Monocerin and Ziganein: Phytotoxins from Pathogenic Fungus *Exserohilum monoceras* Inu-1

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Two phytotoxic compounds were isolated from a culture of *Exserohilum monoceras* Inu-1, a fungal pathogen of Barnyard grass. The structure was determined by spectroscopic analyses including 2D NMR experiments. During the isolation procedure, the toxic components were monitored by the assay using Italian ryegrass (*Lolium multiflorum* Lam.), a host plant of the pathogen. The compounds inhibited the root growth of the host plant seedlings at a level of 100 ppm. While no substantial inhibition was observed even at 300 ppm in non-host plant seedlings such as lettuce and tomato.

Key words : *monocerin, ziganein, phytotoxin,* Exserohilum monoceras *Inu-1.*

Among the many known ways in which plant pathogenic fungi cause disease in plants, phytotoxins produced by the fungi are the most direct means and are considered to be one of the major factors in the mechanism.[1] In particular, pathogenic fungi which cause necrotic spots on the leaves of host plants produce intriguing phytotoxic metabolites. Phytotoxins are also of interest from the viewpoint of development of new types of plant growth regulator,[2,3] as many pesticides have been developed from lead-compounds of natural origin.

In previous studies,[4-8] the author isolated various phytotoxins from the culture of some pathogenic fungi and elucidated the mode of action of these compounds.

Exserohilum monoceras Inu-1 is a pathogenic fungus which affects barnyard grass (*Echinochloa crusgalli* P. Beauv). Fungal infections are characterized by dark brown irregular spots (0.6~3.0 mm) on the leaves of the host. This observation is consistent with the involvement of toxin(s) of some type, which are produced by the pathogenic fungus. In this paper, the author reports the isolation and the structural determination of two phytotoxins isolated from a culture of *E. monoceras* Inu-1, which showed toxic activity on Italian ryegrass (*Lolium multiflorum* Lam.).

Materials and Methods

Fungus and plant. A strain of *E. monoceras* Inu-1 was kindly supplied by Dr. M. Tsuda of Pesticide Research Institute, Kyoto University, Japan. The seeds of Italian ryegrass used for the bioassay were purchased from Takii seed Co (Kyoto, Japan). The seeds of barnyardgrass (*Echinochloa crusgalli* P. Beauv), bulrush (*Scirpus juncoides* ROXB.), and rice (*Oryza sativa* L.) were obtained from Department of Agronomy, Chungnam National University. The seeds of lettuce and tomato were purchased from Hungnong Seed Co (Seoul, Korea).

Phytotoxicity assay: Inhibitory activity of root elongation of seedlings.[9] A specified amount of the test compound dissolved in MeOH was applied to a Petri dish (35 mm in diameter) in which two layers of Whatman No. 2 filter paper were laid. After the solvent was evaporated, one ml of water containing 0.5% Tween 20 (Nakarai Chemicals Ltd., Kyoto, Japan) was added to the dish to dissolve the test compound. Uniformly germinated seeds were selected, with roots ca. 5 mm in length, and placed in the dish (9 seeds per plate) and incubated under dark condition at 26 °C for 72 hrs. The inhibitory activity was evaluated by comparing the root length to that of the control.

Spectral measurements. Optical rotations were measured on a JASCO model J-5 in MeOH solution at 21°C. The IR spectra were determined on a Pye Unicam SP 3200 Infra-Red Spectrophotometer. Electron ionization mass spectra (EI-MS) were obtained on a Hitachi M-80A. The ^{1}H and ^{13}C NMR spectra were determined on a JEOL 300 (300 MHz for ^{1}H and 75 MHz for ^{13}C) in $CDCl_3$, using the tetramethylsilane (TMS) signal as an internal standard. The characters q, t, d and s denote methyl, methylene, methine and quaternary carbon, respectively. High performance liquid chromatography (HPLC) were performed on Hitachi L-6200 apparatus equipped with L-4200H UV-Vis detector using a Cosmosil ODS-18 column (20 i.d.×250 mm).

Isolation of phytotoxins. The fungus *E. monoceras* Inu-1 was cultured on potato-sucrose agar plates (750 plates, 90 mm in diameter) in the dark at 26°C for 14 days. The culture plates were soaked in acetone, and the acetone extract was filtered and concentrated *in vacuo* at 40°C. The

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concentrated aqueous residue was reextracted with a mixture of *n*-hexane and EtOAc (1:1, v/v) and the solvent was removed *in vacuo* to obtain a crude extract (4.6 g). This material was subjected to silica gel (Kiesel gel 60, 70~230 mesh, 230 g) column chromatography, using *n*-hexane containing increasing portions of EtOAc to afford 9 fractions of 250 ml each. Fractions 2~3 and 5~7 showed strong phytotoxic activity against Italian ryegrass seedlings. Fraction 2 (2.4 g), obtained from *n*-hexane-EtOAc (90:10), was further purified on HPLC with the solvent system of 80% MeOH, to afford five pure active compounds. Of the five compounds, two (retention time 12.86, 25.27 min) were structurally characterized.

Physicochemical data. Compound 1; Yield 38 mg; $[\alpha]_D^{21}$ = +45(c=1.0) in MeOH; IR(CCl_4) 3490, 1678, 1610, 1580, 1510 cm^{-1}; EI-MS *m/z* (intensity, %) 308(M^+, 100), 265(61), 209(49), 192(22), 179(21), 163(16); ^{1}H-NMR ($CDCl_3$, δ): 0.92(3H, t, *J*=7.28 Hz), 1.40(2H, m), 1.58(2H, m), 2.18(1H, m), 2.59(1H, m), 3.90(3H, s), 3.95(3H, s), 4.12 (1H, m), 4.54(1H, d, *J*=3.13 Hz), 5.05(1H, m), 6.59(1H, s), 11.28(1H, s, OH); ^{13}C-NMR($CDCl_3$, δ): 14.0(q), 19.1(t), 38.1 (t), 39.1(t), 56.3(q), 60.7(q), 74.5(d), 78.7(d), 81.3(d), 102.1 (s), 104.5(d), 131.3(s), 137.4(s), 156.2(s), 158.7(s), 167.8(s)

Compound 2; orange needles; Yield 16 mg; $[\alpha]_D^{21}$ = + 31.3(c=0.8) in MeOH; IR(KBr) 3508, 1698, 1200cm^{-1}; EI-MS *m/z* (intensity, %) 254(M^+, 11), 185(10), 149(52), 69(58); ^{1}H-NMR($CDCl_3$, δ): 2.46(3H, s), 7.09(1H, d, *J*=1.44 Hz), 7.29(1H, dd, *J*=7.53, 1.20 Hz), 7.65(1H, m), 7.69(1H, d, *J*=7.53 Hz), 7.83(1H, dd, *J*=7.53, 1.20 Hz) 11.99(1H, s, OH) 12.11(1H, s, OH); ^{13}C-NMR($CDCl_3$, δ): 21.21(q), 113.70(s), 115.84(s) 119.88(d), 121.31(d), 124.32(d), 124.51(d), 133.25 (s), 133.61(s), 136.90(d), 149.30(s), 162.38(s), 162.68(s), 181.93(s), 192.49(s)

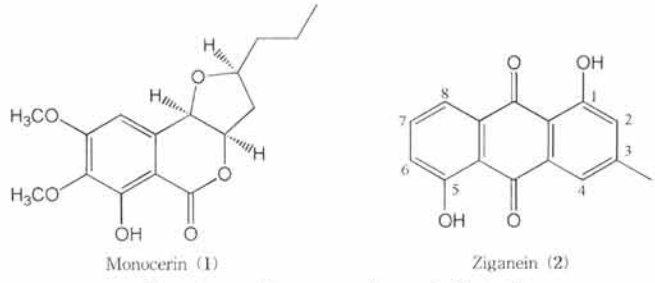
Results and Discussion

Compound 1. EI-MS data of **1** gave the molecular ion at *m/z* 308. The ^{1}H NMR spectrum of **1** gave signals assignable to twenty protons, and ^{13}C NMR spectrum with an aid of INEPT experiment showed the presence of one methyl group, two methoxy groups, three methylene, four methine groups and six non proton-bearing carbon atoms. The relationship between the proton and the carbon signals was determined based on the analysis of the correlation signals in ^{13}C-^{1}H COSY spectrum. The ^{1}H NMR spectrum of **1** showed signals attributable to a hydrogen-bonded phenolic group (δ_H 11.28), two methoxy groups (δ_H 3.95, 4.10), a benzenoid proton (δ_H 6.59), and a primary methyl group (δ_H 0.92). The IR spectrum indicated the presence of OH (3490 cm^{-1}) and carbonyl (1678cm^{-1}) groups. The molecular formula ($C_{16}H_{20}O_6$) indicated the degree of unsaturation of **1** to be 7. Since one carbonyl group and one benzene ring, as described above, **1** was considered to have three-ring structure including a benzene ring.

The deduced planar structure was identical with that of monocerin.[10,11] The stereochemistry of these compounds could be same, because specific optical rotations data of **1** were in good agreement with those of monocerin.[10]

Compound 2. The molecular formula of **2** was found to be $C_{15}H_{10}O_4$ by HRMS (obsd. *m/z* of 254.0564, calcd. 254.0579). The IR spectrum indicated the presence of OH (3508 cm^{-1}) and carbonyl (1698 cm^{-1}) groups. The ^{1}H NMR spectrum of **2** gave signals assignable to ten protons, and ^{13}C NMR spectrum with an aid of INEPT experiment showed the presence of one methyl group, five methine groups and nine non proton-bearing carbon atoms.

The ^{1}H NMR spectrum showed five aromatic protons and the characteristic downfield signals for the chelated hydroxyl groups at δ 11.99 and δ 12.11, respectively. It further showed a methyl group attached to aromatic ring at δ 2.46 (3H, s). Two quaternary signals at δ_C 181.93 and 192.49 suggested the carbonyl group of **2** to be attributable to ketone form. According to these data compound **2** was characterized as ziganein, 1,5-dihydroxy-3-methyl-anthraquinone, reported earlier to be found in roots of *Digitalis orientalis*.[12]



Monocerin (1) Ziganein (2)

Structure of monocerin and ziganein

Monocerin and ziganein completely inhibited the root elongation of the host plant seedlings, as well as causing root necrosis at a level of 100 ppm (Table 1). On the other hand, no substantial inhibition was observed even at 300 ppm in non-host plant seedlings such as lettuce and tomato. These results suggest that these phytotoxins play important role not only in the expression of disease symptoms by this fungus, but also in the host selection.

Table 1. Inhibitory activity of root elongation of seedlings by phytotoxins isolated from *Exserohilum monoceras* Inu-1.

Plant	Conc. of monocerin (mg/l)			Conc. of ziganein (mg/l)		
	50	75	100	50	75	100
Ryegrass	+	+	+++	+	+++	+++
Barnyardgrass	+	++	+++	++	++	+++
Bulrush	++	++	+++	+	++	+++
Rice	+	+	++	+	+++	+++
Lettuce	+	+	+	+	+	+
Tomato	+	+	+	+	+	+

The inhibitory activity was evaluated by comparing the root length to that of the control.
+++, above 91%; ++, 90-31%; +, below 30%.

Monocerin was first described by Aldridge and Turner[11] as a product of *E. monoceras* Leonard et Suggs (=*Helminthosporium monoceras* Drechsler) which protected wheat against powdery mildew (*Erysiphe graminis* D.C. ex Merat). Grove and Pople[13] identified the same compound as a constituent of the entomogenous fungus *Fusarium larvarum* Fuckel and demonstrated its insecticidal properties. Thus, monocerin is known to possess a broad spectrum of biological activity. Ziganein is reported as a rare pigment and possess antimicrobial and antitumour activities found earlier in the roots of *Digitalis orientalis, D. schischkii*[14] and *Cassia italica*.[15] Further investigation is required to elucidate the mode of action of these compounds.

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