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TRYPTOPHOL, A PHYTOTOXIN PRODUCED BY *DRECHSLERA NODULOSUM*

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Key Word Index—*Drechslera nodulosum*; *Eleusine indica*; Gramineae; goosegrass; tryptophol; phytotoxin; tryptophan.

Abstract—Tryptophol was identified as a major metabolite in the culture medium of *Drechslera nodulosum*. This compound produced necrotic lesions on goosegrass at a concentration of 6.2×10^{-4} M in two different leaf bioassay tests. Tryptophol also caused lesions on a number of other monocots and dicots, but generally higher concentrations were required. Tryptophol was also detected in the leaves of goosegrass infected by *D. nodulosum*, but not in uninfected goosegrass.

INTRODUCTION

Goosegrass (*Eleusine indica* L.), a tufted annual grass, is one of the most serious weedy grasses in the world [1]. Its range extends from South Africa to Japan and the northern border of the United States. More than 60 countries report it as a weed problem in at least 46 different crops. A seedling blight of goosegrass was described by Luttrell [2]. In addition, he also reported that spotting, flecking of the leaves and the development of 'sooty heads' in the inflorescences were other manifestations of plant infections caused by *Drechslera nodulosum* (syn. *Helminthosporium nodulosum*). Since many of these symptoms, especially those of leaf spotting and flecking, are reminiscent of the involvement of one or more phytotoxins in disease development, we cultured the pathogen and began a systematic study of its phytotoxic metabolites. Here we report the occurrence of tryptophol (**1**) [3] as a phytotoxin produced by *D. nodulosum* and implicate it in the disease process.

RESULTS AND DISCUSSION

Culture filtrates of *D. nodulosum* were exhaustively extracted with chloroform and the majority of the biological activity resides in this fraction. The biologically active component was purified by flash chromatography followed by high pressure liquid chromatography. The yield was *ca* 2.5 mg/l.

The phytotoxin was identified as tryptophol **1** by conventional spectroscopic techniques and chromatographic identities with authentic tryptophol (Aldrich Co.). The high resolution mass spectrum of the phytotoxin found *m/z* of 161.0840 for $C_{10}H_{11}N_1O_1$ (M^+, 161.0840 calc.). The phytotoxin absorbed at λ_{max}^{EtOH} of 279 and 224 nm which is identical to authentic tryptophol. Furthermore, the ^{1}H NMR spectrum of the phytotoxin was identical to that of authentic tryptophol (see Experimental). Each possessed equivalent R_fs on TLC; silica gel 60, F-254 0.25 mm of 0.42 in chloroform–methanol (9:1) and 0.25 in dichloromethane–acetone (9:1) as detected with anisaldehyde–sulphuric acid–methanol (3:1:16) spray.

Tryptophol may have some role in disease development since it induces symptoms in the host plant closely resembling those in naturally and artificially infected plants [2]. In two bioassay tests, one involving a puncture wound in the leaf blade, and the other a direct placement of a turgid leaf into a phytotoxin solution, there was the subsequent development of greyish lesions which develop into brown necrotic spots on treated leaves about 48 hr after treatment. The more effective assay was the direct placement of a toxin solution on a leaf. Tryptophol did not show host selectivity among the tested plants (Table 1) at 6.2×10^{-3} M; however, some plants showed no symptoms and at 3.1×10^{-3} M; and no plants except 4-week-old goosegrass responded at 6.2×10^{-4} M (Table 1). All tested plants gave an identical response with authentic tryptophol as obtained with the natural phytotoxin.

If a phytotoxin is involved in a host–parasite interaction, it should be demonstrable in plant tissue infected by the organism. Leaves of goosegrass infected with *D. nodulosum* were dried and extracted with methanol. The compound, which has the same retention time as that of authentic tryptophol on HPLC, has an *m/z* of 161 $[M]^+$ (27.9%) and 130 as a parent peak by GC-MS (Varian VG analytical instrument, glass capillary column DB-5, 30 M × 0.25 mm ID) which is identical to tryptophol. By using a

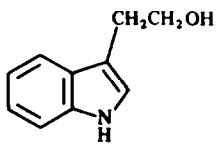

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Table 1. The reaction of several plants to various concentrations of tryptophol, a phytotoxin from *D. nodulosum*, a pathogen of goosegrass. Testing was done by the cut leaf assay technique by placing detached leaves in tryptophol solutions for 2 days at 22°

Plant	Common name	Tryptophol* 6.2×10^{-4} M	3.1×10^{-3} M	6.2×10^{-3} M
Monocots				
Eleusine indica L. Gaerth	Goosegrass (4 weeks)	++	++	++
Eleusine indica L. Gaerth	Goosegrass (10 weeks)	−	−	++
Sorghum vulgare L.	Grain sorghum	−	+	+
Hordeum vulgare L.	Barley	−	+	+
Digitaria ischaemum Schreb.	Crabgrass	−	−	++
Alopecurus aequalis Sobol	Foxtail	−	−	++
Echinochloa crus-galli L.	Barnyard grass	−	+	++
Poa annua L.	Bluegrass	−	−	+
Brachiaria sp.	Signal grass	−	−	++
Dicots				
Chenopodium album L.	Lambsquarters	−	−	++
Cirsium arvense L.	Canada thistle	−	+	+

*Notations: ++ = dead leaf, + = necrotic flecking or spotting, − = no visible symptoms (see assay methods).

standardized curve for quantitation of peak area on HPLC, we estimated that tryptophol was present at 3.4–3.5 μg/0.45 g dry wt leaves. Tryptophol could not be detected in uninfected plant material. At this point it is difficult to determine that the quantities of tryptophol present in infected plant material are great enough to produce some of the disease symptoms. Considering the problems involved in localization or compartmentation of the compound in the tissue, its metabolism in a dynamic host–pathogen interaction, and the difficulties involved in accurately determining the amounts of it that are present in tissue, it certainly is not to be discounted as having some role in this plant disease.

The origin of tryptophol is probably via tryptophan via decarboxylation and deamination reactions [3]. In fact, incubation of the 2-week-old mycelium of *D. nodulosum* with tryptophan 1-^{14}C for 14 hr yielded no detectable radioactivity in the 0.8 mg of tryptophol that was recovered. However, there were 0.24 nCi/5 μmol of tryptophol recovered in a comparable mycelial mat that had been administered tryptophan 3-^{14}C. These observations are consistent with the proposed reactions since the C-1 would be lost and the C-3 would be retained. Further labelling and enzyme studies are needed to further elaborate these points.

Although tryptophol is a well known compound as a natural product in plants and frequently reported as a byproduct of indoleacetic acid metabolism in plants and microorganisms, it has never been reported as a phytotoxin [3–5]. The sensitivity of 4-week-old goosegrass to tryptophol is consistent with the reported severity of *D. nodulosum* on younger tissues (Table 1) [2].

EXPERIMENTAL

Fungal culturing and isolation of toxin. D. nodulosum was supplied by Dr. E. S. Luttrell, University of Georgia, Athens. Cultures were maintained on potato dextrose agar and cultured in a modified M-1-D medium (7) with shaking (200 rpm) under low light intensity 7 μEinsteins/min^2/sec at 26° for 3 weeks. After filtering through glass wool, the filtrate was evaporated to 0.5 vol. and exhaustively extracted successively (2–3 ×) with equal volumes of chloroform which was pooled and evaporated under vacuum at 40°. The chloroform fraction, possessing the biological activity, was subjected to flash chromatography (E. Merck, silica gel 60, 230–400 mesh, 100 g), elution with $CHCl_3$–MeOH (19:1); LH-20 chromatography (Pharmacia, 65 g) elution with MeOH, and then HPLC (E. Merck) RF-18, 4.6 × 250 mm, UV detection at 254 nm, elution with MeCN–H_2O (13:7); flow rate 0.8 ml/min.

Tryptophol (1). Relevant ^{1}H-NMR signals (Brucker WM-250, $CDCl_3$ as solvent, TMC as internal std). δ8.08 (1H, *br s*, N_1-H) 7.62 (1H, *d*, J = 7.5 Hz, C_7-H) 7.37 (1H, *d*, J = 7.5 Hz, C_4-H) 7.18 (1H, *t*, J = 7.5 Hz, C_6-H) 7.15 (1H, *t*, J = 7.5 Hz, C_5-H) 7.08 (1H, *s*, C_2H) 3.90 (2H, *t*, J = 6.4 Hz, C_1-H) 3.03 (2H, *t*, J = 6.4 Hz, C_2-H) 1.65 (1H, *s*, OH).

Bioassays. Extracts, or individual compounds, were dissolved in 2% EtOH and a droplet (1 μl) placed over a puncture wound made in the leaf blade [7]. The leaves were incubated in a moist chamber for 48–72 hr at 23° under intermittent light (7 μEinsteins/m^2/sec). In another test, detached leaves were placed in test tubes containing the compounds to be tested under continuous fluorescent light (7 μEinsteins/m^2/sec) for 48 hr at 23°. Leaves were evaluated and scored for symptom severity.

Plant extracts. D. nodulosum was inoculated as spores and mycelial fragments on to plants which were 4 weeks old growing at 25° in a growth chamber with 12 hr/12 hr [light (170 μEinsteins/m^2/sec)/dark]. Severely infected leaves were harvested, ground in a Waring blender in 80% MeOH, let stand for 3 days at 23°, and filtered. The filtrate was evaporated under red. pres. The extract (dry wt 4.5 mg/0.45 g dry wt of leaves). The extract was subjected to preparative TLC in $CHCl_3$–MeOH (9:1), the band at R_f 0.40–0.45 was eluted with $CHCl_3$–MeOH (2:1) and subjected to HPLC (see toxin isolation).

Radioactivity methods. Equivalent mycelial mats (*ca* 0.37 g dry wt) 3 weeks of age were administered tryptophan 1-^{14}C 50.9 mCi/mmol (10 μCi) or tryptophan 3-^{14}C 56.8 mCi/mmol (10 μCi). The mats were incubated for 14 hr at 23° and then extracted and the tryptophol obtained by the proscribed techniques. Radioactivity was determined by liquid scintillation counting techniques and cpm converted to dpm by use of a quench correction curve.

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