Brief Communications

4-Methoxy-3-methylgoniothalamin from marine-derived fungi of the genus *Penicillium*

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The new styrylpyrone derivative, *viz.* 4-methoxy-3-methylgoniothalamin (**1**), and the known compound sulochrin were isolated from the marine-derived fungi *Penicillium glabrum* and *P. implicatum*. The structure of compound **1** was determined by X-ray diffraction, 2D NMR spectroscopy, and high-resolution mass spectrometry. It was shown for the first time that sulochrin increases expression of the protein Hsp70.

Key words: marine-derived fungus *Penicillium glabrum*, *Penicillium implicatum*, styryl lactones, diphenyl ketones, protein Hsp70.

Recent studies have shown that marine fungi are promising sources of both new and known biologically active substances. Marine and terrestrial ecological forms of fungi of the genus *Penicillium* can produce compounds belonging to different classes. However, only two classes of compounds, *viz.*, pyrenes and aromatic polyketides exhibiting immunosuppressive activity, have been isolated from the fungus *P. glabrum*.[1,2] Three polyketide metabolites have been isolated earlier from *P. implicatum*. In continuation of the search for promising producers of biologically active substances among marine microscopic fungi, we found that the strains of the fungi *P. glabrum* and *P. implicatum* isolated from stems and rhizome of the seagrass *Zostera marina* collected in the Peter the Great Gulf (Sea of Japan) synthesized compounds with antibacterial activity. In the present study, we report data on the isolation and identification of a new styrylpyrone derivative (**1**) and the known diphenyl ketone sulochrin (**2**). To prepare metabolites, the fungi were cultured on a rice medium at 22 °C for three weeks. In parallel, the cultures of the fungi

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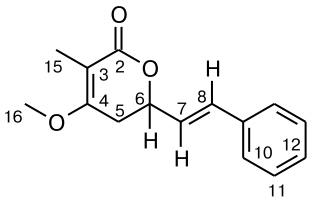
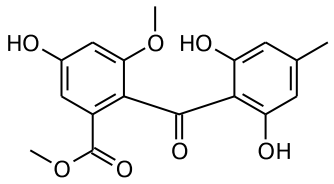
with the biomass medium were extracted with ethyl acetate. The extracts were concentrated to dryness and successively treated with hexane and ethyl acetate. The ethyl acetate extracts of both fungi were separated on silica gel, resulting in the isolation of individual compounds **1** and **2**.



1



2

The molecular formula of 4-methoxy-3-methylgoniothalamin (**1**) was determined as $C_{15}H_{16}O_3$ by high-resolution electron-impact mass spectrometry and was then confirmed by the analysis of the ^{13}C NMR spectra.

The ^1H NMR spectrum shows three multiplets of the five-proton intensity at δ 7.2—7.5, which are indicative of the presence of the monosubstituted benzene ring (Table 1). The doublets of doublets at δ 6.43 and 6.73 with equal spin-spin coupling constants (16.0 Hz) are characteristic of protons in the ethylene group with the double bond in the *trans* configuration. The DEPT and HSQC spectra of compound **1** confirmed the presence of one methyl group (δ_C 8.8), one methoxy group (δ_C 55.7), one methylene group (δ_C 28.7), and eight methine groups (δ_C 74.4, 127.0, 126.6 (2), 128.1, 128.7 (2), 131.9). The other four signals (δ_C 100.5, 135.8, 167.0, 167.3) were assigned to quaternary carbon atoms. The HMBC, COSY, and NOE experiments suggest that compound **1** is closely related to the known styrylpyrone goniothalamin.

The single-crystal X-ray diffraction study confirmed the relative configuration of compound **1** (Fig. 1, Tables 2, 3, and 4).

Therefore, compound **1** has the structure (*E*)-4-methoxy-3-methyl-6-styryl-5,6-dihydro-2*H*-pyran-2-one.[3]

It should be noted that styrylpyrones of the goniothalamin type (to which the compound under investigation belongs) have been earlier isolated only from higher plants of the families *Annonaceae*, *Dipterocarpaceae*, *Cucurbitaceae*, and *Lauraceae*.

The molecular formula of compound **2** was determined as $C_{17}H_{16}O_7$ by high-resolution electron-impact mass spectrometry and was then confirmed by the analysis of the ^{13}C NMR spectra. Compound **2** was identified as sulochrin based on a comparison of the ^1H and ^{13}C NMR spectroscopic data with the data published in the literature.[4] It should be noted that sulochrin has not been isolated earlier from the fungus *P. implicatum*.

We studied the biological activity of compounds **1** and **2**. 4-Methoxy-3-methylgoniothalamin (**1**) and sulochrin (**2**) at concentrations of 4.1 and 3.0 μmol L^{-1}, respectively, did not exhibit cytotoxic activity against mouse splenic lymphocytes and did not display hemolytic activity against mouse erythrocytes.

4-Methoxy-3-methylgoniothalamin (**1**) did not exhibit cytotoxic activity against human erythroleukemia cells K562 (IC_{50} >> 600 μmol L^{-1}) and did not cause the enhancement of the expression of the heat shock protein

Table 1. NMR spectroscopic data for compound **1** (300 MHz, DMCO-d_6, δ, *J*/Hz)

Atom	^{13}C	DEPT	^1H	HMBC	NOE
2	167.3	C			
3	100.5	C			
4	167.0	C			
5	28.7	α-CH_2			
		β-CH_2	2.99 (ddd, J = 2.2, J = 4.3, J = 17.5)	3, 4, 6, 7	6, 16, 5β
			2.76 (ddq, J = 11.2, J = 17.6, J = 2.0)	3, 4, 6, 7	7, 5α
6	74.4	CH	5.01 (m)	4, 5, 7, 8	5α, 7, 8
7	127.0	CH	6.43 (dd, J = 6.2, J = 16.0)	5, 6, 9	5β, 6, 10, 14
8	131.9	CH	6.73 (dd, J = 0.8, J = 16.0)	6, 9, 10, 14	6, 10, 14
9	135.8	C			
10, 14	126.6	CH	7.40 (m)	8, 9, 10, 12, 14	
11, 13	128.7	CH	7.36 (m)	9, 11, 13	
12	128.1	CH	7.28 (m)	10, 14	
15	8.8	CH_3	1.64 (s)	2, 3, 4	
16	55.7	CH_3	3.82 (s)	4	5α

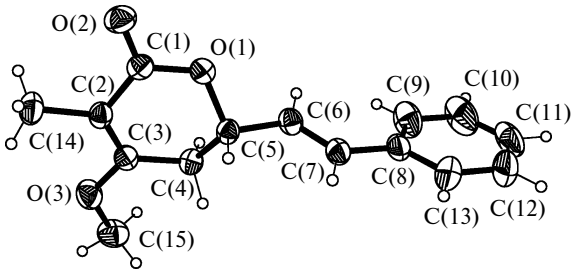


Fig. 1. Molecular structure of compound **1**; the atoms are represented as probability displacement ellipsoids ($p = 50\%$).

Hsp70 in cells. Sulochrin (**2**) exhibited weak cytotoxic activity against the cell line K562 ($IC_{50} = 680$ μmol L^{-1}). The use of sulochrin at nontoxic concentrations (0.1—100.0 μmol L^{-1}) led to the enhancement of the expression of the protein Hsp70 exhibiting protective chaperone activities in cells.

Compounds **1** and **2** at concentrations of 6.4 and 12.0 μmol L^{-1}, respectively, did not show antimicrobial properties against the test cultures used.

Table 2. Crystallographic characteristics and the X-ray data collection and structure refinement statistics for compound **1**

Parameter	Characteristics
Empirical formula	$C_{15}H_{16}O_3$
Molecular weight	244.28
Crystal system	Monoclinic
Space group	$P2_1$
a/Å	4.8664(4)
b/Å	11.3000(9)
c/Å	12.2241(10)
β/deg	96.707(2)
V/Å^3	667.61(9)
Z	2
d_{calc}/g cm^{-3}	1.215
μ/mm^{-1}	0.084
F(000)	260
Crystal dimensions/mm^3	0.44×0.34×0.05
θ-Scan range/deg	2.46—30.01
Number of measured reflections	4935
Number of independent reflections	1974
R_{int}	0.0203
Number of reflections with $I > 2\sigma(I)$	1737
Method for refinement squares based on F^2	Full-matrix least-
Number of refined parameters	165
GOOF по F^2	1.062
R factors ($I > 2\sigma(I)$)	
R_1	0.0394
wR_2	0.1032
R factors (based on all I_{hkl})	
R_1	0.0466
wR_2	0.1086
Residual electron density/e Å^{-3}, ρ_{max}/ρ_{min}	0.226/−0.150

Table 3. Selected interatomic distances (d) in the structure of **1**

Bond	d/Å	Bond	d/Å
O(1)—C(1)	1.365(1)	C(2)—C(14)	1.502(2)
O(1)—C(5)	1.459(1)	C(3)—C(4)	1.491(2)
O(2)—C(1)	1.212(1)	C(4)—C(5)	1.519(2)
O(3)—C(3)	1.347(1)	C(5)—C(6)	1.494(2)
O(3)—C(15)	1.437(2)	C(6)—C(7)	1.317(2)
C(1)—C(2)	1.460(2)	C(7)—C(8)	1.472(2)
C(2)—C(3)	1.351(2)		

Table 4. Selected bond angles (ω) in the structure of **1**

Angle	ω/deg
C(1)—O(1)—C(5)	117.51(9)
C(3)—O(3)—C(15)	119.22(11)
O(1)—C(1)—O(2)	116.93(11)
O(1)—C(1)—C(2)	119.22(10)
O(2)—C(1)—C(2)	123.75(12)
C(1)—C(2)—C(3)	119.31(11)
C(1)—C(2)—C(14)	117.17(12)
C(3)—C(2)—C(14)	123.22(12)
O(3)—C(3)—C(2)	117.20(11)
O(3)—C(3)—C(4)	120.57(11)
C(2)—C(3)—C(4)	122.22(11)
C(3)—C(4)—C(5)	110.70(10)
O(1)—C(5)—C(4)	110.92(9)
O(1)—C(5)—C(6)	106.70(9)
C(4)—C(5)—C(6)	111.98(10)
C(5)—C(6)—C(7)	123.09(13)
C(6)—C(7)—C(8)	126.68(14)

Experimental

Low-temperature (173 K) **X-ray diffraction data** were collected from a plate-like single crystal on a SMART 1000 CCD diffractometer according to a standard procedure (Mo-Kα radiation, graphite monochromator, ω-scanning technique with a step of 0.2° and the exposure time of 30 s per frame). The crystallographic parameters and the structure refinement statistics for compound **1** are given in Table 2.

The structure was solved by direct methods and refined by the full-matrix least-squares method with anisotropic displacement parameters for nonhydrogen atoms. The hydrogen atom were positioned geometrically and refined using a riding model. The absorption correction was applied based on equivalent reflections taking into account the crystal habit. All calculations were carried out with the use of the SHELXTL/PC program package.[5] Selected interatomic distances and bond angles are given in Tables 3 and 4, respectively.

The crystallographic data were deposited with the Cambridge Crystallographic Data Centre (CCDC 780155).

The melting points were determined on a Leica VMTG instrument (Germany). The optical rotation was measured on a Perkin Elmer 343 polarimeter (Germany). The UV spectra

were recorded on an UV-1601 PC Shimadzu spectrophotometer (Japan) in methanol. The IR spectra were measured on a Bruker OPUS Vector-22 spectrophotometer in chloroform. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX-300 spectrometer (300.13 and 75.47 MHz, respectively) with $(CD_3)_2SO$ (^{13}C, 30.5 ppm; ^{1}H, 2.49 ppm) and $(CD_3)_2CO$ (^{13}C, 29.9 ppm; ^{1}H, 2.05 ppm) as the internal standards. The high-resolution mass spectra were obtained on an AMD 604 S spectrometer (Germany). Column chromatography was carried out on silica gel L (40/100 μm, Chemapol, Czechoslovakia). Thin layer chromatography was performed on silica gel plates (10.0×5.0 cm) (5—17 μm, Sorbfil, Russia).

Cultivation of fungi. The fungi were isolated from the stem surface (*Penicillium glabrum*) and rhizome (*Penicillium implicatum*) of the seagrass *Zostera marina* collected in coastal waters of the Trinity Bay (the Peter the Great Gulf, the Sea of Japan). The cultivation of the fungi was carried out at 22 °C in ten 1-L flasks; each flask contained sodium tartrate (0.005 g), the yeast extract (0.01 g), rice (10 g), KH_2PO_4 (0.005 g), and seawater (20 mL).

Extraction and isolation of compounds 1 and **2**. The extraction and isolation from both fungi were carried out according to the same procedure. The fungal mycelium grown on the medium was twice extracted with ethyl acetate (2×1.0 L). The extract was concentrated, and the dry residues (1.5 g of *P. glabrum*, 1.8 g of *P. implicatum*) were dissolved in an ethanol—water system (1 : 4). The resulting solutions were extracted with hexane (3×50 mL) and ethyl acetate (3×50 mL). The ethyl acetate extracts were concentrated to dryness and the residues (0.5 g of *P. glabrum,* 0.7 g of *P. implicatum*) were repeatedly chromatographed on columns (2×8 cm) with silica gel using the hexane—ethyl acetate system (stepwise gradient, 1 : 0 → 1 : 1). Individual compounds **1** and **2** were isolated from the extracts of the fungi *P. glabrum* and *P. implicatum* (**1**, 57.0 and 177.0 mg; **2**, 42.0 and 203.0 mg, respectively).

4-Methoxy-3-methylgoniothalamin (1). Plate-like white crystals, m.p. 217—218 °C (hexane—ethyl acetate, 9 : 1), $[\alpha]^{20}_D$ −129.5 (*c* 0.30, EtOH), $C_{15}H_{16}O_3$. UV (EtOH), λ_{max}/nm (log ε): 292 (3.03), 252 (4.48), 206 (4.47). IR ($CHCl_3$), ν/cm^{-1}: 3008, 2947, 2857, 1696, 1648, 1220. EI-MS, *m*/*z* (I_{rel} (%)): 244 $[M]^+$ (67), 200 $[M - CO_2]^+$ (26), 185 $[M - CO_2 - CH_3]^+$ (27), 141 (21), 125 (27), 112 (100), 84 (31), 44 (41). High-resolution EI-MS. Found: *m*/*z* 244.1091 $[M]^+$. $C_{15}H_{16}O_3$. Calculated: 244.1099.

The NMR spectroscopic data for compound **1** are given in Table 1.

Sulochrin (2). Yellowish-white powder, m.p. 249—251 °C (hexane—ethyl acetate, 9 : 1, see Ref. 4), $C_{17}H_{16}O_7$. EI-MS, *m*/*z* (I_{rel} (%)): 332 $[M]^+$ (53), 301 (52), 300 (40), 272 (28), 269 (74), 209 (53), 151 (100). ^{13}C NMR (acetone-d_6), δ: 22.0, 52.3, 56.6, 104.2, 108.7, 108.9, 111.5, 128.0, 129.8, 148.3, 158.4, 159.1, 163.0, 166.8, 200.7. ^{1}H NMR (acetone-d_6), δ: 2.18 (s, 3 H); 3.65 (s, 3 H); 3.69 (s, 3 H); 6.18 (s, 2 H); 6.71 (d, 1 H, J = 2.2); 7.01 (d, 1 H, J = 2.2), 8.94 (s, 1 H), 10.95 (s, 2 H) (see Ref. 4).

Biological activity assays. The antimicrobial activity of the compounds was determined against the Gram-positive bacteria *Bacillus subtilis* (KMM 430) and *Staphylococcus aureus* (ATCC 21027), the Gram-negative bacteria *Pseudomonas aeruginosa* (KMM 433) and *Escherichia coli* (ATCC 15034), and the yeast fungi *Candida albicans* (KMM 455) according to a known procedure.[6]

The hemolytic activity against mouse blood erythrocytes was determined according to a procedure described earlier.[7]

The human erythroleukemia cell line K562 was obtained from the Institute of Cytology of the Russian Academy of Sciences (St.-Petersburg). The cell culture was loaded and the influence of the compounds on the expression of the heat shock protein Hsp70 was determined by protein immunoblot according to a procedure described in the literature.[8] The cytotoxic activity was assayed for mouse splenic lymphocytes with the use of Trypan blue as a nuclear stain according to a known procedure[9] and for the mouse cell line K562 by the colorimetric method with the use of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT).[10]

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