Isolation of Acetyl T-2 Toxin from Fusarium poae

FRANK N. KOTSONIS, ROBERT A. ELLISON,* AND EUGENE B. SMALLEY

School of Pharmacy and the Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin 53706

Received for publication 18 April 1975

Acetyl T-2 toxin (3,4,15-triacetoxy-8-isovaleroxy-12,13-epoxy-Δ9-trichothecene) was isolated and characterized as a naturally occurring emetic trichothecene from liquid cultures of Fusarium poae (NRRL 3287). Acetyl T-2 toxin was shown to be much less toxic than T-2 toxin in pigeon assays.

Emesis has frequently been reported as a distinct toxicosis associated with the contamination of cereals by Fusarium species (5). Researchers in Japan (7, 8) and the U.S. (2) have implicated Fusarium-elaborated trichotheccenes as causal agents. Their hypothesis has been supported by the identification of emetic trichothecenes in field samples of moldy cereals (3, 9).

Early investigations of moldy cereal emesis carried out by Prentice and Dickson (4) demonstrated that several Fusarium species including F. poae (NRRL 3287) (= F. tricinctum, according to Synder and Hansen (6)) produced emetic substances. They reported the presence of two emetic substances. One was free from harmful side effects at doses of 100 μg or less in pigeons, and the second one caused emesis and death at a comparable dose. In an earlier examination of F. poae (NRRL 3287) metabolites, we isolated T-2 toxin (3-hydroxy-4,15-diacetoxy-8-isovaleroxy-12,13-epoxy-Δ9-trichothecene) and suggested it as the lethal emetic described by Prentice and Dickson (4). We report here our reexamination of F. poae (NRRL 3287) metabolites and the isolation and characterization of acetyl T-2 toxin (Fig. 1).

F. poae was grown as an agitated culture (300 rpm, New Brunswick Gyrotary Shaker) in 14 2-liter flasks, each containing 500 ml of Richards solution (0.1 M potassium nitrate nitrogen source), for 4 weeks.

The contents of the 14 culture flasks were filtered through Whatman no. 2 paper, and the filtrate was adjusted to pH 9.0 with saturated sodium carbonate followed by five extractions with chloroform. The chloroform layers were combined, dried over sodium sulfate, and evaporated in vacuo (40 C) to give an oily residue (861 mg) that was biologically active. Additional active material (86 mg) was obtained by extraction of the mycelial residue with water for 8 h followed by chloroform extraction as before.

Column chromatography of the material recovered from the filtrate (662 mg) on silica gel (Merck E.M., 120 g, 2.7 by 46 cm) was effected with ethyl acetate-Skelly B (85:15) with 1.0-ml (tubes 1 to 232) and 1.7-ml (tubes 233 to 300) fractions being collected. The eluate was combined into 6 fractions (A → F) for bioassay. Fraction D (tubes 200 to 287, 145 mg), clearly the most active fraction, was purified by preparative thin-layer chromatography on 2-mm Brinkman F-254 silica gel plates with ethyl acetate. The isolated oil (59 mg) was nearly pure T-2 toxin (2). Fraction B (tubes 134–163, 113 mg) was rechromatographed on silica gel (20 g, 1.2 by 58 cm) with ethyl acetate-Skelly B (85:15) and 0.5-ml fractions were collected. Tubes 37 to 80 afforded a crude material which was tentatively identified as a trichothecene. Further purification of this material by column chromatography was affected on silica gel (20 g, 1.2 by 58 cm) with toluene-ethyl acetate (4:1), and 0.5-ml fractions were collected. The eluate from tubes 95 to 135 was combined, dried with sodium sulfate, and evaporated in vacuo (40 C). The resulting oil (7 mg) proved to be identical on mixed thin-layer chromatography (ethyl acetate-ethanol [6:1], Rf = 0.74; ethyl acetate, Rf = 0.59; toluene-ethyl acetate [3:1], Rf = 0.22), nuclear magnetic resonance spectroscopy (1H and 13C, Bruker Spectrospin H X 90E) (Fig. 2)

![Fig. 1. Structure of acetyl T-2 toxin (R1 = R2 = Ac), T-2 toxin (R1 = H, R2 = Ac), and HT-2 toxin (R1 = R2 = H).](http://aem.asm.org/Downloaded from http://aem.asm.org/)

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Fig. 2. Proton magnetic resonance spectrum of acetyl T-2 toxin (1,000 Hz wide) in dimethyl sulfoxide-\(d_6\) with tetramethyl-silane (TMS) internal standard.

Fig. 3. Carbon magnetic resonance spectrum of acetyl T-2 toxin (5,000 Hz wide) in dimethyl sulfoxide-\(d_6\) with dimethyl sulfoxide-\(d_6\) internal standard.
and 3), and mass spectroscopy (Finnegan 1015 Spectrometer) (Fig. 4) with an independently prepared sample of acetyl T-2 toxin (1).

The emetic activity of the column fractions and the acute oral toxicity of acetyl T-2 toxin were determined by a previously described method (2). Fifteen pigeons (300 to 400 g) received pure acetyl T-2 toxin at five dose levels (0.44, 1.02, 2.76, 6.76, and 18.20 mg/kg), and were observed 1.5 h for emesis and 5 days for any additional toxic effects. No deaths were observed at any of the five doses. However, slight emesis was observed at the highest dose (18 mg/kg), where two of three pigeons vomited for 5 min approximately 45 min after oral feeding. By comparison, under identical conditions, T-2 toxin had a TD₅₀ equal to 0.72 mg/kg (non-lethal emetic dose) and a mean lethal dose equal to 2.75 mg/kg with pigeons.

The low toxicity of acetyl T-2 toxin, plus its thin-layer chromatographic characteristics have led us to believe that it may be the non-lethal emetic described earlier by Prentice and Dickson. Whether or not the dramatic drop in toxicity observed with acetylation of the 3-hydroxy position of T-2 toxin is a general phenomenon among trichothecenes is not known.

Recently, we found that T-2 toxin is converted to HT-2 toxin (3,4-dihydroxy-15-acetoxy-8-isovaleroxy-12,13-epoxy-A³-trichothecene) in F. poae (NRRL 3287) liquid cultures (manuscript submitted for publication). The isolation of acetyl T-2 toxin from the same strain of F. poae, and the recent work by Yoshizawa and Morooka (10) showing that the 3-acetoxy group of 3α,7α,15-triacetoxy-12,13-epoxytrichothe-9-ene-one is particularly susceptible to enzymatic hydrolysis by Fusarium solani, have led us to suspect that acetyl T-2 toxin is the immediate biogenetic precursor of T-2 toxin. If this is the case, any observed T-2 toxicosis associated with

F. poae (= F. tricinctum) contamination of field samples may well be dependent upon the relative efficiency of C-3 deacetylation in acetyl T-2 toxin.

We thank Gary Girdaukas (School of Pharmacy) for mass spectra and James Blackbourn (School of Pharmacy) for nuclear-magnetic resonance spectroscopy. F. poae (NRRL 3287) was obtained from Northern Regional Research Laboratory, Peoria, Ill.

This work was funded by the graduate school and by the College of Agricultural and Life Sciences of the University of Wisconsin, Madison.

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