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Comparative Studies on Microbial and Chemical Modifications of Trichothecene Mycotoxins

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The microbial modification of several trichothecene mycotoxins by trichothecene-producing strains of *Fusarium nivale* and *F. solani* was studied. These results were compared with the corresponding chemical modifications. The growing mycelia of *Fusarium* spp. did not convert 4β-acetoxy-3α,7α,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one (fusarenon) into 3α,4β,7α,15-tetrahydroxy-12,13-epoxy-trichothec-9-en-8-one (nivalenol), whereas 3α,4β,7α,15-tetraacetoxy-12,13-epoxytrichothec-9-en-8-one (tetraacetylnivalenol) was deacetylated to yield 3α-hydroxy-4β,7α,15-triacetoxy-12,13-epoxytrichothec-9-en-8-one (4,7,15-triacetylnivalenol), which was resistant to further deacetylation. T-2 toxin was transformed into HT-2 toxin, and 8α-(3-methylbutyryloxy)-3α,4β,-15-triacetoxy-12,13-epoxytrichothec-9-en-8-one (T-2 acetate) was transformed into HT-2 toxin via T-2 toxin. Chemical modification with ammonium hydroxide converted tetraacetylnivalenol into fusarenon via 4,7,15-triacetylnivalenol. 3α,-7α,15-Triacetoxy-12,13-epoxytrichothec-9-en-8-one (triacetyldeoxynivalenol) gave deacetylation products lacking the C-7 or C-15 acetyl group in addition to 7α,15-diacetoxy-3α-hydroxy-12,13-epoxytrichothec-9-en-8-one (7,15-diacetyldeoxynivalenol). These results demonstrate the regio-selectivity in microbial modification of trichothecenes. Based on the results and available knowledge concerning the transformation of trichothecenes, mechanisms for biological modifications of these mycotoxins are postulated.

The 12,13-epoxytrichothecenes forms an important group of mycotoxins produced by several fungal species. These compounds show wide-ranging biological activity, and it was suggested that the structural differences of the 12,13-epoxytrichothecene nucleus affect the selectivity and specificity of their biological activity, including toxicity to fungi, protozoan, insects, yeasts, plants, animals, and various mammalian tissue cultures (1, 7, 18). Moreover, it was reported that the structure modification severely affects the selectivity of inhibitory action on different phases of protein synthesis (3, 15, 16), in addition to the inhibition of eukaryotic protein synthesis (2, 13).

However, very little is known about the biological transformation of trichothecenes and its significance in biological activity. In a previous paper (19), we attempted to elucidate the mode of microbial transformation of deoxynivalenol and its derivatives by trichothecene-producing strains of *Fusarium nivale*, *F. roseum*, and *F. solani*, and the regio-selective transformation of these compounds was demonstrated.

This paper reports the microbial modifications of nivalenol, T-2 toxin, and their derivatives by growing mycelia of *F. nivale* and *F. solani*. These results were compared with the chemical transformation of these mycotoxins, including deoxynivalenols.

MATERIALS AND METHODS

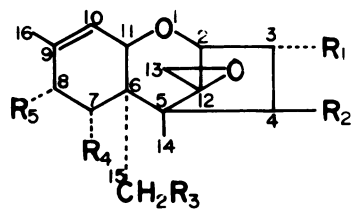
Microorganisms and chemicals. Compounds used are listed in Table 1. Two fungal strains, *F. nivale* and *F. solani*, previously described (19), were used. Growing mycelia of the fungus that had been grown on peptone-supplemented Czapek-Dox medium (pH 6.8) in a shaking flask at 20 C for 3 days were washed three times with 0.67 M phosphate buffer (pH 6.8). Deoxynivalenol (VII) and its derivatives were prepared as previously described (10, 17, 19). Fusarenon (II) and T-2 toxin (XIV) were isolated from peptone-supplemented Czapek-Dox media of *F. nivale* and *F. solani*, respectively, by the procedure described by Ueno et al. (11, 14). Tetraacetylnivalenol (VI) and T-2 acetate (XV) were synthesized from fusarenon and T-2 toxin, respectively.

Reaction conditions and trichothecene determination. Microbial conversion of tricho-

TABLE 1. *Chemical structures and thin-layer chromatography of trichothecene derivatives*

Compound	R_1	R_2	R_3	R_4	R_5	Name	R_f value[a]		
							(i)	(ii)	(iii)
I	OH	OH	OH	OH	=O	Nivalenol	0.01	0.44	0.08
II	OH	OAc	OH	OH	=O	Fusarenon (4-acetylnivalenol)	0.20	0.70	0.35
III	OAc	OAc	OH	OH	=O	3,4-Diacetylnivalenol	0.62	0.83	0.58
IV	OH	OAc	OH	OAc	=O	4,7-Diacetylnivalenol	0.26	0.70	0.41
V	OH	OAc	OAc	OAc	=O	4,7,15-Triacetylnivalenol	0.52	0.84	0.51
VI	OAc	OAc	OAc	OAc	=O	3,4,7,15-Tetraacetylnivalenol	0.91	0.86	0.69
VII	OH	H	OH	OH	=O	Deoxynivalenol	0.09	0.62	0.21
VIII	OAc	H	OH	OH	=O	3-Acetyldeoxynivalenol	0.40	0.89	0.54
IX	OH	H	OH	OAc	=O	7-Acetyldeoxynivalenol	0.09	0.64	0.24
X	OAc	H	OH	OAc	=O	3,7-Diacetyldeoxynivalenol	0.40	0.78	0.53
XI	OH	H	OAc	OAc	=O	7,15-Diacetyldeoxynivalenol	0.40	0.80	0.44
XII	OAc	H	OAc	OAc	=O	3,7,15-Triacetyldeoxynivalenol	0.85	0.84	0.67
XIII	OH	OH	OAc	H	$OCOCH_2CH(CH_3)_2$	HT-2 toxin	0.17	0.69	0.21
XIV	OH	OAc	OAc	H	$OCOCH_2CH(CH_3)_2$	T-2 toxin	0.59	0.83	0.53
XV	OAc	OAc	OAc	H	$OCOCH_2CH(CH_3)_2$	T-2 acetate	0.94	0.86	0.76

[a] Solvent systems: (i) chloroform-methanol (97:3, vol/vol); (ii) chloroform-methanol (5:1, vol/vol); (iii) ethyl acetate-toluene (3:1, vol/vol).



thecenes by intact mycelia of *F. nivale* or *F. solani* was performed in sugar-free Czapek-Dox medium under the same conditions as previously described (19). Chemical transformation of trichothecenes was performed in methanolic ammonium hydroxide at 5 C for an appropriate period, and methanol was evaporated in vacuo. Residual product was detected on thin-layer chromatography (TLC) plates using Kieselgel GF_{254} (Merk AG) or gas-liquid chromatography (GLC) as trimethylsilylated derivatives. Solvents used for chromatography were (i) chloroform-methanol (97:3, vol/vol), (ii) chloroform-methanol (5:1, vol/vol), and (iii) ethyl acetate-toluene (3:1, vol/vol). Gas chromatography equipment and operating conditions were as previously described (19). Transformation product was isolated with preparative TLC plates or silica gel column chromatography (see below). Physicochemical properties of trichothecenes were determined with the apparatus used previously (19).

RESULTS

Partial hydrolysis of nivalenols by Fusarium spp. After incubating fusarenon with growing mycelia of *F. nivale* or *F. solani* for 24 h, deacetylation into nivalenol was not detected on TLC. No transformation of fusarenon occurred in the culture filtrate.

On the other hand, 3,4,7,15-tetraacetylnivalenol (t_R, 6.6 min; R_f, 0.91 in solvent [i]) was partially deacetylated by intact mycelia of both fungal species to give similar products (R_f, 0.52 in solvent [i]; t_R of the trimethylsilylated derivative was 7.4 min). Transformation by *F. nivale* was slow and the half-life of the tetraacetate was approximately 4 h, whereas the reaction by *F. solani* was very fast and the half-life of the substrate was approximately 2.5 h (Fig. 1). Further incubation of the product with both fungal mycelia to 24 h did not give the following deacetylation product. In the culture filtrate of both fungal species, the tetraacetate was not converted into any other deacetylation product, indicating the participation of intracellular enzymes in this microbial transformation.

For isolation of the deacetylation product, the tetraacetate (100 mg) was incubated with the mycelia of *F. solani* at 25 C for 24 h in sugar-free Czapek-Dox medium (100 ml). The filtrate from the reaction mixture was extracted three times with equal amounts of ethyl acetate. The extract (99 mg) was purified with preparative TLC plates using solvent (iii) to give an amorphous solid (55 mg). R_f: 0.51 in solvent (iii); infrared υ_{max}^{KBr} cm^{-1}: 3,480, 1,750, 1,700; mass spectrum (m/e): 438 (M^+); proton magnetic resonance $\delta_{Me_4Si}^{CDCl_3}$: 0.93 (3H, s), 1.89 (3H, d),

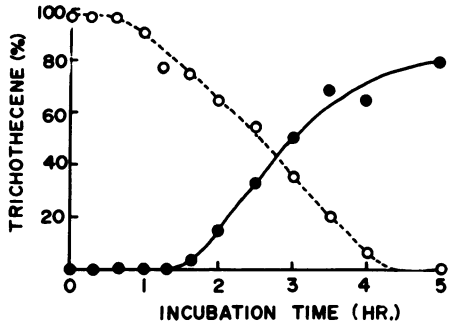


FIG. 1. *Time course of the deacetylation of 3,4,7,15-tetraacetylnivalenol by intact mycelia of F. solani. The fungus was cultured on sugar-free Czapek-Dox medium. Symbols: O, 3.4,7,15-tetraacetylnivalenol; ●, 4,7,15-triacetylnivalenol transformed.*

1.78, 2.18, and 2.24 (each 3H, s), 2.73 and 3.09 (each 1H, d), 3.81 (1H, d), 4.27 (1H, dd), 4.26 and 4.52 (each 1H, d), 4.80 (1H, d), 5.31 (1H, d), 6.11 (1H, s), and 6.64 (1H, dd). The proton at C-3 (δ, 5.25) of the substrate shifted to lower magnetic field (δ, 4.27) in the deacetylation product; therefore a hydroxyl group of the product was located at C-3 of the trichothecene nucleus. From these results, the product was identified as a new trichothecene derivative, 3α - hydroxy - 4β,7α,15 - triacetoxy - 12,13 - epoxytrichothec-9-en-8-one (IV).

Partial hydrolysis of T-2 toxin and its acetate by Fusarium spp. By incubating T-2 toxin with growing mycelia of *F. nivale* or *F. solani* for 24 h, although the transformation rate was very low, the substrate was deacetylated into HT-2 toxin (XIII) and a more polar substance (unidentified), whereas T-2 acetate, 8α - (3 - methylbutyryloxy) - 3α,4β,15 - tri - acetoxy - 12,13 - epoxytrichothec - 9 - en - 8 - one (XV), was partially deacetylated by intact mycelia of both fungal species. The substrate was converted quantitatively into T-2 toxin (R_f, 0.59 in solvent [i]) within 2 h by *F. solani* and within 5 h by *F. nivale*. When the reaction mixture was further incubated to 24 h, T-2 toxin gave more polar products (R_f, 0.17 and 0.10 in solvent [i]), one of which was assumed to be HT-2 toxin (Fig. 2).

Partial hydrolysis of triacetyldeoxynivalenol (XII) by ammonium hydroxide. Triacetyldeoxynivalenol (200 mg) was reacted with 10% methanolic ammonium hydroxide (5 ml) at 5 C for 20 min, and the reaction mixture was immediately concentrated in vacuo. Residual product was chromatographed on preparative TLC plates with solvent (i). Sixty milligrams of the substrate (R_f, 0.85) was recovered, and two products were obtained, products A (95 mg; R_f, 0.40) and B (14 mg; R_f, 0.09). From the results of infrared and proton magnetic resonance spectroscopies, product B was identified as 3α,7α,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one (deoxynivalenol). Product A was further chromatographed on TLC with solvent (iii) to isolate two products, A-1 (43 mg; R_f, 0.44) and A-2 (40 mg; R_f, 0.53). The former product was recrystallized from ethyl acetate-petroleum ether to give 27 mg of pure crystals (plates): mp 146 to 147 C. Analysis found: C, 59.76%; H, 6.37%. Calculated for $C_{19}H_{24}O_8$: C, 59.99%; H, 6.34%. Its infrared spectrum was identical with that of authentic 7,15-diacetyldeoxynivalenol (XI). The latter product was recrystallized from *n*-pentane-ethyl ether to give pure crystals (hexagonal plates; 11 mg): mp 217 to 219 C. Analysis found: C, 59.84%; H, 6.33%. Calculated for $C_{19}H_{24}O_8$: C, 59.99%; H, 6.34%. Proton magnetic resonance $\delta^{CDCl_3}_{Me_4Si}$: 6.08 (1H, s, C-7), about 5.2 (1H, m, C-3), 2.18 and 2.24 (each 3H, s, two acetyl groups), and 3.93 (2H, s, C-15). From these results, product A-2 was confirmed as 3α,7α - diacetoxy - 15 - hydroxy - 12,13-epoxytrichothec-9-en-8-one (X, 3,7-diacetyldeoxynivalenol). Proton magnetic resonance spectroscopy of amorphous solids (8.2 mg) obtained from the mother liquors that remained after the crystallization of product A-2 revealed the presence of 3-acetyldeoxynivalenol (VIII) and 3,7-diacetyldeoxynivalenol.

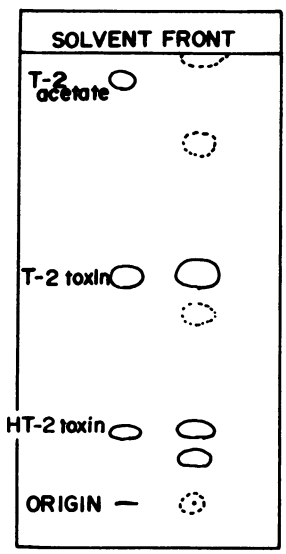


FIG. 2. *Thin-layer chromatography on Kieselgel G of deacetylation products from the reaction of T-2 acetate with intact mycelia of F. solani. The fungus was cultured on sugar-free Czapek-Dox medium. Chloroform-methanol (97:3, vol/vol) was used.*

Partial hydrolysis of tetraacetylnivalenol by ammonium hydroxide. Tetraacetylnivalenol (70 mg) was reacted with 10% methanolic ammonium hydroxide (6 ml) at 5 C for 70 min, and the reaction mixture was concentrated in vacuo. Residual product was purified on TLC plates by multiple developing with solvent (i) (Fig. 3). Two major products, C (R_f, 0.52) and F (R_f, 0.20), and five minor products, A (R_f, 0.91), B (R_f, 0.62), D (R_f, 0.26), E (R_f, 0.23), and G (R_f, 0.07), were obtained. Products C and F were identified as 4,7,15-triacetylnivalenol and 4-acetylnivalenol (fusarenon), respectively. Product B was identified as 3α,4β-deacetoxy-7α,15-dihydroxy-12,13-epoxytrichothec-9-en-8-one (III, 3,4-diacetylnivalenol) from its proton magnetic resonance spectrum, $\delta^{CDCl_3}_{Me_4Si}$: 2.18 (two acetyl groups), about 3.65 (C-15), 4.04 (C-2), 4.76 (C-11), 4.80 (C-7), 5.28 (C-3), and 6.04 (C-4). The proton magnetic resonance spectrum of product D in $CDCl_3$ gave signals at δ2.18 and 2.22 (acetyl groups); δ3.67 and 4.15 (C-15); δ3.79 (C-2): δ about 4.3 (C-3); δ4.94 (C-11); and δ6.07 (C-7). These observations show that product D is 4β,7α-diacetoxy-3α,15 - dihydroxy - 12,13 - epoxytrichothec - 9-en-8-one (IV, 4,7-diacetylnivalenol).

DISCUSSION

It has been proposed that 4,15-diacetylnivalenol is converted into nivalenol via 4-acetylnivalenol during the incubation of *F. nivale* in peptone-supplemented Czapek-Dox medium (6, 12). Grove (8) reported that nivalenol and fusarenon are not true metabolites of *F. nivale* because they are easily converted from 4,15-diacetylnivalenol by nonbiological hydrolysis. We have shown here that the 4β-acetyl group of fusarenon was resistant to microbial deacetylation. These facts suggest that nivalenol may be produced by nonbiological hydrolysis in the culture broth of the fungus.

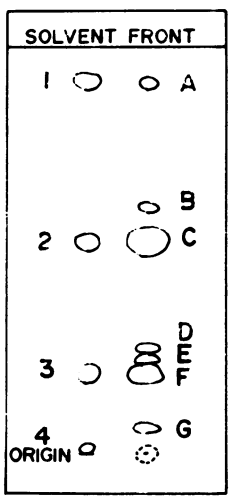


FIG. 3. *Thin-layer chromatography on Kieselgel G of the deacetylation products from the partial hydrolysis of 3,4,7,15-tetraacetylnivalenol by ammonium hydroxide. Authentic standard samples: (1) 3,4,7,15-tetraacetylnivalenol; (2) 4,7,15-triacetylnivalenol; (3) 4-acetylnivalenol (fusarenon); (4) nivalenol. Chloroform-methanol (97:3, vol/vol) was used.*

By incubating tetraacetylnivalenol with the intact mycelia to elucidate the mode of ester cleavage in nivalenols, the lability of C-3 ester bond was shown as previously described in the microbial transformation of triacetyldeoxynivalenol (19). The stability of ester groups other than C-3 ester in nivalenols could be explained as follows: (i) the stability of C-7 ester was in good agreement with the results as shown in the microbial transformation of deoxynivalenols (19); (ii) a C-15 primary ester may be sterically influenced by substituents at the 7α and 3α positions; and (iii) the stability of the 4β-ester group was readily anticipated from the result of fusarenon, and, moreover, 12,13-ethylene oxide ring and a C-14 methyl group protected the 4β-ester bond from the enzymatical elimination. The same transformation pattern as mentioned above was also observed by the chemical transformation of nivalenols as shown in Fig. 3. However, it should be emphasized that the regio-selective transformation of nivalenols was clear, in contrast to the corresponding chemical transformation (Fig. 4).

In a previous paper, we showed that triacetyldeoxynivalenol was converted into 7-acetyldeoxynivalenol via 7,15-diacetyldeoxynivalenol by the growing mycelia of *Fusarium* spp. (19). By contrast with these microbial transformations, the corresponding chemical transformation gave deacetylation products lacking the C-7 or C-15 acetyl group, indicating no significant difference in the chemical elimination of these two ester groups (Fig. 5).

T-2 acetate was microbiologically converted into T-2 toxin by eliminating the C-3 acetyl group, followed probably by deacetylation of the C-4 ester to yield HT-2 toxin. The same conversion of T-2 toxin was demonstrated with supernatant fractions of both human and bovine liver homogenates by Ellison and Kotsonis (5). These results showed that the C-15 ester bond is more stable in T-2 type than in nivalenols. The resistance of the C-15 ester group in T-2 toxins to the biological elimination derived from the steric effect of a bulky substituent at the 7α position; therefore the 4β-ester bond was preferentially hydrolyzed

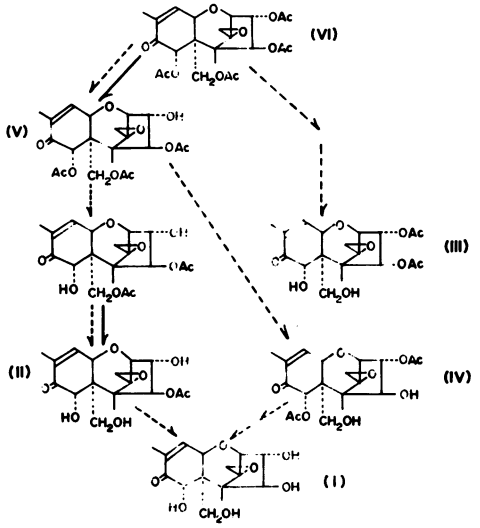


FIG. 4. *Proposed pathways for the biological and chemical transformations of nivalenols. Symbols: ——, Biological transformation; -----, chemical transformation.*

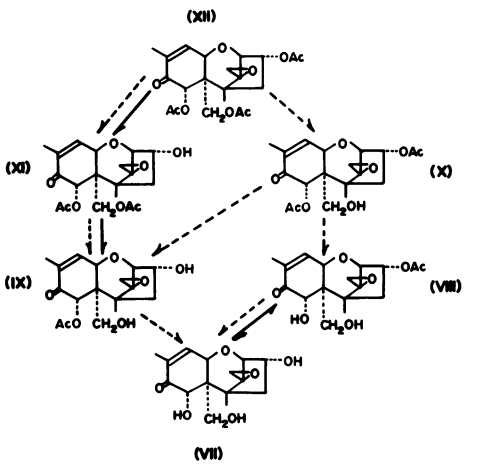


FIG. 5. *Proposed pathways for the biological and chemical transformations of deoxynivalenols. Symbols: ——, Biological transformation; -----, chemical transformation.*

by microbial enzymes (Fig. 6). The above hypothesis was also proved by the result of diacetoxyscirpenol lacking substituents at the C-7 and C-8 positions, in which the C-15 ester was eliminated in preference to the 4β-ester (4).

The observations presented here suggest that the substituent at C-7 or C-8 and its spacial bulk may play an important role in the stereoselective cleavage of several ester bonds on the trichothecene nucleus. Based on our results and

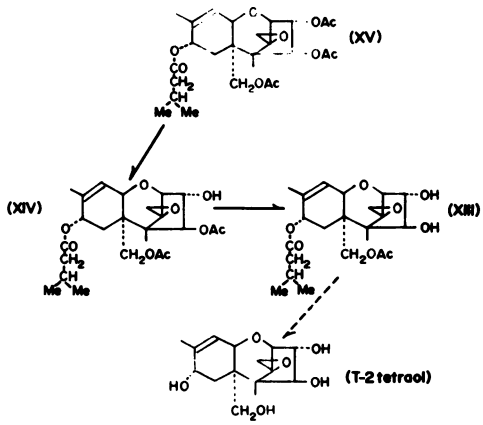


FIG. 6. *Proposed pathways for the biological transformation of T-2 toxin and its derivatives.*

on available knowledge concerning the transformation of trichothecenes (4–6, 9, 12), we propose the following classification concerning the influence of structure alterations in the molecule on the mode of biological transformation. (i) In diacetoxyscirpenol lacking a substituent at C-7 or C-8, secondary esters at C-3 and C-4 are readily eliminated in preference to the C-15 ester. (ii) In nivalenols and deoxynivalenols having relatively small substituents at C-7 and C-8, the C-3 ester is initially followed by the C-15 ester, whereas the esters at C-4, C-7, and C-8 are stable. (iii) In T-2 toxins having a bulky group at C-7 or C-8, the C-3 ester is initially hydrolyzed followed by the C-4 ester, whereas esters at C-7, C-8, and C-15 are comparatively resistant to the biological hydrolysis.

This classification explains not only the influence of structure modification on biological transformations but also the contribution of substituents including the ethylene oxide ring to the biological activity of trichothecenes. Recently, the following hypotheses on the latter problem were presented. Wei et al. (15, 16) reported that the ability of a 12,13-epoxytrichothecene to inhibit initiation of the elongation-termination steps of protein synthesis depends on the presence of oxygen-containing substituents at C-3, C-4, and C-15 in the molecule. Cundliffe et al. (3) reported that nivalenol, T-2 toxin, and verrucarin A are potent and highly selective inhibitors of polypeptide chain elongation in eukaryotes, whereas trichodermin inhibits chain elongation and/or termination, and suggested that the presence of substituent at C-15 may be important in determining the precise modes of action of these compounds.

The hypothesis presented here may explain

the above suggestions concerning biological activity, but further investigation is required to explain this problem.

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LITERATURE CITED

1. Bamburg, J. R., and F. M. Strong. 1971. 12,13-Epoxytrichothecenes. *In* S. Kadis, A. Ciegler, and S. J. Ajl (ed.), Microbial toxins, vol. 7, Fungal toxins. Academic Press, Inc., New York.
2. Barbacid, M., and D. Vazquez. 1974. Binding of [acetyl-^{14}C]trichodermin to the peptidyl transferase centre of eukaryotic ribosomes. Eur. J. Biochem. **44**:437–444.
3. Cundliffe, E., M. Cannon, and J. Davies. 1974. Mechanisms of inhibition of eukaryotic protein synthesis by trichothecene fungal toxins. Proc. Natl. Acad. Sci. U.S.A. **71**:30–34.
4. Dawkins, A. W. 1966. Phytotoxic compounds produced by *Fusarium equiseti*. Part II. The Chemistry of diacetoxyscirpenol. J. Chem. Soc. **1966**:116–123.
5. Ellison, B. A., and F. N. Kotsonis. 1974. In vivo metabolism of T-2 toxin. Appl. Microbiol. **27**:423–424.
6. Fujimoto, Y., Y. Morita, and T. Tatsuno. 1972. Recherches toxicologiques sur les substances toxiques de *Fusarium nivale:* etude chimique des toxins principales, nivalenol, fusarenon-X et nivalenol-4,15-di-O-acetate. Chem. Pharm. Bull. **20**:1194–1203.
7. Grove, J. F. 1969. The cytotoxicity of some transformation products of diacetoxyscirpenol. Chem. Commun. **1969**:1473–1478.
8. Grove, J. F. 1970. Phytotoxic compounds produced by *Fusarium equiseti*. Part V. Transformation products of 4β,15 - diacetoxy - 3α,7α - dihydroxy - 12,13 - epoxytrichothec - 9 - en - 8 - one and the structure of nivalenol and fusarenone. J. Chem. Soc. **1970**: 375–378.
9. Horvath, I., and J. M. Varga. 1961. Enzymatic inactivation of trichothecin and crotocin. Nature (London) **192**:88.
10. Morooka, N., N. Uratsuji, T. Yoshizawa, and H. Yamamoto. 1972. Studies on the toxic substances in barley infected with *Fusarium* spp. Jpn. J. Food Hyg. **13**:368–375.
11. Ueno, Y., K. Ishii, K. Sakai, S. Kanaeda, H. Tsunoda, T. Tanaka, and M. Enomoto. 1972. Toxicological approaches to the metabolites of *Fusaria*. IV. Microbial examination on the "beam hulls poisoning of horses" with the isolation of toxic trichothecenes, neosolaniol and T-2 toxin, of *Fusarium solani* M-1-1. Jpn. J. Exp. Med. **42**:187–203.
12. Ueno, Y., Y. Ishikawa, K. Amakai, M. Saito, and H. Tsunoda. 1970. Environmental factor influencing the production of fusarenon-X, a cytotoxic mycotoxin of *Fusarium nivale* Fn 2B. Chem. Pharm. Bull. **18**:304–312.
13. Ueno, Y., M. Nakajima, K. Sakai, K. Ishii, N. Sato, and N. Shimada. 1973. Comparative toxicology of trichothec mycotoxin: inhibition of protein synthesis in animal cells. J. Biochem. (Tokyo) **74**:285–296.
14. Ueno, Y., I. Ueno, K. Amakai, Y. Ishikawa, H. Tsunoda, K. Okubo, M. Saito, and M. Enomoto. 1971. Toxicological approaches to the metabolites of *Fusaria*. II. Isolation of fusarenon-X from the culture filtrate of *Fusarium nivale* Fn 2B. Jpn. J. Exp. Med. **41**:507–519.
15. Wei, C., B. S. Hansen, M. H. Vanghan, Jr., and C. S. McLaughlin. 1974. Mechanism of action of the mycotoxin trichodermin, a 12,13-epoxytrichothecene. Proc. Natl. Acad. Sci. U.S.A. **71**:713–717.
16. Wei, C., and C. S. McLaughlin. 1974. Structure-function relationship in the 12,13-epoxytrichothecenes, novel inhibitors of protein synthesis. Biochem. Biophys. Res. Commun. **57**:838–844.
17. Yoshizawa, T., and N. Morooka. 1973. Deoxynivalenol and its monoacetate: new trichothecene mycotoxins from *Fusarium roseum* and moldy barley. Agric. Biol. Chem. **37**:2933–2934.
18. Yoshizawa, T., and N. Morooka. 1974. Studies on the toxic substances in the infected cereals. (III). Acute toxicities of new trichothecene mycotoxins: deoxynivalenol and its monoacetate. Jpn. J. Food Hyg. **15**:261–269.
19. Yoshizawa, T., and N. Morooka. 1975. Biological modification of trichothecene mycotoxins: acetylation and deacetylation of deoxynivalenols by *Fusarium* spp. Appl. Microbiol. **29**:54–58.