Isolation of Acetyl T-2 Toxin from Fusarium poae

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Acetyl T-2 toxin (3,4,15-triacetoxy-8-isovaleroxy-12,13-epoxy-Δ9-trichothe- 
cene) was isolated and characterized as a naturally occurring emetic trichothe- 
cene 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 trichothe- 
cenes as causal agents. Their hypothesis has 
been supported by the identification of emetic trichothe- 
cenes 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, ac- 
cording 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 exami- 
nation of F. poae (NRRL 3287) metabolites, we 
isolated T-2 toxin (3-hydroxy-4,15-diacetoxy-8- 
isovaleroxy-12,13-epoxy-Δ9-trichothe- 
cene) and suggested it as the lethal emetic described by 
Prentice and Dickson (4). We report here our 
reexamination of F. poae (NRRL 3287) metab- 
olites 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 Rich- 
rards 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 resi- 
due (861 mg) that was biologically active. Ad- 
ditional active material (96 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 re- 
covered 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 com- 
bined into 6 fractions (A → F) for bioassay. 
Fraction D (tubes 200 to 287, 145 mg), clearly 
the most active fraction, was purified by prepara- 
tive thin-layer chromatography on 2-mm 
Brinkman F-254 silical 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 trichothe- 
cene. 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 ac- 
etate-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).](image-url)
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 (nonlethal 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 nonlethal 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-D-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-epoxytrichothecene-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.

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