Fungal Metabolite from Members of the Genus *Rhizopus*

D. P. THOMPSON,¹ * A. W. SMALLEY² AND BRODERICK E. ERIBO¹

Biology Department¹ and Chemistry Department,² Southern University, Baton Rouge, Louisiana 70813

Received 18 November 1980/Accepted 9 March 1981

A fluorescent metabolite present in seven members of the genus *Rhizopus* was isolated. This compound appeared green before spray treatment and purple after spray treatment with *p*-anisaldehyde in visible light. Subsequent purification and structural elucidation of the isolated compound yielded 1-[2,6,10,14-tetramethyl-17-carbomethyl heptadecyl]-1-[2,6,10,14-tetramethyl-17-methanoyl heptadecyl]-benzene.

A number of fluorescent metabolites were detected in seven members of the genus *Rhizopus* by Thompson and Smalley (5). These metabolites were separated by thin-layer chromatography and analyzed by their chromatographic behavior and reaction to spray reagents. The data obtained from these analyses, when compared with those of known mycotoxins, did not reveal the presence of any of the known mycotoxins. This study describes the isolation, purification, and structural elucidation of one fluorescent metabolite present in all seven fungi.

Species of *Rhizopus* used in this work were obtained from the American Type Culture Collection (Rockville, Md.) and the Department of National Health and Welfare (Ottawa, Ontario, Canada). Stock cultures were maintained at 5°C on slants of potato-dextrose agar enriched with 0.2% yeast extract. The cultural procedures employed were those previously described by van Walbeek et al. (7). The fungi were grown in mycological broth enriched with 0.5% yeast extract and with 15% sucrose plus 2% yeast extract for 14 days at 27°C.

The extraction procedures described by Armbricht et al. (1) were used to extract the secondary metabolites. The secondary metabolites were separated by thin-layer chromatography (5). The procedures of Steyn (4) and Scott et al. (3) were employed to detect the presence of nonfluorescent materials with chromatogenic reagents.

The chloroform extracts were spotted on 0.500-mm thin-layer chromatography plates (Redi-Coat 5-8172, Aflasil, Supelco, Bellefonte, Pa). Plates were developed with benzene-methanol-acetic acid (24:4:1, vol/vol/vol) and chloroform-methanol (99:1, vol/vol). The zone containing the metabolite was scraped from the plates, eluted with methanol, and rechromatographed on thin-layer chromatography plates for further purification. The zone was once again

scraped from the thin-layer chromatography plates and eluted with methanol. Final purification was achieved by crystallization from petroleum ether-ethyl acetate. The melting point of the metabolite was determined in sealed capillaries with an electrothermal melting point apparatus. Ultraviolet spectra in methanol were taken with a Hitachi spectrophotometer (Arthur Thomas Co.). The infrared spectra were measured with a Nicolet 7000 series dual-beam Fourier transform-infrared spectrophotometer. The gas chromatograph-mass spectrograms were obtained on a Finnigan 4000 series instrument equipped with a 2000 INCOS 2000 series data system. Nuclear magnetic resonance spectra were determined with a Varian model EM-390 MHz spectrometer. Liquid chromatograms were obtained on a Waters model 204 liquid chromatograph equipped with a C-18 reversed-phase column, ultraviolet detector, and integrator.

The R_f values $\times 100$ of the metabolite developed with benzene-methanol-acetic acid and with chloroform-methanol were 61 and 73, respectively. The fluorescent metabolite appeared green before spray treatment and purple after spray treatment with *p*-anisaldehyde in visible light. The melting point of the metabolite ranged from 125 to 128°C. The ultraviolet spectrum showed $\lambda_{\max}$ at 210nm ($\Sigma_{\max}$ 21,100 [Fig. 1]). As shown in Fig. 2, the liquid chromatogram exhibited a single narrow peak at retention time of 0.69 min, which indicates a single compound. The presence of a single compound in the data from the liquid chromatogram was in agreement with the results obtained from the thin-layer chromatography plates. The nuclear magnetic resonance spectrum (Fig. 3) exhibited a singlet at δ 10 (phenol H), a multiplet at δ 2.7 (CH_2H), and a multiplet at δ 2.0 (CH_3). The infrared spectrum showed characteristic absorption peaks at 3,610 cm^{-1} (M, OH), 1,230 cm^{-1} (s, C—O), and 730 to 790 cm^{-1} (strong). Peaks in the re-

gion of 1,500 to 2,000 cm^{-1} exhibited an absorption pattern characteristic of an orthodisubstituted benzene (2). As shown in Fig. 4, the gas chromatography-mass spectrometry data revealed alkyl fragments containing 22, 18, and 16 carbon atoms (spectrum no. 1,866, 1,843, and 1,678, respectively) and a disooctyl benzene frag-

FIG. 1. Ultraviolet spectra of the metabolite (—) and aflatoxin G_2 (-----).

FIG. 2. Liquid chromatography of the fluorescent metabolite.

FIG. 3. Nuclear magnetic resonance spectrum of the fluorescent metabolite.

FIG. 4. Gas chromatography mass spectrum of the fluorescent metabolite.

FIG. 5. Structure of the fluorescent metabolite.

ment (spectrum no. 2,334). The structure of the metabolite, 1-[2,6,10,14-tetramethyl-17-carbomethyl heptadecyl]-2-[2,6,10,14-tetramethyl-17-methanoyl heptadecyl]benzene, is shown in Fig. 5.

During this investigation, there was no attempt to determine the probable origin of the isolated metabolite; however, it may be derived from intermediates of the shikimic acid pathway (7).

The isolation of this fluorescent metabolite is of interest since it may have antimicrobial properties. Studies on the possible antimicrobial properties of the metabolite are presently under way and will be reported on in the near future.

This work was supported by Public Health Service grant RR08025-08 from the National Institutes of Health.

We thank D. Imhoff, J. Ryall, L. Feycer, V. O. Brandt, and J. B. Sigrest for their technical assistance.

LITERATURE CITED

1. Armbrrecht, B. H., F. A. Hodges, H. R. Smith, and A. A. Nelson. 1963. Mycotoxins. I. Studies on aflatoxin derived from contaminated peanut meal and certain strains of *Aspergillus flavus*. *J. Assoc. Off. Agric. Chem.* 46:805-817.
2. Conley, R. T. 1972. Infrared spectroscopy, 2nd ed. p. 112-121. Allyn & Bacon, Inc., Boston.
3. Scott, P. M., J. W. Lawrence, and W. van Walbeek. 1970. Detection of mycotoxins by thin-layer chromatography: application to screening of fungal extracts. *Appl. Microbiol.* 20:839-842.
4. Steyn, P. S. 1969. The separation and detection of several mycotoxins by thin-layer chromatography. *J. Chromatogr.* 45:473-475.
5. Thompson, D. P., and A. W. Smalley. 1980. Separation of fluorescent compounds in members of the genus *Rhizopus* by thin-layer chromatography. *J. Chromatogr.* 187:399-408.
6. Turner, W. B. 1971. Fungal metabolites, p. 33-61. Academic Press, Inc., New York.
7. van Walbeek, W., P. M. Scott, and F. S. Thatcher. 1968. Mycotoxins from food-borne fungi. *Can. J. Microbiol.* 14:131-137.