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Tremorgenic Mycotoxins from *Aspergillus caespitosus*

H. W. SCHROEDER,* R. J. COLE, H. HEIN, JR., AND J. W. KIRKSEY

Agricultural Research Service, Southern Region, College Station, Texas 77840, and Dawson, Georgia 31742*

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Two tremorgenic mycotoxins were isolated from *Aspergillus caespitosus*, and identified as verruculogen and fumitremorgin B. They were produced at the rate of 172 and 325 mg per kg, respectively, on autoclaved cracked field corn.

Chloroform extracts of cultures of *Aspergillus caespitosus* Raper and Thom were dried, suspended in corn oil, and injected into the crop of day-old cockerels. Severe tremors developed in 15 to 20 min. Subsequently, two compounds with tremorgenic activity were separated and identified.

Two cultures of *A. caespitosus*, no. 4 (isolated from cottonseed) and NRRL 1929, used in these experiments were found to produce essentially the same kinds and quantities of secondary metabolites. The fungi were grown on autoclaved cracked field corn in 500-ml Erlenmeyer flasks incubated at 25 C for 12 to 14 days. The cultures were comminuted in 70% aqueous acetone in a blender and filtered through Whatman no. 4 filter paper. After a cleanup procedure (2) the aqueous solution was extracted with chloroform, adsorbed on silica gel (particle size <0.08 mm), placed in a Butt tube and eluted in sequence with benzene and 3% methanol in methylene chloride. The second fraction contained the bulk of the tremorgenic compounds although some was eluted in the first fraction. The procedure was repeated with both fractions to increase the concentration of the tremorgins and to reduce the amount of other compounds. These fractions were further separated on silica gel columns prepared and developed with 1.5% acetone in chloroform. The tremorgenic compounds were eluted from the column in overlapping bands. Several columns were required for adequate separation.

The toxins were detected by thin-layer chromatography. Thin-layer chromatography plates of EM Silica Gel 60 HR (0.50 mm thick) were spotted and developed in two solvent systems: (i) diethyl ether and (ii) acetone-methylene chloride (5:95 [vol/vol]). The R_f's $\times$ 100 of verruculogen were 63 and 46, respectively, compared to 67 and 38 for fumitremorgin B. After development, the plates were sprayed with an ethanolic solution of $AlCl_3$ (20%), then heated for 10 min. The first tremorgenic compound eluting from the column (A) fluoresced greenish blue under short wave ultraviolet light and the second (B) bright blue. The compounds were initially crystallized from a mixture of hexane, diethyl ether, and methylene chloride. Subsequent crystallizations were from benzene-ethanol solution (1:1 [vol/vol]) to yield colorless crystals (mp A = 234 C; B = 210 to 212 C). Yields were 172 mg of crystals per ml of A and 325 mg of B per kg.

The ultraviolet spectra of both tremorgens suggested a 6-0 methoxy-indole chromophore [A λ_{max}^{ETOH} 227 (ϵ_{max}, 47,800), 227 (11,000), and 294 nm (9,800); B = 226 (67,000), 275 (12,000), and 295 nm (9,000)]. In both cases, the carbonyl region of the infrared spectra showed typical diketopiperazine absorptions of 1,655 (A) and 1,665 nm/cm (B) in addition to OH and/or indole adsorptions in the region of 3,460 to 3,420 nm/cm.

Co-chromatography of the tremorgens on thin-layer chromatography with authentic standards of several known tremorgens provided presumptive evidence that compound A was verruculogen and compound B was fumitremorgin B.

Comparisons of the physical and spectroanalytical properties for compound A and B with those for verruculogen and fumitremorgin B, respectively, confirmed their identity with those previously reported tremorgens.

Verruculogen was identified as a metabolite of *Penicillium verruculosum* by Cole et al. (1) in 1972 and fumitremorgin B was isolated from *A. fumigatus* by Yamazaki et al. in 1971 (3). Their isolation from *A. caespitosus*, a fungus of divergent phylogenic relationship, suggests that they might also be the metabolites of other species of *Penicillium* and *Aspergillus*.

The close structural similarity of the fumitremorgins and verruculogen and the coexistence of these tremorgens in fungal culture extracts strongly suggests a common biosynthetic origin.

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