Journal of Natural Products
Vol. 48, No. 1, pp. 139-141, Jan-Feb 1985

THE IDENTIFICATION OF A MAJOR PHYTOTOXIC COMPONENT FROM *ALTERNARIA MACROSPORA* AS αβ-DEHYDROCURVULARIN

DAVID J. ROBESON,*[1] GARY A. STROBEL,

Department of Plant Pathology, Montana State University, Bozeman, MT 59717

and RICHARD N. STRANGE

Department of Botany and Microbiology, University College, London, Gower St., WCIE 6BT, UK

Many species of *Alternaria* Nees ex Fr. (Dematiaceae) are of economic importance as plant pathogens of major crop species. Members of the genus *Alternaria* are known to produce a range of phytotoxic compounds (1,2) which are chemically diverse and which possess a broad range of biological activities and metabolic effects (3-6). *Alternaria macrospora* Zimm. causes a leaf spot and twig blight disease of cotton (*Gossypium hirsutum*). The fungus was first reported by Zimmerman (7) in 1904, and it has subsequently been recorded on cotton in China, Africa, India, South America, and Israel, as well as in the USA (8-11).

In some cases, *A. macrospora* has been reported to be a weak pathogen affecting mainly plants under stress (9, 12) and yet as being severe (11,13) and destructive (10) in other instances and locations. The noxious weed, spurred anoda (*Anoda cristata* [L.] Schlect) is also attacked by *A. macrospora*, which has consequently been proposed as a potential biocontrol agent (13). Symptom expression on cotton takes the form of brown necrotic spots on leaves, stems, and bolls. The lesions, which are more numerous on the leaves than on the other plant parts, are characterized by a purple halo. In severe cases of infection, lesions coalesce, resulting in defoliation with concomitant dramatic reduction in yield (9,11).

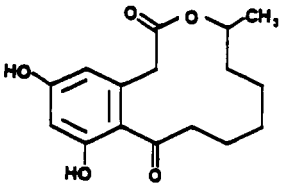
There have been at least three independent reports discussing the production of putative phytotoxic metabolite(s) by *A. macrospora* (10,14,15) although, to date, no toxic compounds have been described from the chemical viewpoint in terms of structural identification. In the earliest of these communications, Balasubramanian and Bhama (10) applied culture filtrates of the pathogen grown on Richard's medium and observed the appearance of brown necrotic lesions on leaves after 16 h. A toxic fraction was partially purified by these authors, but no analytical data on the compound(s) responsible for the observed phytotoxicity were presented (10). Some preliminary data on the biological characterization of a putative toxin was presented in a later publication (14). However, in this case, crude culture filtrate was used for the bioassays, and no attempt was made to fractionate the liquid culture into its various components. Inhibition of seed germination of nine cotton varieties after treatment with neat culture filtrate was observed to range from 70-100%, and culture filtrate sprayed on seedlings resulted in the development of necrotic spots one week after treatment (14). Desai and Hegde (15) also examined the phytotoxic effect of *A. macrospora* culture filtrate and reported inhibition of root elongation as well as other effects. Again, no attempt was made by these authors to purify and characterize chemically the compounds responsible for the toxic effect.

RESULTS AND DISCUSSION

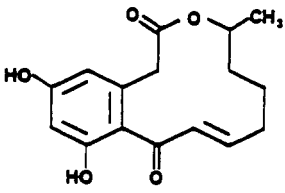
When *A. macrospora* was grown in pure culture in modified Czapek-Dox broth for 16 days, a $CHCl_3$ extract of the culture filtrate yielded two phytotoxic fractions. These components of the fun-

[1]Present address: ARCO Plant Cell Research Institute, 6560 Trinity Court, Dublin, CA 94568-2685.

gal culture were readily purified by tlc ($CHCl_3$-EtOH, 25:1) (16) and identified using standard spectral techniques as curvularin (**1**) and the more abundant α,β-dehydrocurvularin (**2**) [4,5,6,7-tetrahydro-11, 13-dihydroxy-4-methyl-2H-3-benzoxacyclododecin-2, 10(1H)-dione]. The maximum yield of α,β-dehydrocurvularin was produced by liquid cultures of *A. macrospora* after incubation for 16 days, at which time the concentration was of the order of 0.5 g/liter. A rapid decrease in the concentration of **2** to ca. 0.1 g/liter occurred after incubation for a further 24 h. In comparison, the maximum levels of **1** observed were only ca. 20 mg/liter.



1



2

α,β-Dehydrocurvularin was previously known as a product of certain fungal species (16), including *Alternaria cucumerina* (17) (the etiological agent of leaf spot of cucurbits). The phytotoxicity, as well as the antimicrobial activity, of **1** and **2** has already been noted (16). From the stereochemical viewpoint, measurements of optical rotation demonstrated that the same enantiomer of **2** was produced by *A. macrospora* as was isolated from *A. cucumerina* by Starratt and White (17) and from *Alternaria cinerariae* (16). Activity of the toxic components from *A. macrospora* was first observed following their application to the adaxial surfaces of cotton leaves (cv. San Joaquin C-1) over hypodermic needle punctures. Upon application of 10 μg and 0.5 μg of **2**, lesions became apparent after 16 h and 4 days, respectively. A more quantitative and sensitive bioassay was later performed, which utilized protoplasts prepared from cucumber (*Cucumis sativus*) cotyledons. After incubation in the presence of **1** or **2** for 3 h at 25°, treated protoplasts were scored for viability, using fluorescein diacetate as a 'staining' reagent as previously described (18). Using this technique, LD_{50} values were determined to be 21 and 15 μg/ml for **1** and **2**, respectively. The lethality of **2** in the 15 ppm range (ca. 5×10^{-5}M) towards cucumber protoplasts was of interest since this same toxic compound is also produced by the cucumber leaf spot pathogen *A. cucumerina* (17). However, as yet we have no evidence for or against the possible involvement of **1** or **2** in disease caused by either pathogen.

EXPERIMENTAL

CULTURE OF *A. MACROSPORA* AND ISOLATION OF **1** AND **2**.—Isolates of *A. macrospora* and *A. cucumerina* were kindly provided by Dr. David Netzer, Institute of Plant Protection, Bet Dagan, Israel. Cultures were maintained on V-8 juice agar and grown in modified Czapek-Dox broth as previously described (16). Liquid culture of the fungi and isolation of **1** and **2** were performed as by Robeson and Strobel (16). Identification of **1** and **2** was made by comparing the physical data with that of authentic samples obtained, as described previously (16). Spectral data for **1** (17,19) and **2** (17,20) have been given in the literature: $[\alpha]^{25}D$ −85° (*c* 1.5, EtOH).

BIOASSAYS.—*Cotton leaves.*—Cotton (cv. San Joaquin C-1) was grown in a growth chamber with a 14-h photoperiod at 25°/20°. The first true leaves, ca. 5 cm long, were excised and placed abaxial side down in a petri plate. Four droplets (10 μl or 5 μl) of solutions of **1** or **2** in concentrations ranging from 1000 to 10 ppm in 2% aqueous EtOH were applied to each half of the leaf. Treated leaves were incubated at 23° with a 12 h photoperiod and examined frequently for macroscopically visible symptoms.

Cucumber protoplasts.—Protoplasts were pre-

pared from cotyledons of cucumber as described in (18). Protoplasts treated with **1** or **2** were incubated for 3 h at 25°. Counting of incubated protoplasts, determination of percentage viability as compared with control values, and calculation of LD_{50} values were performed, in duplicate, as previously described (18).

ACKNOWLEDGMENTS

This work was supported by grants from the Montana Agricultural Experiment Station, BARD 1-31-79, the National Science Foundation (USA), and the Science Research Council (UK).

LITERATURE CITED

1. G.E. Templeton, in: "Microbial Toxins VIII, Fungal Toxins," Ed. by S. Kadis, A. Ciegler, and S.J. Ajl, New York: Academic Press, 1972, pp. 169-192.
2. S. Nishimura and K. Kohmoto, *Ann. Rev. Phytopathol.*, **21**, 87 (1983).
3. D.J. Harvan and R.W. Pero, in "Mycotoxins and Other Fungal Related Food Problems, Advances in Chemistry Series 149," Ed. by J.V. Rodricks, Washington, DC: American Chemical Society, 1976, pp. 344-355.
4. J.A. Steele, T.F. Uchytil, R.D. Durbin, P. Bhatnagar, and D.H. Rich, *Proc. Natl. Acad. Sci. USA.* **73**, 2245 (1976).
5. P. Park, S. Nishimura, K. Kohmoto, and H. Otani, *Ann. Phytopath. Soc. Japan*, **47**, 488 (1981).
6. D.J. Robeson and G.A. Strobel, *Phytochemistry*, **23**, 1597 (1984).
7. A. Zimmermann, *Berichte über land-u, Fortwirtschaft von Deutsch Ostafrica*, **2**, 24 (1904).
8. C.G. Hansford, *Proc. Linn. Soc. Lond.*, **1**, 34 (1943).
9. G.L. Sciumbato and J.A. Pinckard, *Plant Dis. Reptr.*, **58**, 201 (1974).
10. R. Balasubramanian and K.S. Bhama, *Curr. Sci.*, **46**, 426 (1977).
11. S. Hadas and T. Jakoby, *Phytoparasitica*, **9**, 252 (1981).
12. R.J. Hillocks, *Trop. Pest Management*, **27**, 1 (1981).
13. H.L. Walker and G.L. Sciumbato, *Plant Sci. Lett.*, **22**, 71 (1981).
14. P. Padmanaban and P. Narayanasamy, *Madras Agric. J.*, **65**, 33 (1978).
15. S.A. Desai and R.K. Hegde, *Mysore J. Agric. Sci.*, **14**, 330 (1980).
16. D.J. Robeson and G.A. Strobel, *Z. Naturforsch. Teil C*, **36**, 1081 (1981).
17. A.N. Starratt and G.A. White, *Phytochemistry*, **7**, 1883 (1968).
18. R.N. Strange, D.J. Pippard, and G.A. Strobel, *Physiological Plant Pathology*, **20**, 359 (1982).
19. H. Gerlach, *Helv. Chim. Acta*, **60**, 3039 (1977).
20. J.F. Grove, *J. Chem. Soc., (C)*, 2261 (1971).

Received 10 August 1984