Note

The Structure of Zinnolide, a New Phytotoxin from *Alternaria solani*

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Although a number of phytotoxins have been isolated from *Alternaria solani*,¹⁾ a causal fungus of the early blight disease of tomato and potato, the presence of other phytotoxins has been suggested.²⁾ Very recently, we reported the structural determination of three new phytotoxins, solanapyrones A, B and C, from the culture filtrate of the fungus.^{3,4)} In our continuing search for isolation of phytotoxins, we have further examined the culture filtrate of the fungus and isolated a new phytotoxin named zinnolide (**1**) through the procedure described in EXPERIMENTAL.

Zinnolide (**1**), a colourless solid, mp 171~173°C, has the molecular formula $C_{15}H_{18}O_5$ from its high resolution mass spectrum. The IR spectrum showed absorption bands at 3200 cm^{-1} due to a hydroxyl group and at 1720 cm^{-1} due to a carbonyl group and at 1600 cm^{-1} due to a phenyl group. Since the ^1H NMR spectrum exhibited characteristic signals at δ 2.69 (3H, s), 2.75 (3H, s), 4.45

(2H, d, $J=7\text{ Hz}$) and 5.35 (1H, br.t, $J=7\text{ Hz}$) ascribable to a prenyl moiety, at δ 2.13 (1H, s) assignable to a methyl group on an aromatic nucleus, and at δ 3.90 (3H, s) attributable to a methoxyl group, it was deduced that the phytotoxin had a closely related structure to that of zinniol (**2**), which has been isolated from the phytopathogenic fungus *Alternaria zinniae*.⁵⁾ Extensive comparison between zinnolide (**1**) and zinniol (**2**) by the ^1H NMR spectrum revealed that two hydroxymethyl groups in **2** were completely missing in **1**. Instead, the presence of a lactol moiety (δ 6.60, 1H, O-CHOCO) was deduced in zinnolide **2**. In fact, reduction of **1** with LiAlH_4 in ether afforded a diol, whose spectral data were completely identical with those of authentic (**2**) in all respects. Therefore, two possible structures **1** or **1a** were tentatively assigned to zinnolide. The lower field shift (δ 6.85) of the aromatic proton (7-H) of **1** compared with that (δ 6.66) of **2** indicates that the lactone carbonyl was located at a *peri* position to 7-H and effectively deshields the proton. This fact suggests the preferable structure **1** for zinnolide. It is known that chromic acid oxidation of zinniol (**2**) yields two isomeric phthalides **3** (mp 85~86°C) and **4** (mp 106~108°C).⁵⁾ In order to confirm the correct structure, zinnolide (**1**) was treated with NaBH_4 in THF to yield a phthalide mp 84~86°C. The spectroscopic data of the phthalide were identical with those of previously reported phthalide **3**. Since this phthalide must therefore have the structure **3**, it follows that structure **1** represents zinnolide.

The phytotoxic effects of **1** on lettuce seedlings were examined by a growth test. Zinnolide (**1**) showed a 34.5% inhibition to the growth of the hypocotyl and 22.0% inhibition to the growth of the root at 100 ppm. The toxicity was rather weak compared with other phytotoxic metabolites, *i.e.*, solanapyrones, from the same fungus.

EXPERIMENTAL

Melting points (mp) were measured on an MP-35 Yanaco Micro Melting Point Apparatus and are uncorrected. IR spectra were taken on a Hitachi 285 spectrometer. NMR spectra were recorded on an R-22 Hitachi High Resolution NMR Spectrometer (90 MHz) and a JEOL FX 100 Spectrometer (100 MHz), and MS were run on JEOL JMS-D 300 and JEOL JMS-DISG-2 Mass Spectrometers.

Cultures and isolation. The fungus was grown in 500 ml flasks containing 150 ml of potato-sucrose medium at 25°C for 25 days in the dark. The culture filtrates (15 liters from 100 flasks) were concentrated *in vacuo* to 1.5 liters below 40°C, and the concentrate was acidified with 2N-HCl to pH 3.5 and extracted five times with CHCl_3 (5 liters). The combined extracts were dried over anhydrous MgSO_4 , and concentrated *in vacuo* to give a dark red residue (2.74 g), which was chromatographed on a silicic acid column (120 g, Wako gel C-200) eluting with benzene-EtOAc (9:1) to give fractions A-J. Fraction E

(155 mg) showed rather weak phytotoxic activity on the growth of lettuce seedlings. This fraction was chromatographed on a silicic acid column (Kiesel gel 60), eluting with benzene–EtOAc (95:5) and further eluting with benzene–EtOAc (80:20) to yield an eluate. The eluate was chromatographed by HPLC (HITACHI GEL 3019) using MeOH and AcOH (1.5%)-MeOH as the eluant to yield zinnolide (102 mg) as a colorless solid, mp 171~173°C. UV $\lambda_{\max}^{\text{EtOH}}$ nm(ϵ): 220 (32000), 260 (6600); IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3200, 1720, 1600, 1120, 1095; ¹H NMR (90 MHz) $\delta_{\text{TMS}}^{\text{CDCl}_3}$: 1.69 (3H, s, =C–CH₃), 1.75 (3H, s, =C–CH₃), 2.13 (3H, s, ArCH₃), 3.90 (3H, s, OCH₃), 4.45 (2H, d, $J=7$ Hz, –CH₂O), 5.35 (1H, br.t, $J=7$ Hz, =C–H), 6.60 (1H, s, –OCHO–), 6.85 (1H, s, Ar–H). EI-MS m/z 278 (M^+), 245, 224, 210, 193, 69, HR-MS m/z 278.1167; calcd. for C₁₅H₁₈O₅, m/z 278.1154.

Reduction of zinnolide (1) to zinniol (2). To a solution of zinnolide (20 mg, 0.072 mmol) in anhydrous ether (0.4 ml) was added dropwise a suspension of LiAlH₄ (8 mg, 0.211 mmol) in anhydrous ether (0.1 ml), and the mixture was refluxed for 4 hr under a nitrogen atmosphere. After adding EtOAc and then 2N-HCl, the reaction mixture was extracted three times with EtOAc. The combined extracts were washed with brine, dried over anhydrous Na₂SO₄ and concentrated *in vacuo* to give a residue, which was chromatographed on a silicic acid column to yield zinniol (7 mg, 36.6%), whose spectral data were identical with those of an authentic sample. IR $\nu_{\max}^{\text{neat}}$ cm⁻¹: 3350, 2900, 1600, 1580, 1450, 1410; ¹H NMR (100 MHz) $\delta_{\text{TMS}}^{\text{CDCl}_3}$: 1.74 (3H, s, =C–CH₃), 1.80 (3H, s, =C–CH₃), 2.15 (3H, s, Ar–CH₃), 3.75 (3H, s, OCH₃), 4.51 (2H, d, $J=6.6$ Hz, –CH₂–), 4.66 (2H, s, –CH₂O), 5.46 (1H, br. t, $J=6.6$ Hz, =CH), 6.67 (1H, s, Ar–H). EI-MS m/z 266 (M^+), 198, 180, 163, 152.

Reduction of zinnolide (1) to phthalide (3). A solution of zinnolide (10 mg, 0.036 mmol) and sodium borohydride (5.5 mg, 0.145 mmol) in anhydrous THF was stirred for 1 hr at room temperature. After adding water, the reaction mixture was extracted three times with EtOAc. The combined extracts were washed with brine, dried over anhydrous Na₂SO₄ and concentrated *in vacuo* to give a

residue, which was chromatographed on a silicic acid column using benzene–EtOAc (3:1) as the eluant to yield phthalide (8 mg, 87.9%), whose spectral data were identical with those of the authentic sample 3: white solid, mp 84~86°C (lit.⁵ mp 85~86°C). UV $\lambda_{\max}^{\text{EtOH}}$ nm(ϵ): 256 (7100), 302 (3400), IR $\nu_{\max}^{\text{neat}}$ cm⁻¹: 2900, 1760, 1610, 1110; ¹H NMR (100 MHz) $\delta_{\text{TMS}}^{\text{CDCl}_3}$: 1.75 (3H, s, =C–CH₃), 1.80 (3H, s, =C–CH₃), 2.21 (3H, s, Ar–CH₃), 3.88 (3H, s, OCH₃), 4.57 (2H, d, $J=6.6$ Hz, –CH₂–), 5.38 (2H, s, CH₂), 5.49 (1H, br. t, $J=6.6$ Hz, =CH), 7.08 (1H, s, Ar–H). EI-MS m/z 262 (M^+), 224, 194, 165, 69; HR-MS m/z 262.1209; calcd. for C₁₅H₁₈O₄, m/z 262.1204.

Bioassay

Growth inhibition of lettuce seedlings. An EtOAc solution (1 ml) containing a sample (0.3 mg) was poured on to a sheet of filter paper (7 cm diameter, Toyo-Roshi No.2) in a Petri dish (9 cm diameter). After removing the solvent in a desiccator, 3 ml of a Tween-80 solution (100 ppm) was added to the dish to make a 100 ppm solution. Lettuce seeds (Great Lake) were placed on the filter paper and germinated in the dark at 23°C for 3 days.

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