Purification and Partial Characterization of Host-Specific Toxins Produced by *Periconia circinata*

T. J. Wolpert and L. D. Dunkle

Research assistant and research plant pathologist, Science and Education Administration, U.S. Department of Agriculture, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907.

Portion of a thesis submitted by the senior author in partial fulfillment of the requirements for the MS degree, Purdue University. Joint contribution of the Science and Education Administration, U. S. Department of Agriculture and Purdue University Agricultural Experiment Station, West Lafayette, IN 47907. Journal Article 7868 of the Purdue University Agricultural Experiment Station.

We thank M. Hermodson, Department of Biochemistry, Purdue University, for assistance with high-performance liquid chromatographic analyses and for the amino acid analyses.

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or imply exclusive approval over other products that also may be suitable.

Accepted for publication 1 March 1980.

ABSTRACT

WOLPERT, T. J., and L. D. DUNKLE. 1980. Purification and partial characterization of host-specific toxins produced by *Periconia circinata*. Phytopathology 70:872-876.

Host-specific toxins isolated from culture filtrates of *Periconia circinata* inhibited root growth of the susceptible sorghum genotype by 50% at 1 ng/ml and had no effect on root growth of the near-isogenic resistant genotype at 2 μg/ml. Purity of the isolated products was assessed by thin-layer chromatography (TLC), thin-layer electrophoresis (TLE), and high-performance liquid chromatography (HPLC) of the toxins or their dansyl derivatives. Two toxic substances were separated by preparative TLC; each contained only aspartic acid and two components with properties of polyamines. HPLC resolved each of those toxic fractions into two compounds, a biologically active one and an inactive one. In addition to aspartic acid, both active compounds contained the same polyamine, and both inactive compounds contained another electrophoretically distinct polyamine. The Periconia toxins are low-molecular-weight, acidic compounds containing multiple residues of aspartic acid and one or more residues of a polyamine, which is responsible for ninhydrin reactivity and apparently responsible for selective biological activity.

Additional key words: milo disease, Periconia root rot, *Sorghum bicolor*, selective pathotoxin.

Pathogenicity of certain fungal pathogens is determined by host-specific toxins (8) (selective pathotoxins [13]), metabolic products which, at low concentrations, affect only the hosts of the pathogen and produce the symptoms of the disease in the absence of the pathogen. Thus, these toxins are assumed to elicit the biochemical responses of the host to the pathogen. The toxin-producing fungi and their hosts provide convenient systems for studies of host-parasite interactions, because the mechanism of pathogenesis may be deduced from the mode of action of the toxin.

Periconia circinata (Mangin) Sacc., causal agent of milo disease (4), produces a substance toxic only to genotypes of sorghum (*Sorghum bicolor* [L.] Moench) that are susceptible to the pathogen (11,12). For studies on the mechanisms of susceptibility and resistance, the toxin must be free of contaminating substances that may induce responses not directly or necessarily involved in the initial stages of pathogenesis and in the host response to the pathogen. The objectives of this study were to develop a procedure for purifying the toxin produced by *P. circinata,* to establish criteria for assessing the purity of the isolated product, and to describe some of the chemical properties of the toxin.

MATERIALS AND METHODS

Plant material. Near-isogenic lines of Colby milo differing only in the allelic form of the semidominant *Pc* gene for susceptibility to *P. circinata* (12) were used to assess toxin activity.

Bioassays. A seedling bioassay (6) was used to determine toxic activity of fractions during purification. Seedlings were grown in 25-ml beakers containing 20 ml of nutrient solution (5) and incubated at room temperature (~24 C) under continuous light (7,000 lx). Five- to 7-day-old seedlings were incubated in 20 ml of a solution of the test material for 48 hr and evaluated for foliar wilting and necrosis (2).

Root growth inhibition bioassays (2) were used to quantify toxin activity. Seeds were germinated at 25 C for 24 hr. Seedlings with roots 2–3 mm long were placed in 9-cm-diameter petri dishes containing 15 ml of 0.01 M KH_2PO_4 or solutions of toxin in 0.01 M KH_2PO_4 and incubated in the dark at 25 C for 48 hr. To determine activity, lengths of roots of susceptible and resistant seedlings incubated in test solution were compared with roots of seedlings incubated in 0.01 M KH_2PO_4.

Toxin production. Single-spore isolates of *P. circinata* were obtained from roots of susceptible sorghum (6) and maintained on potato-dextrose agar. For toxin production the fungus was grown in standing culture at room temperature (~24 C) for 10 days in 400-ml prescription bottles containing 100 ml of modified-Fries' medium (MF) supplemented with 0.1% yeast extract (7). After 10 days the medium was replaced with 100 ml of MF without yeast extract, and the cultures were incubated for an additional 15 days (2). Removal of the yeast extract improved purification by eliminating several contaminating peptides and had no apparent effect on growth of the fungus. The culture filtrate (CF) was obtained by filtering the culture medium through four layers of cheese cloth.

Toxin purification. CF (2–6 L) was concentrated 20-fold in vacuo at 35 C. In all cases of in vacuo concentration, the sample was cooled to 4 C until vacuum was established and again before vacuum was released. The concentrated filtrate was allowed to stand at 4 C for 24 hr. Insoluble material was removed by filtration, and the active supernatant was deproteinized by the addition of an equal volume of methanol and allowed to stand at 4 C for 24 hr. The inactive precipitate was removed either by filtration or centrifugation. The methanol-soluble portion was concentrated 50-fold (with respect to original volume) to remove the methanol and allowed to stand at 4 C for 24 hr. The soluble portion was then adsorbed to activated charcoal (Norit A, Sigma Chemical Co., St. Louis, MO 63178) (2.5 g/L of original CF) which was washed

thoroughly by filtration with approximately 2 L of deionized water. Toxic activity was eluted from the charcoal with 500–1,000 ml of 10% (v/v) aqueous pyridine until the eluate was devoid of pigment (7). The pyridine eluate was then loaded directly onto a column (3 × 40 cm) of QAE-Sephadex (Q25-120) (Sigma Chemical Co.) which had been equilibrated with 0.1 N acetic acid/pyridine buffer (APB), pH 3.25. After the sample was applied, the column was washed with 500 ml of APB, pH 3.25, and toxic activity was eluted with 500 ml of 1.0 N aqueous acetic acid. The acetic acid eluate was concentrated 400-fold (with respect to volume of CF) and applied to a smaller (1.5 × 20 cm) column of QAE-Sephadex prepared and equilibrated the same as the larger column. Toxic activity was eluted with a 200 ml continuous gradient of 0.1 to 1.0 N aqueous acetic acid. Fifty 3-ml fractions were collected and a 250 μl sample was removed from each even-numbered fraction and dried under a cool air stream to remove acetic acid. Dried samples were immediately solubilized in 20 ml of deionized water, and activity was determined with the seedling bioassay.

Active fractions were combined and concentrated 2,000- to 4,000-fold. The concentrated preparation was subjected to preparative thin-layer electrophoresis (TLE) by applying 2,000-ml equivalents of CF (0.5 to 1.0 ml of sample) on silica gel G plates along a 15-cm path parallel to and 4 cm from the cathode. The plate was sprayed until saturated with pyridine/acetate buffer (PAB), pH 6.5, and electrical contact was made with buffer-saturated Whatman #3 paper strips (18 × 5 cm) to electrode tanks containing PAB. The plate was subjected to a constant 400 V for 3.5 hr at 10 C. After electrophoresis, the plate was air-dried and the ninhydrin-positive, active band was detected by spraying a section at the edge of the plate. The remainder of the band was then removed and toxin was eluted with 50% (v/v) aqueous methanol by filtering the silica gel on a medium-grade fritted glass filter. The 50% methanol eluate was concentrated in vacuo to 2,000-fold. To determine the dry weight of toxin preparations, aliquots of concentrated solution were dried on preweighed aluminum pans and weighed on a Cahn Model 25 Electrobalance, (Cahn Instruments, Cerritos, CA 90701).

Analytical procedures. Dansylation and thin-layer chromatography (TLC) on polyamide sheets were performed according to the procedures described by Gray (3). The dansyl amino acids were purchased from Sigma Chemical Co. The mono- and di-dansyl derivatives of spermidine were prepared (3) and purified by TLE at pH 6.5.

Solvents for TLC on silica gel G were prepared as follows: solvent A—propanol/acetic acid/water (100:3:200, v/v); solvent B—*n*-butanol/pyridine/acetic acid/water/ethyl acetate (125:25:50:50:50, v/v); and solvent C—*n*-butanol/pyridine/acetic acid/water (120:80:24:96, v/v).

Volatile buffers for TLE were prepared as follows: pH 6.5—3 ml glacial acetic acid + 120 ml pyridine per 2,000 ml (PAB); pH 2.75—0.2 M acetic acid; pH 4.4—9 ml glacial acetic acid + 16 ml pyridine per 1,000 ml.

High-performance liquid chromatography (HPLC) was performed on a Varian 5000 liquid chromatograph with a 250 × 4.6-mm column of Whatman ODS (5-μm particles). The column was maintained at 40 C and eluted at a flow rate of 1 ml/min with 0.1% (v/v) H_3PO_4 and acetonitrile ("glass distilled" grade; Burdick and Jackson Laboratories, Inc., Muskegon, MI 49442). The gradient of 0.1% H_3PO_4 (A) and acetonitrile (B) was programmed to give the following mixtures: from 100% A at 0 time linearly to 90% A and 10% B by 2 min; linearly to 70% A and 30% B by 25 min; and linearly to 100% B by 27 min. The column effluent was monitored by absorbance at 206 nm, or at 223 nm for highly concentrated preparations.

Toxin preparations were hydroloyzed in 6 N HCl at 110 C for 24 hr. Amino acid analyses were performed on a Durrum D500 Analyzer according to standard procedures.

RESULTS

Toxin purified through the preparative TLE step selectively inhibited root growth of susceptible seedlings by 50% at a concentration of 1 ng/ml (Fig. 1), but had no effect on root growth of resistant seedlings at concentrations up to 2 μg/ml, the highest concentration tested. On columns of Sephadex G-10 equilibrated and eluted with 5% acetic acid buffer, toxic activity was detected in a single peak slightly after the exclusion volume, indicating a molecular weight of ~700. Toxin eluted near the void volume of Bio-Gel P2 columns when water was used as the eluant, suggesting a molecular weight of approximately 1,800. This artifically high molecular weight estimate may have resulted from ionic interactions between the negatively charged toxin and the negatively charged gel.

In amino acid analyses of acid-hydrolyzed toxin preparations, aspartic acid was the only amino acid detected. Untreated (nonhydrolyzed) toxin preparations were devoid of free aspartic acid, and chromatographic and electrophoretic comparisons indicated that the toxin migrated differently from both aspartic acid and polyaspartate.

Toxin at this stage of purification migrated as a single electronegative, ninhydrin-positive compound at pH 6.5 and as a weakly electropositive compound at pH 2.75. When toxin preparations were reacted with dansyl chloride (3) to form the dns-toxin derivative, a single fluorescent spot was detected by TLC on silica gel G and polyamide supports. Further, a single fluorescent compound, co-migrating with several mono-dns-polyamines (eg, ϵ-dns-lysine; mono-dns-cadaverine; mono-dns-spermidine), was detected when dns-toxin preparations were acid hydrolyzed and chromatographed in two dimensions on a polyamide support (3).

The foregoing observations suggested that the product was a single toxic compound. However, preparative TLC of the fraction purified through TLE at pH 6.5 resolved two active compounds with R_f's of 0.29 (toxin I) and 0.40 (toxin II) in solvent B. Based on ninhydrin reactivity, weight, and biological activity, toxin I was present in greater amount. Therefore, most analyses were done on that toxin.

Analysis and characteristics of toxin I. TLC of toxin I on silica gel G resulted in a single ninhydrin-positive spot with the following R_f's: 0.45 in solvent A; 0.29 in solvent B; and 0.39 in solvent C. TLE analysis at pH 6.5 and pH 2.75, likewise, indicated a single compound. In all instances, biological activity coincided with ninhydrin reactivity.

Reaction of toxin I with trinitrobenzene sulfonic acid to form the trinitrophenyl (TNP) derivative completely abolished activity. Removal of the TNP moiety by treatment with 1N NH_4OH for 5 min at room temperature restored toxic activity following neutralization of the solution.

We have stored toxin I preparations as water solutions for several months at 4 C without detectable loss of activity. However,

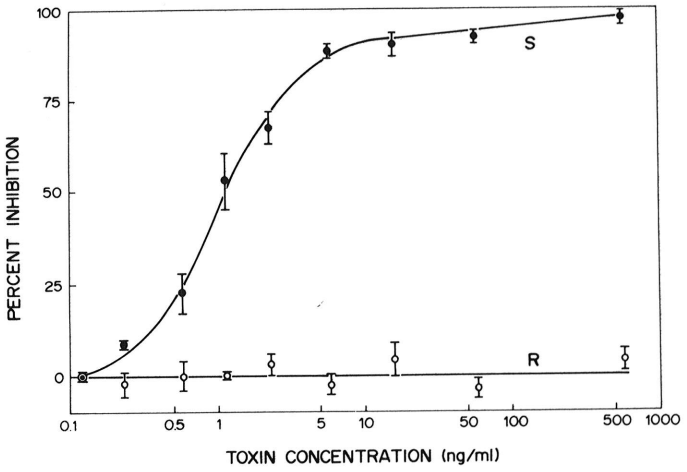
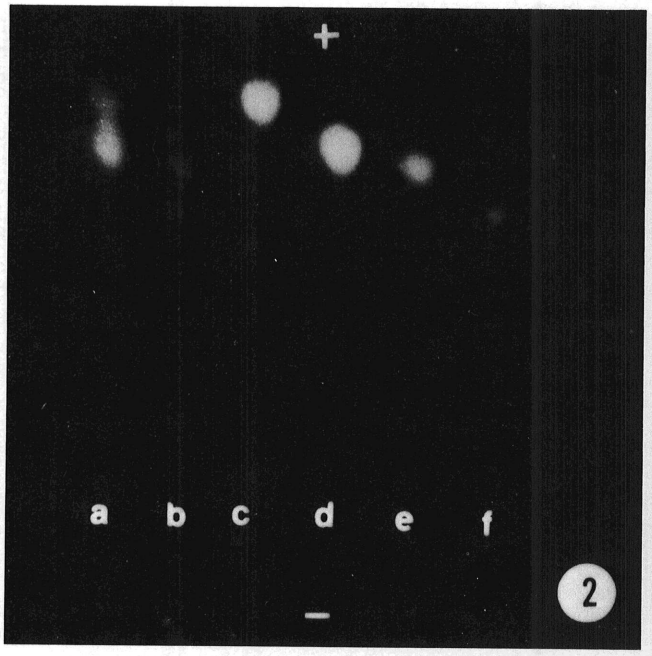


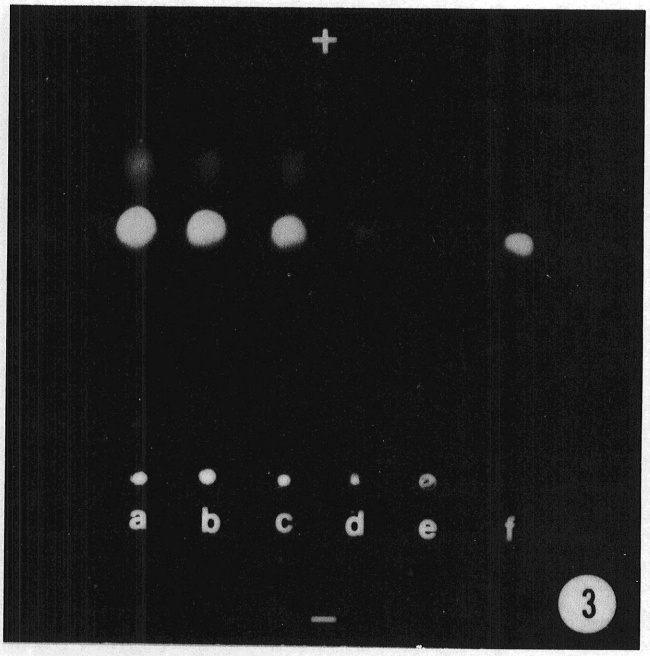
Fig. 1. Root growth inhibition bioassay of toxin with near-isogenic sorghum cultivars susceptible (S, •) and resistant (R, o) to *Periconia circinata*. Each data point is the average of five measurements, and the vertical bars represent the range of means obtained in separate bioassays of toxin purified through preparative thin-layer electrophoresis.

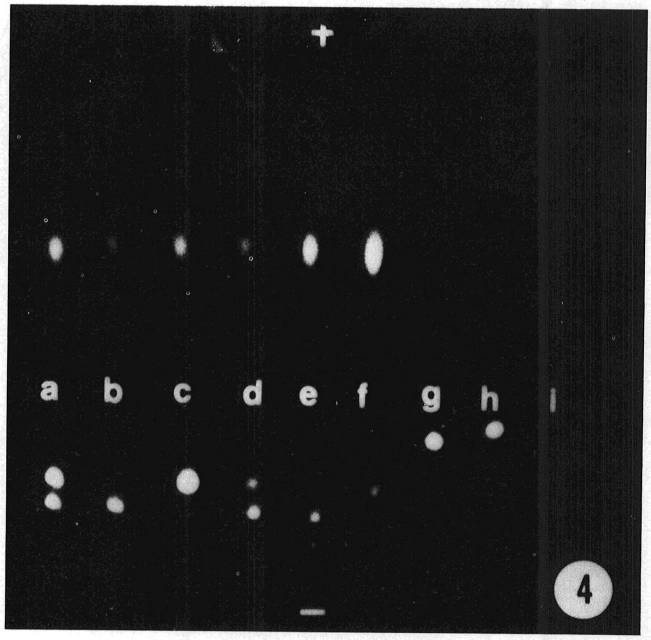
toxin I was sensitive to mild alkaline hydrolysis. Incubating the toxin in dilute NH_4OH, pH 12, for 24 hr at room temperature reduced toxic activity nearly 200-fold and cleaved the toxin into four ninhydrin-positive fragments, none of which co-migrated with the untreated toxin.

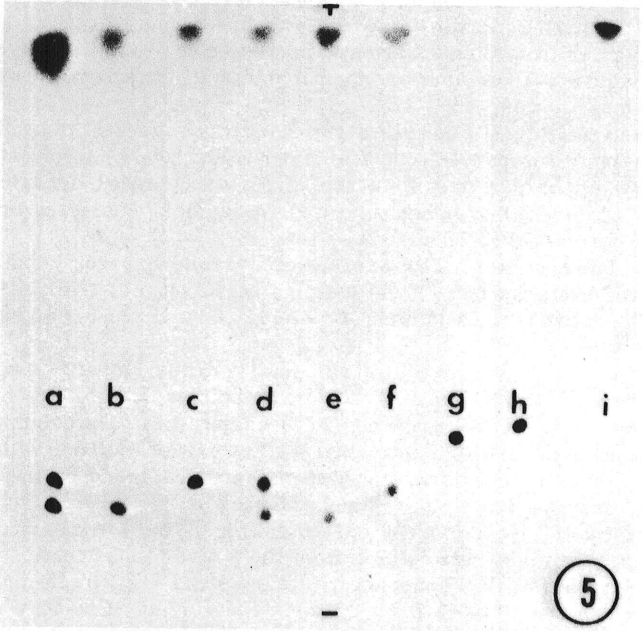
Incubation of 200 μg of toxin I with pronase or proteinase K (Sigma Chemical Co.) under conditions that resulted in the complete hydrolysis of 3 mg of casein had no effect on toxic activity or on the chromatographic and electrophoretic properties of the toxin.

Preliminary studies to determine the composition of the toxin were done by dansylating the acid hydrolyzate or hydrolyzing the dns-toxin derivative and analyzing by TLE and two-dimensional TLC on polyamide. Dansylated acid hydrolyzate of toxin I yielded α-dns-aspartate and a dns-component with chromatographic and electrophoretic properties similar to those of di-dns-spermidine (Fig. 3) and di-dns-cadaverine. When dns-toxin I was acid hydrolyzed and subjected to TLE at pH 6.5, two fluorescent









Figs. 2–5. 2, Thin-layer electrophoretogram (pH 6.5, 400 V, 10 C, 10.5 hr) showing fluorescence of dansylated preparations of (a) toxin I and (b) toxin II from preparative thin-layer chromatography and of compounds (c) Ia, (d) Ib, (e) IIa, and (f) IIb, which were purified by high-performance liquid chromatography and then dansylated prior to thin-layer electrophoresis. Letters indicate the origins. **3,** Thin-layer electrophoretogram (pH 4.4, 400 V, 10 C, 4.25 hr) showing fluorescence of a–d) dansylated hydrolyzates (ie, the di-dns-components) of compounds from HPLC: (a) Ia; (b) Ib; (c) IIa; and (d) IIb; compared with (e) di-dns-spermidine standard; and (f) α-dns-aspartate standard. The large diffuse spot in a thru d migrating ahead of the α-dns-aspartate is dns-hydroxide, a side product of the dansylation reaction. Letters indicate the origins. **4,** Thin-layer electrophoretogram (pH 6.5, 400 V, 10 C, 2.25 hr) showing fluorescence of (a–f) the acid hydrolyzed dansyl derivatives (ie, the mono-dns-components) of: (a) toxin I; (b) compound Ia; (c) compound Ib; (d) toxin II; (e) compound IIa; (f) compound IIb; compared with (g) and (h) mono-dns-spermidines A and B; and (i) aspartic acid. Dansyl hydroxide is present in hydrolyzates a thru f. Letters indicate the origins. **5,** Thin-layer electrophoretogram of the same materials described in Fig. 4 showing ninhydrin reactivity of the mono-dns-derivatives and of the non-derivatized aspartic acid, which also is present in the hydrolyzates (a–f).

electropositive compounds were observed (Fig. 4). Compounds with similar electrophoretic behavior were produced by dansylating spermidine and preparing the mono-dns-derivatives, spermidine A (N-[3-aminopropyl]-1, dns-4-butanediamine) and spermidine B (dns-N-[3-aminopropyl]-1,4-butanediamine) (Fig. 4). Furthermore, the two dns-compounds from the hydrolyzed dns-toxin I as well as the mono-dns-spermidines A and B reacted with ninhydrin (Fig. 5) and were capable of further reaction with dns-Cl to form the electrophoretically neutral di-dns-derivatives (Fig. 3). The only other ninhydrin-reactive compound detected in the hydrolyzed dns-toxin I preparations was aspartic acid (Fig. 5). No α-dns-aspartate was detected when the acid hydrolyzed dns-toxin I was analyzed by TLE at pH 6.5 and pH 4.4 or by TLC on polyamide. Thus, the reactivity of the toxin with ninhydrin and with dns-Cl apparently is due to a free amino group associated with a polyamine and not due to the α-amino group of aspartic acid.

High-performance liquid chromatography (HPLC) of toxin I resolved two compounds (Fig. 6). Only the predominant compound (Ia) was biologically active. Dansylation of each compound and electrophoretic analysis of the hydrolyzates established the association of a single dns-component with compound Ia and an electrophoretically different dns-component with the minor compound Ib (Fig. 4). Mixtures of the two dns-components from compounds Ia and Ib reproduced the fluorescent pattern obtained from toxin I preparations prior to HPLC. Thus, HPLC was the only procedure that was capable of separating the active compound from the very similar but inactive compound in their native (non-derivatized) states.

TLE of the dns-derivative of toxin I for a longer time (6.5–10.5 hr) resolved two fluorescent compounds (Fig. 2), an electronegative, intensely fluorescent one and a less negative, less fluorescent one. When hydrolyzed and analyzed electrophoretically, each of these dns-compounds was found to contain the same mono-dns-components described above for compounds Ia and Ib.

Analysis and characteristics of toxin II. As with toxin I, TLE of the dns-derivative of toxin II for 10.5 hr resolved two fluorescent compounds (Fig. 2). TLE analysis of acid hydrolyzed dns-toxin II produced the same pattern of two fluorescent electropositive components as toxin I (Fig. 4). HPLC of toxin II separated two compounds, a major, biologically active compound (IIa) and a minor, inactive one (IIb) (Fig. 7). Each of the compounds contained a single mono-dns-component (Fig. 4) and each contained aspartic acid (Fig. 5). The dns-components associated with the active compounds Ia and IIa were electrophoretically identical, and those associated with the inactive compounds Ib and IIb were identical (Fig. 4).

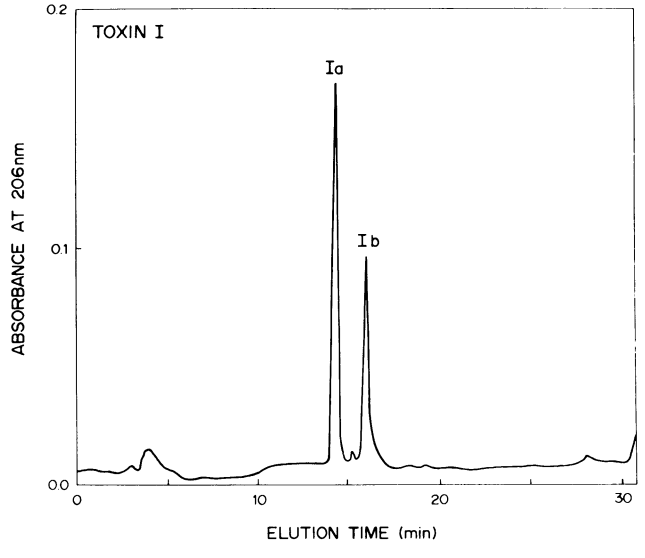


Fig. 6. Elution profile from high-performance liquid chromatography of toxin I on a column of Whatman ODS eluted at 40 C with a linear gradient of acetonitrile and 0.1% H_3PO_4 at a flow rate of 1 ml/min.

DISCUSSION

The toxic products isolated from culture filtrates of *P. circinata* satisfied a number of criteria that we required as evidence of a pure product. A single ninhydrin-positive compound was detected by TLC and TLE of each toxin (toxins I and II); a single fluorescent compound was produced by reaction of each toxin with dansyl chloride; a single dns-component was detected following acid hydrolysis of the dns-derivative of each toxin; biological activity was associated with a single UV-absorbing peak from preparations analyzed by HPLC; and the products were selectively active in bioassays at very low concentrations.

Toxic activity was abolished or greatly reduced by treatments that altered chromatographic and electrophoretic behavior of the isolated products as well as by treatments used to chemically modify the compounds. These observations together with the coincidence of biological activity and ninhydrin reactivity indicate that the products detected and analyzed are the toxic substances.

Amino acid analysis of a mixture of compounds Ia, Ib, IIa, and IIb indicated that aspartic acid was the only amino acid present. Chromatographic analyses of acid hydrolyzates of each of the purified compounds confirmed the presence of aspartic acid. However, analysis of acid hydrolyzed dns-toxin derivatives failed to detect dns-asparate but showed the presence of a fluorescent dns-substance that was also ninhydrin-positive. Hydrolysis of toxin followed by dansylation resulted in the formation of α-dns-aspartate and a di-dns-derivative, neither of which was ninhydrin positive. Thus, the residue in the toxin responsible for reactivity to ninhydrin and dansyl chloride was assumed to be a polyamine (or diamine). The polyamine residue may be responsible for toxicity of the molecule because the constituent polyamines are the only apparent differences between the active compounds, Ia and IIa, and their inactive counterparts, compounds Ib and IIb.

Toxic compounds Ia and IIa, while having the same electrophoretic properties and containing the same polyamine, have different R_f's on TLC (0.29 vs 0.40) and elute from the HPLC columns at distinctly different times (14 min vs 20 min). Differences in molecular weights due to the number of aspartic acid residues may affect solubility and account for these differences.

Some evidence suggests that the toxins are cyclic peptides. They are resistant to pronase, proteinase K, and a number of other proteases; and no α-dns-aspartate is formed by dansylation, suggesting that the α-amino group is involved in a peptide bond. If this is the case, the polyamines may be attached by an amide linkage through the β-carboxyl group of aspartic acid. However, similar results would be obtained if the toxins were linear, N-blocked peptides containing polyamines linked to the β-carboxyl group of aspartic acid or the α-carboxyl group of the peptide. Our hydrolysis

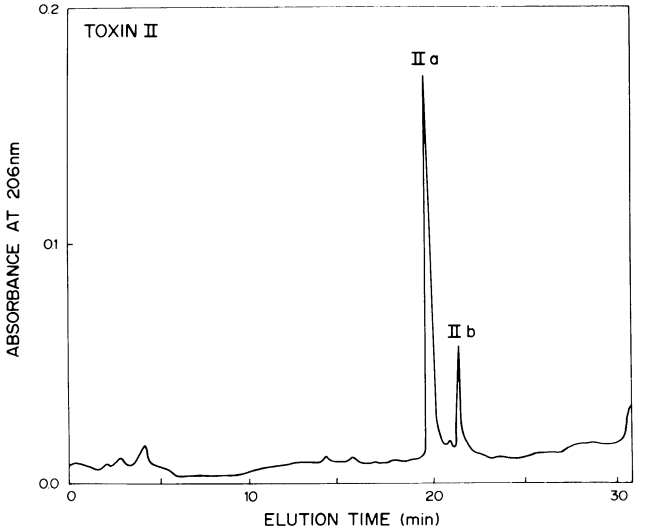


Fig. 7. Elution profile from high-performance liquid chromatography of toxin II as described in the Fig. 6 legend.

and dansylation data are not consistent with the possibility that the polyamines are associated with a linear peptide molecule through a linkage involving the α-amino group of aspartic acid, similar to that in the aspergillomarasmines and the toxin produced by *Pyrenophora teres* (1).

In their studies on the Periconia toxin, Pringle and Scheffer (7,9,10) concluded that the toxin was a peptide comprised of 6 moles of alanine, 4 moles of aspartic acid, 2 moles of glutamic acid, and 2 moles of serine. They assessed purity of the product by countercurrent distribution, ion-exchange chromatography, and paper chromatography (9). Their toxin preparations were selectively active in root growth inhibition bioassays at 0.1 to 1.0 μg/ml (7,9). They also presented evidence for a second, less-polar toxin with nearly equal activity on a weight basis (10). Our toxin preparations purified through the QAE-Sephadex step were active at 0.5 μg/ml (2) and found by amino acid analysis to contain aspartic acid and smaller quantities of alanine, glycine, glutamic acid, and serine. Thus, the subsequent electrophoretic and chromatographic procedures apparently removed inactive, contaminating peptides comprised of the aforementioned amino acids. It is possible that the preparations analyzed by Pringle and Scheffer (9), likewise, contained similar peptides.

Although the possibility cannot be eliminated that additional toxins are produced by *P. circinata*, we have no evidence that there are others. All fractions of eluates from column chromatographic steps and all zones of thin-layer chromatographic plates were bioassayed routinely. No active fractions were detected other than those described above. During the course of this study, several sources or lots of yeast extract were used to prepare the growth medium, and two single-spore isolates of *P. circinata* were used. Characteristics of the two toxins and the relative quantities produced were not affected by those variations, suggested by Pringle and Scheffer (10) to be parameters that may influence toxin production and composition.

LITERATURE CITED

1. BACH, E., S. CHRISTENSEN, L. DALGAARD, P. O. LARSEN, C. E. OLSEN, and V. SMEDEGARD-PETERSEN. 1979. Structures, properties and relationship to the aspergillomarasmines of toxins produ by *Pyrenophora teres*. Physiol. Plant Pathol. 14:41-46.
2. DUNKLE, L. D. 1979. Heterogeneous reaction of shattercane to *Periconia circinata* and its host-specific toxin. Phytopathology 69:260-262.
3. GRAY, W. R. 1972. End group analysis using dansyl chloride. Pages 121–138 in: C .H. W. Hirs and S. N. Tinasheff, eds. Methods in Enzymology. Vol. 25. Academic Press, New York, NY. 724 pp.
4. LEUKEL, R. W. 1948. *Periconia circinata* and its relation to milo disease. J. Agric. Res. 77:201-222.
5. ODVODY, G. N., and L. D. DUNKLE. 1979. Charcoal stalk rot of sorghum: Effect of environment on host-parasite relations. Phytopathology 69:250-254.
6. ODVODY, G. N., L. D. DUNKLE, and L. K. EDMUNDS. 1977. Characterization of the *Periconia circinata* population in a milo disease nursery. Phytopathology 67:1485-1489.
7. PRINGLE, R. B., and R. P. SCHEFFER. 1963. Purification of the selective toxin of *Periconia circinata*. Phytopathology 53:785-787.
8. PRINGLE, R. B., and R. P. SCHEFFER. 1964. Host-specific plant toxins. Annu. Rev. Phytopathol. 2:135-156.
9. PRINGLE, R. B., and R. P. SCHEFFER. 1966. Amino acid composition of a crystalline host-specific toxin. Phytopathology 56:1149-1151.
10. PRINGLE, R. B., and R. P. SCHEFFER. 1967. Multiple host-specific toxins from *Periconia circinata*. Phytopathology 57:530-532.
11. SCHEFFER, R. P., and R. B. PRINGLE. 1961. A selective toxin produced by *Periconia circinata*. Nature 191:912-913.
12. SCHERTZ, K. F., and Y. P. TAI. 1969. Inheritance of reaction of *Sorghum bicolor* (L.) Moench to toxin produced by *Periconia circinata* (Mang.) Sacc. Crop Sci. 9:621-624.
13. WHEELER, H., and H. H. LUKE. 1963. Microbial toxins in plant disease. Annu. Rev. Microbiol. 17:223-242.