Chemistry of Natural Compounds, Vol. 45, No. 5, 2009

DEHYDRODECALIN DERIVATIVE FROM MARINE ISOLATE OF THE FUNGUS *Wardomyces inflatus*

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UDC 577.115:528.281

In connection with the program to discover biologically active compounds in extracts of marine isolates of microscopic fungi, we tested 1000 strains of marine fungi isolated from marine sediments of Sakhalin Bay for the presence in their extracts of compounds with antibiotic activity that were active in early embryogenesis of the sea urchin *Strongylocentrotus intermedius*. One of them, *Wardomyces inflatus* (Marchal) Hennebert, synthesized such compounds. This fungus is usually parasitic on terrestrial plants [1, 2] and was isolated by us for the first time from deep (50 m) marine sediments.

Mycelium of the fungus with medium was extracted twice with EtOAc. The extract was evaporated. The solid was dissolved in EtOH:H_2O (1:4) and extracted (2×) successively with hexane, $CHCl_3$, and BuOH. The $CHCl_3$ extract was evaporated in vacuo. The dry solid (700 mg) was chromatographed over a column (25 × 2 cm) of SiO_2. Compound **1** (27 mg) was eluted by hexane:EtOAc (85:15). The UV spectrum of the compound had no absorption bands. Its IR spectrum showed absorption bands characteristic of hydroxyls (3621, 3568 cm^{-1}). The PMR and ^{13}C NMR spectra of **1** (Table 1) was consistent with two quaternary C atoms (δ_C 52.6, C-1; 215.7, C-9) and eight methines (δ_H 1.94–δ_C 52.3, C-2; δ_H 5.70–δ_C 123.9, C-3; δ_H 6.0–δ_C 126.1, C-4; δ_H 2.14–δ_C 39.0, C-4a; δ_H 3.43–δ_C 75.3, C-5; δ_H 4.04–δ_C 69.6, C-6; δ_H 1.74–δ_C 30.5, C-8; δ_H 1.93–δ_C 43.0, C-8a; δ_H 1.12–δ_C 37.1, C-1′), two of which were bound to C-5 and C-6, which were oxygenated, and two of which were located on the C-3–C-4 double bond.

The position of the resonances for the C-3 and C-4 protons and their SSCC indicated that they were situated *cis* to each other on the double bond. Besides these resonances, spectra of **1** contained resonances for four methyls (δ_H 0.59–δ_C 22.4, CH_3-12; δ_H 0.75–δ_C 12.5, CH_3-3′; δ_H 0.93–δ_C 19.2, CH_3-4′; δ_H 1.25–δ_C 19.4, CH_3-13), the multiplicity of which indicated that one of them was bound to a quaternary C atom; two, to a tertiary; and one, to a secondary.

The chemical structure was determined from the ^1H–^1H COSY and HSQC spectra incorporating HMBC correlations that indicated that **1** was a derivative of *trans*-dehydrodecalin. The relative stereochemistry of the molecule was proved based on SSCC of protons and NOESY data (Table 1). NOESY correlations between H-8a, Ha-7, H-5, and CH_3-12 indicated that these protons and methyl group were located on one side of the molecular plane. The SSCC for H-8a indicated that this proton was *trans* to H-8 and H-4a. The axial location of CH_3-13 was proved based on NOESY correlations of the methyl protons and H-8 and H-4a. Protons of CH_3-13 and H-2 were *cis* according to NOESY correlations between them and H-2 and H-8.

The absolute configuration of **1** was established by the literature method [3]. Weak-field shifts of resonances for the C-5 and C-11 protons in the PMR spectrum of **1** compared with the position of these resonances in PMR spectra of α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) esters **1a** and **1b** showed that the MTPA esters formed through the C-5 and C-11 hydroxyls. The configuration of C-5 was determined as *S* based on the difference of the chemical shifts for the *S*- and *R*-MTPA esters (Fig. 1). Considering this and the aforementioned NOESY correlation, the configurations for C-1, C-2, and C-8a were established as *S*; C-4a, C-6, C-8, and C-1′, as *R*.

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0009-3130/09/4505-0753

TABLE 1. PMR and ^{13}C NMR Spectra of **1** ($CDCl_3$, δ, ppm, J/Hz)

C atom	δ_C	δ_H	NOESY	HMBC
1	52.6 (C)			
2	52.3 (CH)	1.94 m	13, 10, 4′, 1′	2′, 1′, 8a, 3, 4, 9
3	123.9 (CH)	5.70 (ddd, J = 2.7; 4.6; 10.5)	4′	9, 4a, 1, 5
4	126.1 (CH)	6.00 (dt, J = 10.5; 2.0)	5, 4a	4a, 8a, 5, 2
4a	39.0 (CH)	2.14 (tq, J = 10.4; 2.4)	4, 13, 8	5, 6, 1, 8a, 1′, 8
5	75.3 (CH)	3.43 (dd, J = 3.2; 10.9)	4, 8a, 7	4a, 6, 4, 7
6	69.6 (CH)	4.04 (q, J = 3.0)	7	8, 4a, 5
7	41.3 (CH_2)	Ha – 1.51 (ddd, J = 2.8; 12.1; 14.3)	5, 6, 12, 8a	12, 8, 8a
		Hb – 1.84 (dt, J = 14.3; 2.9)	6, 12	12, 8, 8a, 1, 5, 6
8	30.5 (CH)	1.74 m	13, 4a	8a, 7, 4a, 12, 6
8a	43.0 (CH)	1.93 (t, J = 10.0)	12, 5, 2′, 7	13, 8, 4a, 7, 1, 5, 9
9	215.7 (C)			
10	41.1 (CH_2)	2.85 (ddd, J = 3.9; 7.2; 18.8)	13, 12	9, 11, 13
		2.66 (ddd, J = 3.6; 6.2; 18.8)	13, 2	9, 11
11	58.0 (CH_2)	3.83 (ddd, J = 4.0; 6.1; 11.6)		9, 10
		3.89 (ddd, J = 3.5; 7.2; 11.5)		9, 10
12	22.4 (CH_3)	0.59 (d, J = 6.9)	10, 7, 8a	8a, 7, 8
13	19.4 (CH_3)	1.25 s	10, 2, 4a, 8	1, 8a, 9, 2
1′	37.1 (CH)	1.12 m	2	1, 2′, 13, 3′, 3, 2
2′	24.5 (CH_2)	0.75 m		
		1.47 m	8a	3′, 1′, 4′, 2
3′	12.5 (CH_3)	0.75 m		1′, 2′,
4′	19.2 (CH_3)	0.93 (d, J = 6.8)	2, 3	1′, 2′, 2

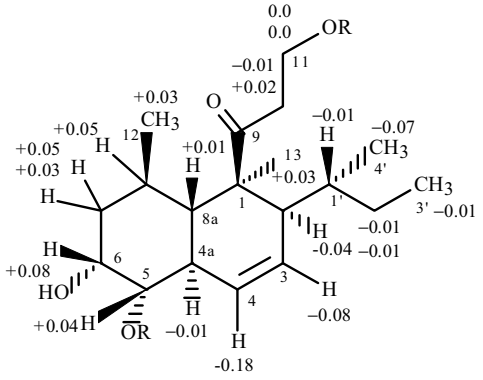

Fig. 1. Structure of **1** (R = H) and difference in chemical shifts of protons ($\Delta\delta = \delta S - \delta R$) in PMR spectra of MTPA esters **1a** [R = (*S*)-MTPA] and **1b** [R = (*R*)-MTPA].

The results indicated that **1** was identical to eujavanicol A, which was isolated from the soil fungus *Eupenicillium javanicum* [4]. It was surprising that the main metabolite of fungi isolated from different ecological niches and belonging to different genera was the same compound. Marine microbes more frequently undergo chemical adaptation to the habitat conditions, as a result of which metabolites with unusual structural skeletons are synthesized or metabolites previously isolated from terrrestrial microbes undergo chemical modification. An example of such adaptation is the isolation of an ester of **1** from marine isolate of *Trichoderma harzianum*, which is associated with the sponge *Mycale cecilia* [5]. Compound **1** was isolated from a fungus of the genus *Wardomyces* for the first time. Known metabolites from this genus are xanthones [6] and hydroxylated sclerosporins [7], which are cadalane-type sesquiterpenes [8].

Compound **1** exhibited high cytotoxic activity against developing embryos of the sea urchin *S. intermedius*. It inhibited 100% division of fertilized urchin ova at blastomer stage four at a concentration of 4.5 μg/mL.

PMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker DRX-500 spectrometer; mass spectra, in Varian MAT 371 (70 eV) and Varian 311 A (70 eV) spectrometers. IR spectra in $CHCl_3$ solutions were obtained on a Specord M 82 instrument (Carl Zeiss, Jena). Optical rotation was measured on a Perkin—Elmer 141 polarimeter. TLC was performed on Sorbfil Silica Gel STKh-1A plates (ZAO Sorbpolimer, Russia) using toluene:*i*-PrOH (6:1); column chromatography, over silica gel L (40/100 μm, Czechoslovakia) using hexane:EtOAc.

Cultivation of Fungus. The strain was isolated from soil collected at a depth of 50 m in Sakhalin Bay of Okhotsk Sea. Fungus was cultivated for 20 d at 22°C in six 1-L flasks, each of which contained medium consisting of unhopped beer wort (50 mL), washed agar (5 g), and seawater (200 mL).

Compound 1, $C_{19}H_{32}O_4$, yellow oil, $[\alpha]_D$ +57° (*c* 0.33, $CHCl_3$). Mass spectrum (EI, *m*/*z*, *I*rel, %): 324 (10) $[M]^+$, 306 (35) $[M - H_2O]^+$, 288 (25) $[M - 2H_2O]^+$, 268 (17), 250 (20), 233 (56), 215 (40), 177 (94), 159 (100), 142 (58), 119 (59), 105 (40), 93 (39), 73 (88). IR spectrum ($CHCl_3$, ν, cm^{-1}): 3621, 3568, 3014, 2963, 2930, 2878, 1693, 1602, 1462, 1383, 1351, 1067, 1041.

Preparation of MTPA Esters of 1. A solution of **1** (4 mg) in dry pyridine was treated with several crystals of 4-dimethylaminopyridine (DMAP) and (*R*)-MTPA chloride (35 μL), stirred at room temperature for 1 h, and evaporated. The solid was chromatographed over a column of silica gel with elution by hexane:EtOAc (97:3) to afford (*S*)-MTPA ester **1a** (6 mg). (*R*)-MTPA ester **1b** was prepared analogously using (*S*)-MTPA chloride. Mass spectra (EI) of **1a** and **1b** had peaks with *m*/*z* 755 $[M - 1]^+$.

5,11-Di-(*S*)-MTPA-ester 1a. PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.52 (3H, d, J = 7.0, Me-12), 0.71 (1H, m, H-2′), 0.72 (3H, m, Me-3′), 0.84 (3H, d, J = 7.0, Me-4′), 1.03 (1H, m, H-1′), 1.20 (3H, s, Me-13), 1.44 (1H, m, H-2′), 1.55 (1H, ddd, J = 2.7, 11.8, 14.5, H-7a), 1.75 (1H, m, H-8), 1.82 (1H, dt, J = 14.2, 2.9, H-7b), 1.87 (1H, m, H-2), 2.05 (1H, t, J = 10.1, H-8a), 2.45 (1H, br.t, J = 12.0, H-4a), 2.78 (1H, dt, J = 18.7, 6.6, H-10), 3.00 (1H, dt, J = 18.7, 6.2, H-10), 4.20 (1H, q, J = 2.8, H-6), 4.53 (1H, dt, J = 11.3, 6.5, H-11), 4.61 (1H, dt, J = 11.3, 6.3, H-11), 4.91 (1H, dd, J = 2.9, 11.6, H-5), 5.34 (1H, br.d, J = 10.5, H-4), 5.59 (1H, ddd, J = 2.7, 4.6, 10.5, H-3).

5,11-Di-(*R*)-MTPA-ester 1b. PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.49 (3H, d, J = 7.0, Me-12), 0.72 (1H, m, H-2′), 0.73 (3H, m, Me-3′), 0.91 (3H, d, J = 7.0, Me-4′), 1.04 (1H, m, H-1′), 1.19 (3H, s, Me-13), 1.45 (1H, m, H-2′), 1.50 (1H, ddd, J = 2.8, 11.8, 14.6, H-7a), 1.70 (1H, m, H-8), 1.79 (1H, dt, J = 14.6, 2.9, H-7b), 1.91 (1H, m, H-2), 2.04 (1H, t, J = 10.2, H-8a), 2.46 (1H, br.t, J = 12.2, H-4a), 2.79 (1H, dt, J = 18.7, 6.0, H-10), 2.98 (1H, dt, J = 18.7, 6.6, H-10), 4.12 (1H, q, J = 2.9, H-6), 4.53 (1H, dt, J = 11.3, 6.0, H-11), 4.61 (1H, dt, J = 11.2, 6.6, H-11), 4.87 (1H, dd, J = 2.8, 11.6, H-5), 5.52 (1H, br.d, J = 10.5, H-4), 5.67 (1H, ddd, J = 2.6, 4.7, 10.5, H-3).

Determination of Cytotoxic Activity of 1. Biotesting using reproductive cells of sea urchin *S. intermedius* was carried out by the literature method [9].

ACKNOWLEDGMENT

The work was supported by grants of the RFBR (No. 09-04-00388, 08-04-00289) and the RAS Presidium Program "Molecular and Cellular Biology."

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