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BIOLOGICALLY ACTIVE METABOLITES FROM THE MARINE ISOLATE OF THE FUNGUS *Myceliophthora lutea*

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*The new compound isoacremine D (**1**) and acremine A (**2**) that was previously unknown for marine organisms were isolated from the marine isolate of the fungus* Myceliophthora lutea. *It was found that acremine A in $CHCl_3$ was converted through the action of light into spirocompounds called by us spiroacremines A (**3**) and B (**4**). The structures of the compounds were elucidated based on NMR spectroscopic and high-resolution mass spectrometric data. The absolute stereochemistry of the spiroacremines was determined by Mosher's method. It was shown that isoacremine D exhibited antimicrobial activity against* Staphylococcus aureus *at a concentration of 200 μg/mL and cytotoxic activity against embryos of the sea urchin* Strongylocentrotus nudus. *It was found that **1–4** exhibited cytotoxic activity against sea urchin sperm cells.*

Keywords: acremines, marine isolate of the fungus *Myceliophthora lutea*, NMR data, 1-oxaspiro[4,5]decenes.

Facultative and obligate marine microscopic fungi are sources of biologically active secondary metabolites [1–3]. In continuation of the search for producers of biologically active compounds among marine isolates of microscopic fungi, we determined that the fungus *Myceliophthora lutea* Costantin [4], which was isolated from marine sediments of Sakhalin Bay (Sea of Okhotsk), synthesizes compounds with antibacterial and cytotoxic activities. Little is known about secondary metabolites from fungi of the genus *Myceliophthora* although these fungi are often encountered and can be isolated from various substrates [5–8]. Herein we present data on the isolation and identification of known acremine A (**2**) [9] and previously unknown compounds called by us isoacremine D (**1**) and spiroacremines A (**3**) and B (**4**). The last two compounds are apparently artifacts of the isolation process.

The fungus was cultivated on solid medium for 21 d at 22°C in order to produce the metabolites. The dried EtOAc extract of the biomass produced a $CHCl_3$ extract, separation of which over silica gel and Sephadex LH-20 afforded pure **1** and **2** (Fig. 1) in addition to **3** and **4**, which were not present in the starting total EtOAc extract according to TLC (Fig. 2).

The empirical formula of **1** was determined as $C_{12}H_{14}O_3$ on the basis of the high-resolution mass spectrum and NMR spectra. The PMR spectrum of **1** contained three 3H-singlets in the range 1.55–2.27 ppm, indicative of three tertiary methyls in its structure. The DEPT and HSQC spectra of **1** confirmed the presence of three methyls (δ_C 17.6, 31.0, 31.0 ppm) and also indicated that three methine groups were present (δ_C 101.2, 106.5, 113.4). The remaining six C atoms were classified as quaternary (δ_C 165.9, 153.0, 150.8, 128.6, 123.1, 69.7). Figure 1 shows the key HMBC-correlations of **1**.

The position of methyl 1″ (δ_H 2.27 ppm) on C(6) was established by HMBC-correlations of C-1″ with C-5 (δ_C 153.0 ppm), C-6 (δ_C 123.1), and C-7 (δ_C 113.4). Singlets for H_3C-2′ (δ_C 31.0, δ_H 1.55 ppm) and H_3C-3′ (δ_C 31.0, δ_H 1.55) were assigned to geminal methyls on the basis of mutual HMBC-correlations with each other and correlations of the methyl protons with quaternary C-1′ (δ_C 69.7), which was bonded to oxygen.

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TABLE 1. NMR Spectral Data for **1**/Acremine D [9] (500 MHz, acetone-d_6, δ, ppm, J/Hz)

C atom	δ_C	DEPT	δ_H	HMBC	COSY	NOE
2	165.9/165.3	C				
3	101.2/99.6	CH	6.45/6.44 (c)	3a, 7a, 2	7	4, 2′, 3′, 1′-OH
3a	128.6/135.1	C				
4	106.5/104.7	CH	6.92/6.91 (c)	3, 5, 6, 7a	7, 1″	3, 2′, 3′, 5-OH
5	153.0/150.0	C				
6	123.1/122.3	C				
7	113.4/111.8	CH	7.15/7.15 (c)	1″, 3a, 5, 7a	1″, 3, 4	2′, 3′
7a	150.8/144.3	C				
1′	69.7/77.8	C				
2′	31.0/27.3	Me	1.55/1.54 (c)	1′, 2	1′-OH	3, 1′-OH
3′	31.0/27.3	Me	1.55/1.54 (c)	1′, 2	1′-OH	3, 1′-OH
1″	17.6/16.0	Me	2.27/2.26 (c)	5, 6, 7	4, 7	7, 2′, 3′
1′-OH			4.25 (c)	3′, 1′, 2′, 2	2′, 3′	3, 2′, 3′, 1′-OH
5-OH			7.93 (c)	4, 5, 6	7	4

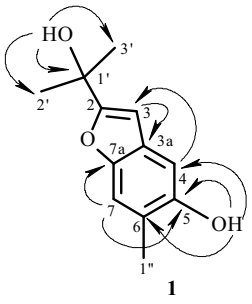

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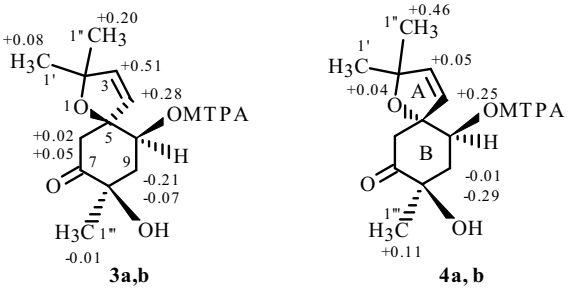
Fig. 1



3a,b **4a, b**

Fig. 2

Fig. 1. Key HMBC-correlations of **1**.

Fig. 2. Differences in proton chemical shifts ($\Delta\delta = \delta_S - \delta_R$) of MTPA-esters of spiroacremines A (**3a, b**) and B (**4a, b**).

The resonances of C-2 and C-7a in the ^{13}C NMR spectrum of **1** (δ_C 165.9 and 150.8) indicated that these atoms were bonded to oxygen. The positions of two hydroxyls on C-1′ and C-5 were inferred from HMBC-correlations of the hydroxyl protons with the corresponding C atoms (Fig. 1, Table 1). Coupling of HC-4 (δ_H 6.92 ppm) and C-3 (101.2) in the HMBC-spectrum of **1** in addition to NOE-coupling of HC-4 and HC-3 confirmed fully the structure shown in Fig. 1. Thus, **1** was 2-(1′-hydroxy-1′-methylethyl)-6-methylbenzofuran-5-ol.

A compound with this same formula, acremine D, was recently reported by Italian researchers [9]. The PMR spectra of isoacremine D and acremine D coincided (Table 1). However, the ^{13}C NMR spectra of **1** and acremine D were significantly different. This fact and the different melting points of isoacremine D (260–262°C) and acremine D (142–145°C) were indicative of a new compound.

The empirical formula of **2** ($C_{12}H_{18}O_4$) was established by high-resolution mass spectrometry (observed $[M]^+$ with *m/z* 226.1221; calcd $[M]^+$ 226.1205) and PMR and ^{13}C NMR spectral data (Table 2). The similar resonances of protons and C atoms in PMR and ^{13}C NMR spectra of **2** and acremine A, which was isolated from the terrestrial fungus *Acremonium byssoides* [9, 10], in addition to an x-ray structure analysis of **2** indicated that these two compounds were identical. Thus, **2** was 4*S*,6*R*-dihydroxy-3-[*E*-3′-hydroxy-3′-methylbut-1-enyl]-6-methylcyclohex-2-en-1-one.

Two new compounds, spiroacremines A (**3**) and B (**4**) formed upon storage of **2** in $CHCl_3$ in the light for a week. However, such conversions did not occur upon storage of **2** in $CHCl_3$ in the dark. Figure 3 shows the probable pathway of the transformations. Apparently a photochemical reaction that changed the configuration of the double bond occurred through the action of light. This made further cyclization possible. Chloroform, which becomes acidic in light, was the proton donor required for the cyclization. The hydroxyl oxygen of the side chain attacked the electron-poor enone C-5 to form the spirocompounds (Fig. 3).

TABLE 2. NMR Spectral Data for **2** (500 MHz, δ, ppm, J/Hz)

C atom	δ_C		DEPT	δ_H	
	methanol-d_4	acetone-d_6		methanol-d_4	acetone-d_6
1	203.0	201.4	C		
2	123.0	123.3	CH	5.98 (s)	5.92 (s)
3	161.3	160.3	C		
4	67.0	66.7	CH	4.62 (dd)	4.62 (m)
5	46.5	46.1	CH_2	2.34 (dd)	2.30 (dd, J = 4.5, J = 13.8)
				2.07 (dd)	2.10 (dd, J = 13.7, J = 6.6)
6	74.2	74.0	C		
1′	124.6	125.1	CH	6.42 (d)	6.45 (d, J = 16.0)
2′	148.0	148.6	CH	6.66 (d)	6.67 (d, J = 16.0)
3′	71.6	71.5	C		
4′	29.6	*	Me	1.33 (s)	1.30 (s)
5′	29.6	*	Me	1.33 (s)	1.30 (s)
1″	24.7	25.4	Me	1.28 (s)	1.24 (s)

*Signal overlapped by signal of acetone-d_6.

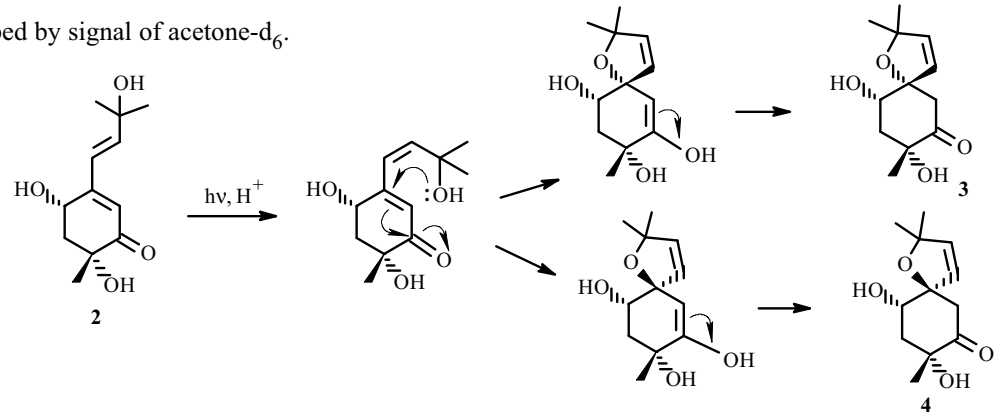


Fig. 3. Probable formation pathway of spiroacremines A and B.

The empirical formula of **3** was determined from high-resolution mass spectral and PMR and ^{13}C NMR spectral data. An analysis of ^1H and ^{13}C DEPT spectra of **3** proved that C-7 was a ketone (δ_C 211.2 ppm); that three quaternary C atoms were bonded to oxygen functional groups at C-2 (δ_C 90.0), C-5 (δ_C 95.7), and C-8 (δ_C 76.5); that two methylenes occurred at C-6 (δ_C 49.2) and C-9 (δ_C 44.6); that a single hydroxymethylene was present at C-10 (δ_C 70.4); and that three methyls were situated at C-1‴ (δ_C 25.9, δ_H 1.39), C-1′ (δ_C 28.6, δ_H 1.31), and C-1″ (δ_C 29.9, δ_H 1.30). The spectral data indicated that **3** had a single double bond at C-3 (δ_C 138.4, δ_H 5.88) and C-4 (δ_C 128.9, δ_H 5.61) and were consistent with a bicyclic structure for the compound (Table 3). The ^1H–^1H COSY NMR spectrum enabled two spin–spin systems to be identified. These corresponded to C-1′/C-3/C-4 and C-1‴/C-9/C-10 groupings in **3**. HMBC-correlations of the H_3C-1‴ protons with oxygenated quaternary C-8, methylene C-9, and carbonyl C-7 indicated that C-8 was bonded to C-7, C-9, and C-1‴. HMBC-correlations of H_2C-6 with C-5, C-7, and C-10 determined that C-6 was bonded to C-7 and C-5 of cyclohexane ring B in **3**. Correlations of proton HC-3 (δ_H 5.88) with C-5 (δ_C 95.7) in addition to cross peaks between HC-6β (δ_H 2.37) and C-8 (δ_C 76.5) and protons H_2C-6 and C-4 confirmed that **3** contained a spirocyclic system. The SSCC between HC-3 and HC-4 ($J_{3,4}$ = 5.9 Hz) was typical of a *cis*-double bond in a five-membered ring [11] (ring A). The relative configurations of the asymmetric C atoms in **3** were determined based on NOESY experiments and SSCC of the corresponding protons.

Proton HC-3 (δ_H 5.88) showed NOESY cross peaks with both HC-4 (δ_H 5.61) and methyl protons of C-1″ (δ_H 1.31) and C-1′ (δ_H 1.30) whereas H-4 had cross peaks with HC-6α (δ_H 2.95) and H-10 (δ_H 4.0) (Table 3). These protons, in turn, were coupled to each other and with protons of the C-1‴ methyl (δ_H 1.39). These data and the SSCC of HC-10 and HC-9 confirmed that the six-membered ring in **3** had a *pseudo*-chair conformation in which HC-6α (δ_H 2.95), HC-10, and H_3C-1‴ were axial. The absolute configuration of **3** was established by Mosher's method [12]. Esterification of **3** with (*S*)- and (*R*)-MTPA-Cl at the C-10 hydroxyl formed (*S*)- and (*R*)-MTPA esters **3a** and **3b**, respectively. The differences in the chemical shifts ($\Delta\delta = \delta_S - \delta_R$) in proton spectra of the esters (Fig. 2) were consistent with the 10*S*-configuration. Therefore, C-5 and C-8 should have had the *S*- and *R*-configurations, respectively, taking into account the previously found NOESY-couplings in **3** (Fig. 2). Thus, **3** was (5*S*,8*R*,10*S*)-8,10-dihydroxy-2,2,8-trimethyl-1-oxaspiro[4,5]dec-3-en-7-one.

TABLE 3. NMR Spectral Data for **3** (500 MHz, CD_3OD, δ, ppm, J/Hz)

C atom	δ_C	DEPT	δ_H	NOESY	HMBC
2	90.0	C			
3	138.4	CH	5.88 (d, J = 5.9)	4, 1′, 1″	2, 5, 1′, 1″
4	128.9	CH	5.61 (d, J = 5.9)	3, 6α, 10	2, 5
5	95.7	C			
6	49.2	α-CH_2	2.95 (d, J = 14.4)	4, 10, 1‴	7, 4, 5, 10
		β-CH_2	2.37 (d, J = 14.4)	1″	7, 4, 5, 8, 10
7	211.2	C			
8	76.5	C			
9	44.6	α-CH_2	2.04 (dd, J = 12.6, J = 4.7)	1‴	5, 10, 1‴, 7, 8
		β-CH_2	2.13 (d, J = 12.0)		5, 10, 1‴, 7, 8
10	70.4	CH	4.00 (dd, J = 11.4, J = 4.7)	1‴, 4, 6α	4, 5, 8, 9
1′	28.6	Me	1.31 (s)	3	1″, 3, 2
1″	29.9	Me	1.30 (s)	3, 6β	1′, 3, 2
1‴	25.9	Me	1.39 (s)	10, 6α, 9α	9, 7, 8

TABLE 4. NMR Spectral Data for **4** (500 MHz, CD_3OD, δ, ppm, J/Hz)

C atom	δ_C	DEPT	δ_H	NOESY	HMBC
2	90.0	C			
3	138.3	CH	5.93 (d, J = 6.0)	1′	4, 2, 5, 1, 1″
4	128.4	CH	5.83 (d, J = 6.0)	1′, 9β, 6β	3, 2, 5
5	96.3	C			
6	47.7	α-CH_2	2.38 (d, J = 13.7)	1″, 1‴, 10	7, 4, 5, 8, 10
		β-CH_2	3.19 (d, J = 13.7)	1″, 4	7, 4, 5, 8, 10
7	209.9	C			
8	76.5	C			
9	42.5	α-CH_2	2.13 (dd, J = 14.4, J = 3.7)	1‴, 10	5, 10, 1‴, 7, 8
		β-CH_2	1.92 (dd, J = 14.4, J = 6.1)	1‴, 4, 10	5, 10, 1‴, 7, 8
10	74.4	CH	3.73 (dd, J = 3.6, J = 6.1)	1‴, 9β, 6α, 9α	4, 5, 6, 8, 9
1′	29.2	Me	1.28 (s)	3, 4	3, 2
1″	29.7	Me	1.29 (s)	6α, 6β	3, 2
1‴	24.2	Me	1.26 (s)	9β, 6α, 9α, 10	9, 7, 8

Compound **4** had molecular formula $C_{12}H_{18}O_4$, which was confirmed by high-resolution electron-impact mass spectrometry. An analysis of PMR and ^{13}C NMR spectra, ^1H–^1H COSY, HSQC, and HMBC spectra for **4** (Table 4) and a comparison of them with the data for **3** indicated that **4** had the same spiro[4,5]decene skeleton. The differences in chemical shifts of protons in the (*S*)- and (*R*)-MTPA esters (**4a** and **4b**) proved the absolute configuration of C-10 as *S*. The 1D NOE-correlations of HC-10 in **4b** with protons of the C-8 methyl and with HC-6α confirmed that these protons and H_3C-1‴ were axial and that C-8 had the *R*-configuration in **4**. A 1D NOE-correlation of H-4 with HC-9β in **4a** and **4b** indicated that C-5 had the *R*-configuration. Thus, it was found that **4** was (5*R*,8*R*,10*S*)-8,10-dihydroxy-2,2,8-trimethyl-1-oxaspiro[4,5]dec-3-en-7-one.

The SSCC for protons on C-9 and C-10 [(14.4; 3.7), (14.4; 6.1) and (3.6; 6.1)] indicated that the cyclohexane ring of spiroacremine B was slightly distorted (Table 4). Introduction of a bulky substituent in the 10-position during preparation of the MTPA esters caused ring B to adopt a *pseudo*-chair conformation. This was evident in the SSCC for the C-9 and C-10 protons (see Experimental).

Compound **1** exhibited antimicrobial activity against *Staphylococcus aureus* at a concentration of 200 μg/mL. The activity of **1** at a concentration of 50 μg/mL on developing sea urchin embryos was such that only 35–50% of them passed the blastula stage. Compounds **2**–**4** at the studied concentrations did not exhibit antimicrobial activity against Gram-positive and Gram-negative bacteria and did not show cytotoxic activity against developing sea urchin embryos. The fertilizing ability of sea urchin spermatozoids decreased by 50% upon incubation with **1**–**4** at concentrations of 40, 50, 15, and 30 μg/mL, respectively.

EXPERIMENTAL

Melting points were determined on a Leica VMTG instrument (Germany). Optical rotation was measured on a Perkin–Elmer Model 343 polarimeter (Germany). UV spectra were taken in MeOH on a CECIL, CE 7250, 7000 spectrophotometer (England). IR spectra were recorded in $CHCl_3$ on a Bruker OPUS Vector-22 spectrophotometer. PMR and ^{13}C NMR spectra were measured on a Bruker DRX-500 (500 and 125.6 MHz) spectrometer with TMS internal standard. High-resolution electron-impact mass spectra were taken on an AMD 604 S spectrometer (Germany). Column chromatography was carried out over silica gel L (40/100 μm, Chemapol, Czechoslovakia) and Sephadex LH-20 (Pharmacia Biotech, Sweden). Silica-gel plates (4.5 × 6.0 cm, 5–17 μm, Sorbfil, Russia) were used for TLC.

Cultivation of Fungus. The fungus *Myceliophthora lutea* was isolated from marine sediments of Sakhalin Bay (Sea of Okhotsk, 32 m depth) and cultivated for three weeks on standing at 22°C in six 1-L flasks, each of which contained medium consisting of wort (50 mL), agar (5 g), and seawater (200 mL).

Extraction and Isolation of 1–4. Fungal mycelium was extracted with EtOAc (2.5 L). The solvent was evaporated. The solid (2.5 g) was treated with H_2O:EtOH (4:1, 200 mL). The resulting suspension was extracted successively with hexane (200 mL × 2), $CHCl_3$ (200 mL × 2), and *n*-BuOH (100 mL × 2). The $CHCl_3$ extract was evaporated. The solid (900 mg) was chromatographed over a column (20 × 2 cm) of silica gel using hexane:EtOAc (95:5, 90:10, 85:15, 80:20). Gel filtration over Sephadex LH-20 in $CHCl_3$ afforded pure **1** (5 mg), **2** (57 mg), **3** (15 mg), and **4** (9 mg). Compounds **3** and **4** were not present in the starting EtOAc extract and were formed during the isolation process. This was confirmed experimentally.

Conversion of Acremine A (2) to Spiroacremines A (3) and B (4). Acremine A (16 mg) was dissolved in $CHCl_3$ (5 mL) and left on a table. Two compounds in addition to acremine A were observed in the solution after a week according to TLC. The solution was evaporated. The resulting mixture was separated using column chromatography over silica gel and hexane:EtOAc (95:5–80:20) to afford **1** (8 mg), **3** (5 mg), and **4** (3 mg). Longer storage (about one month) of acremine A in $CHCl_3$ solution transformed it completely to the spiroacremines.

Isoacremine D (1), $C_{12}H_{14}O_3$, white crystals, mp 260–262°C (hexane:EtOAc, 9:1). UV spectrum (EtOH, λ_{max}, nm): 203, 249, 295 (log ε 4.22, 3.96, 3.60). IR spectrum ($CHCl_3$, ν, cm^{-1}): 3605, 2928, 1737, 1602. High-resolution mass spectrum (EI): found: *m*/*z* 206.0949 $[M]^+$; calcd: 206.0943. Table 1 presents NMR spectral data for **1**.

Acremine A (2), $C_{12}H_{18}O_4$, white crystals, mp 121–123°C (hexane:EtOAc, 9:1), $[\alpha]_D^{20}$ +12.1° (*c* 0.31, EtOH). UV spectrum (EtOH, λ_{max}, nm): 281 (log ε 4.05). IR spectrum ($CHCl_3$, ν, cm^{-1}): 3467, 3330, 1681, 1639, 1598. High-resolution mass spectrum (EI): found: *m*/*z* 226.1221 $[M]^+$; calcd: 226.1205. Table 2 presents NMR spectral data for **2**.

Spiroacremine A (3), $C_{12}H_{18}O_4$, white crystals, mp 128–131°C (hexane:EtOAc, 8:1), $[\alpha]_D^{20}$ +18° (*c* 0.3, EtOH). UV spectrum (EtOH, λ_{max}, nm): 217, 281 (log ε 2.92, 2.10). IR spectrum ($CHCl_3$, ν, cm^{-1}): 3571, 3494, 2979, 2932, 1721, 1602. High-resolution mass spectrum (EI): found: *m*/*z* 226.1209 $[M]^+$; calcd: 226.1205. Table 3 presents NMR spectral data for **3**.

Preparation of (*S*)- and (*R*)-MTPA (methoxy trifluoromethyl phenyl acetic acid) Esters of Spiroacremine A (3a and 3b). A solution of **3** (2 mg) in Py (200 μL) was treated with several crystals of 4-dimethylaminopyridine and (*R*)-MTPA-Cl (20 μL). The resulting mixture was left for 1 h at room temperature. The solvent was evaporated. The solid was chromatographed over silica gel using hexane:EtOAc (95:5) to afford the (*S*)-MTPA ester **3a** (3 mg). The (*R*)-MTPA ester **3b** was prepared in the same manner using (*S*)-MTPA-Cl.

(*S*)-MTPA Ester (3a). PMR spectrum (CD_3OD, δ, ppm, J/Hz): 5.89 (1H, d, J = 5.7, H-3), 5.66 (1H, d, J = 5.7, H-4), 5.49 (1H, dd, J = 5.4, 11.6, H-10), 3.49 (3H, s, OMe), 3.14 (1H, d, J = 14.7, H-6α), 2.31 (1H, d, J = 14.7, H-6β), 2.15 (1H, dd, J = 5.1, 12.0, H-9α), 2.11 (1H, t, J = 12.0, H-9β), 1.48 (3H, s, Me-1‴), 1.23 (3H, s, Me-1′), 1.03 (3H, s, Me-1″), 7.39-7.57 (5H, m, Ph). Mass spectrum (EI): *m*/*z* 442 $[M]^+$.

(*R*)-MTPA Ester (3b). PMR spectrum (CD_3OD, δ, ppm, J/Hz): 5.38 (1H, d, J = 5.7, H-3), 5.38 (1H, m, J = 5.7, H-4), 5.49 (1H, dd, J = 4.8, 12.0, H-10), 3.58 (3H, s, OMe), 3.12 (1H, d, J = 14.7, H-6α), 2.26 (1H, d, J = 14.7, H-6β), 2.22 (1H, dd, J = 4.8, 11.7, H-9α), 2.32 (1H, t, J = 12.0, H-9β), 1.49 (3H, s, Me-1‴), 1.15 (3H, s, Me-1′), 0.83 (3H, s, Me-1″), 7.38–7.49 (5H, m, Ph). Mass spectrum (EI): *m*/*z* 442 $[M]^+$.

Spiroacremine B (4), $C_{12}H_{18}O_4$, white crystals, mp 142–145°C (hexane:EtOAc, 8:1), $[\alpha]_D^{20}$ +4.2° (*c* 0.24, EtOH). UV spectrum (EtOH, λ_{max}, nm): 215, 281 (log ε 3.37, 2.39). IR spectrum ($CHCl_3$, ν, cm^{-1}): 3600, 3500, 2977, 2929, 1719, 1602. High-resolution mass spectrum (EI): found: *m*/*z* 226.1195 $[M]^+$; calcd: 226.1205. Table 4 presents NMR spectral data for **4**.

Preparation of (*S*)- and (*R*)-MTPA Esters of Spiroacremine B (4a and 4b). A solution of **4** (2 mg) in Py (200 μL) was treated with several crystals of 4-dimethylaminopyridine and (*R*)-MTPA-Cl (20 μL). The resulting mixture was left for 1 h at room temperature. The solvent was evaporated. The solid was chromatographed over silica gel using hexane:EtOAc (93:7) to afford the (*S*)-MTPA ester **4a** (3 mg). The (*R*)-MTPA ester **4b** was prepared in the same manner using (*S*)-MTPA-Cl.

(*S*)-MTPA Ester (4a). PMR spectrum (CD_3OD, δ, ppm, J/Hz): 5.98 (1H, d, J = 6.0, H-3), 5.99 (1H, d, J = 6.0, H-4), 5.42 (1H, dd, J = 5.3, 11.5, H-10), 3.49 (3H, s, OMe), 3.13 (1H, d, J = 15.8, H-6β), 2.71 (1H, d, J = 15.8, H-6α), 2.50 (1H, t, J = 12.0, H-9β), 2.36 (1H, dd, J = 12.5, 5.4, H-9α), 1.66 (3H, s, Me-1‴), 1.20 (3H, s, Me-1′), 1.30 (3H, s, Me-1″), 7.39–7.57 (5H, m, Ph). Mass spectrum (EI): *m*/*z* 442 $[M]^+$.

(*R*)-MTPA Ester (4b). PMR spectrum (CD_3OD, δ, ppm, J/Hz): 5.93 (1H, d, J = 5.9, H-3), 5.74 (1H, m, J = 5.9, H-4), 5.14 (1H, dd, J = 4.1, 12.8, H-10), 3.49 (3H, s, OMe), 2.51 (1H, t, J = 12.4, H-9β), 2.65 (1H, dd, J = 12.1, 4.1, H-9α), 1.57 (3H, s, Me-1‴), 0.74 (3H, s, Me-1′), 1.26 (3H, s, Me-1″), 7.39–7.57 (5H, m, Ph). Resonances for H-6α and H-6β overlapped with those of the solvent. Mass spectrum (EI): *m*/*z* 442 $[M]^+$.

Crystallographic data for **2** are deposited at the Institute of Chemistry, FEB, RAS. Copies of these data can be obtained at the address: Russian Federation, 690022, Vladivostok, Prosp. 100-Letiya Vladivostoka, 159; fax: (4232) 31 18 89; e-mail: pumalych@ich.dvo.ru.

Determination of Biological Activity. Antimicrobial activity of the isolated compounds was determined against Gram-positive bacteria *Bacillus subtilis* (KMM 430) and *Staphylococcus aureus* (ATCC 21027), Gram-negative bacteria *Pseudomonas aeruginosa* (KMM 433) and *Escherichia coli* (ATCC 15034), and yeast fungus *Candida albicans* (KMM 455) by the literature method [13].

Gametes of the sea urchin *Strongylocentrotus nudus* were used for the test of inhibition of fertilizing ability of spermatozoids and the test of fertilized eggs. The usual procedure was used for this [14].

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