Solaniol, a Toxic Metabolite of *Fusarium solani*

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*Fusarium solani* M-1-1 isolated from moldy bean hulls produces T-2 toxin, diacetoxyscirpenol, and a new toxic trichothecene, solaniol, in Czapek-Dox-peptone medium.

In the Tokachi, Kitami, and Hidaka districts of Hokkaido, the northern island of Japan, which are known for the breeding of horses and the cultivation of beans, an intoxication of unknown origin, characterized by disturbance of the central nervous system, has occasionally been observed in horses during the winter and spring of the past several decades. Because of the seasonal coincidence of accidents and the use of bean hulls as feed, bean hulls contaminated with toxic agent(s) were suspected as the possible cause of the intoxication (3).

As reported in this paper, an investigation on toxicogenic microflora of bean hulls was carried out. It resulted in the isolation of *Fusarium solani* M-1-1 which was found to produce T-2 toxin, diacetoxyscirpenol, and, in addition, a new trichothecene, which we named solaniol.

Fifteen specimens of bean hulls collected on a farm near Obihiro City in Hokkaido were invaded with fungi which consisted of *F. roseum* and *F. solani*. Fungal isolates were grown in 200-ml portions of Czapek-Dox-peptone medium (30 g of sucrose, 10 g of peptone, 2 g of NaNO₃, 1 g of K₂HPO₄, 0.5 g of KCl, 0.5 g of MgSO₄, 0.01 g of FeSO₄, in 1 liter of deionized water) in stationary 500-ml Erlenmeyer flasks at 25 °C for 12 days. At the end of this incubation period, crude toxin was prepared from the culture filtrate by the charcoal adsorption method (6, 10). A toxicity test with mice and rabbit reticulocytes (7, 8, 12) revealed that, of 26 isolates tested, 11 synthesized toxic material, the highest yields being from *F. solani* M-1-1. This fungus was grown without shaking at 25 °C for 12 days in 100 Fernbach flasks, each containing 250 ml of Czapek-Dox-peptone medium. From 20 liters of pooled culture filtrates, 13 g of the crude toxin was obtained. The death rate of mice administered intraperitoneally with the crude toxin was one of three and three of three with doses of 2 and 5 mg/10 g, respectively. Furthermore, the uptake of 14C-leucine in the reticulocytes was completely inhibited by 10 µg of the crude toxin per ml. In the fatal cases, the pathological findings were characterized by extensive cellular degeneration and karyorrhexis of the bone marrow, spleen, thymus, lymph nodes, and crypt cells of the small intestine of mice.

The isolation procedure of the toxic principle(s) is shown in Fig. 1. The crude toxin was divided into four parts, and each part (3 g) was chromatographed on a silica gel column (4 by 45 cm) with n-hexane-ethyl acetate (1:3 to 1:7), followed by ethyl acetate, ethyl acetate-methanol (5:1), and methanol. Fractions II and IV were lethal to mice in a dose of 250 µg/10 g and positive in the reticulocyte bioassay. The crystallization of the former fraction with benzene-n-hexane yielded 160 mg of colorless needles. The melting point was 150 to 151 °C; the infrared (IR) spectrum and nuclear magnetic resonance (NMR) spectrum were identical with those of T-2 toxin of *F. tricinctum* (1). Fraction III, which was lethal to mice at 1 mg/10 g and positive in the reticulocyte bioassay, and trace amounts of T-2 toxin and diacetoxyscirpenol (2, 5) were detected by thin-layer chromatography (TLC) analysis.

The toxic fraction IV was rechromatographed on a silica gel column with acetone-n-hexane (1:1). The purified fraction (IV-2, 400 mg) yielded 200 mg of crystals from ethyl acetate-n-hexane; melting point was 171 to 172 °C. Analysis: calculated for C₁₈H₂₀O₅: C, 59.65; H, 6.85; O, 33.49; found: C, 59.78; H, 6.66; O, 33.56; M⁺ ion m/e 382. The IR spectrum (KBr; Fig. 2) showed a hydroxyl group (ν₅max = 3450 cm⁻¹) and an ester group (ν₅max = 1735 and 1250 cm⁻¹). The NMR spectrum in D₂Cl₃ (Fig. 3) showed an angular methyl group (δ 0.85, 3H s), an allylic methyl group (δ 1.88, 3H s), acetyl groups (δ 2.03 and 2.14, each 3H s), an epoxide ring (δ 2.80 and 3.07, each 1H d J = 4 cps), and an olefinic proton (δ 5.67, 1H d J = 6 cps). The NMR spectrum also
Crude toxin (13 g)

Silica gel column

\( n\)-Hexane-ethyl acetate (1:3 to 1:7),
Ethyl acetate, ethyl acetate-methanol (5:1),
and methanol

|    | I   | II  | III | IV  | V   | VI  |
|----|-----|-----|-----|-----|-----|-----|
| Yield (mg) | 110 | 400 | 220 | 600 | 2700| 5900|
| Death rate of mice injected intraperitoneally with 250 \( \mu \)g/10 g (%) | 0 | 100 | 0 | 100 | 0 | 0 |
| Activity of \( ^{14} \text{C}-\text{leucine} \) uptake (%) | 7.4 | 0.3 | 1.0 | 1.1 | 18.9 | 59.9 |

**Fig. 1. Isolation procedure of the mycotoxins of *Fusarium solani.*

**Fig. 2. Infrared spectrum of fraction IV-2, solaniol.**

revealed that two hydroxyl groups at 4\( \beta \) and 15 were acetylated. Alkaline hydrolysis of the toxin in 1 \( \text{N} \) \( \text{NH}_2\text{OH}-\text{methanol} \) yielded scirpen tetraol (1), identified by direct comparison of its IR absorption spectrum and TLC with those of the product obtained from T-2 toxin by alkaline hydrolysis in 2 \( \text{N} \) \( \text{NH}_2\text{OH}-\text{methanol} \). \( R_F \) values on Silica Gel G were 0.35 (ethyl acetate-\( n \)-hexane, 3:1) and 0.5 (acetone-\( n \)-hexane, 1:1). The toxin exhibited light-blue fluorescence under an ultraviolet lamp when heated at 100 C after being sprayed with 20\% \( \text{H}_2\text{SO}_4 \).

These results offer the chemical structure of this toxin as 3\( \alpha \), 8\( \alpha \)-dihydroxy-4\( \beta \), 15-diacetoxy-12, 13 epoxy-\( \Delta^4 \)-trichothecene (Fig. 4). This compound lacks isovaleryl residue in T-2 toxin and is a newly isolated fungal metabolite of *F. solani*, which we name solaniol.

The \( \text{LD}_{50} \) of solaniol is 14.5 mg/kg in male mice of ddys strain when administered intraperitoneally. In fatal cases, marked cellular degeneration and karyorrhexis were observed in the actively dividing cells of the thymus, lymph nodes, spleen, bone marrow, intestine, and testes. These morphological changes seem to reflect the so-called "radiomimetic" biological property (4) of solaniol. The uptake of \( ^{14} \text{C}-\text{leucine} \) in the reticulocytes was inhibited 50\% by solaniol of an estimated 0.25 \( \mu \)g/ml concentration. Minimum dose for skin-irritant toxicity to rabbit skin was approximately 1.0 \( \mu \)g. These data revealed that the biological features of the new mycotoxin are very
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