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Toxicological Approaches to the Metabolites of *Fusaria*.[1,2] IX. Isolation of Vomiting Factor from Moldy Corn infected with *Fusarium* Species

Kenji Ishii, Yukio Ando, and Yoshio Ueno

Laboratory of Microbial Chemistry, Faculty of Pharmaceutical Sciences, Tokyo University of Science[3]

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A sample of moldy corn infected with *Fusarium* species was analyzed both chemically and biologically to elucidate the vomiting factor. With the aid of rabbit reticulocyte and ducking bioassays, 26 mg of deoxynivalenol, one of trichothecene mycotoxin, was isolated from 3.5 kg of the corn sample. The toxin was extracted with aqueous methanol and purified using solvent partitions and chromatographic separations.

Ingestions of moldy cereals infected with fungi *Fusarium* and *Gibberella* species have sporadically caused intoxications in men and animals, such as vomition and refusal of food and feed, as reviewed by the authors.[4,5] In 1972, this food-born intoxication was broken out in the Midwest of the United States, where a huge amount of corn was polluted by *Fusarium* spp. because of a low temperature and high humidity during the harvest time. The vomition and refusal of feed were observed in swine after ingestion of the moldy corn.

We received a sample of the moldy corn from C.W. Hesseltine, the Northern Regional Reseach Laboratory (Peoria, Illinois), in order to elucidate causative agents. By employing the rabbit reticulocyte and duckling methods which are specific bioassay tools for detecting trichothecene mycotoxins and vomiting factor, respectively, we had already demonstrated that the toxic trichothecenes are responsible for the vomition caused by ingestion of the moldy corn.[6] By examining the sample, no known trichothecenes such as T-2 toxin, neosolaniol, fusarenon-X and nivalenol were detected at that time in the toxic fractions. Later on experiments, however, proved the presence of deoxynivalenol in this corn sample, which constitutes the present communication.

Procedure for the extraction and fractionation of causative factor(s) was carried out with the previous method[6] which was slightly modified as follows (Fig. 1). The ground corn (3.5 kg) was extracted three times with 8 liters of methanol–water (9:1). The combined aqueous methanol solution was concentrated to 1.2 liters and extracted three times with 0.4 liter portions of *n*-hexane to remove oily materials. The aqueous methanol solution was then extracted three times with 0.7 liters portions of chloroform. The combined chloroform solution was evaporated to dryness and redissolved in 0.5 liters of hot methanol to give the methanol-soluble fraction (40 g). The dried powder was extracted under reflux with 0.5 liter of acetone for three hours. The dried acetone extract (7.8 g) was then chromatographed on a silica gel column (4 by 70 cm) with 2 liters of benzene-acetone (2:1) followed by 1 liter of acetone and 1.5 liters of methanol. The eluates were monitored by TLC and divided into six

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3) Location: *12, Ichigaya-Funagawaramachi, Shinjuku-ku, Tokyo, 162, Japan.*
4) Y. Ueno, Y. Ishikawa, M. Nakajima, K. Sakai, K. Ishii, H. Tsunoda, M. Saito, M. Enomoto, K. Ohkubo, and M. Umeda, *Japan. J. Exp. Med.*, **41**, 257 (1971).
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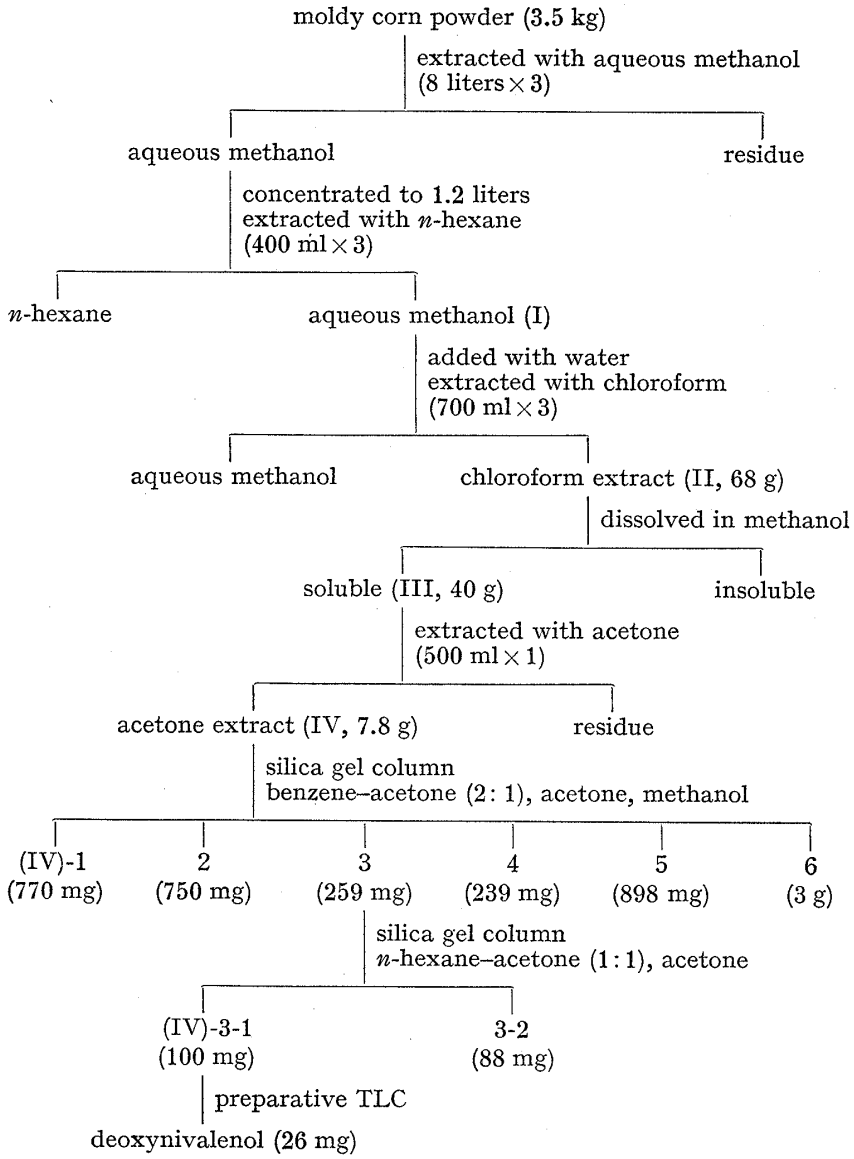

Fig. 1. Fractionation of Vomiting Factor from the Moldy Corn

fractions, on which presence of trichothecene mycotoxins and vomiting factor(s) was checked by the rabbit reticulocyte method[7,8] and the duckling method,[3,4] respectively.

As summerized in Table I, the vomiting responce to ducklings was demonstrated in the four fractions, among which the fraction IV-3 was the most effective to ducklings. The reticulocyte assay also proved the presence of trichothecene(s) in the fraction IV-1, 3 and 4. The TLC analysis with the standard trichothecenes revealed the presence of deoxynivalenol. Further purification by column chromatography on silica gel with *n*-hexane–acetone (1: 1) and preparative TLC with chloroform–methanol (7: 1) resulted in 26 mg of pure deoxynivalenol. The last compound was confirmed by measurement of NMR spectrum.

These biological and chemical analyses strongly supported that the vomition caused by the ingestion of the moldy corn was ascribable to the contamination of deoxynivalenol, one of trichothecene mycotoxins of *Fusarium*.[9] Productivity of trichothecenes by *Fusarium* spp. isolated from the corn sample is under investigation.

7) Y. Ueno, M. Hosoya, and Y. Ishikawa, *J. Biochem.*, **66**, 419 (1969).
8) Y. Ueno and N. Shimada, *Chem. Pharm. Bull.* (Tokyo), **22**, 2744 (1974).
9) T. Yoshizawa and N. Morooka, *Agr. Biol. Chem.*, **37**, 2933 (1973).

Table I. Bioassays of Vomiting Factor and Trichothecene

Fraction	Yield (g)	Ducklings		Reticulocytes[a] % of the control	TLC
		Dose (mg/kg, s.c.)	Vomition		
IV-1	0.770	500	−	30	
2	0.750	500	+	69	
3	0.259	300	++	16	deoxynivalenol
4	0.239	500	+	58	
5	0.898	500	+	100	
6	3.0	500	−	84	

a) inhibition of protein synthesis at concentration of 100 μg/ml

As presented in Table I, another fraction, IV-5, exhibited the vomition to ducklings without an inhibitory effect to the reticulocyte bioassay. This finding may indicate a possible contamination of non-trichothecene compound(s) which is able to induce the vomition to ducklings.

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Stereochemistry of $\Delta^{1,4}$ Unsaturation in Microbial Transformation of Cholesterol[1]

Toshio Nambara, Shigeo Ikegawa[2a] and Motohiko Kato[2b]

Pharmaceutical Institute, Tohoku University[2a] and Central Research Laboratories, Kikkoman Shoyu Co., Ltd.[2b]

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The stereochemistry of hydrogen loss from C-2 and C-4 in cholesterol during microbial degradation into androsta-1,4-diene-3,17-dione has been studied. Each of the substrates, epimeric 2- and 4-deuteriocholesterols (I—IV), was incubated with respiring cultures of *Arthrobacter simplex*. Determination of the labeled isotope retained in the biotransformation products revealed that metabolic transformation of cholesterol into $\Delta^{1,4}$-3-ketosteroid proceeds by a stereospecific removal of 4β- and 2β-hydrogens.

In recent years considerable attentions have been focused on an interesting finding that some microorganisms are capable of transforming cholesterol into androsta-1,4-diene-3,17-dione (ADD) with elimination of the side chain.[3] Although the metabolic route has been

1) This paper constitutes Part III of the series entitled "Studies on Microbial Transformation Products derived from Steroids."; Part II: T. Nambara, S. Ikegawa, and H. Hosoda, *Chem. Pharm. Bull.* (Tokyo), **21**, 2794 (1973).
2) Location: a) *Aobayama, Sendai*; b) *Noda-shi, Chiba.*
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