αβ-Dehydrocurvularin and Curvularin from *Alternaria cinerariae*

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Secondary metabolite production by the phytopathogen *Alternaria cinerariae* has been investigated resulting in the isolation and identification of αβ-dehydrocurvularin and curvularin as products of this fungus. The phytotoxicity of these compounds is demonstrated.

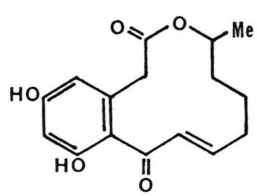
The genus *Alternaria* Nees ex Fr. (Dematiaceae) contains a number of species pathogenic to economically important crop plants [1]. Perhaps not unrelated to this is the fact that the *Alternaria* are a rich source of biologically active secondary metabolites [2], several of which are phytotoxic [3]. In fact, with the possible exception of the genus *Helminthosporium* Link ex Fries, more phytotoxins are known to be produced by the *Alternaria* than any other fungal genus [4–6]. They include toxins both host-specific (*eg.* phytoaltarnarin A [3]) and non host-specific (*eg.* zinniol [7]) in their action. *Alternaria cinerariae* Hori and Enjoji (syn. *Alternaria senecionis* Neergaard) [8] is a pathogen of the ornamental cineraria (*Senecio cruentus* (Masson) DC. (Compositae)). The fungus causes damping-off of seedlings and may be responsible for a severe leaf-spot on cineraria [1, 9]. A strong attack on lettuce has also been observed during infection experiments [1]. Secondary metabolite production by this fungus was investigated as part of a screening process of phytopathogenic fungi in search of compounds with potential for use as herbicides.

The fungus was maintained on potato dextrose agar and grown in shake culture for periods of two to three weeks. After incubation for fifteen days $CHCl_3$ extracts of the culture filtrate, when dried *in vacuo* and taken up in 50% EtOH, yielded a pale yellow crystalline product as needles. Recrystallization from petrol-EtOAc afforded pale yellow plates (mp. 224°), the molecular formula of which was $C_{16}H_{18}O_5$ (high resolution MS-290.1161). It was identified, by comparison (UV, TLC, IR, and mixed mp.) with an authentic specimen, as αβ-dehydrocurvularin (**1**). The yield of crystalline **1** in 15 day old culture filtrate was ca. 70 mg/l.

TLC of the $CHCl_3$ extract of 15 day old culture filtrate confirmed that αβ-dehydrocurvularin was the only major component. However, a second major component was detected in extracts of culture filtrate after incubation for a further two days. The rapid accumulation of this compound and its molecular weight (292) indicated that it was derived directly from, and was structurally related to, dehydrocurvularin (**1**). Indeed, it was identified as curvularin (**2**) by direct comparison (UV, TLC, ^{1}H-NMR, MS) with an authentic specimen prepared by reduction of **1** with Zn dust in HOAc [10]. Curvularin can be isolated from the culture filtrate of *A. cinerariae* at a concentration of at least 24 mg/l. It was previously known as a metabolite of two *Penicillium* species [11, 12]. Also αβ-dehydrocurvularin and curvularin have been reported to co-occur in a *Curvularia* species [13] and in an unidentified fungus [14]. While three other *Alternaria* species, as well as the *Alternaria* state of *Pleospora scirpicola* (DC.) Karst. (syn. *Cercospora scirpicola* (Fuckel) van Zinderen Bakker) are also known to produce αβ-dehydrocurvularin [15–17], this is the first report of the occurrence of curvularin in the genus *Alternaria.*

1, but not **2**, exhibited antifungal activity (against *Cladosporium herbarum* (Pers.) Link ex S. F. Gray) using the standard TLC bioassay technique of Homans and Fuchs [18]. Similarly Musgrave [19] reported, lack of antifungal (*Penicillium digitatum*), and antibacterial activity of curvularin. In contrast, curvularin was found to suppress several species of fungi and *Bacillus subtilis* (on seeds of *Phaseolus mungo*) [20]. The antibacteria, activity of αβ-dehydrocurvularin has already been noted [21].

In a prior investigation [10] neither culture filtrate (of *Alternaria cucumerina* from which **1** was isolated) nor its $CHCl_3$ extract expressed activity



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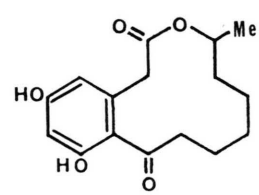

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against cut seedlings of cucumber or squash, and there have been no previous reports on the phytotoxicity of either **1** or **2**. However, both $\alpha\beta$-dehydrocurvularin and its hydrogenation product do possess phytotoxic activity, causing stem collapse and vascular necrosis of cut zinnia (*Zinnia elegans* Jacq. (Compositae)) seedlings at concentrations down to 0.1 and 0.33 mg/ml respectively within 24 h. Similarly, both metabolites caused necrosis of cuttings of Canada thistle (*Cirsium arvense* (L.) Scop. (Compositae)) at 0.33 mg/ml within 16 h. Both **1** and **2** appeared to be less active against oats (*Avena byzantina* cv. Coast Black); no visually apparent damage resulting from treatment with either compound, at a concentration of 0.33 mg/ml, for 24 h. However, after incubation for a further 40 h both compounds had caused chlorosis at the lowest concentration tested (0.1 mg/ml). In addition **1**, but not **2**, resulted in severe "water-soaking" symptoms after the same incubation period. Curvularin and $\alpha\beta$-dehydrocurvularin are thus non-specific in their phytotoxic action.

Experimental

Fungal culture

A. cinerariae was obtained from the American Type Culture Collection-ATCC No. 11784 [22] and grown in modified Czapek-Dox liquid medium of the following compostion: 100 g sucrose, 2 g casein hydrolysate, 1.5 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g KCl, 0.5 g $MgSO_4$, 0.01 g $FeSO_4$, H_2O to 1 l, pH adjusted to 6.0. One 1 volumes in 2 l conical flasks were incubated at 24° on a gyratory shaker at 200 rpm.

TLC

TLC was performed using silica gel as adsorbent. The TLC bioassay utilized $CHCl_3$:MeOH, 25:1 as solvent system: (**1**) R_f 0.28; (**2**) R_f 0.14. R_fs for (**1**) and (**2**) in two additional solvent systems were (i) Me_2CO: hexane, 2:1, R_f 0.57 and R_f 0.56 respectively; and (ii) *n*-pentane:Et_2O:HOAc, 75:25:3, R_f 0.11 and 0.38 respectively. Preparation of **2** was performed on prep. TLC plates (2.0 mm) by applying the residue after crystallization of **1** or $CHCl_3$ extracts of late culture filtrate. Development in $CHCl_3$:MeOH, 100:8 afforded **1** (R_f 0.50) and **2** (R_f 0.36) which were eluted from the adsorbent with MeOH.

Plant assays

Concentrations of **1** and **2** at 1.0, 0.33, and 0.1 mg/ml were prepared in 2% aqueous EtOH. Cut stems of zinnia (cv. Giant Double Scarlet), petioles of Canada thistle, or leaves of oats (cv. Coast Black) were placed in the liquid (2 cuttings per concentration for each species) and incubated at ambient temperature (ca. 25°). Control cuttings were placed in a solution of 2% EtOH in H_2O and appeared normal after the termination of the assay (up to 5 days).

Instrumentation

UV, IR, MS, and NMR spectra were recorded on a Beckman model 25, Perkin Elmer 237, AEI-MS 30 or Finnigan 4000, and on a Bruker WM 250 FT respectively. Melting points were determined on a Fisher-Johns or an Electrothermal apparatus.

*Reduction of $\alpha\beta$-dehydrocurvularin (**1**)*

1 (50 mg) in HOAc (5 mls) was stirred gently with Zn dust (160 mg) for 36 h at ambient temperature to give **2** plus unchanged **1**, separated by prep. TLC, as described above, to yield 20 mg of curvularin (**2**) and **1** (14 mg).

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