

Pollen Growth Regulator, Fusanolide A, and a Related Metabolite from *Fusarium* sp.

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Z. Naturforsch. **57b**, 239–242 (2002); received September 19, 2001

Fusanolide, Pollen, Growth Regulator

Fusanolides A (**1**) and B (**2**) were isolated from cultures of the fungus *Fusarium* sp. as pollen growth regulators and their structures were established by spectroscopic evidence. **1** completely inhibited pine pollen germination and tea pollen tube growth at a concentration of 300 mg/l, but **2** showed no inhibitory effect on them at the same concentration.

Introduction

We have investigated fungal metabolites as pollen growth regulators using bioassay methods with pine and tea pollen grains, because the regulators may be useful for developing new herbicides and as tools to analyze plant reproductive functions in higher plants. So far, we have isolated and characterized naphthoquinones [1], vulculic acid [2], hericerine [3], emeniveol [4], isofunicone [5], and simplicissin [6] as pollen growth and germination inhibitors. In the course of our screening search

for new pollen growth regulators, we found the presence of the regulator in the cultural metabolites of *Fusarium* sp.. Bioassay-guided fractionation led to isolation of the active compound (**1**) and related one (**2**). In this report, we describe the structures and biological activities of **1** and **2**.

Results and Discussion

Fusanolides A (**1**) and B (**2**) were isolated from the culture filtrate of a 21-day-old stationary culture of *Fusarium* sp. in a malt extract medium.

Table 1. ¹³C (125 MHz) and ¹H (500 MHz) NMR data for **1** and **2**.

Position	1 ^a		2 ^a	
	¹³ C ^b	¹ H ^c	¹³ C	¹ H
1	170.9 (s)		171.0 (s)	
2	114.8 (d)	5.59 (d, 11.2, 1H)	123.7 (d)	5.90 (dd, 12.2, 1.0, 1H)
3	146.2 (d)	6.65 (dd, 12.2, 11.2, 1H)	138.8 (d)	5.83 (dd, 12.2, 2.4, 1H)
4	127.3 (d)	7.35 (dd, 15.0, 12.2, 1H)	73.0 (d)	4.65 (ddd, 7.8, 2.4, 1.0, 1H)
5	147.4 (d)	6.17 (ddd, 15.0, 7.7, 7.3, 1H)	131.8 (d)	5.65 (dd, 15.7, 7.2, 1H)
6	32.9 (t)	2.26 (m, 2H)	139.5 (d)	5.55 (dd, 15.7, 8.3, 1H)
7	24.9 (t)	1.49 (m, 4H)	73.7 (d)	4.12 (ddd, 11.2, 8.3, 3.4, 1H)
8	38.7 (t)	1.49 (m, 4H)	44.8 (t)	1.73 (ddd, 13.7, 11.2, 11.2, 1H) 1.90 (ddd, 13.7, 3.4, 1.9, 1H)
9	67.9 (d)	3.82 (ddq, 7.0, 6.5, 6.4, 1H)	71.0 (d)	5.25 (ddq, 11.2, 1.9, 6.4, 1H)
10	23.5 (q)	1.22 (d, 6.4, 3H)	22.4 (q)	1.21 (d, 6.4, 3H)

^a **1** in CDCl₃ and **2** in CD₃OD;

^b the s, d, t and q in the ¹³C NMR spectral data show multiplicities;

^c the d, m and q, and the numbers in parentheses in the ¹H NMR spectral data show multiplicities and coupling constants in Hz.

The molecular formula of **1** was determined by MS and the ^{13}C and ^1H NMR spectra to be $\text{C}_{10}\text{H}_{14}\text{O}_2$. The IR absorption band at 1692 cm^{-1} and one signal at $\delta = 170.9$ in the ^{13}C NMR spectrum indicated the presence of a conjugated carbonyl carbon. The ^{13}C and ^1H NMR spectra of **1** (Table 1) indicated the presence of one methyl, three methylene, one *O*-substituted aliphatic methine, and four olefinic methine groups. Two olefinic protons at $\delta = 5.59$ and 6.65 indicated the *Z* geometry by their coupling constants ($J = 11.2$ Hz). On the other hand, the coupling constants ($J = 15.0$ Hz) of two olefinic protons at $\delta = 6.17$ and 7.35 showed the *E* geometry. Detailed analysis of ^1H - ^1H COSY and homospin-decoupling experiments led to the partial structure corresponding to C-2 – C-10 in **1**. From these results, the planar structure of **1** was established to be (2*Z*, 4*E*)-2,4-decadiene-9-olide and the compound was named fusanolide A (Fig. 1).

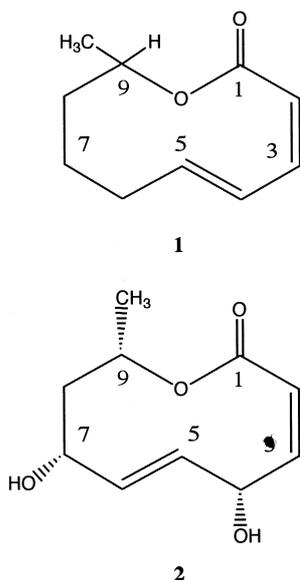


Fig. 1. Structures of fusanolides A (**1**) and B (**2**).

The molecular formula of **2** was determined by MS and elemental analysis to be $\text{C}_{10}\text{H}_{14}\text{O}_4$. The IR absorption band at 1717 and 1641 cm^{-1} and one signal at $\delta = 171.0$ in the ^{13}C NMR spectrum indicated the presence of an α , β -unsaturated carbonyl carbon. A diacetyl derivative of **2** was obtained by treatment of **2** with acetic anhydride-pyridine. This chemical evidence indicated that two hydroxy groups were present in **2**. The ^{13}C

and ^1H NMR spectra of **2** (Table 1) indicated the presence of one methyl, one methylene, three *O*-substituted aliphatic methine, and four olefinic methine groups. Two olefinic protons at $\delta = 5.55$ and 5.65 indicated the *E* geometry by their coupling constants ($J = 15.7$ Hz). On the other hand, the coupling constants ($J = 12.2$ Hz) of two olefinic protons at $\delta = 5.83$ and 5.90 showed the *Z* geometry. Detailed analysis of ^1H - ^1H COSY and homospin-decoupling experiments led to the partial structure corresponding to C-2 – C-10 in **2**. The relative stereochemistry of **2** was deduced from the ^1H NMR coupling constants and ROESY correlations (Fig. 2). The stereochemistry of 4-H was assigned to be β with equatorial orientation in the view of two coupling constants of 4-H ($J = 2.4$ and 7.2 Hz) and NOEs between 4-H and 5-H [7–10].

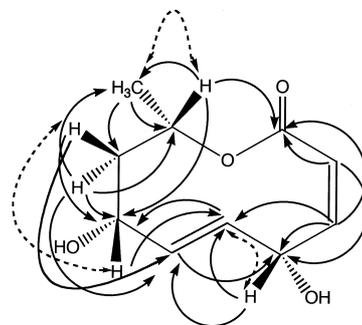


Fig. 2. Key HMBC (\rightarrow) and ROESY ($\leftarrow\leftarrow\leftarrow$) correlations observed for **2**.

The coupling constants between 7-H and 8- H_2 ($J = 3.4$ and 11.2 Hz) indicated that 7-H was β with axial orientation [10]. The coupling constants between 9-H and 8- H_2 ($J = 1.9$ and 11.2 Hz) indicated that 9-H was also β with axial orientation. These results indicated that the relative configurations at C-4, C-7 and C-9 were $4R^*$, $7R^*$ and $9S^*$, respectively. From these results, **2** was established to be ($4R^*$, $7R^*$, $9S^*$)-(2*Z*, 5*E*)-4,7-dihydroxy-2,5-decadiene-9-olide and the compound was named fusanolide B (Fig. 1). Compounds **1** and **2** were the ten-membered lactone such as pyrenolides [11, 12] and diploidalides [13–15].

Plant growth activities of **1** and **2** toward pine and tea pollen were examined (Fig. 3). With pine pollen, **1** and **2** showed no inhibitory effect on the germination at concentrations of 3 and 30 mg/l. **1** completely inhibited the germination at a concentration of 300 mg/l, but **2** showed no inhibitory ef-

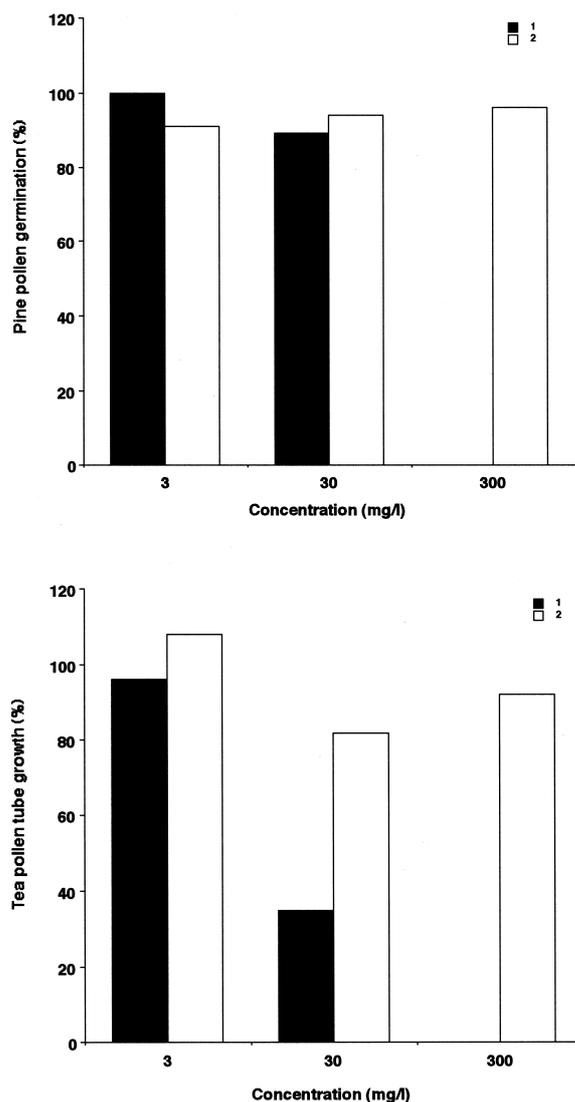


Fig. 3. Effects of **1** and **2** on pine pollen germination and tea pollen tube growth.

fect on that at the same concentration. With tea pollen, **1** inhibited the pollen tube growth to 35% of control at a concentration of 30 mg/l and completely inhibited that at a concentration of 300 mg/l. On the other hand, **2** showed no inhibitory effect on the pollen tube growth at the concentration from 3 mg/l to 300 mg/l, respectively. These results suggest that the long conjugated system such as α , β - γ , δ -unsaturated carbonyl group in **1** may play an important role in promoting inhibitory activity.

Experimental Section

Melting points were determined using a Yanagimoto micromelting point apparatus. Optical rotations were determined on a HORIBA SEPA-200 polarimeter. UV and IR spectra were recorded on a Hitachi 100-50 and a JASCO FT/IR-7000 spectrometer, respectively. All the NMR spectra including HMQC, HMBC and ROESY spectra were obtained on a JEOL JNM-ESP 500 NMR spectrometer. The MS spectrum was taken on a Hitachi RMU-6U spectrometer.

Isolation and purification of fusanolides A (**1**) and B (**2**)

Fusarium sp. was cultured stationarily in a malt extract medium at 24 °C for 21 days. The culture broth (20 l) was filtered, and the filtrate was adjusted to pH 2.0 with 2 N HCl, before being extracted twice with EtOAc. The combined solvents were concentrated *in vacuo*, and the resulting residue was fractionated by column chromatography on silica gel (hexane-acetone). The active fraction eluted with 20% acetone was further fractionated by column chromatography on silica gel (hexane-EtOAc) and purified by preparative TLC developing with benzene-EtOAc(3:7, v/v) to afford 36 mg of **1** (R_f : 0.39, benzene-EtOAc, 1:1, v/v) as yellow oil. The fraction eluted with 40% acetone was recrystallized from EtOAc to afford 813 mg of **2** (R_f : 0.21) as colorless needles.

Analytical and spectroscopic data of **1** and **2**: Fusanolide A (**1**)

$[\alpha]_D^{20}$ -4.9° (c 1.0, MeOH). – UV/vis (EtOH): λ_{max} ($\lg \epsilon$) = 250 nm (3.73). – IR (KBr): $\bar{\nu}$ = 2930 (C=C), 1692 (O-C=O), 1638, 1603 (C=C), 1434, 1379, 1262, 1096, 1005, 802 cm^{-1} . – MS (EI): m/z (%) = 166 (25) [M^+], 152 (19), 124 (35), 98 (27), 82 (100).

Fusanolide B (**2**)

M.p. 180–182 °C. – $[\alpha]_D^{20}$ $+25.0^\circ$ (c 0.2, MeOH). – UV/vis (EtOH): λ_{max} ($\lg \epsilon$) = 208 nm (3.33). – IR (KBr): $\bar{\nu}$ = 3294 (OH), 2984 (C=C), 1717 (O-C=O), 1641 (C=C), 1402, 1220, 1115, 1029, 987, 948, 747 cm^{-1} . – MS (EI): m/z (%) = 198 (8) [M^+], 138 (29), 126 (25), 100 (42), 96 (74), 84 (100). – $C_{10}H_{14}O_4$ (198.22): calcd. C 60.59, H 7.12; found C 60.59, H 7.12.

Acetylation of **2**

2 (10 mg) was acetylated with acetic anhydride (0.1 ml) and pyridine (0.2 ml) for 24 h at room

temperature. Purification by preparative TLC in benzene-EtOAc (3: 7, v/v) gave a diacetyl derivative of **2** (9 mg) as colorless oil.

Diacetyl derivative of **2**: ^1H NMR (270.05 MHz, CDCl_3): δ = 1.25 (d, J = 6.5 Hz, 3H), 1.89 (m, 2H), 2.03 (s, 3H), 2.07 (s, 3H), 5.28 (m, 2H), 5.76 (m, 4H), 5.95 (d, J = 15.2 Hz, 1H). – MS (EI): m/z (%) = 282 (3) [M^+], 222 (9), 178 (65), 158 (98), 140 (100), 99, (96), 85 (80).

Bioassay for pine pollen germination

Pollen grains of *Pinus thunbergii* Parl. were collected from an open flower, dried in a desiccator over silica gel and stored in a refrigerator. The grains were sown with a paintbrush on a 1.5% agar medium containing 10% sucrose and the compound to be tested at various concentrations on a

microscopic slide, and then incubated in a moist chamber at 24 °C in the dark. After cultivation for 3 days, the number of germinated grains was measured and compared with that of an untreated control [1].

Bioassay for tea pollen tube growth

Pollen grains of *Camellia sinensis* O. Kuntze were collected from an open flower, dried in a desiccator over silica gel and stored in a refrigerator. The grains were sown with the edge of a cover glass on a 1.5% agar medium containing 10% sucrose, 10 ppm boric acid and the compound to be tested at various concentrations on a microscopic slide, and then incubated in a moist chamber at 24 °C in the dark. After cultivation for 12 h, the length of pollen tube was measured and compared with that of an untreated control [16].

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