Antifungal Compounds from the Root and Root Exudate of Zea mays

Sun Park, Yuuko Takano, Hideyuki Matsuura, and Teruhiko Yoshihara

Laboratory of Bio-organic Chemistry, Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Kita 9, Nishi 9, Kitaku, Sapporo 060-8589, Japan

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A maize plant (Zea mays) planted in a test tube was found to inhibit the growth of the soil-borne plant pathogen, Fusarium oxysporum f. sp. melongenae. The antifungal compounds, 6-methoxybenzoxazolinone and 6,7-dimethoxybenzoxazolinone, were isolated from an ethanol extract of Zea mays roots, and (6R)-7,8-dihydro-3-oxo-α-ionone and (6R,9R)-7,8-dihydro-3-oxo-α-ion were isolated from the root exudate.

Key words: Zea mays; antifungal activity; Fusarium oxysporum f. sp. melongenae; Cephalosporium gregatum

Farmers tend not to grow identical crop species in the same field for several years. This is because continuous cropping results in the accumulation of populations of specific plant pathogens, and leads to a decline in crop yield and quality. Farmers, therefore, attempt to minimize the accumulation of soil-borne pathogens by crop rotation with specific crop species.1) Farmers tend not to grow identical crop species in the same field for several years. This is because continuous cropping results in the accumulation of soil-borne plant pathogens by crop rotation with specific crop species.1) Zea mays (maize) is commonly used in the control of soil-borne diseases such as tomato Fusarium wilt by F. oxysporum and brown stem rot of adzuki bean by Cephalosporium gregatum. We report here the inhibition of growth of Fusarium oxysporum f. sp. melongenae by chemical factors from maize, and provide a structural elucidation of the antifungal compounds. In addition, we evaluate the antifungal activity of these compounds against Fus. oxysporum and C. gregatum.

An ethanol extract of Z. mays roots (5 kg) was separated by silica gel column chromatography to afford two known compounds, 6-methoxybenzoxazolinone (MBOA, 1, 2.4 mg)2) and 6,7-dimethoxybenzoxazolinone (DMBOA, 2, 11.5 mg),3) together with the common antifungal compounds, p-hydroxybenzaldehyde, p-ethyl coumarate, and syringaldehyde.

In order to analyze the root exudate, maize seeds (4 seeds/pot) were grown in 6 pots each filled with 15 liters of vermiculite and activated charcoal (2:1, v/v) for three months. A sample of the soil mixture (90 liters) was taken in 3 stainless steel columns (φ39 cm; 39 cm height). The exudate of Z. mays that had been adsorbed to the vermiculite and activated charcoal was successively extracted with 70% aq. EtOH, EtOH, EtOAc, and n-hexane (100 liters each). The EtOH (448 mg) and EtOAc (1.98 g) eluates were combined and partitioned with EtOAc to give a residue (1.7 g). This residue was purified by a series of chromatographic techniques to afford compounds 3 (8.9 mg) and 4 (4.1 mg).

Compound 3 was isolated as a yellow oil, [α] D 23 +48.3° (c 0.41, CHCl3). The HREIMS data confirmed its molecular formula to be C15H20O2 (found, 208.1461; calcd., 208.1464). The 1H-NMR spectral data of 3 exhibited an olefinic methyl group at δH 2.00 (H-13) and a methylene group at δH 2.05 (H-2a) and 2.37 (J = 17.4 Hz, H-2b). The signals of δH 1.02 (H-11), 1.07 (H-12) and 2.16 (H-10) were attributed to the methyl groups. Since these spectral data, together with the optical rotation value, agreed closely with previously reported data,4)–6) the chemical structure of 3 was determined to be (6R)-7,8-dihydro-3-oxo-α-ionone.

Compound 4 was obtained as a colorless oil. The HREIMS data for 4 gave the molecular formula of C13H12O2 (found, 210.1643; calcd., 210.1620). The 1H-NMR spectral data were similar to those of 3, except for the resonances at δH 2.52~2.56 (2H, m, H-8), 3.77 (1H, m, J = 6.2 Hz, H-9) and 2.16 (3H, s, H-10). Since these spectral data, together with the 13C-NMR data and optical rotation value, [α] D 23 44.6° (c 0.26, CHCl3), agreed closely with previously reported data,7)–9) the chemical structure of 4 was determined to be (6R,9R)-7,8-dihydro-3-oxo-α-ionone.

To determine the inhibitory effect of Z. mays against soil-borne pathogens in vivo, the number of the soil mixtures affected by Z. mays was counted. After soaking in 0.1% NaClO for 30 min, Z. mays seeds (1 seed/test tube) were sown in test tubes containing sterilized vermiculite (40 ml) and maintained for 2 weeks. A spore suspension was adjusted to a concentration of 3.5 × 107 cfu/ml and inoculated (20 ml) into each test tube. The soil sample obtained from one test tube was suspended in sterilized water (40 ml), and CFUs of this soil suspension were determined by the 10-fold dilution method.10) Eight days after inoculating with F. oxysporum, we observed that the population of the colonies from the Z. mays samples had not changed, whereas those of the control sample

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1. To whom correspondence should be addressed. Tel: +81-11-706-2495; Fax: +81-11-706-2505; E-mail: yoshihara@chem.agr.hokudai.ac.jp

Abbreviation: CFU, colony forming unit
had increased. This data suggests that the inhibitory effect was due to antifungal compounds from the roots of *Z. mays* (Fig. 1).

The antifungal activity was measured by TLC bioautography. Compounds 1 and 2 showed inhibitory activity against *F. oxysporum* growth at a dose of 5 μg, whereas compounds 3 and 4 were ineffective at the same dose. All compounds showed an inhibitory effect against *F. oxysporum* growth at a dose of 25 μg. In order to investigate the effect of the isolated compounds on spore germination, spores of *C. gregatum* (2 × 10⁵ cfu/ml) were incubated in a potato dextrose broth medium (1 ml) with these isolated compounds (0.1, 1, 10, or 100 mg/liter) at 25 °C for 3 days. The addition of compounds 3 and 4 (each at 1 mg/liter) respectively inhibited spore germination of *C. gregatum* by about 70% and 50%.

We found that planting *Z. mays* suppressed the colonization of *F. oxysporum*. It was suggested that some antifungal compounds were partly responsible for this inhibitory effect on the growth of the fungus. In our attempt to identify these antifungal compounds, we isolated 6-methoxybenzoxazolinone (1) and 6,7-dimethoxybenzoxazolinone (2) from an ethanol extract of the roots. These compounds have been identified from several Graminea plants such as maize, wheat and rye, and have also been reported for host plant resistance. Compounds 3 and 4, which possess an α-ionone skeleton, have been isolated from tobacco (*Nicotiana tabacum* L.), but this is the first report of their isolation from corn (*Z. mays*). These two compounds are usually isolated as ionone-glycosides; for example, the 9-O-glucoside type from mature fruit of *Capparis spinosa* and *Nicotiana* species and the 4-O-glucosyl type from the fruit of raspberry (*Rubus idaeus*).

We have demonstrated in this study the inhibitory effect on the growth of a soil-borne pathogen in vivo, and suggest that this inhibitory effect was due to antifungal compounds 1–4 derived from the roots of *Z. mays*. We hope this preliminary data will lead to a better understanding of crop rotation and change in soil flora.

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**References**


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