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Toxic Substances Produced by *Fusarium* I: Trichothecene Derivatives from Two Strains of *Fusarium oxysporum* f. sp. *carthami*

Keyphrases □ *Fusarium oxysporum*—cultured, trichothecenes isolated, phytotoxic and dermatitic properties evaluated □ Phytotoxins—trichothecenes isolated from *Fusarium oxysporum*, activity evaluated □ Fungi—*Fusarium oxysporum* cultured, trichothecenes isolated, phytotoxic and dermatitic properties evaluated

To the Editor:

Fusarium oxysporum Schlecht. f. *carthami* Klisiewicz & Houston is involved in the typical wilt disease of safflower (*Carthamus tinctorius* Linn.) (1, 2). However, the nature of the substance or substances responsible for the phytotoxic effects has not been evaluated previously. Since food materials infected with a species of *Fusarium* have often contained substances that produce high mammalian toxicity (3), the presence of *F. oxysporum* in safflower is thus cause for alarm.

Although previous investigations with other forms of *F. oxysporum* furnished biologically active 12,13-epoxytrichothecenes (3, 4), there is no report on this form of the fungus producing any toxic substance in artificial media or the host tissue. The present investigation was designed to isolate and study the substances produced by the fungus in artificial media that are responsible for the pathogenic property.

Two strains (weakly parasitic and virulent) were collected from Varanasi, India (2), and their identity was confirmed by the Commonwealth Mycological Institute, Kew, England [CMI (IMI-166917 and IMI-186539)]. These strains were separately grown in Richard's solution (200 ml) (5) in still culture flasks (1 liter) at 21° for 21 days.

When sprayed on safflower plants, the culture filtrates caused severe scorching of foliage accompanied by marked retardation of stem growth and frequently death of the plants. Even in high dilution (1:100), the culture filtrates inhibited root elongation of 2-day-old seedlings. There was no inhibition of germination of safflower seeds when sown in a medium containing the culture filtrates, but the seedlings showed the usual toxic symptoms.

The effects of the intracellular toxins (from the mycelium) were more severe than those of the extracellular ones (from the culture filtrates). For the extraction of the intracellular toxins, the mycelia were first washed and then macerated with water in a high-speed blender. The extract was passed through a bacteria-proof filter. The effect of the filtrate was tested on the host plant in the usual way.

The toxic substances were isolated from the culture filtrates by solvent extraction. In a typical experiment, the culture filtrate (5 liters) from the weakly parasitic strain at the natural pH (about 3.8) was successively extracted with chloroform (3 liters) and ethyl acetate (3 liters). The aqueous mother liquor was then concentrated to about 500 ml under reduced pressure and again extracted with hot ethyl acetate (3 liters). The three extracts were processed separately.

Evaporation of the solvent from the chloroform extract afforded a brown oil (0.88 g) which, in very low concentration (1–2 ppm), produced phytotoxic effects similar to those shown by the culture filtrates. It showed the presence of about six trichothecene derivatives by TLC (fluorescence under UV light, characteristic Ehrlich-reagent positive spots) (6).

A solution of the oil in ether–hexane (2:1, 50 ml) was filtered and set aside. Colorless crystals (58 mg) resulted, mp 158–160°; $[\alpha]_D^{22}$ +18° (c 0.52, ethyl alcohol); UV: λ_{max} (ethyl alcohol) only an end absorption; mass spectra: *m/e* 366 (M^+). The melting point, optical rotation, and spectral properties of this compound were indistinguishable from those of diacetoxyscirpenol (4β,15-diacetoxy-12,13-epoxytrichothec-9-en-3α-ol) (6).

The oily residue obtained from the ether–hexane mother liquor was dissolved in benzene (10 ml) and chromatographed over a magnesium silicate column (1.2 × 14 cm). Elution with chloroform and chloroform–methanol (98:2) yielded several 20-ml fractions containing a toxic material, as indicated by a rat skin bioassay (7). The residue from the concentrated eluates, when crystallized from hexane–benzene, gave colorless needles (33 mg), mp 148–150°; $[\alpha]_D^{22}$ +17.8° (c 0.48, ethyl alcohol), +16.5° (c 0.52); UV: λ_{max} (ethyl alcohol) only an end absorption; IR: ν_{max} (potassium bromide) 3400 (br), 1722, and 1245 cm^{-1}; mass spectra: *m/e* 466 (M^+, relative intensity 0.5%), 364 ($M^+ - C_5H_{10}O_2$, 22%), 322 (2.5), 305 (2), 304 (7), 291 (14), and 121 (100). The melting point, optical rotation, and spectral properties of this compound were indistinguishable from those of T-2 toxin (4β,15-diacetoxy-8-isovaleroxy-12,13-epoxytrichothec-9-en-3α-ol) (8).

No significant difference was observed in the amount of chloroform extractives or in the types of chemical constituents isolated from the culture filtrates of the virulent strain (IMI-186539) of the fungus.

The dermatitic properties of the trichothecene mycotoxins, diacetoxyscirpenol, T-2 and HT-2 toxins, nivalenol, and fusarenone, have been used as the basis for semiquantitative biological tests (7). Doses of 0.05, 0.1, and 0.2 mg of the residue from the chloroform extract were tested on albino rats (80–120 g, bred from CDRI strains) according to the method of Wei *et al.* (7). Two rats were used at each dose level. On the 2nd day, edema was noticed on all six rats. It became progressively severe, developing into a heavy scab and hemorrhaging by the 4th day. The animals that received 0.1 or 0.2 mg of the total toxins died within 6 days. Smaller doses (100–500 μg) of the chloroform extract left pinkish scars on the treated spots.

The chloroform extract of the culture filtrates of the fungus also showed prolonged emetic activity in pigeons at nonlethal concentrations on oral and intravenous administrations. The mean toxic values for this substance were 1.4 and 0.3 mg/kg, respectively.

The biological effects of the toxic substance from *F. oxysporum* are analogous to those reported for 12,13-epoxytrichothecenes (3, 9). The present study has additional significance because this is a seedborne disease of safflower. Furthermore, similar trichothecene derivatives, found in the culture filtrates of the fungus, also were detected in the infected safflower seeds. Work is now in progress to characterize and bioassay the remaining components in the chloroform and ethyl acetate extracts and in the infected seeds.

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BOOKS

REVIEWS

Emulsions and Emulsion Technology, Part II (Surfactant Science Series, Vol. 6). Edited by KENNETH J. LISSANT. Dekker, 270 Madison Ave., New York, NY 10016, 1974. 971 pp. 16 × 23.5 cm. Price $48.50.

This text consists of five chapters covering *extensively* the various aspects of emulsion technology.

Chapter 9 dealing with emulsion polymerization is well organized, tracing the subject from its beginning with the technique of polymerizing liquid monomers in bulk and expanding the theory to include in detail the mechanism of polymerization of hydrophilic (in the presence and absence of surfactants) and hydrophobic monomers. The behavior of additives such as initiators, protective colloids, organic solvents, plasticizers, and the like including their role in emulsion polymerization is given careful attention.

Chapter 10 deals with emulsions in the paper-making industry, devoting special consideration to surface roughness and permeability of paper as well as emulsion stability. The role of emulsions in the process of making paper with a controlled sensitivity to water, referred to as sizing, is given thorough coverage. The extensive use of emulsions in coatings applied to paper is discussed in addition to other topics such as foam control, bubble, and encapsulated coatings.

Chapter 11 deals with emulsions in the graphic arts and points out the limited amount of research in this area. The first part of the chapter discusses prevention or remedy of emulsification that is deleterious to the production of a graphic arts product. The latter part of the chapter addresses itself to the creation of emulsification products or processes that facilitate or improve function or output in printing, duplication, copying, photography, or recording.

Chapter 12 moves on to discuss hydraulic fluid emulsions. The authors point out the advantages of the hydraulic system of power transmission and indicate requirements necessary for the successful operation of the hydraulic system. A good review of emulsion rheology is provided as well as general stability considerations for emulsion systems. A discussion of the basic components of any hydraulic system and methods for testing stability, viscosity, lubricity, *etc.*, concludes this section.

The final chapter is probably the most significant and relevant for the pharmaceutical scientist. It is very well organized, covering an initial section on emulsion theory, prediction of types of emulsions and their identification. The author does a very thorough job of discussing microemulsions. The HLB system and the selection, classification, and mechanism of emulsifying agents are covered in such detail that this section could serve as an excellent reference on the subject. Equipment generally used for emulsification and milling is covered to a lesser degree with some pictorial and di-