

# Isolation of the Emetic Principle from *Fusarium*-Infected Corn

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A mycotoxin responsible for vomiting in swine has been isolated from *Fusarium*-contaminated field corn. The compound was tentatively identified as a trichothecene, 3,7,15-trihydroxy-12,13-epoxy-trichothene-9-en-8-one, and has been given the trivial name vomitoxin.

During 1972, because of unusually wet weather, much corn produced in some parts of the U.S. Corn Belt was infected in the field by *Fusarium graminearum* Schw. (perfect stage, *Gibberella zeae*), a species associated with various mycotoxicoses (6). Reports soon followed that swine were refusing to eat this contaminated grain or had vomited after eating small quantities.

Incidences of vomiting by swine when fed moldy corn infected with *Fusarium* species were reported in 1928 (7) and in 1966 (2). Reports of emesis resulting from consumption of other moldy cereal grains both in animals and humans have been recorded periodically since 1916 (3-5, 9, 12). Meager and often conflicting data are available concerning the chemistry of the mycotoxin involved. Prentice and Dickson (10) partially purified a *Fusarium*-emetic principle from liquid medium. Ueno et al. (13) isolated an emetic trichothecene from *F. nivale*, fusarenon-X, which caused cats and ducklings to vomit. Our preliminary report covers the isolation of the emetic factor from the 1972 crop corn and its tentative structure assignment.

From a Northwest Ohio farm we obtained samples of infected corn that had caused vomiting in swine. The predominant fungus on this corn was *F. graminearum*, but representatives of *F. moniliforme*, *F. nivale*, *Cephalosporium* sp., and various *Penicillium* sp. were also found (R. J. Bothast, personal communication).

Each fraction during the isolation procedure was assayed for emetic activity by intubation into pigs of an amount equivalent to 500 g of feed. Vomiting occurred within 25 min with active fractions. Ground corn (4 kg) was blender-extracted twice with butanol (2 liters/kg)

for 5 min, and the corn residue was washed with hexane, dried in a vented hood, and then blender-extracted twice with 40% aqueous methanol (2 liters/kg) for 5 min. The aqueous methanol extracts were combined and concentrated to a small volume (500 to 700 ml). Three volumes of cold absolute ethanol were added to this concentrate, and the mixture was refrigerated for 4 h. A tan precipitate was removed by filtration. This solution was again concentrated to a small volume (500 to 700 ml) and centrifuged to remove a finely suspended material, and the clear supernatant fluid was treated batchwise first with a cationic exchange resin (Bio-Rad 50 W-X8, <sup>2</sup>H<sup>+</sup> form) and then with an anionic exchange resin (Amberlite IRA-400, OH<sup>-</sup> form). The remaining neutral solution was freeze-dried to a tan solid (43 to 48 g) which was suspended in chloroform and applied to a Florisil column (8 by 16 cm, 60 to 100 mesh) pre-saturated with chloroform. The column was washed with 2 liters of chloroform, and then the crude emetic factor was eluted first with 3.6 liters of chloroform-methanol (50:50 vol/vol) and then with 2.6 liters of chloroform-methanol (25:75 vol/vol); methanol-chloroform extracts were combined and evaporated to dryness. This residue was dissolved in a small amount of methanol, and the solution was added to acetone to precipitate sugars. Upon flash evaporation of the supernatant fluid, a yellow viscous oil (fraction A) remained (1.98 to 2.5 g/4 kg of corn). Preparative thin-layer chromatography (TLC) of fraction A on precoated plates containing a fluorescent indicator (silica gel F-254; 2-mm thick, Brinkmann Instruments Co.) in solvent system I (chloroform-methanol-water, 80:20:0.1) revealed nine spots under shortwave (250 nm) ultraviolet (UV) light.

Only material eluted from band 6 (counting

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from the origin) (fraction B) with methanol caused emesis in swine. Band 6 turned to yellowish brown upon being sprayed with anisaldehyde reagent (11) and heated at 110 C. TLC analysis of band 6 did not give spots corresponding to the known tricothecenes, T-2 and fusarenon-X (emetic,  $R_f$  0.45; T-2,  $R_f$  1.0; fusarenon-X,  $R_f$  0.66).

Fraction B was purified further by preparative TLC to give 50 mg of solids (fraction C). Fraction C was dissolved in methanol and applied to a Sephadex LH-20 column (2.4 by 200 cm) presaturated with methanol. The column was eluted with methanol, and 2.2-ml fractions were collected. Fractions containing the band absorbing at 254 nm after development on silica gel F-22 were pooled and evaporated to dryness. A solid (fraction D) weighing 33 mg was recovered, which TLC analysis

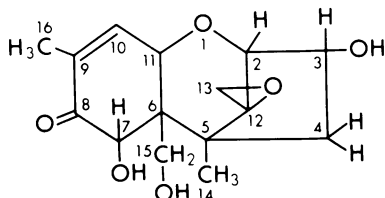


FIG. 1. Proposed structure for the emetic factor, vomitoxin.

showed had one major spot and several minor spots. Fraction D was extracted with chloroform to give 12 mg of a compound (fraction E) which gave only one spot on TLC analysis.

A tentative structure of 3,7,15-trihydroxy-12,13-epoxy-trichothe-9-en-8-one (Fig. 1) was established for the vomition factor based on infrared (IR) and nuclear magnetic resonance (NMR) spectra. We propose the trivial name of vomitoxin for this substance. A substance possessing similar spectrophotometric properties has been described by Morooka et al. (8), but neither an empirical or structural formula was presented.

A molecular weight of 296.157 and an empirical formula of  $C_{15}H_{20}O_6$  were determined by high-resolution mass spectrometry (Fig. 2). The IR showed a band at  $1,680\text{ cm}^{-1}$ , indicative of an  $\alpha,\beta$ -unsaturated ketone (NMR showed no aldehyde proton), and also a hydroxyl band at  $3,400\text{ cm}^{-1}$ . The UV spectrum showed weak absorption at 287 nm, and the main absorption at 217 nm also indicates an  $\alpha,\beta$ -unsaturated ketone. The mass spectrum fragmentation patterns for the parent alcohols of tricothecenes have been well established (1). In the spectrum of vomitoxin, the parent ion ( $m/e$  296) loses methyl, water, hydroxymethyl, or formaldehyde to give rise to peaks at  $m/e$  281, 278, 265,

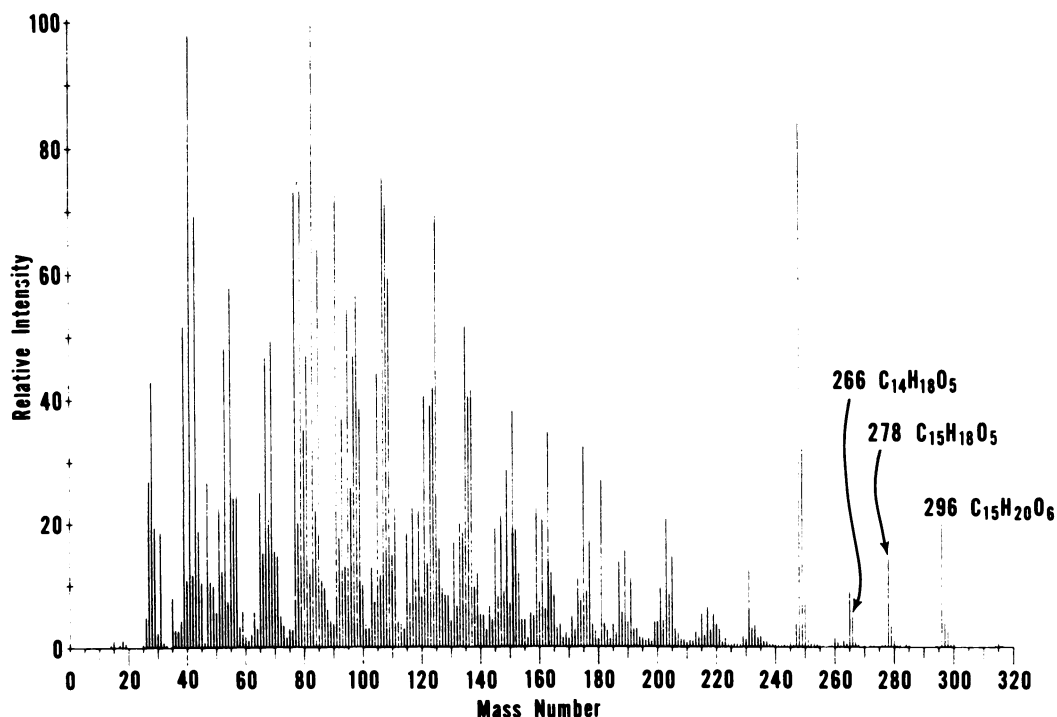
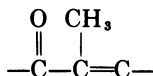


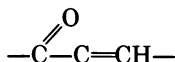
FIG. 2. Mass spectrum of the emetic factor. Nuclide 90 mass spectrometer.

and 266, respectively, which correspond to two less mass units than for the fragmentation pattern observed for the alcohol, T-2-tetrol. In addition, the major ion ( $m/e$  248), which would correspond to loss of both water and formaldehyde, would favor a compound containing a ketone at C-8, an OH at C-7, and a hydroxymethyl at C-6.

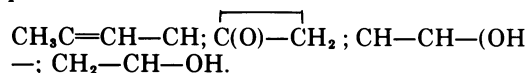
The NMR spectrum of vomitoxin in  $\text{CDCl}_3$  (tetramethylsilane internal standard, Varian 100) gave signals quite characteristic for 12, 13-epoxytrichothecenes: a signal at  $\delta$  1.13s for a tertiary methyl;  $\delta$  1.88d vinyl methyl with an oxygenated substituent, e.g.,



$\sigma$  3.09q epoxide protons;  $\sigma$  6.58dd vinyl proton, e.g.,



$\sigma$  3.60d methine. In addition to these moieties, the presence of the following structural parts was supported by results of spin-decoupling experiments:



Work is in progress to substantiate the proposed structure.

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