Minor Metabolites of *Fusarium roseum* (ATCC 28114)

Roy Greenhalgh,* Rose-Marie Meier, Barbara A. Blackwell, J. David Miller, Alan Taylor, and John W. ApSimon

The main product produced by *Fusarium roseum* (ATCC 28114) in liquid culture is 3-acetyldeoxynivalenol. In addition, several minor metabolites have been isolated from the crude fungal extract, including butenolide, culmorin, zearalenone, and four trichothecenes. These compounds were characterized by ^1H and ^{13}C NMR. They were assigned the structures 3-acetoxy-12,13-epoxytrichothec-9-ene (isotrichodermin), 3-acetoxy-15-hydroxy-12,13-epoxytrichothec-9-ene, 3-acetoxy-15-hydroxy-12,13-epoxytrichothec-9-en-8-one, and 3,15-diacetoxy-7,8-dihydroxy-12,13-epoxytrichothec-9-ene. The latter compound was readily transformed into 3,15-diacetyldeoxynivalenol on oxidation with manganese dioxide. A partial biosynthetic scheme for the formation of 3-acetyldeoxynivalenol from isotrichodermin is proposed.

Secondary metabolite formation by fungi is influenced by environmental and genetic factors, which accounts for the diverse nature of the mycotoxins reported for each species. Early experiments with *Fusarium roseum* indicated that DAS, neosolaniol, HT-2, and T-2 (Ueno et al., 1973) and zearalenone (Ishii et al., 1974) were produced. By contrast, a specific isolate, *F. roseum* (ATCC 28114), cultured on rice gave deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-AcDON), and traces of 3,15-diacetyldeoxynivalenol (Yoshizawa et al., 1978). Recently, this strain has been shown to produce mainly 3-AcDON and DON in liquid culture, together with small amounts of DAS, zearalenone, butenolide, and T-2 (Miller et al., 1983a). This strain has been identified as *Fusarium graminearum* Schwabe. Most Canadian isolates of *F. graminearum*, however, produce 15- and not 3-acetyl derivatives of DON.

In the field, corn inoculated with Canadian *F. graminearum* (*roseum*) produced DON, some 15-AcDON, and zearalenone (Miller et al., 1983b). Other trichothecenes reported to be formed by this species include 3-lactyldeoxynivalenol (Chun et al., 1982) and 4,7-dideoxynivalenol (Bennett et al., 1981).

The availability of appreciable amounts of crude fungal extract from liquid cultures of *F. roseum* (ATCC 28114) produced by the large-scale process for 3-AcDON (Greenhalgh et al., 1984) afforded the opportunity of isolating and characterizing several minor metabolites. In this paper, structures for these compounds are suggested and their biogenetic significance is discussed.

METHODS AND MATERIALS

^1H and ^{13}C nuclear magnetic resonance spectra (NMR) were recorded at 250 and 62.8 MHz, respectively, on a Bruker WM 250 NMR spectrometer. Chemical shifts are referenced to $CDCl_3$ at 77.0 ppm for ^{13}C and 7.24 ppm for ^1H and reported relative to Me_4Si. Mass spectra (MS) were determined (a) by using a Finnigan MAT 312 mass spectrometer with the FAB probe, the samples were applied in glycerine and (b) by direct insertion into the source of a Du Pont 21-110b instrument. Precise mass measurements were obtained by peak matching with an ion in the spectrum of perfluorokerosene as a standard. TLC data were obtained with the solvent system ethyl acetate–hexane (3:1) by using 250-μL silica gel TLC plates (Whatman).

F. roseum (ATCC 28114) was cultured, the cultures were harvested, and the broth was extracted as described by Greenhalgh et al. (1984). Some of the oil (8.5 g) from the methanol phase after it had been partitioned and concentrated was dissolved in methylene chloride and passed through a Florisil column (169 g). The column was eluted with 750-mL volumes of (A) methylene chloride, (B) 0.5 methanol in methylene chloride, and (C) 5% methanol in methylene chloride. Fraction A afforded an oil (5.5 g) from which 3-AcDON (2.9 g), mp 184–186 °C, was obtained. Fractions B and C also afforded yellow oils weighing 1.24 and 1.89 g, respectively.

The oil (1.69 g), which remained in fraction A after removal of 3-AcDON, was chromatographed on silica gel (20 g, Kieselgel 60, 70–230 mesh). The column was eluted first with 10% toluene in methylene chloride (250 mL) and then with 0.5% methanol in methylene chloride (250 mL), 1.5% methanol in methylene chloride (250 mL), 3% methanol in methylene chloride (250 mL), and finally 13% methanol in methylene chloride. Fractions (50 mL) were collected and monitored by TLC; fractions 6–10, 11–12, 13–17, and 18–20 were combined to give mixtures A1 (80 mg), A2 (740 mg), A3 (552 mg), and A4 (178 mg), respectively. Further purification of mixtures A2 and A3 was accomplished using a Chromatatron (Model No. 7924, Harrison Research, Inc.) with a 2-mm silica gel plate (Kieselgel 60).

Mixture A2. A sample (520 mg) was eluted with 20% ethyl acetate in hexane, and 10-mL fractions were collected. A compound (37 mg), R_f 0.83, was obtained, which on rechromatography with 15% ethyl acetate in hexane gave colorless crystals (I): mp 97–97.5 °C; $[\alpha]^{24}_D$ + 9.5° (*c* 0.09, $CHCl_3$); MS *m/z* 293.174 (M + 1), calcd for $C_{17}H_{25}O_4$ 293.168; NMR δ 0.74 (3 H, H-15), 0.80 (3 H, H-14), 1.0–2.0 (6 H, H-7, H-4, H-8 m), 1.71 (3 H, H-16), 2.11 (3 H, Ac-CH_3), 2.84, 3.07 (2 H, H-13, J_{ab} = 4.0 Hz), 3.73 (1 H, H-2, $J_{2,3}$ = 4.6 Hz), 3.96 (1 H, H-11, $J_{10,11}$ = 5.5 Hz), 5.15 (1 H, H-3, $J_{2,4}$ = 10.0 Hz, $J_{2,3}$ = 4.6 Hz), 5.44 (1 H, H-10, $J_{10,11}$ = 5.5 Hz, $J_{10,16}$ = 1.5 Hz). Changing the solvent to 30% ethyl acetate in hexane gave a material (13.5 mg), R_f 0.63, which was recrystallized from 20% ethyl acetate in hexane to give colorless prisms (II): mp 181–183 °C; $[\alpha]^{26}_D$ +10.2° (*c* 0.8, $CHCl_3$); MS *m/z* 309.167 (M + 1), calcd for $C_{17}H_{25}O_5$ 309.162; NMR δ 0.90 (3 H, H-14), 1.71 (3 H, H-16), $J_{16,10}$ = 1.5 Hz), 2.09 (3 H, Ac-CH_3), 1.73–2.30 (6 H, H-7, H-4, H-8 m), 2.84, 3.07 (2 H, H-13, J_{ab} = 4.0 Hz),

Chemistry and Biology Research Institute, Agriculture Canada, Ottawa, Ontario K1A 0C6 Canada (R.G., B.A.B., and J.D.M.), Carleton University Ottawa, Ontario K1S 5B6, Canada (R.-M.M. and J.W.A.), and Atlantic Regional Laboratory, National Research Council, Halifax, Nova Scotia B3H 3Z1, Canada (A.T.).

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3.47, 3.66 (2 H, H-15, J_{ab} = 11.6 Hz, $J_{5,7}$ = 4.9, 3.2 Hz), 3.72 (1 H, H-2, $J_{2,3}$ = 4.68 Hz), 3.96 (1 H, H-11, $J_{10,11}$ = 5.28 Hz), 5.3 (1 H, H-3, $J_{2,3}$ = 4.6 Hz, $J_{3,4}$ = 10.0, 5.0 Hz), 5.46 (1 H, H-10, $J_{10,16}$ = 1.5 Hz, $J_{10,11}$ = 5.3 Hz). A second compound (11 mg), R_f 0.46, was obtained on continued elution with this solvent system, which was rechromatographed with 20% ethyl acetate–hexane to give colorless needles (III): mp 202–203 °C; $[\alpha]^{26}_D$ +41.7° (*c* 0.4, $CHCl_3$); MS *m/z* 323.149 (M + 1), calcd for $C_{17}H_{23}O_6$ 323.150; NMR δ 0.83 (3 H, H-14), 1.80 (3 H, H-16, $J_{16,10}$ = 1.4 Hz), 2.12 (3 H, Ac-CH_3), 2.40, 2.17 (2 H, H-4, J_{ab} = 12.8 Hz, $J_{4,3}$ = 4.4 Hz), 2.86, 2.42 (2 H, H-7, J_{ab} = 16.8 Hz), 2.86, 3.09 (2 H, H-13, J_{ab} = 3.9 Hz), 3.64 (2 H, H-15), 3.81 (1 H, H-2, $J_{2,3}$ = 4.6 Hz), 4.52 (1 H, H-11, $J_{11,10}$ = 5.7 Hz), 5.18 (1 H, H-3, $J_{3,2}$ = 4.6 Hz, $J_{3,4}$ = 10.2, 4.4 Hz), 6.55 (1 H, H-10, $J_{10,11}$ = 5.7 Hz, $J_{10,16}$ = 1.4 Hz).

Mixture A3. A sample (164 mg) was chromatographed with a mixture of 50% ethyl acetate in hexane to give colorless needles (102 mg), R_f 0.37. Recrytallization from tetrahydropyran–cyclohexane yielded 87 mg of culmorin (IV): mp 178–179 °C; $[\alpha]^{24}_D$ −15 ± 4° (*c* 0.6, $CHCl_3$); MS *m/z* 238.1922 (M), calcd for $C_{15}H_{26}O_2$ 238.1922; NMR δ 0.70 (3 H, H-13), 0.86 (3 H, H-12), 0.90 (3 H, H-14), 1.02 (3 H, H-15), 1.3–2.1 (6 H, H-3, H-4, H-5 m), 1.64 (2 H, H-10, $J_{10,11}$ = 6.8 Hz), 1.74 (1 H, H-7, $J_{7,8}$ = 5.0 Hz), 1.90 (1 H, H-1, $J_{1,11}$ = 4.5 Hz), 3.86 (1 H, H-8, $J_{2,8}$ = 8.0 Hz), 4.35 (1 H, H-11, $J_{10,11}$ = 6.8, $J_{11,1}$ = 4.5 Hz).

Further elution of the plate with 50% ethyl acetate in hexane gave a second compound (50 mg), R_f 0.31, which on recrystallization from ether gave needles (47 mg) of the diacetate (V): mp 190–192 °C; $[\alpha]^{26}_D$ +7.2° (*c* 0.07, $CHCl_3$); MS *m/z* 383.179 (M + 1), calcd for $C_{19}H_{27}O_8$ 383.171; NMR δ 1.12 (3 H, H-14), 1.86 (3 H, H-16, $J_{10,16}$ = 1.0 Hz), 2.02 (3 H, Ac-CH_3), 2.10 (3 H, Ac-CH_3), 2.13, 2.34 (2 H, H-4, $J_{4,3}$ = 11.1, 4.6 Hz), 2.71 (1 H, 8-OH, $J_{8,OH}$ = 8.0 Hz), 3.0 (1 H, 7-OH, $J_{7,OH}$ = 9.7 Hz), 3.09, 3.20 (2 H, H-13, J_{ab} = 4.3 Hz), 3.77 (1 H, H-2, $J_{2,3}$ = 4.4 Hz), 3.99 (1 H, H-8, $J_{7,8}$ = 5.8, $J_{8,OH}$ = 7.5 Hz), 4.14, 4.44, (2 H, H-15, J_{ab} = 12.3 Hz), 4.32 (1 H, H-11, $J_{11,10}$ = 5.8 Hz), 4.50 (1 H, H-7, $J_{7,OH}$ = 9.6 Hz, $J_{7,8}$ = 5.4 Hz), 5.15 (1 H, H-3, $J_{3,2}$ = 4.4 Hz, $J_{3,4}$ = 4.5, 11.1 Hz), 5.58 (1 H, H-10, $J_{10,11}$ = 5.8 Hz, $J_{10,16}$ = 0.9 Hz).

The aqueous raffinate (6 L) from the broth (pH 9) was acidified (pH 5.5) and extracted with methylene chloride (3 × 5 L), the combined extracts were concentrated, and the residue (0.143 g) was digested with *n*-butyl acetate (5 mL). The colorless crystals obtained (45 mg) were recrystallized from *n*-butyl acetate to give butenolide (2-acetamido-5-hydroxyfuran, VI) as needles: mp 118–120 °C; MS *m/z* 141.0409 (M), calcd for $C_6H_7NO_3$ 141.0426; NMR δ 2.08 (3 H), 6.2 (1 H, *J* = 6 Hz), 6.7 (1 H), 7.25 (1 H, *J* = 6 Hz). Zearalenone (VII) (30 mg), mp 164 °C, $[\alpha]^{24}_D$ −189°, was obtained from the mother liquors.

Oxidation of V. The material (5 mg, 0.014 M) was dissolved in ethyl acetate (0.6 mL), activated MnO_2 (57.2 mg) added, and the mixture stirred for 24 h. Further additions of 67.2 and 50.1 mg of MnO_2 were made at 24 and 48 h, respectively. The reaction mixture was filtered, and the filtrate was taken to dryness. The crude product was purified by TLC on silica gel plates, solvent ethyl acetate–hexane (1:1), to give 2.8 mg of crystalline material, mp 120–121 °C [lit. mp 119–120 °C (Yoshizawa et al., 1978)]; MS *m/z* 381.159 (M + 1), calcd for $C_{19}H_{25}O_8$ 381.155; NMR δ 1.10 (3 H, H-14), 1.88 (3 H, CH_3-Ac), 2.13 (3 H, CH_3-Ac), 2.22 (2 H, H-4, $J_{3,4}$ = 4.6, 10.9 Hz, 3.13 (2 H, H-13, J_{ab} = 4.2 Hz), 3.77 (1 H, 7-OH, J_{OH-H} = 1.74 Hz), 3.19 (1 H, H-2, *J* = 4.4 Hz), 4.25 (2 H, H-15, J_{ab} = 3.3, 15.3 Hz), 4.70 (1 H, H-11, $J_{11,10}$ = 5.8 Hz), 4.81 (1 H, H-7, J_{7-OH} = 1.7 Hz), 5.22 (1 H, H-3, $J_{3,4}$ = 10.9 Hz, $J_{2,3}$ = 4.6 Hz), 6.57 (1 H, H-10, J_{10-11} = 5.8 Hz, $J_{10,16}$ = 1.4 Hz).

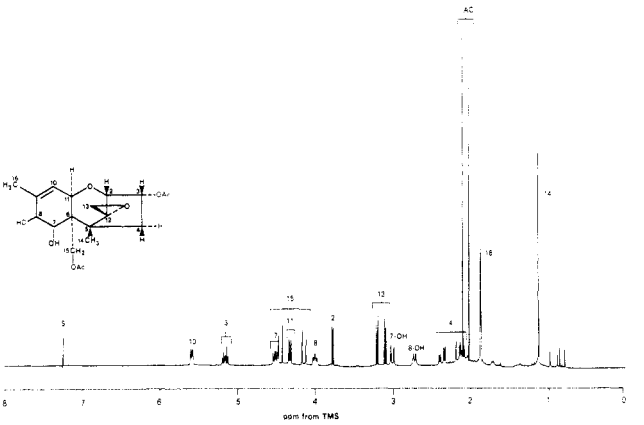

Figure 1. ^1H (250-MHz) spectrum of 3,15-diacetoxy-7,8-dihydroxy-12,13-epoxytrichothec-9-ene.

RESULTS AND DISCUSSION

Chromatography of mixture A3 gave culmorin and crystalline material that eluted with ethyl acetate–hexane (1:1). The ^1H NMR of this material showed it to be a mixture of two trichothecene diacetates; only V was obtained in pure state. The ^1H NMR spectrum (Figure 1) shows resonances typical of epoxide (C-13), methyl (C-14), and allylic methyl (C-16) moieties in a trichothecene ring system (Bamberg and Strong, 1971). The chemical shifts of these moieties, together with the AB content of the H-13 protons, reflect the degree of substitution in the rings. In this case, the resonances resemble those found in deoxynivalenol (DON), (H-13, 3.07, 3.15 ppm, J_{ab} = 4.3 Hz; H-14, 1.12 ppm; H-16, 1.86 ppm, *J* = 1.2 Hz) (Blackwell et al., 1984), suggesting that the compounds are structurally similar. Comparison of the chemical shift of other resonances enabled spectral assignments to be made for H-4 and H-2. The characteristic H-3 multiplet and the H-15 AB quartet in DON are shifted downfield in V, suggesting acetoxy moieties at these positions. This is corroborated by the H-3 and H-15 chemical shifts (5.22 and 4.20 ppm) in 3,15-diacetyldeoxynivalenol, together with two methyl singlets at 2.16 and 1.92 ppm, respectively (Yoshizawa et al., 1978). The upfield shifts of the H-11 and H-10 resonances relative to those of DON (4.66 ppm, *J* = 5.9 Hz, and 6.56 ppm, *J* = 5.9 and 1.5 Hz, respectively) and the lack of UV absorption at 218 mμ is consistent with the absence of a ketone group at the C-8 position. The multiplets, 4.50 and 3.99 ppm (Figure 1), are part of an AB system (J_{ab} = 5.8 Hz) and were assigned to H-7 and H-8, respectively. This assignment was confirmed by observation of the coupling between the H-8 and H-16 resonances in a long-range COSY spectrum [$^1H/^1H$ shift correlation (Bax and Freeman, 1981)]. In CD_3OD, these multiplets collapsed into two doublets (3.84 and 4.34 ppm) and the two doublets at 3.0 and 2.71 ppm disappeared. The latter two resonances are due to the C-7 OH and C-8 OH protons, which exchange in CD_3OD but not $CDCl_3$, as was the case for the C-7 OH in DON. The AB system of H-15 also appears as a singlet (4.30 ppm) in CD_3OD instead of an AB quartet.

The ^{13}C NMR spectrum of V (Figure 2) shown as spin-echo spectrum with echo delay $1/J_{CH}$ (Brown et al., 1981) shows resonances for 19 carbon atoms, 4-CH_3, 5-O-CH, 1-=CH, 6-quaternary C including two keto groups, and 3-CH_2 according to the multiplicity determination. The chemical shift assignments, given in Table I, are based on unambiguously assigned spectral data of other tricho-

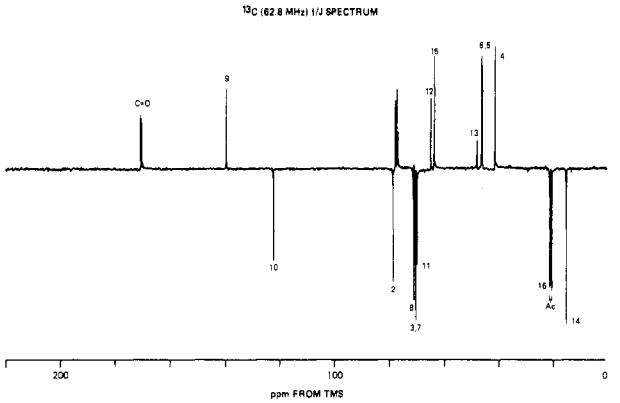

Figure 2. ^{13}C (62.8-MHz) spin-echo ($1/J$) spectrum of 3,5-diacetoxy-7,8-dihydroxy-12,13-epoxytrichothec-9-ene.

thecenes (Blackwell et al., 1984). The reduced intensity of the resonance assigned to C-13 is due to the high value of the epoxide J_{CH} (175 Hz). The ^1H and ^{13}C NMR data of V, the absence of UV absorption together with an empirical formula of $C_{19}H_{28}O_8$, are compatible with the structure 3, 15-diacetoxy-7,8-dihydroxy-12,13-epoxytrichothec-9-ene.

The ^1H spectra of I, II, and III also indicate them to be trichothecenes. The ^{13}C chemical shifts for C-3 (Table I) suggest that they are substituted with an acetoxy moiety at this position. Since I has an empirical formula ($C_{17}H_{24}O_4$), it can be the only substitution in the molecule. The functionality of the carbon atoms in trichothecenes can be established from the ^{13}C spectrum by using the correlations established by Cole and Cox (1983). Thus, C-9 and C-10 chemical shifts of approximately 140 and 119 ppm, respectively, are consistent with C-8 being a methylene group. The absence of a methylene resonance at 64 ppm and the appearance of a resonance at 15 ppm indicate that C-15 is a methyl group. The ^1H spectrum shows two

Table I. 62.8-MHz ^{13}C Chemical Shifts Assignments for DON and Three Minor Trichothecene Metabolites[a] from *F. roseum*

carbon	chemical shift, ppm from Me_4Si				
	I	II	III	V	DON[b]
2	78.2	78.2	78.1	78.5	80.6
3	71.7	71.5	71.2	70.4	68.6
4	38.6	39.4	38.5	41.3	43.0
5	45.3	45.3	45.0	46.0	46.0
6	40.2	44.2	47.5	46.2	52.1
7	24.4	20.8	38.3	70.2	70.2
8	28.2	28.5	198.7	71.0	202.3
9	139.5	140.5	138.3	139.3	135.7
10	119.4	119.5	137.7	122.1	138.5
11	71.5	68.3	68.2	69.7	74.4
12	65.2	65.2	65.3	64.6	65.7
13	48.4	48.5	48.3	47.8	47.2
14	11.0	12.3	11.2	15.0	13.9
15	15.9	62.8	64.1	63.3	61.4
16	23.1	23.1	15.3	21.1	14.9
C=O (Ac)	170.6	170.6	170.5	170.4, 170.0	
CH_3 (Ac)	20.9	20.8	20.9	20.9, 20.3	

[a] I = 3-acetoxy-12,13-epoxytrichothec-9-ene; II = 3-acetoxy-15-hydroxy-12,13-epoxytrichothec-9-ene; III = 3-acetoxy-15-hydroxy-12,13-epoxytrichothec-9-en-8-one; V = 3,15-diacetoxy-7,8-dihydroxy-12,13-epoxytrichothec-9-ene. [b] DON = deoxynivalenol; unambiguous assignments.

methyl resonances (H-15, 0.74 ppm, and $AcCH_3$, 2.11 ppm) and a complex of multiplets (0.9–2.1 ppm) for H-7, H-8, and H-4. The ^1H and ^{13}C NMR and MS data are compatible with the structure 3-acetoxy-12,13-epoxytrichothec-9-ene (I).

Comparison of the ^1H NMR spectra of I and II indicates the main difference to be replacement of the 3 H singlet (0.74 ppm) in I by a 2 H, double quartet (3.57 ppm) in II. This implies that the methyl group at C-15 has been oxidized to a primary alcohol in II. The MS is in agreement with this hypothesis, the data being compatible with the

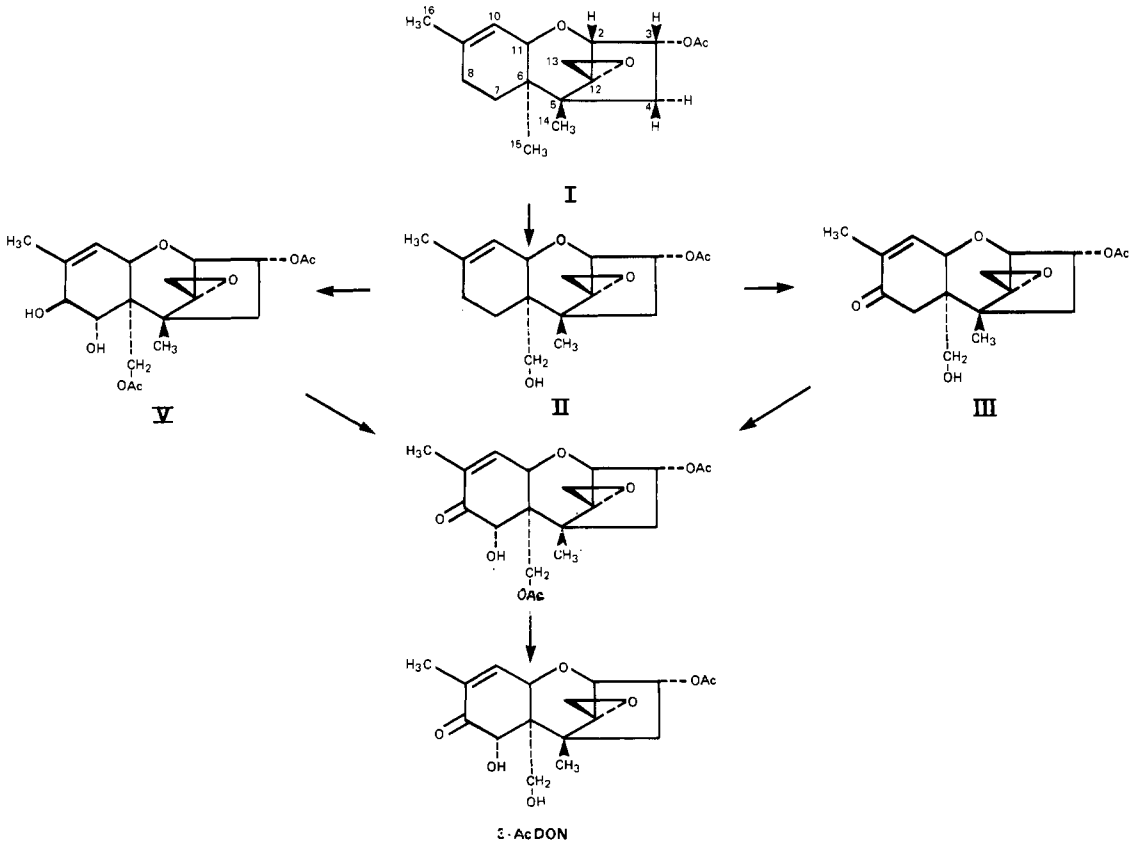

Figure 3. Proposed final stages of the biosynthetic scheme for 3-acetoxydeoxynivalenol by *F. roseum*.

structure 3-acetoxy-15-hydroxy-12,13-epoxytrichothec-9-ene (II, 15-deacetylcalonectrin). Gardner et al. (1972) first reported isolating 15-deacetylcalonectrin from *Calonectrin nivalis* Schaffnit.

In addition to the 3-acetoxy moiety in III, the ^{13}C NMR spectrum shows C-8 as a ketone group, which is corroborated by C-9 and C-10 chemical shifts of approximately 135 and 138 ppm, respectively, and a C-15 as a $-CH_2OH$ group with a shift of approximately 63 ppm. The H-3 and CH_3-Ac chemical shifts in the ^1H spectrum confirm the presence of a 3-acetoxy moiety. The H-15 resonance appears as a broad singlet as a result of either accidental equivalence or rotational averaging. The H-7 protons also exhibit an AB character (J_{ab} = 16.8 ppm), which is greater than that of T-2 or DAS (Cole and Cox, 1983). With an empirical formula ($C_{17}H_{22}O_6$), the spectra data of III indicate the structure to be 3-acetoxy-15-hydroxy-12,13-epoxytrichothec-9-en-8-one. Bennett et al. (1981) isolated the corresponding deacetyl analogue from corn inoculated with *Fusarium* cultures (NRRL 6206 and NRRL 6207). The NMR spectrum of this latter compound reported H-7 as a doublet (2.87 ppm, J = 15.6 Hz). Both the deacylation of AcDON and acylation of DON by growing mycelia have been reported, the rate being dependent on the fungal strain (Yoshizawa and Morooka, 1975). Under the growth conditions used with *F. roseum*, 3-AcDON was not deacylated, suggesting the absence of the particular enzyme system. The addition of 3-AcDON to homogenates of corn did, however, result in its hydrolysis. Similarly, in field studies involving the inoculation of corn with *F. graminearum*, DON rather than AcDON is the main product (Miller et al., 1983b).

In addition to the trichothecenes, three other mycotoxins were isolated, namely, butenolide, zearalenone, and culmorin. The latter gave a parent ion, m/z 238, by direct insertion MS; however, its FAB/MS spectrum showed only cluster ions m/z 477 (2M + H), 331 (M + H + glycerol), 221 (M + H - H_2O), and 203 (M + H - $2H_2O$). The ^1H NMR spectrum was identical with that described for culmorin (Barton and Werstiuk, 1968). This secondary metabolite was isolated initially from a strain of *Fusarium culmorum* (Ashley et al., 1937).

The major and minor metabolites of *F. roseum* discussed in this paper have in common an acetoxy group at the 3-position of the trichothecene ring. Since this is the only exocyclic oxygen substituent in I, it suggests that I could be a biosynthetic precursor for 3-AcDON, with the other more highly oxygenated metabolites as intermediates (Figure 3). While 3,15-diacetyldeoxynivalenol was not isolated in these experiments, it is known to be produced by this isolate (Yoshizawa et al., 1978). It could be formed from V, as was demonstrated chemically.

Machida and Nozoe (1972) isolated 12,13-epoxytrichothec-9-ene and its 4,8-dihydroxy analogue and proposed them as intermediates in the biosynthesis of trichothecin by *Trichothecium roseum*. This implies that 12,13-epoxytrichothec-9-ene could be a common intermediate for all trichothecene mycotoxins. Although oxidation of the trichothecene ring at positions 7, 8, and 15 is well documented (Tamm and Breitenstein, 1980), that of positions 3 and 4 is not. Recently, Ichinoe et al. (1983) showed that *Gibberella zeae* (*F. graminearum*) produced either nivalenol (3,4-oxygenated) or deoxynivalenol (3-oxygenated) metabolites. The fact that *F. roseum* also mainly produces 3-oxygenated trichothecenes suggests that the 3- and 4-positions of the trichothecene ring are the primary sites for biological oxidation. Specificity resulted from the presence of systems in the particular fungus. Further oxidation of the trichothecene ring at positions 7, 8, and 15 appears to be a more general reaction and common to all fungal species. Experiments are being carried out to determine the incorporation of oxygen by *F. culmorum* in shake culture.

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Registry No. I, 91423-90-4; II, 38818-66-5; III, 91632-02-9; V, 91632-03-0; 3-AcDON, 50722-38-8; butenolide, 497-23-4; culmosin, 18374-83-9; zearalenone, 17924-92-4; 3,15-diacetyldeoxynivalenol, 56676-60-9.

LITERATURE CITED

Ashley, J. N.; Hobbs, B. C.; Raistrick, H. *Biochem. J.* **1937**, *31*, 385–392.

Bamburg, J. R.; Strong, F. M. In "Microbial Toxins"; Kadis, S.; Ciegler, A.; Ail, S. J., Eds.; Academic Press: New York, 1971; pp 207–292.

Barton, D. H. R.; Werstiuk, N. H. *J. Chem. Soc. C* **1968**, 148–155.

Bax, A.; Freeman, R. *J. Magn. Reson.* **1981**, *44*, 542–547.

Bennett, G. A.; Peterson, R. E.; Platter, R. D.; Shotwell, O. L. *J. Am. Oil Chem. Soc.* **1981**, 1002A–1005A.

Blackwell, B. A.; Greenhalgh, R.; Bain, A. *J. Agric. Food Chem.* **1984**, *32*, 1078–1083.

Brown, D. W.; Nakashima, T. T.; Rabenstein, D. L. *J. Magn. Reson.* **1981**, *45*, 302–308.

Chun, X. Y.; Xuan, H.; Yusheng, C. *Acta Microbiol. Sin. (Engl. Transl.)* **1982**, *22*, 35–39.

Cole, R. J.; Cox, R. H. In "Trichothecenes, Chemical, Biological and Toxicological Aspects"; Ueno, U., Ed.; Elsevier: Amsterdam, 1983; pp 39–46.

Gardner, D.; Glen, A. T.; Turner, W. B. *J. Chem. Soc., Perkin Trans. 1* **1972**, 2576–2578.

Greenhalgh, R.; Hanson, A. W.; Miller, J. D.; Taylor, A. *J. Agric. Food Chem.* **1984**, *32*, 945–948.

Ichinoe, M.; Kurata, H.; Sugiura, Y.; Ueno, Y. *Appl. Environ. Microbiol.* **1983**, *46*, 1364–1369.

Ishii, K.; Sawano, M.; Ueno, Y.; Rsunoda, H. *App. Microbiol.* **1974**, *27*, 625–628.

Machida, Y.; Nozoe, S. *Tetrahedron* **1972**, *28*, 5113–5117.

Miller, J. D.; Taylor, A.; Greenhalgh, R. *Can. J. Microbiol.* **1983a**, *29*, 1171–1178.

Miller, J. D.; Young, J. C.; Trenholm, H. L. *Can. J. Bot.* **1983b**, *1*, 3080–3087.

Tamm, Ch.; Breitenstein, W. In "The Biosynthesis of Mycotoxins"; Steyn, P. S., Ed.; Academic Press: New York, 1980; pp 69–101.

Ueno, Y.; Sato, N.; Ishii, K.; Sakai, K.; Tsunoda, H.; Enomoto, M. *Appl. Microbiol.* **1973**, *25*, 699–704.

Yoshizawa, T.; Morooka, N. *Appl. Microbiol.* **1975**, *29*, 54–58.

Yoshizawa, T.; Shirota, T.; Morooka, N. *J. Food Hyg. Soc. Jpn.* **1978**, *19*, 178–184.

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