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# A NEW SPIROTRYPROSTATIN FROM THE MARINE ISOLATE OF THE FUNGUS *Aspergillus fumigatus*

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*The new spirocyclic diketopiperazine alkaloid spirotryprostatin F was isolated from the marine isolate of the fungus* Aspergillus fumigatus*. The structure of the compound was established using on NMR spectroscopy, high-resolution mass spectrometry, and acid hydrolysis. Spirotryprostatin F in low and ultralow doses ($10^{-6}$–$10^{-17}$ M) exhibited stimulating action on the growth of sprout roots of soy [*Glycine max *(L.) Merr.], buckwheat (*Fagopyrum esculentum *Moench), and corn (*Zea mays *L.). The dose-effect curve had a bimodal character.*

**Keywords:** spirotryprostatin, marine isolate of the fungus *Aspergillus fumigatus*, NMR spectroscopy, soy, buckwheat, and corn sprout roots.

Recent studies have shown that marine isolates of fungi and micromycetes are promising sources of both new and known biologically active compounds [1]. Marine and terrestrial ecoforms of the fungus *Aspergillus fumigatus* are capable of producing compounds with an amazing variety of structures. Terpenoids, peptides, indole alkaloids, anthraquinones, and other compounds have been identified in its various extracts. The metabolites of this fungus exhibit antibacterial, fungicidal, insecticidal, cytotoxic, and several other types of activity [2]. In continuation of the search for producers of biologically active compounds among marine isolates of microscopic fungi, we studied *A. fumigatus* isolated from soft coral *Sinularia* sp. Herein data on the isolation and structural identification of the new diketopiperazine alkaloid spirotryprostatin F (**1**) are reported and its phytoregulating activity at low and ultralow concentrations is studied.

The fungus was cultivated for 21 d in a specially modified rice medium [3]. The dry EtOAc extract of the culture was separated successively on a column of silica gel and then by normal-phase HPLC to produce pure **1**.

The ESI-MS of **1** contained a peak for the cationic molecule at *m*/*z* 450 $[M + Na]^+$. The molecular formula of **1** was determined as $C_{22}H_{25}N_3O_6$ based on ES-TOF high-resolution mass spectral data and was confirmed by $^{13}C$ NMR spectral analysis. The UV spectrum of **1** had maxima at $\lambda_{max}$ 220 (log 4.24) and 274 (log 3.44) nm that were characteristic for absorption of a substituted benzene ring. The IR spectrum exhibited absorption bands at 3444 (NH), 1715 (γ-lactam CO), 1686, and 1636 $cm^{-1}$ (CO, amide I). The PMR spectrum of **1** contained resonances for a tri-substituted benzene ring (δ 6.97, d, J = 8.5 Hz, H-4; 6.56, dd, J = 2.3, 8.5 Hz, H-5; 6.45, d, J = 2.3 Hz, H-7); an olefinic proton (δ 4.89, m); an N–H proton (δ 8.1, s), a methoxyl (δ 3.79, s, $OC\underline{H}_3$), and two methyls (δ 1.62, s, 3<u>H</u>-21 and 1.11, s, 3<u>H</u>-22) in addition to resonances for several methine and methylene groups. DEPT and HSQC spectra of **1** confirmed the presence of two methyls (δ 18.0 and 25.3) and a methoxyl (δ 55.5, $OC\underline{H}_3$) and indicated the presence of three amide carbonyls (δ 181.3, C-2; 169.0, C-11; 165.1, C-17), an $sp^2$ C atom bonded to O (δ 160.7, C-6), four $sp^2$ (δ 97.4, C-7; 107.6, C-5; 121.6, C-19; 127.3, C-4), and three $sp^3$ (δ 75.5, C-8; 60.7, C-12; 57.5, C-18) methines, several quaternary C atoms and three methylenes.

Resonances of H and C atoms in NMR spectra of **1** practically coincided with the corresponding resonances in spectra of spirotryprostatins C-E, which were recently isolated by Chinese researchers [4], with the exception of the C-2 and C-7a resonances. Figure 1 illustrates COSY-45 spectra and HMBC correlations, which established the complete primary structure of **1**. ROESY correlations between H-4 and H-8 and H-19 and between 9-OH and H-12 and H-18 showed that the first three protons were located on one side of the molecule whereas 9-OH, H-12, and H-18 were situated in the opposite direction.

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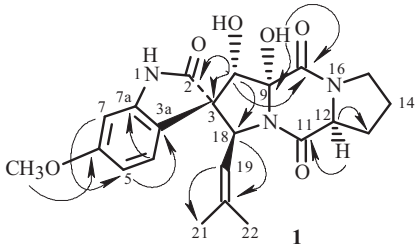
TABLE 1. NMR Spectra of Spirotryprostatin F (**1**) ($CDCl_3$, δ, ppm, J/Hz)

| Atom | $\delta_C$ (DEPT) | $\delta_H$ | HMBC | NOESY |
|---|---|---|---|---|
| 1 (N<u>H</u>) | | 8.10 s | 3, 3a, 7a | 7 |
| 2 | 181.3 (C) | | | |
| 3 | 61.2 (C) | | | |
| 4 | 127.3 (CH) | 6.97 (d, J = 8.4) | 3, 5, 6, 7, 7a, 18* | 8, 18*, 19, 21 |
| 5 | 107.6 (CH) | 6.56 (dd, J = 2.3, 8.5) | 3, 3a, 6, 7, 7a* | 4, 6-OC<u>H</u>$_3$ |
| 6 | 160.7 (C) | | | |
| 7 | 97.4 (CH) | 6.45 (d, J = 2.3) | 3*, 3a, 4, 5, 6, 7a | 1 (N<u>H</u>), 6-$OCH_3$ |
| 8 | 75.5 (CH) | 4.82 s | 2, 3, 3a, 17, 18 | 4 |
| 9 | 87.1 (C) | | | |
| 11 | 169.0 (C) | | | |
| 12 | 60.7 (CH) | 4.61 (dd, J = 7.3, 9.6) | 11, 13, 14* | 9-O<u>H</u>, 13$\alpha$, 14$\alpha$ |
| 13 | 27.6 ($CH_2$) | $\alpha$: 2.38 m | 11, 12, 14, 15 | 12 |
| | | $\beta$: 2.10 m | 11, 12, 14, 15 | |
| 14 | 23.2 ($CH_2$) | $\alpha$: 1.97 m | 12*, 13, 15 | 12 |
| | | $\beta$: 2.05 m | 12, 13, 15 | |
| 15 | 45.1 ($CH_2$) | 3.59 m | 12, 13, 14, 17 | |
| 17 | 165.1 (C) | | | |
| 18 | 57.5 (CH) | 4.92 m | 2, 8, 9*, 19, 20, 21* | 4*, 9-O<u>H</u>, 21, 22 |
| 19 | 121.6 (CH) | 4.89 m | 3*, 18*, 20, 21, 22 | 4, 21, 22 |
| 20 | 139.1 (C) | | | |
| 21 | 25.3 ($CH_3$) | 1.62 s | 19, 20, 22 | 19 |
| 22 | 18.0 ($CH_3$) | 1.11 s | 19, 20, 21 | |
| 6-OC<u>H</u>$_3$ | 55.5 ($CH_3$) | 3.79 s | 6 | 5, 7 |
| 9-O<u>H</u> | | 7.11 s | 9, 17 | 8*, 12, 18 |
| 3a | 117.8 (C) | | | |
| 7a | 142.2 (C) | | | |

*Weak HMBC and NOESY correlations.




**1**

Fig. 1. Structure and key HMBC correlations of **1**.

Acid hydrolysis of **1** gave L-proline, which was identified by Marfey's method using L-FDAA derivatives [5]. Thus, the absolute stereochemistry of the new metabolite from *A. fumigatus* was established as 3*S*,8*S*,9*R*,12*S*,18*S* (Fig. 1). It was called spirotryprostatin F (**1**).

Phytoregulating activity was found for **1** at low and ultralow concentrations on the growth of sprout roots of soy [*Glycine max* (L.) Merr.], buckwheat (*Fagopyrum esculentum* Moench), and corn (*Zea mays* L.) (Fig. 2). However, the studied test cultures reacted differently to the action of the alkaloid. This compound showed the most pronounced stimulating action on the growth of corn sprout roots at concentrations of $10^{-16}$ (109%), $10^{-14}$ (120%), $10^{-12}$ (115%), and $10^{-7}$ (116%) M; soy, $10^{-13}$ (117%), $10^{-10}$ (114%), $10^{-8}$ (115%), and $10^{-6}$ (115%) M; buckwheat, $10^{-10}$ (117%), $10^{-8}$ (111%), and $10^{-6}$ (111%) M. Dead zones were typically observed for this alkaloid between the stimulating doses where the effect corresponded to the control.

The positive control was heteroauxin, which exhibits both stimulating and inhibiting activity on the growth of roots of the test cultures.

Our results agreed somewhat with the literature. Thus, it was observed in one of the early studies that a preparation exhibited the same activity at doses differing by six orders of magnitude ($10^{-13}$ and $10^{-7}$ M) for the action of a hydroperoxide herbicide on plant cell culture whereas the effect was missing at intermediate concentrations [6]. Analogous trends were noted for the action of ultralow doses of triethanolamine derivatives [7] and diterpene glycosides [8] on the growth of root sprouts of monocotyledonous and dicotyledonous plants.

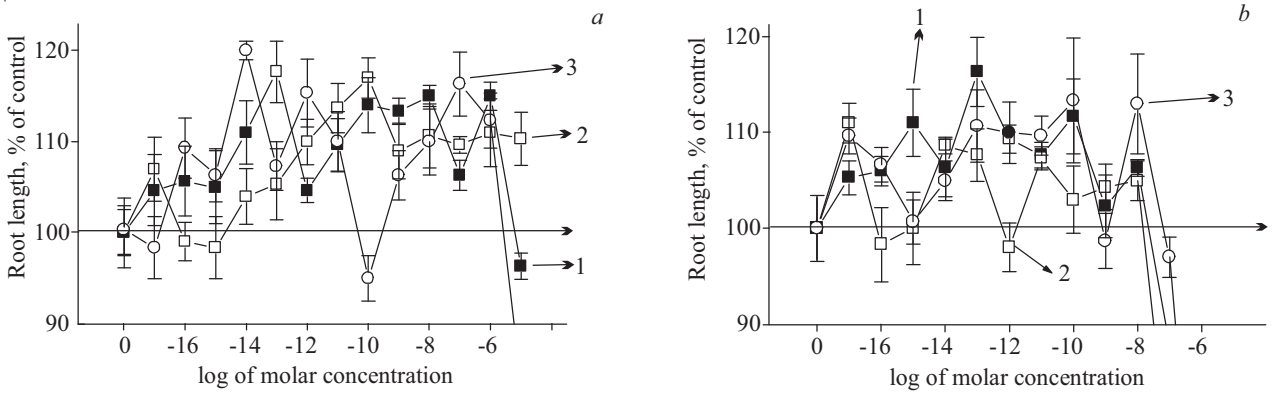



Fig. 2. Effect of spirotryprostatin F (*a*) and heteroauxin (*b*) on growth of sprout roots of soy (1), buckwheat (2), and corn (3).

The study of the action of ultralow doses is promising because it can provide a basis for creating new methods of applying biologically active compounds in plant cultivation.

## EXPERIMENTAL

Optical rotation was measured on a Perkin–Elmer 343 polarimeter (Germany). UV spectra were recorded in MeOH on a Shimadzu UV-1601 PC spectrophotometer (Japan). PMR and $^{13}C$ NMR spectra were recorded in $CDCl_3$ with $Me_4Si$ internal standard on a Bruker DRX-700 spectrometer (700 and 175.6 MHz, respectively). IR spectra were obtained in $CCl_4$ on a Bruker OPUS Vector-22 spectrophotometer. HMBC correlations were optimized for 8 Hz, NOESY (350 ms). ESI-TOF mass spectra were taken on an Agilent 6510 LCQ-TOF spectrometer (USA). The potential in the capillary, nozzle, and skimmer and the collision energy were 3500, 215, and 65 V, and 35 V, respectively. Column chromatography used silica gel L (40/100 μm, Chemapol, Czechoslovakia). HPLC was performed on an Agilent 1100 Series chromatograph (USA).

**Cultivation of *A. fumigatus* (KMM 4631).** The strain was isolated from soft coral *Sinularia* sp. (Kunashir Is., Kuril Islands, 52 m depth). Fermentation was carried out at 25°C in 10 Ehrlenmeyer flasks (500 mL) containing rice (20 g), yeast extract (20 mg), $KH_2PO_4$ (10 mg), and natural seawater (40 mL).

**Isolation of Spirotryprostatin F.** Fungus mycelium together with medium was ground and extracted (×3) with EtOAc. The extract was evaporated. The solid (1.2 g) was chromatographed over a column (7 × 13 cm) of silica gel with elution successively by hexane and hexane:EtOAc systems (stepwise gradient, 5:1→1:4). The fraction (210 mg) eluted by the 1:4 system was separated by HPLC over a Hypersil Si column (25 cm × 4.6 mm, 5 μm) using $CHCl_3$:*i*-PrOH:MeOH (100:0.5:1.0) to afford pure spirotryprostatin F (28 mg).

**Spirotryprostatin F.** $C_{22}H_{25}N_3O_6$, pale-yellow powder, $[\alpha]_D^{20}$ –52° (*c* 0.1, $CHCl_3$). UV spectrum (MeOH, $\lambda_{max}$, nm, log ε): 220 (4.24), 274 (3.44), 295 (3.36). IR spectrum ($CCl_4$, ν, $cm^{-1}$): 3444, 2927, 2855, 2360, 1747, 1715, 1686, 1458. ESI-HR-MS (*m*/*z*): 450.1657 $[M + Na]^+$; calcd: 450.1636. Table 1 presents the NMR spectra.

**Acid Hydrolysis of Spirotryprostatin F.** Compound **1** (1.5 mg) was placed in a glass ampul and dissolved in HCl solution (6 N, 0.8 mL). The ampul was evacuated, heated at 105°C for 24 h, and cooled. The reaction mixture was diluted with doubly distilled $H_2O$ and concentrated *in vacuo*. The absolute configuration of the amino acid was determined by Marfey's method [5].

**Determination of Phytoregulating Activity.** We studied sprout roots of soy (*G. max*), buckwheat (*F. esculentum*), and corn (*Z. mays*). Seeds from the 2010 harvest were obtained from Primorskii Agricultural Research Institute, RAAS (Ussuriisk, Russia). We used a sprouting scheme in rolls of filter paper. Dry seeds were spread on strips of filter paper (12 × 42 cm) that were previously moistened with test solution, rolled, placed into beakers with a small amount of test solution (100 mL), and left for 3 d in a thermostat at 26–27°C. After incubation, the length of the main root of the sprouts was measured. The controls were sprouts of the same culture grown in distilled $H_2O$. The positive control was heteroauxin. Test results were estimated as the arithmetic mean of three repeated tests (20 seeds in each) and were expressed in percent of the controls (M ± se). Results were processed statistically using the ORIGIN 7.0 computer program. The significance of the results between control and test samples was estimated using the Student *t*-criterion ($p < 0.05$).

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