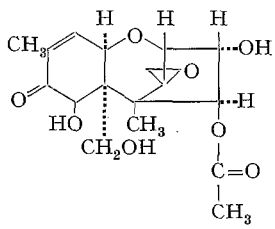
Fusarenon-X, a Toxic Principle of *Fusarium nivale* - Culture Filtrate

A new metabolite with marked cytotoxic activity has been isolated from a culture broth of the fungus *Fusarium nivale*.

Numerous intoxications in animals fed scabby grains prompted us to isolate the toxic principle(s) from cereal grains infected with Fusaria, and nivalenol (mp 222 to 223 °C)[1,2] and fusarenon (mp 78–80 °C)[3] were isolated from rice grains polluted by *F. nivale* Fn 2B which was isolated from actually damaged wheat[3].

To obtain enough of the toxic agents for chemical and toxicological tests, a study on the liquid culture of the fungus was attempted; *F. nivale* Fn 2B was grown on Czapek medium supplemented with 10 g/L of peptone at 25–27 °C for 2 weeks. As indicator, a lethal effect on mice and a reticulocyte bioassay[4] were used. The toxin in the broth was absorbed on active carbon, followed by elution with methanol. After evaporation of the solvent, 5 volumes of chloroform were added to the methanol extract. The methanol-chloroform soluble fraction, herein referred to as crude toxin, was chromatographed on Kieselgel. Development with chloroform-methanol (97:3 to 5:1) yielded a highly toxic fraction eluted before the fraction of nivalenol. Rechromatography on Kieselgel with chloroform-acetone (5:1) gave a white powder. This material, when crystallized from dichloro-methane-*n*-pentane, gave hexagonal bipyramides: mp 91–92 °C; $[\alpha]_D^{25} = +58.0$ (*c* 1.0 in methanol). Found, C 57.62, H 6.22, O 36.16. Calculated for $C_{17}H_{22}O_8$ (mass spectrum), C 57.62, H 6.22, O 36.16. UV-spectrum showed end absorption only, and no fluorescence was exhibited. The crystals were freely soluble in chloroform, methanol, ethylacetate, soluble in water, insoluble in *n*-hexane and *n*-pentane, and gave a single spot at 0.89 (chloroform-methanol, 5:1), 0.19 (chloroform-methanol, 97:3) or 0.37 (ethylacetate-*n*-hexane) on TLC with Kieselgel G. By ammonolysis in methanol, this compound changed to nivalenol ($C_{15}H_{20}O_7$), and by acetylation with acetic anhydride in pyridine, gave tetra-acetylnivalenol ($C_{23}H_{28}O_{11}$). Based on these results and IR- and NMR-spectra, the structure of the toxin, named Fusarenon-X[5], is 3,7,15-trihydroxyscirp-4-acetoxy-9en-8one, as shown in the Figure.



Fusarenon-X.

Yields of fusarenon-X and nivalenol from 10 l of the culture filtrate were around 200 mg and 10 mg, respectively.

Toxicological and pathological examinations with the crude toxin and fusarenon-X gave the following results: With the crude toxin, mice died 24–48 h after i.p. administration of 0.1–0.3 g/kg, and the observed findings were hypertrophy and heamorrhage in the intestine, atrophy and ecchymosis in the thymus, hyperaemia in the periphery of the liver lobules. Pathological findings were degeneration and karyorexis in the mucosal epithelia of small intestine, lymph follicles, thymus, spleen, ovary, bone marrow, and heamorrhage in the heart muscle. A subchronic experiment revealed that rabbits died of subarachinoidal heamorrhage 28 days after p.o. administration of 5 mg/kg twice a week, and karyorexis in the ovum, heamorrhage and necrosis in the heart muscle were noted. With fusarenon-X, mice died 36–48 h after i.p. administration of 8 mg/kg, and i.p. LD_{50} of the toxin was 3.3 mg/kg in male mice of ddS strain. Pathological changes of the damaged tissues were similar as a whole to those caused by the crude toxin. The most noticeable changes were the cellular destruction and karyorexis in the bone marrow and intestinal mucosa.

As to the biochemical effect, 0.5 µg/ml of the toxin inhibited the uptake of C^{14}-leucine in rabbit reticulocytes and that of C^{14}-leucine and C^{14}-thymidine in Ehrlich ascites tumour without affecting the uptake of C^{14}-uracil, and interfered with the poly U-directed synthesis of polyphenylalanine in both cells. These results indicate that fusarenon-X is a potent inhibitor of protein synthesis in animal cells, as is nivalenol[4,6].

KEYL et al.[7] and YATES et al.[8] isolated a toxic butenolide from a culture filtrate of *F. nivale*, and DAWKINS[9] and BAMBURG et al.[10] isolated a cytotoxic diacetoxyscirpenol from *F. equiseti* and *F. tricinctum*. Comparing the biological and chemical properties of the toxin reported here to those of the two mycotoxins referred to above, it seems that fusarenon-X is closely related to the latter toxin[11].

Zusammenfassung. Fusarenon-X, ein toxisches Produkt aus dem Kulturfiltrat von *Fusarium nivale* wurde isoliert, näher charakterisiert und dessen Struktur ermittelt.

Y. UENO, I. UENO, T. TATSUNO, K. OHOKUBO and H. TSUNODA

Microbial Chemistry,
Faculty of Pharmaceutical Sciences,
Science University of Tokyo,
Department of Pharmacology, University of Tokyo,
Institute of Physical and Chemical Research,
Department of Pathology,
Nippon Veterinary and Zootechnical College and
Food Research Institute,
The Ministry of Agriculture and Forestry,
Tokyo (Japan), 15 April 1969

[1] T. TATSUNO, Y. UENO, I. UENO, Y. MORITA, M. HOSOYA, M. SAITO, M. ENOMOTO, K. OHOKUBO, S. MOROOKA, S. NAKANO and H. TSUNODA, Folia pharmac. jap. *64*, 121 (1968).
[2] T. TATSUNO, M. SAITO, M. ENOMOTO and H. TSUNODA, Chem. pharm. Bull., Tokyo *16*, 2519 (1968).
[3] H. TSUNODA, N. TOYAZAKI, S. MOROOKA, N. NAKANO, H. YOSHIYAMA, K. OHOKUBO and M. ISODA, Proc. Food Res. Inst. *23*, 89 (1968).
[4] Y. UENO, M. HOSOYA, Y. MORITA, I. UENO and T. TATSUNO, J. Biochem., Tokyo *64*, 479 (1968).
[5] Y. UENO, K. SAITO and H. TSUNODA, UJNR Conference on Toxic Microorganisms, Honolulu, October 1968, Proc. Hawaiian Conference on Toxic Microorganisms, in press (1969).
[6] Y. UENO and K. FUKUSHIMA, Experientia *24*, 1032 (1968).
[7] C. A. KEYL, J. C. LEWIS, J. J. ELLIS, S. G. YATES and H. L. TOOKEY, Mycopath. Mycol. Appl. *31*, 327 (1967).
[8] S. G. YATES, H. J. TOOKEYS and J. J. ELLIS, Tetrahedron Letters *7*, 621 (1967).
[9] A. W. DAWKINS, J. chem. Soc. (C) *116* (1966).
[10] J. R. BAMBURG, N. V. RIGGS and F. M. STRONG, Tetrahedron *24*, 3329 (1968).
[11] This investigation was partly aided by the Cancer Research Grant (1968) from the Ministry of Welfare.