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SHORT COMMUNICATION

IDENTIFICATION OF SOME METABOLITES OF *ALTERNARIA CUCUMERINA* (E. & E.) ELL.*

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AS PART of a general study of the structure and mode of action of phytotoxic fungal metabolites, we have investigated the products formed by *Alternaria cucumerina* (E. & E.) Ell. which causes leaf spot disease on cucurbits, especially muskmelon and watermelon.[1] Combined benzene and ether extracts of the dried mycelium of *A. cucumerina* gave substances which we have identified as alternariol monomethyl ether and alternariol.[2] Alternariol monomethyl ether, the major component of the mixture, yielded a diacetate and both compounds gave alternariol trimethyl ether which was identical with an authentic specimen. These substances, in proportions varying with the strain,[3,4] have been isolated from the mycelium of *A. dauci*[3] and *A. tenuis*.[2] Mannitol crystallized from the methanol extract of the mycelium.

A chloroform extract of the culture filtrate yielded a crystalline substance identified as $\alpha\beta$-dehydrocurvularin by direct comparison with an authentic specimen. Catalytic hydrogenation or reduction by zinc dust in acetic acid gave a compound exhibiting properties in agreement with those reported for curvularin.[5,6] $\alpha\beta$-Dehydrocurvularin has been reported to be a minor product formed together with curvularin by a *Curvularia* sp.[5,6]

Neither the crude culture filtrate nor the residue from the chloroform extract showed phytotoxic properties when tested on cut seedlings of cucumber and squash. This was likewise the case in tests with culture filtrates from other strains of the fungus grown on a variety of liquid media.

EXPERIMENTAL

Melting points were determined on a Kofler hot stage and are uncorrected. U.v. spectra of substances dissolved in 95 per cent ethanol were recorded using a Beckman DK spectrophotometer and i.r. spectra were obtained for KBr discs on a Perkin–Elmer Model 21 spectrometer. Kieselgel (Camag) containing fluorescent indicator was used for thin-layer chromatography (TLC). Microanalyses were done by Dr. C. Daesslé, Montreal.

Organism and Cultural Conditions

Isolates of *Alternaria cucumerina*, kindly provided by Dr. E. G. Simmons (U.S. Army Natick Laboratories, Natick, Mass.), were maintained on potato–dextrose agar slants. A pathogenic strain (QM 7448), isolated in Florida from a muskmelon leaf, was selected for growth in liquid culture.

* Contribution No. 377.

[1] C. R. JACKSON, *Phytopathol.* **49**, 731 (1959).
[2] H. RAISTRICK, C. E. STICKINGS and R. THOMAS, *Biochem. J.* **55**, 421 (1953).
[3] G. G. FREEMAN, *Phytochem.* **5**, 719 (1966).
[4] T. ROSETT, R. H. SANKHALA, C. E. STICKINGS, M. E. U. TAYLOR and R. THOMAS, *Biochem. J.* **67**, 390 (1957).
[5] O. C. MUSGRAVE, *J. Chem. Soc.* 4301 (1956).
[6] H. D. MUNRO, O. C. MUSGRAVE and R. TEMPLETON, *J. Chem. Soc.* (c) 947 (1967).

Small pieces of agar inoculum from the slant culture were placed in a 200 ml Erlenmeyer flask containing 25 ml of a potato–sucrose medium. The medium consisted of an extract from potato (100 g/l) and 3 per cent sucrose. The pH was adjusted to 6·0 before sterilization. After 1 week the culture was blended aseptically for 5–10 sec with 25 ml of sterile water. Aliquots of this mycelial suspension were used to inoculate each 150 ml of medium in Roux bottles. These were kept at 26° for 14 days at which time the contents were filtered through cheesecloth. The black and gelatinous mycelial mats were air-dried.

Extraction of the Mycelium

Mycelium (79 g) of *A. cucumerina* was extracted (Soxhlet apparatus) successively with light petrol (35–60°; 2 days), benzene (4 days), ether (4 days), and methanol (3 days). Removal of the solvent from the light petrol extract gave an oil (1·02 g) which was not investigated further. The benzene extract (1·38 g) and the ether extract (0·78 g) were shown by TLC to be almost identical and were combined. Oily material was removed by shaking with light petrol and the residue (420 mg) was separated by the method of Freeman[3] to give alternariol monomethyl ether (245 mg), alternariol (13 mg), and mixed crystals (57 mg) of alternariol and the monomethyl ether. The methanol extract was concentrated and, after cooling to 0°, a crystalline substance (1·17 g), m.p. 161–163°, was deposited slowly. This was identified as mannitol (mixture m.p., i.r.).

Alternariol monomethyl ether. Alternariol monomethyl ether crystallized from ethanol as needles, m.p. 265–267°. Acetylation gave diacetylalternariol monomethyl ether,[2] m.p. 164–165°, and methylation gave alternariol trimethyl ether[2] identical with that obtained from alternariol.

Alternariol. Alternariol crystallized from aqueous ethanol as needles, m.p. approx. 350° with decomposition, λ_{max} 256, 287, 299, 335 nm (ϵ 44,600, 9320, 9800, 10,465). (Found: C, 64·93; H, 4·02. Calc. for $C_{14}H_{10}O_5$: C, 65·12; H, 3·90 per cent.) Methylation of alternariol by the method of Raistrick *et al.*[2] gave alternariol trimethyl ether, m.p. 163–165°, identified by direct comparison (TLC (chloroform), i.r., u.v., mixture m.p.) with an authentic specimen.

Extraction of the Culture Filtrates

The culture filtrate from 125 bottles was extracted with chloroform (4 × 700 ml) by vigorous stirring for 15 min each time. The chloroform extracts were washed with water, dried, and the solvent removed *in vacuo* to yield a crystalline residue (900 mg).

αβ-Dehydrocurvularin. The crude material from the culture filtrate was recrystallized from methanol–acetonitrile containing a few drops of acetic acid. αβ-Dehydrocurvularin (362 mg) separated as plates, m.p. 230–232°; $[\alpha]_D$ −85° (*c*, 1·3 in ethanol); λ_{max} 226, 297 nm (ϵ 14,940, 5535), λ_{infl} 331 nm (ϵ 5090); ν_{max} 3428, 3320, 1718, 1632, 1597 cm^{-1}. (Found: C, 66·30; H, 6·06; O, 27·33. Calc. for $C_{16}H_{18}O_5$: C, 66·20; H, 6·25; O, 27·56 per cent.) This compound was shown by direct comparison to be identical (TLC) (chloroform–methanol; 19:1 v/v), i.r., u.v., mixture m.p.) with authentic αβ-dehydrocurvularin.[5,6] Hydrogenation of αβ-dehydrocurvularin over palladized charcoal or over palladium on barium sulfate followed by recrystallization of the product from chloroform–methanol gave curvularin as plates, m.p. 205–207°; $[\alpha]_D$ −39° (*c*, 1·5 in ethanol); λ_{max} 221, 269, 303 nm (ϵ 11,050, 6065, 4870) and λ_{infl} 232 nm (ϵ 9310); ν_{max} 3295, 1697, 1656, 1608 cm^{-1}. (Found: C, 65·83; H, 6·56. Calc. for $C_{16}H_{20}O_5$: C, 65·74; H, 6·90 per cent.) Curvularin was the major product when αβ-dehydrocurvularin (56 mg) in glacial acetic acid (5 ml) was stirred with zinc dust (170 mg) for 36 hr at room temperature.

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