**Note**

Importance of the Ketone Function for the Phytotoxicity of Spiciferone A, a Phytotoxin Produced by the Fungus Cochliobolus spicifer

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Spiciferone A is a major phytotoxin produced by an isolate of Cochliobolus spicifer Nelson (D-5), a pathogen of leaf spot disease in wheat. In addition to spiciferone A, five of its derivatives, including spiciferones B and C, were tested for their phytotoxicity by using wheat cotyledon protoplasts. The results indicate that the ketone function at C-7 as well as the methyl at C-2 are essential to the phytotoxicity of spiciferone A.

A strain of Cochliobolus spicifer Nelson (D-5), a pathogen of leaf spot disease in wheat, has been isolated from diseased wheat in Kagawa, Japan and identified by Dr. M. Ichino of the National Institute of Hygienic Sciences in Tokyo. The isolates produced several phytotoxins, which have been isolated and characterized by our group.\(^1\) Spiciferone A (1) is a major phytotoxin, which produced similar botchy spots on cotyledons of wheat (*Triticum aestivum* L. cv. Ushio-komugi) to those of the disease symptom, suggesting that this toxin may play an important role in the pathogenesis of this fungus. Spiciferones B (2) and C (3) are minor metabolites structurally related to spiciferone A, spiciferone C being less phytotoxic to wheat cotyledons than spiciferone A, while spiciferone B was not phytotoxic.\(^2\) These findings suggest that the methyl on the \(\gamma\)-pyrone ring, especially at C-2, would probably be involved in the mechanism of action of spiciferone A. To explore those structural units essential to the phytotoxicity of spiciferone A in more detail, three derivatives of spiciferone A were prepared and tested, together with spiciferones A, B, and C, for their phytotoxicity. In the present study, wheat cotyledon protoplasts were used to evaluate the phytotoxicity instead of wheat cotyledons themselves. The protoplast bioassay is more reproducible and quantitative, and thus more reliable.\(^3\) Moreover, a much smaller amount of a sample is needed to determine the phytotoxicity by this bioassay. In this paper, we describe the phytotoxicity of several derivatives of spiciferone A, and then discuss a key structural unit of the molecule in its phytotoxicity.

Spiciferones A, B, and C were isolated from a culture filtrate of C. spicifer (D-5) as described previously.\(^1\) Hydrogenation of 1 over PdO\(_2\) in EtOAc afforded dihydrospiciferone A (4) as colorless needles (EtOH).\(^3\)\(^1\) NaBH\(_4\) reduction (THF, 20 min, 0°C) of 4 gave two compounds, which were separated by HPLC (column, Partisil-5 ODS-2, 10 \(\times\) 250 mm; solvent, 60% MeOH in water containing 1% AcOH; flow rate, 1.5 ml/min; detector, 258 nm). The retention times by HPLC and the yields of the minor component (5) and the major one (6) were 31 and 36 min, and 14% and 59%, respectively. The physicochemical properties of 5 were as follows: \([\alpha]_D^{20} + 77^\circ\) (c 0.2 EtOH); UV \(\lambda_{max}\) (EtOH) nm (e): 257 (8800), 222 (6000 sh), 215 (6600), 204 (4900 sh); IR \(\nu_{max}\) (film) cm\(^{-1}\): 3394, 2938, 1656, 1595, 1440, 1384, 1217, 1166, 1061; NMR \(\delta\) (CDCl\(_3\)): 0.79 (3H, t, \(J = 7\) Hz), 1.23 (3H, s), 1.76 (2H, q, \(J = 7\) Hz), 1.70–2.05 (2H, m), 1.92 (3H, s), 2.28 (3H, s), 2.38 (1H, ddd, \(J = 17.6, 10.0, 5.7\) Hz), 2.67 (1H, ddd, \(J = 17.6, 17.6, 5.9\) Hz), 3.90 (1H, dd, \(J = 10.0, 3.3\) Hz); EIMS \(/m/z\) (relative intensity): 236 (77%, M\(^+\)), 221 (56), 219 (28), 208 (22), 207 (48), 191 (29), 189 (58), 177 (71), 43 (100). The physicochemical properties of 6 were as follows: \([\alpha]_D^{20} + 9^\circ\) (c 0.5, EtOH); UV \(\lambda_{max}\) (EtOH) nm (e): 257 (11,100), 222 (7100 sh), 215 (7800), 204 (5000); IR \(\nu_{max}\) (film) cm\(^{-1}\): 3330, 2920, 1654, 1593, 1440, 1386, 1194, 1162, 1068; NMR \(\delta\) (CDCl\(_3\)): 0.97 (3H, t, \(J = 6.5\) Hz), 1.24 (3H, s), 1.78 (2H, q, \(J = 6.5\) Hz), 1.88 (2H, m), 1.92 (3H, s), 2.27 (3H, s), 2.52 (1H, ddd, \(J = 17.6, 6.4, 6.4\) Hz), 2.58 (1H, ddd, \(J = 17.6, 7.0, 7.0\) Hz), 3.82 (1H, dd, \(J = 5.0, 5.8\) Hz); EIMS \(/m/z\) (relative intensity): 236 (45%, M\(^+\)), 221 (31), 219 (20), 207 (30), 191 (20), 189 (32), 177 (40), 43 (100). The \(^1\)H-NMR data indicated that the stereochemistry of the hydroxyl at C-7 was pseudoaxial in the major component (6) and pseudoenatorial in the minor one (5) on the assumption that the conformation of the cyclohexene ring was of chair form.

The phytotoxicity of compounds 1–6 was determined by a bioassay with wheat (*Triticum aestivum* L. cv. Ushio-komugi) cotyledon protoplasts\(^4\) with the results shown in the figure. Spiciferone C (3) was less active than spiciferone A, and spiciferone B (2) had no activity, indicating that the substitution on the \(\gamma\)-pyrone ring of spiciferone A affects its phytotoxicity and also that the methyl at C-2 was essential to the phytotoxicity.

**Fig.** Phytotoxic Activity of Spiciferones and their Derivatives against Protoplasts of Wheat Cotyledons.
of spiciferone A. This is in agreement with the data that were previously obtained with the cotyledon bioassay. In the present protoplast bioassay, dihydrospiciferone A (4) was as active as spiciferone A, while compounds 5 and 6, which had hydroxyls instead of the ketone of spiciferone A, showed no activity. This indicated that the ketone function at C-7 was essential to phytotoxicity of spiciferone A, but that the double bond between C-5 and -6 was not.

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References