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ISOLATION OF CYTOCHALASINS A AND B FROM *ASCOCHYTA HETEROMORPHA*

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A severe foliar blight of oleander (*Nerium oleander* L.) caused by *Ascochyta heteromorpha* (Sch. *et* Sacc.) Curzi, characterized by extensive brown-leaf zonations, was observed in a nursery near Bari during April of 1985. Investigation on the toxic metabolites responsible for the foliar symptoms led to the purification in large amount of cytochalasins B (1-3) and a smaller amount of A (1,2), both known for their biological activity (4).

It is reported that *Helminthosporium dematioideum* and *Phoma* spp. produce cytochalasins A and B (5), and *Hormiscium* spp. producing only cytochalasin B (5). Moreover, it has been reported that the only phytopathogenic fungus, producing cytochalasins, specifically H and J, is *Phomopsis paspalli* (6).

The role of the cytochalasins A and B in the observed oleander leaf blight is under investigation. However, it appears that their importance is very limited because most of the lipophilic phytotoxic activity remains in the organic chromatographic fractions after their purification. Furthermore, a very strong phytotoxic activity was found still in the same culture filtrate after exhaustive CH_2Cl_2 extraction.

EXPERIMENTAL

FUNGUS SPECIES.—*A. heteromorpha* was isolated from brown spots of oleander leaves and was maintained on slants of potato-dextrose-agar in the collection of Istituto Tossine e Micotossine da Parassiti Vegetali del CNR, Bari, Italy.

PRODUCTION, ISOLATION, AND CHARACTERIZATION OF CYTOCHALASINS A AND B.—Single spore cultures of *A. heteromorpha*, freshly reisolated from infected oleander plants, were used. Fungus was cultured in Roux flasks containing a semisynthetic liquid medium (7), incubated at 25° for 21 days. The culture filtrates (9 liters, pH=7) were lyophilized, resuspended in distilled H_2O, and extracted with CH_2Cl_2. The organic extracts were combined, dried, and evaporated under reduced pressure. The residue (1.0 g) was chromatographed on SiO_2 column, which was eluted with a discontinuous EtOAc-*n*-hexane gradient starting with a 60:40 mixture. The fractions (EtOAc-*n*-hexane, 80:20) showing phytotoxic activity gave a residue of 125 mg. This material, after crystallization from EtOAc/*n*-hexane, yielded a white crystalline compound (90 mg, 10 mg for each liter of culture broth) that showed no phytotoxicity when assayed on tomato cuttings. The toxic activity remained in the mother liquors; its nature is under further investigation. The mp (218-220°) of the crystalline compound, its elemental analysis (found: C, 69.95; H, 7.70; N, 2.80%; M, 479, $C_{29}H_{37}NO_5 \times H_2O$), and its uv, ir, ms, ^1H- and ^{13}C-nmr spectra were identical to those of an authentic sample of cytochalasin B and were consistent with the data reported in the literature (1-3).

Reaction of cytochalasin B dissolved in pyridine with Ac_2O under the usual conditions gave a derivative which showed physical and spectral data consistent with those of diacetylcytochalasin B (1,2).

The mother liquors of the cytochalasin B crystallization yielded, by evaporation under reduced pressure, an oily mixture containing cytochalasin A as the main component, together with the other compounds responsible for the phytotoxicity of the mentioned fraction (35.4 mg). The mixture was further purified on a SiO_2 column (eluent: $CHCL_3$-iPrOH, 95:5) giving a homogeneous compound (25 mg). Crystallization from EtOAc/*n*-hexane yielded a white solid (16 mg, 1.8 mg/liter). The mp (181-183°), the ir, ms, uv, and ^1H-nmr data were identical to those of an authentic sample of cytochalasin A and were consistent with data reported in the literature (1,2). Cytochalasin A, compared to B, showed the lack of the signal of H-20 in the ^1H-nmr spectrum and the downfield shift to 203 ppm ($\Delta = 132.6$) of C-20 in the ^{13}C-nmr spectrum.

The oxidation of cytochalasin B by MnO_2 in CH_2Cl_2 afforded a product identical in all respects to cytochalasin A.

Full details on the isolation of the compounds are available upon request to the senior author.

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ESTRAGOLE: AN ACUTE TOXIC PRINCIPLE FROM THE VOLATILE OIL OF THE LEAVES OF *CLAUSENA ANISATA*

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The toxicity of the volatile oil of the leaves of *Clausena anisata* Hook. f. (Rutaceae) to variegated grasshopper *Zonocerus variegatus* L. is reported. The plant species has a strong, aromatic odor compared by some to aniseed and by others to garlic (1). Its uses in some parts of Africa and the Phillipines had earlier been reviewed (1). One of the many uses is the burning of the dried plant to repel mosquitoes. Novak also demonstrated that the volatiles from the leaves of the plant had repellent activities against a tick, *Ixodes ricimus* (2).

Steam distillation of the fresh leaves yielded a strong, sweet-smelling, brownish-yellow oil. Acute toxicity tests on the oil showed that it was toxic to the third nymphal instar of the grasshopper. Chromatography led to the isolation of estragole (the major component of the oil) which was 1½ times more toxic than the crude oil. This represents the first report of the biological activity of this compound. Furthermore, its isolation as the major compound from the volatile oil of the leaves of this plant contradicts the earlier report of anethole as the major constituent (1).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ir and uv spectra were obtained on Pye Unicam Sp3-300 and Sp8-400 spectrometers, respectively. The ^{1}H-nmr spectra were obtained on a Varian FT 80 A spectrometer (80 MHz) and the ^{13}C-nmr spectra were recorded on a Varian FT 80 A spectrometer (20 MHz). $CDCl_3$ was used as solvent with TMS as internal standard.

EXTRACTION AND ISOLATION OF ESTRAGOLE.—Collections were made from plants authenticated as *C. anisata* by Jaiyeola, Department of Botany, University of Ife, Unife, Nigeria. A voucher specimen is deposited in the Herbarium of the department.

Fresh leaves of *C. anisata* (0.2 kg collected from Unife environ in June 1986) were steam distilled for 2 h. The volatile oil (0.54 g; 1 ml) was separated from the condensed steam. The crude oil, which showed significant toxicity (LD_{50} 3780 mg/kg), was further purified by preparative tlc on Si gel $PF_{254+366}$ (hexane-EtOAc, 10:1). The fastest-moving component (Rf 0.78) was collected and extracted from the silica with $CHCl_3$. Concentration of the $CHCl_3$ extract left a colorless oil (70% of the total oil) that was identified as estragole [1-methoxy-4-(2′-propenyl) benzene] by comparison of its spectral data (uv, ir, ^{1}H nmr) with published data (3,4) and the ^{13}C-nmr data: 158.1 C-1, 137.9 C-2′, 132.1 C-4, 129.5 C-3 and 5, 115.4 C-2 and 6, 113.9 C-3′, 55.2—OCH_3, 39.4 C-1′.