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Nivalenol, a Toxic Principle of *Fusarium nivale*

Recently, a number of *Fusarium* species were collected from the damaged wheat, and a biological screening was undertaken to select toxic strains by cultivations of the fungi on unpolished rice at 27° for 3 weeks or on a peptone-supplemented Czapek medium at 27° for 2 weeks. As a result, *F. nivale* Fn 2 was selected for isolation of the toxic agents.[1]

The purpose of this work was to purify the toxic principle, and to elucidate its chemical and biological properties.

For a typical purification run, *F. nivale* Fn 2 was grown on 20 kg unpolished rice at 27° for 3 weeks, and the toxic principle was fractionated by the charcoal methods shown in Fig. 1.

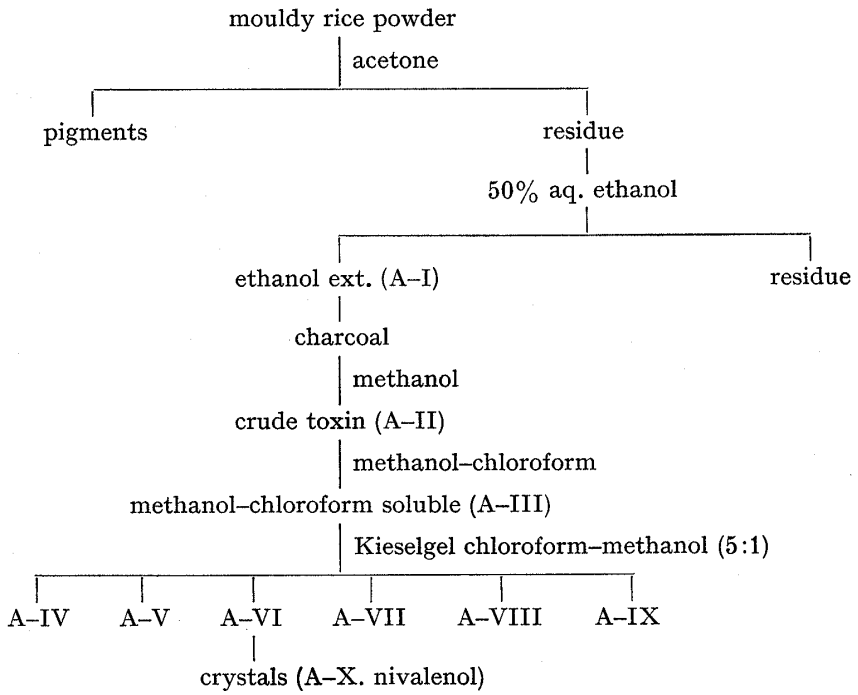

Fig. 1. Chemical Fractionation of Nivalenol from *F. nivale*-mildewed Rice

Toxicity tests were based on killing of mice and assay with rabbit reticulocytes.[2] Non-toxic pigments were removed from the mouldy rice powder by acetone, and the residue was extracted with 50% aq. ethanol to give the toxic fraction (A-I). 1.2 liter of the toxic ethanol extract (A-I) was passed through a charcoal column (6 cm × 60 cm). The materials adsorbed were eluted with 3 liter of methanol to give the crude toxin (A-II). This material was dissolved in 50 ml of hot methanol and 9 volumes of chloroform was added to precipitate non-toxic materials. The soluble fraction (A-III) was chromatographed on Kieselgel (2 cm × 50 cm), chloroform–metanhol (5:1, v/v) system. The highly toxic fraction (A-VI) was separated. Recrystallization of A-VI from methanol yielded white rectangular crystals (A-X) weighing 10—100 mg. After drying over P_2O_5 at 80° under reduced pressure,

1) H. Tsunoda, N. Toyosaki, N. Morooka, N. Nakano, H. Yoshiyama, K. Ohkubo and M. Isoda, *Reports Food Res. Inst.*, **23**, 89 (1968).
2) Y. Ueno, M. Hosoya and T. Tatsuno, *Seikagaku*, **39**, 708 (1967).

the crystals give mp. 222—223° (decomp.), $[\alpha]_D^{24}$ +21.54° (c=1.3, l=1, ethanol), *Anal.* Calcd. for $C_{15}H_{20}O_7$[3]: C, 57.68; H, 6.46; O, 35.86. mol. wt., 312.3. Found: C, 56.29; H, 6.01; O, 37.70.

The crystals gave *Rf* 0.45 on thin-layer chromatography (Kieselgel G, chloroform-methanol, 5:1), and a single peak by gas chromatography. The spot tests gave a brown colour with H_2SO_4, and ammoniacal $AgNO_3$, pink-violet with α-naphthol-H_2SO_4, and were negative to 2,4-dinitrophenylhydrazine and ninhydrin. The pure toxin exhibited a weak UV absorption spectrum at 220 mμ (in methanol) and 260 mμ (in acetonitrile), indicating no aromatic ring in the structure.

With the ethanol extract (A-I), mice died 18—72 hr after intraperitoneal administration of 15—20 mg/10 g, and pathological findings were necrosis and degeneration of the proliferating cells in the gastrointestinal epithelium, bone-marrow, lymph nodes, thymus and testis. These findings were also observed in guinea-pig.[4] With the toxin (A-X), mice died 15—26 hr after intraperitoneal injection of 200 μg/10 g, and LD_{50} of the toxin was 41 μg/10 g in male mice of ddS. Pathological changes of the mice were similar to those of mice given the extract (A-I).

As for biological effects of the toxin, a few micrograms of the toxin per mililiter inhibited uptake of C^{14}-leucine in rabbit reticulocytes and poly-U dependent-polyphenylalanine synthesis in the cell-free system from the same cells.[2] According to unpublished data of Ohtsubo, *et al.*, the toxin inhibits multiplication of Chang's liver cells and HeLa cells, and it was shown to suppress protein and DNA, but not RNA, syntheses of HeLa cells.

The physical and chemical characteristics of our toxic principle differ from that of the sesquiterpene [8-(3-methylbutyryloxy)diacetoxyscirpenol] of Yates, *et al.*,[5] though some similarity was observed. In this respect, the toxin described in this report is a new mycotoxin, to which we gave a name "nivalenol."

From the ethanol extract (A-I), Morooka independently isolated the other toxin, named fusarenon,[1] which we found to be a monoacetyl derivative of nivalenol.

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3) We have reported the molecular formula as $C_{18}H_{28}O_{10}$ in the meeting of the society of Japanese pharmacology——*Folia Pharmacol. Japan*, **64**, 121 § (1967)——, considering the molecular weight which we observed osmometrically. But recently, the high-mass spectrometrical data gave us the molecular weight, 312.31, though, we revise the molecular formula as $C_{15}H_{20}O_7$.

4) K. Ohkubo and M. Isoda, *Bull. Nippon Vet. Zootechnic. College*, **16**, 22 (1967).

5) S.G. Yates, H.L. Tooky, J.J. Ellis and H.J. Burkhardt, *Phytochem.*, **7**, 139 (1968).