Note

Dendrostilbella sp. (85-25),
a New Source of
Trichodermin

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During the course of examining fungi as sources of plant growth regulating compounds we noted that extracts of *Dendrostilbella* sp. (85-25) significantly inhibited the growth of etiolated wheat coleoptiles. The fungus, a phialidic synnematal hyphomycete, isolated from the stump of a loblolly pine (*Pinus taeda*) that had been growing in Athens, Georgia (U.S.A.), was shown to produce the fungal metabolite trichodermin, 4 β -acetoxy-12, 13-epoxy- Δ^9 -trichothecene (1).

The initial cultivation of *D. sp.* (85-25) was carried out on potato dextrose agar slants for 1 week at room temperature and these were stored at 5°C until use. Slants were flooded with sterile distilled water and the organism was transferred to 2.8 liter Fernbach flasks each containing 200 g of shredded wheat medium.¹⁾ Cultures were maintained at 26°C for seven days. Approximately 300 ml of acetone was then added to each flask, the mycelium and substrate were macerated with a Super Dispax Homogenizer, (Tekmar Co., Ohio)* the pulp placed on Whatman No. 1 filter paper in a Büchner funnel and filtered, the filtrate reduced in volume under vacuum at 50°C and then extracted twice with an equal volume of ethyl acetate. The ethyl acetate fraction was dried over anhydrous sodium sulfate, reduced in volume and added to the top of a silica gel (70~230 mesh) chromatography column (9.0 $\times$ 10 cm) slurry packed in benzene. Stepwise elution followed with 1 liter each of benzene, *t*-butyl methyl ether, ethyl acetate, acetone, and aceto-

nitrile. Aliquots of the ether and ethyl acetate fractions completely inhibited the growth of etiolated wheat coleoptiles (the bioassay has been described in detail).²⁾ These fractions were combined, reduced in volume and further fractionated on a silica gel (70~230 mesh) chromatography column (3.5 $\times$ 45 cm) using a linear gradient of benzene (1 liter) to acetone (1 liter); 25 ml fractions were collected. Aliquots (25 μ l) were tested for activity in the wheat coleoptile bioassay, and activity was shown to be present in tubes 37~47. These were combined, reduced in volume and added to a silica gel column (3.5 $\times$ 38 cm) packed in CH₂Cl₂. Chromatography was effected with CH₂Cl₂-acetone (9:1, v/v) after allowing 250 ml of the solvent to pass through the column after addition of the biologically active fraction. Again, 25 ml fractions were collected and growth inhibition activity was determined to be in tubes 38~56, so these were combined and reduced in volume. Further clean up was afforded with another silica gel chromatography column (3.5 $\times$ 46 cm) packed in CH₂Cl₂-acetone (9:1, v/v) and a linear elution gradient of CH₂Cl₂ (1 liter) to CH₂Cl₂-acetone (4:1, v/v) (1 liter). Activity was noted in tubes 35~52. Final purification was made on a C₁₈ reverse phase open column (2.5 $\times$ 17 cm). Packing was obtained from a Waters Associates Prep-Pak 500 C₁₈ cartridge. The solvent phase was acetonitrile-water (3:1, v/v) and bands that absorbed and fluoresced at 366 nm were collected. Void bands were also collected. Tubes 3~5, inclusive, contained material inhibitory to wheat coleoptiles. These were combined, reduced in volume under vacuum, to yield a viscous glass-like material. Fifty-six flasks of shredded wheat medium yielded 2.48 g of metabolite.

The *R_f* value of the metabolite was 0.68~0.71 (authentic trichodermin, 0.68~0.72) on silica gel 60, F-254 (E. M. Laboratories, Inc.) thin-layer chromatography plates developed in toluene-ethyl acetate-formic acid (5:4:1, v/v/v). Developed plates were sprayed with anisaldehyde, and heated at 100°C. The metabolite and trichodermin were slate colored. The typical UV spectrum absorption band of trichodermin [$\lambda_{\text{max}}^{\text{EtOH}}$ 204 nm (log ϵ =3.69)] was observed, and the IR spectrum obtained from thin film on KBr windows, using a 4 $\times$ beam condenser, indicated the values 2970 (s), 1735 (s), 1680 (w), 1432, 1375, 1243, 1220, 1080, 1030, 970, 956, 928, 850, 832, 750, 732, 650, and 639 cm⁻¹ and closely matched those reported for trichodermin.³⁾ Both ¹H and ¹³C NMR spectra were entirely consistent with those obtained previously for trichodermin.³⁾ Direct probe, electron impact mass spectral data show a molecular ion peak (M⁺) at *m/z* 292 and a computer-generated formula C₁₇H₂₄O₄ (found 292.167, reported³⁾ 292.1668). There were prominent ion fragment peaks at *m/z* 277, 217, 189, 149, 135, 121, and 107.

The isolated metabolite significantly inhibited etiolated wheat coleoptiles (*p*<0.01) at 10⁻³, 10⁻⁴, and 10⁻⁵ M

*1 Mention of a firm name does not imply endorsement by the US Department of Agriculture of the products. No discrimination or preference is intended. The use of firm brand names and products is for identification only.

FIG. 1. Effects of Trichodermin in Ethiolated Wheat Coleoptiles (*Triticum aestivum* L., cv. Wakeland).

Initial length of coleoptiles ($\times 3$) was 12 mm. Dotted line: control. Below solid line: significant inhibition ($p < 0.01$).

FIG. 2. Plate: (1) Structure of Trichodermin.

in duplicated bioassays (Fig. 1). Data were statistically analyzed by a multiple comparison procedure.⁴⁾ The phytotoxic properties of trichodermin on *Nicotiana tabacum* L., *Phaseolus vulgaris* L., and *Zea mays* L.

have been reported.⁵⁾

Trichodermin has been found in *Trichoderma viride*,⁶⁾ *T. lignorum*,⁷⁾ *T. polysporum*,⁸⁾ *T. sporulosum*,⁸⁾ and *Hypocrea austrograndis*,⁹⁾ but, to our knowledge, this is the first report of the metabolite in *Dendrostilbella*. The exact role that trichodermin plays in the ecosystem under the conditions that the organism was found is puzzling.

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