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Isolation of Gregatin A from *Phialophora gregata* by Preparative High-Pressure Liquid Chromatography

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A method was developed for the production and purification of gregatin A from *Phialophora gregata* NRRL 13198 cultured on rice at 20°C for 28 days. Liquid extraction followed by high-pressure liquid chromatography afforded 247.0 mg of crystalline gregatin A per kg of rice.

Gregatin A (Fig. 1A) which is produced by *Phialophora gregata* (Allington and Chamberlain) W. Gams (2), is an enantiomer of aspertetronin A (Fig. 1B), which is a known metabolite from cultures of *Aspergillus rugulosus* (1). While both display antimicrobial activity, only gregatin A is associated with the vascular browning symptom of brown stem rot of soybeans (*Glycine max*). Brown stem rot was first reported in Illinois in 1944 (W. B. Allington, Phytopathology **36**:394, 1946), and subsequently in the western, midwestern, eastern, and southeastern regions of the United States, but not outside North America (6). This disease reduces soybean yields under certain environmental conditions (3, 4, 6).

The objective of our study was to develop methods to produce and purify multigram quantities of this phytotoxin for vascular browning studies; we report a rapid procedure for the isolation and purification of gram quantities of gregatin A from *P. gregata* on rice cultures followed by high-pressure liquid chromatography.

P. gregata NRRL 13198 (University of Illinois strain S1) is maintained by the Agricultural Research Service Culture Collection (Northern Regional Research Center). Cultures were maintained on soybean extract agar slants (5 g of cracked soybeans boiled for 1 h in 400 ml of distilled water and then diluted to 1 liter to which 18 g of Bacto-Agar [Difco Laboratories, Detroit, Mich.] was added). To obtain inoculum for production, 6 ml of 1:10,000 sterile aqueous Triton X-100 was added to a 7-day-old slant of *P. gregata* and swirled vigorously. A 2-ml portion of this spore suspension was added to a 300-ml Erlenmeyer flask containing 30 g of Uncle Ben's converted rice (Uncle Ben's, Inc., Houston, Tex.). The rice had been soaked with 13.5 ml of distilled water overnight and autoclaved for 15 min at 121°C. Nine 300-ml Erlenmeyer flasks prepared in this way were incubated at 20°C for 7 days with daily manual agitation to disperse clumps. The growth medium was prepared by the addition of 300 g of Uncle Ben's converted rice and 135 ml of distilled water to each of nine 2.8-liter Fernbach flasks. The contents were mixed thoroughly and soaked overnight. The flasks were autoclaved for 20 min at 121°C, and each was inoculated by adding the contents of one of the 300-ml Erlenmeyer flasks. The flasks were incubated at 20°C for 28 days, with daily manual shaking for the first 4 days to prevent clumping.

The content of each flask was soaked overnight in 1 liter of ethyl acetate, blended for 60 s in an explosion-proof blender, and then filtered through Whatman no. 1 filter paper. The solids were subsequently blended twice for 60 s each time with 1 liter of ethyl acetate and were filtered as described above. The combined organic extracts were concentrated to an oily residue under reduced pressure.

The residue was dissolved in 100 ml of methylene chloride, and chromatography was performed with a Waters Associates Prep 500 chromatograph fitted with a Prep Pak 500 silica column and eluted with methylene chloride-methanol (99.5:0.5, vol/vol) at 250 ml/min. Gregatin A plus other unknown products eluted at between 5.5 and 7.0 min, leaving behind about half of the beginning weight. This eluate was concentrated under reduced pressure and rechromatographed with hexane-ethyl acetate (90:10, vol/vol). Gregatin A appeared to elute at between 10.5 and 14.5 min (5 to 7 column volumes). This portion of eluant was evaporated under reduced pressure and the residue was dissolved in hexane for crystallization. The resulting crystals were removed by filtration and dried to yield 733.6 mg of gregatin A from 2.97 kg of rice. The gregatin A exhibited a single spot on thin-layer chromatography in methylene chloride-methanol (99:1, vol/vol) and produced nuclear magnetic resonance, mass spectral data, and a melting point of 73.1°C (uncorrected) which were consistent with reported values (5).

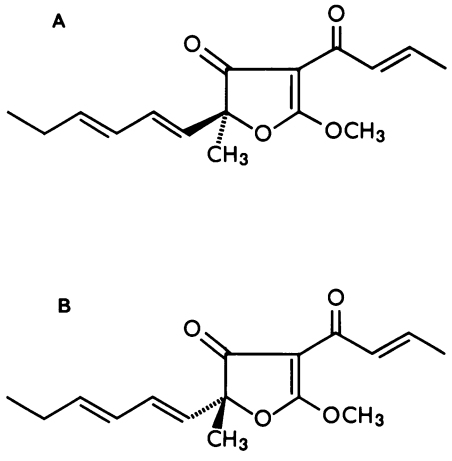

FIG. 1. Structures of gregatin A (A) and aspertetronin A (B).

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LITERATURE CITED

1. **Ballantine, J. A., V. Ferrito, C. H. Hassall, and V. I. P. Jones.** 1969. Aspertetronin A and B, two novel tetronic acid derivatives produced by a blocked mutant of *Aspergillus rugulosus*. J. Chem. Soc. Sect. C **1969**:56–61.
2. **Gams, W.** 1971. Cephalosporium-artige Schimmelpilze (Hyphomycetes). Gustav Fischer Verlag, Stuttgart, Federal Republic of Germany.
3. **Gray, L. E.** 1972. Effect of *Cephalosporium gregatum* on soybean yield. Plant Dis. Rep. **56**:580–581.
4. **Gray, L. E., and J. B. Sinclair.** 1973. The incidence, development, and yield effects of *Cephalosporium gregatum* on soybeans in Illinois. Plant Dis. Rep. **57**:853–855.
5. **Kobayashi, K., and T. Ui.** 1975. Isolation of phytotoxic substances produced by *Cephalosporium gregatum* Allington and Chamberlain. Tetrahedron Lett. **47**:4119–4122.
6. **Weber, C. R., J. M. Dunleavy, and W. R. Fehr.** 1966. Influence of brown stem rot on agronomic performance of soybeans. Agron. J. **58**:519–520.