a-H are both *cis* to the C-10′b~C-10b bond.

Table 3. ^{1}H NMR spectral data of leptosin J (**3**) and derivatives (**4**~**7**), and NOESY correlations for **3** and **6**[a].

H	3	NOESY	4	5	6	NOESY	7
5a	6.31 d (3.4)	5′a	6.39 d (3.6)	6.42 br s	6.14 d (3.6)	5′a	6.16 d (3.5)
6	4.26 br s		4.33 br d (3.6)	4.35 br s	4.65 d (3.6)		4.82 d (3.5)
7	6.23 d (7.8)		6.26 dd (7.5, 0.7)	6.28 br s	6.50 dd (7.5, 0.8)		6.57 d (7.8)
8	7.16 t (7.8)		7.19 td (7.5, 0.7)	7.19 t (7.8)	7.28 td (7.5, 0.8)		7.27 t (7.8)
9	6.92 t (7.8)		6.97 td (7.5, 0.7)	6.97 t (7.8)	6.94 td (7.5, 0.8)		6.89 t (7.8)
10	7.73 d (7.8)	11, 10′, 11′	8.06 dd (7.5, 0.7)	8.06 d (7.8)	7.82 dd (7.5, 0.8)	11, 10′, 11′	7.58 d (7.8)
11	5.22 s	10, 11′	5.23 s	5.26 s	5.30 s	10, 11, 12-SMe	5.05 s
13	3.01 s	14, 15, 16	3.07 s	3.04 s	3.01 s	3-SMe, 14, 15, 16	3.07 s
14	2.67 heptet (6.8)	13	2.78 heptet (6.8)	2.70 heptet (6.8)	2.43 heptet (6.8)	13	2.46 heptet (6.8)
15	1.04 d (6.8)	13	1.12 d (6.8)	1.06 d (6.8)	1.02 d (6.8)	13, 5′a	0.99 d (6.8)
16	1.44 d (6.8)	13	1.47 d (6.8)	1.45 d (6.8)	1.26 d (6.8)	13	1.22 d (6.8)
3-SMe					2.12 s	13	2.13 s
12-SMe					2.02 s	11	2.05 s
1′							3.27 d (12.0)
							3.42 d (12.0)
3′	3.81 d (2.4)	13′	4.28 dd (9.0, 3.5)	4.11 t (3.0)	3.96 m		2.89 dd (5.8, 2.8)
5′a	5.76 br s	5a	5.82 d (5.9)	5.86 br s	5.78 d (5.0)	5a, 15	5.70 d (4.5)
6′	[b]		5.18 d (5.9)	5.17 br s	5.26 d (5.0)		5.16 d (4.5)
7′	6.48 d (8.0)		6.61 dd (7.8, 0.8)	6.58 d (7.8)	6.56 dd (7.8, 0.8)		6.57 d (7.8)
8′	6.99 t (8.0)		7.05 td (7.8, 0.8)	7.03 td (7.8, 1.0)	7.03 td (7.8, 0.8)		7.06 t (7.8)
9′	6.42 t (8.0)		6.40 td (7.8, 0.8)	6.36 t (7.8)	6.40 td (7.8, 0.8)		6.41 t (7.8)
10′	6.04 d (8.0)	10	6.00 dd (7.8, 0.8)	6.02 dd (7.8, 1.0)	5.93 dd (7.8, 0.8)	10	5.93 d (7.8)
11′	5.35 s	10, 11	6.65 s	6.65 s	5.41 d (5.8)	10, 11	4.89 d (7.5)
13′	2.84 s	3′, 14′	3.12 s	3.08 s	3.03 s	14′	2.47 s
14′	3.91 dd (12.5, 2.4)	13′	4.06 dd (10.5, 9.0)	4.62 dd (12.0, 3.0)	3.96 m	13′	4.02 br s
	4.11 d (12.5)		4.52 dd (10.5, 3.5)	4.70 dd (12.0, 3.0)	3.96 m		3.80 dd (11.2, 5.8)
11′-OH	[b]				2.79 d (5.8)		1.92 d (7.5)
14′-OH	[b]				3.49 br s		4.02 br s
11′-OAc			1.52 s	1.49 s			
14′-OAc			2.14 s	2.00 s			

[a] ^{1}H chemical shift values (δ ppm) followed by multiplicity and then the coupling constant (J/Hz) in parentheses.
[b] Not detected.

Table 4. ^{13}C NMR spectral data of leptosin J (**3**) and derivatives (**4**~**6**).

C	3	4	5	6
1	165.37 (q)[a]	165.68 (q)	164.58 (q)	165.40 (q)
3	82.30 (q)	82.30 (q)	82.22 (q)	78.50 (q)
4	167.33 (q)	168.01 (q)	167.22 (q)	162.63 (q)
5a	79.16 (t)	78.83 (t)	78.79 (t)	78.88 (t)
6a	150.06 (q)	149.63 (q)	149.76 (q)	150.86 (q)
7	110.36 (t)	110.13 (t)	110.23 (t)	110.28 (t)
8	131.11 (t)	131.22 (t)	131.16 (t)	130.70 (t)
9	119.83 (t)	120.01 (t)	119.95 (t)	118.86 (t)
10	125.00 (t)	125.41 (t)	125.44 (t)	123.70 (t)
10a	125.80 (q)	125.07 (q)	125.00 (q)	126.39 (q)
10b	64.66 (q)[b]	64.85 (q)	65.10 (q)[b]	64.21 (q)[b]
11	86.38 (t)	87.39 (t)	86.99 (t)	85.49 (t)
12	78.09 (q)	77.81 (q)	77.86 (q)	70.53 (q)
13	30.23 (p)	29.90 (p)	30.11 (p)	29.57 (p)
14	35.83 (t)	35.82 (t)	35.74 (t)	37.15 (t)
15	18.22 (p)	18.63 (p)	18.25 (p)	18.00 (p)
16	18.48 (p)	18.52 (p)	18.35 (p)	18.00 (p)
3-SMe				14.02 (p)
12-SMe				16.44 (p)
1′	161.87 (q)	160.09 (q)	160.42 (q)	162.37 (q)
3′	64.66 (t)	63.37 (t)	61.51 (t)	66.25 (t)
4′	164.89 (q)	164.50 (q)	163.87 (q)	166.20 (q)
5′a	79.44 (t)	79.61 (t)	79.32 (t)	79.44 (t)
6′a	151.88 (q)	152.11 (q)	151.99 (q)	152.05 (q)
7′	110.88 (t)	111.11 (t)	110.06 (t)	110.28 (t)
8′	129.96 (t)	130.49 (t)	130.40 (t)	130.32 (t)
9′	119.65 (t)	119.54 (t)	118.93 (t)	119.66 (t)
10′	126.61 (t)	127.21 (t)	129.40 (t)	126.92 (t)
10′a	120.55 (q)	119.47 (q)	118.82 (q)	119.51 (q)
10′b	64.21 (q)[b]	63.62 (q)	63.72 (q)[b]	64.10 (q)[b]
11′	73.50 (t)	73.23 (t)	73.59 (t)	74.04 (t)
12′	92.62 (q)	90.97 (q)	90.67 (q)	92.37 (q)
13′	31.65 (p)	34.48 (p)	31.75 (p)	32.52 (p)
14′	61.05 (s)	68.26 (s)	62.07 (s)	60.81 (s)
11′-$OCOCH_3$		19.87 (p)	19.88 (p)	
11′-$OCOCH_3$		168.79 (q)	168.71 (q)	
14′-$OCOCH_3$		20.85 (p)	20.65 (p)	
14′-$OCOCH_3$		170.14 (q)	170.02 (q)	

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

[b] Assignments may be interchangeable in each column.

Furthermore, an NOE between 15-H and 5′a-H in **6** demonstrated that the isopropyl-bearing diketopiperazine ring of **6** exists in a chair conformation. These observations allowed assignment of the relative configuration of **6** and consequently **2**. The relative configuration of the C-3′ hydroxymethyl group was established by spectral comparison of **2** with **3** and an NOE experiment for **7** as described below.

Leptosin J (**3**) had properties similar to those of **2** and its molecular formula was the same as **2**. The general features of its UV, IR and NMR specra (Tables 3 and 4) closely resembled those of **2** except that the NMR signals for 3′-H, 13′-H, 14′-H, C-3′ and C-4′ revealed a chemical shift difference relative to those of **2**. In addition, the NOESY spectrum of **3** exhibited cross peaks similar to that of **2** (Tables 2 and 3). These observations showed **3** to be a stereoisomer of **2** at C-3′.

As a general rule, it has been established for a wide variety of six-membered ring systems that a signal

for an *axial* proton appears at higher field than that for an equatorial proton[7]. A reversal of this *axial-equatorial* relationship has been observed in α-bromocyclohexanones[8], steroidal α-haloketones[9], and 3-oxo derivatives of the oleanane[10] and euphane[11],† series of triterpenes. The C-3′ proton signal in **3** was found shifted upfield by 0.14 ppm, relative to that of **2**. It is most likely that the hydroxymethyl-bearing diketopiperazine rings of **2** and **3** exist in a chair conformation, and the application of the latter *axial-equatorial* relationship described above to this case suggested the C-3′ protons for **2** and **3** to be in *axial* and *equatorial* orientations, respectively. This expectation was supported by the fact that irradiation of the C-1′ *axial* proton (δ 3.27) in **7** gave an NOE enhancement to the C-3′ proton, implying that the hydroxymethyl-bearing diketopiperazine ring of **7** exists in a chair conformation. These observations elucidated the stereochemistry of **2** and **3** at C-3′, and consequently led to relative stereostructure **3** for leptosin J.

Cytotoxic activity of leptosins I (**2**) and J (**3**) were examined in the P388 lymphocytic leukemia test system in cell cuture, according to the method reported previously[12]. As a result, both **2** and **3** exhibited significant cytotoxicity (ED_{50} 1.13 and 1.25 μg/ml, respectively).

Experimental

General

MPs were obtained on a Yanagimoto micromelting point apparatus and are uncorrected. UV spectra were recorded on a Shimadzu spectrophotometer and IR spectra on a Perkin-Elmer FT-IR spectrometer 1720X. Optical rotations were obtained on a JASCO ORD/UV-5 spectropolarimeter. CD spectra were recorded on a JASCO J-500A spectrometer. NMR spectra were recorded at 27°C on a Varian XL-300 spectrometer, operating at 300 and 75.4 MHz for ^1H and ^{13}C, respectively, in $CDCl_3$ with TMS as an internal reference. The ^1H-^1H and ^1H-^{13}C COSY spectra were recorded on a Varian XL-300 spectrometer, and the HMBC and NOESY spectra on Varian UNITY-400 and Bruker ARX-500 spectrometers with the usual parameters. FAB-MS was determined using a VG ZAB-SE mass spectrometer in 3-nitorobenzyl alcohol matrix. Liquid chromatography over silica gel (mesh 230~400) was performed in a medium pressure. HPLC was run on a Waters ALC-200 instrument equipped with a differential refractometer (R 401) and Shim-pack PREP-ODS (25 cm × 20 mm i.d.). Analytical TLC was performed on precoated Merck aluminium sheets (DC-Alufolien Kieselgel 60 F_{254}, 0.2 mm), and compounds were viewed under UV lamp and sprayed with 10% H_2SO_4 followed by heating.

Production and Isolation of Leptosins

A strain of *Leptosphaeria* sp. OUPS-4, isolated from the marine alga *Sargassum tortile*, was cultured at 27°C for 3 weeks in a medium (20 liters) containing 2% glucose, 1% peptone and 0.5% yeast extract in artificial seawater adjusted to pH 7.5. As reported previously[5,6], the CH_2Cl_2-MeOH (1:1) soluble fraction (21.5 g) of the mycelium was successively chromatographed on Sephadex LH-20 (CH_2Cl_2-MeOH, 1:1) and silica gel (hexane-CH_2Cl_2-MeOH gradient). The MeOH-CH_2Cl_2 (1:49) elute (Fr. 12 (49.7 mg)) from silica gel column chromatography was purified by HPLC using MeOH-H_2O (4:1) as the eluent to afford **2** (15.4 mg) and **3** (2.1 mg).

Leptosin I Diacetate (4)

Acetylation of leptosin I (**2**) (7.4 mg) with Ac_2O/pyridine followed by chromatography on silica gel with CH_2Cl_2 as the eluent afforded **4** (4.2 mg) as a pale yellow powder; FAB-MS *m/z*: 825 $(MH)^+$. IR ν_{max} (KBr) cm^{-1}: 3522 (OH, NH), 1756 ($OCOCH_3$), 1691, 1679 (CON), 1615, 1595 (Ar. C-C). Its ^1H and ^{13}C NMR data are listed in Tables 3 and 4.

† NUMATA, A. and H. MORITO: Unpublished ^1H NMR data for the 2α (*equatorial*) and 2β (*axial*) protons of methyl kulonate, kulinone and kulactone; δ 2.25 (1H, dt, J = 13.5, 3.5~4.0 Hz, 2α-H), 2.76 (1H, td, J = 13.5, 5.0~5.5 Hz, 2β-H).

Leptosin J Diacetate (**5**)

Leptosin J (**3**) (8.1 mg) was acetylated and purified as summarized above for **4** to afford **5** (5.4 mg) as a pale yellow powder; FAB-MS *m/z*: 825 $(MH)^+$. IR ν_{max} (KBr) cm^{-1}: 3500 (OH, NH), 1755 ($OCOCH_3$), 1690, 1679 (CON), 1612, 1594 (Ar. C-C). Its ^1H and ^{13}C NMR data are listed in Tables 3 and 4.

Formation of the Bis (methylthio) Derivatives **6** and **7** from Leptosin I (**2**)

Leptosin I (**2**) (12.4 mg) was dissolved in a solution (0.27 ml) of pyridine and MeOH (5:8). CH_3I (1 ml) and $NaBH_4$ (5 mg) were added, and the mixture was stirred for 20 minutes at room temperature. The reaction mixture was then diluted with water and extracted with ether. The solvent was evaporated *in vacuo*, and the residue was chromatographed on a silica gel column with a CH_2Cl_2-MeOH gradient as the eluent. The MeOH-CH_2Cl_2 (2:98) eluate gave **6** (5.2 mg) and **7** (1.0 mg). **6** was obtained as a pale yellow powder; FAB-MS *m/z*: 707 $(MH)^+$. IR ν_{max} (KBr) cm^{-1}: 3389 (OH, NH), 1664 (CON), 1611, 1593 (Ar. C-C). Its ^1H and ^{13}C NMR data are listed in Tables 3 and 4. **7** was obtained as a pale yellow powder; FAB-MS *m/z*: 693 $(MH)^+$. IR ν_{max} (KBr) cm^{-1}: 3392 (OH, NH), 1666 (CON), 1608, 1595 (Ar. C-C). Its ^1H NMR data are listed in Table 3.

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