Minor Metabolites of *Fusarium roseum* (ATCC 28114). 2

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

Further workup of the crude chloroform extract of the beer from liquid cultures of *Fusarium roseum* (ATCC 28114) resulted in the isolation of trace amounts of the known trichothecenes 3,15-diacetyl-deoxynivalenol and calonectrin, together with the 7- and 8-hydroxy derivatives of isotrichodermin and calonectrin. In addition, the 11-keto isomer of culmorone and sambucoin were isolated. All these compounds were characterized by ^{1}H and ^{13}C NMR. All the trichothecenes produced by this particular fungal isolate are oxygenated at the 3-position and not the 3,4- or 4-positions, which suggests it to be the primary oxidation site of the trichothecene ring by enzyme systems present in this isolate.

INTRODUCTION

The large-scale production of 3-acetyldeoxynivalenol (ADON) by liquid culturing of *Fusarium roseum* (ATCC 28114) and *Fusarium culmorum* (CMI 14764) has been reported previously (Greenhalgh et al., 1984a). In addition to ADON, the crude fungal extract contained a number of minor secondary metabolites that included butenolide, culmorin, and four trichothecenes (Greenhalgh et al., 1984b). The latter metabolites, i.e. 3-acetoxy-12,13-epoxytrichothec-9-ene (isotrichodermin, ITD), 3-acetoxy-15-hydroxy-12,13-epoxytrichothec-9-ene (deacetylcalonectrin, DEACAL), 3-acetoxy-15-hydroxy-12,13-epoxytrichothec-9-en-8-one (deoxyacetyldeoxynivalenol, DEOADON), and 3,15-diacetoxy-7,8-dihydroxy-12,13-epoxytrichothec-9-ene (dihydroxycalonectrin, DHCAL), were all oxygenated in the C-3 position. This isolate, therefore, can be classified chemotaxonomically as a deoxynivalenol producer (Ichinoe et al., 1983).

Knowledge of the minor fungal metabolites of *Fusaria* assumes greater importance as recent data suggest that the pathogenicity of crude extracts of *Fusarium* isolates from wheat is not necessarily related to the amount of DON or zearalenone present (Chelkowski et al., 1984; Trenholm et al. 1984). Such a phenomenon could be the result of the presence of unknown metabolites or of synergistic effects between metabolites.

Further purification of the crude fungal extract from the culture filtrate of *F. roseum* has yielded trace amounts of other known and unknown trichothecenes. The characterization of these compounds by mass spectrometry (MS) and ^{1}H and ^{13}C nuclear resonance (NMR) is reported and postulation made on their biosynthetic origins.

MATERIALS AND METHODS

Electron impact (EI) and fast atom bombardment (FAB) mass spectrometry (MS) were carried out on a Finnigan MAT 312 mass spectrometer. Accurate mass measurements were determined by peak matching with an ion in the spectrum of perfluorokerosene. ^{1}H and ^{13}C NMR spectra were run on a Bruker WM 250-MHz spectrometer. The ^{1}H spectra were acquired with 16K data points, a 2200-Hz spectral window, 60° pulses, and a 10-s repetition rate. ^{13}C spectra were recorded with 16K data points, a 15-KHz spectral window in a 5-mm fixed-frequency probe with a 45° (6-μs) pulse, and 2.5-s repetition

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

rate. Chemical shifts were referenced to deuteriochloroform at 77.0 and 7.24 ppm, respectively, for ^{13}C and ^{1}H and reported (ppm) relative to tetramethylsilane (Me$_4$Si). ^{13}C chemical shift assignments were made by using the DEPT pulse sequence (Doddrell et al., 1982) to determine the number of directly attached protons corresponding to each resonance. Confirmation of ^{1}H chemical shift assignments was made by ^{1}H homonuclear correlation spectra (COSY). The (90–t_1–45 FID) pulse sequence (COSY-45) was accumulated by using 512 × 1K FID's of 16 scans each. Sine bell windows (shifted π/4 in F_1) were used for a final resolution of 1.4 Hz/point. These spectra were especially useful in resolving overlapping AB systems.

F. roseum (ATCC 28114) was cultured as previously described, (Greenhalgh et al., 1984a). The cultures were harvested and subjected to a purification procedure, which resulted in the isolation of a yellow oil (fraction A). This oil was chromatographed on a silica gel column, and fractions were monitored by thin-layer chromatography (TLC), appropriate fractions being combined to give four samples (A1–A4) (Greenhalgh et al., 1984b). Further purification of these fractions was accomplished on a Chromatotron (Model 7924, Harrison Research Inc.) with a 2-mm silica gel plate (Kiesel gel 60) and Prep TLC plates (20 × 20 cm, Gel 60 F-254, 2 mm thick; BDH Chemicals, Montreal, Quebec). The R_f data reported were obtained with a developing solvent of 75% ethyl acetate in chloroform.

Fraction A2. A sample of the oil (520 mg) was chromatographed on the Chromatotron with 30% ethyl acetate in hexane, and fractions (10 mL) were collected. Appropriate fractions were combined, and the solvent was removed to give six major samples. One was a pale yellow material (28.5 mg), which was further purified by TLC with a developing solvent of 5% methanol in chloroform. A crystalline material was obtained, I: R_f 0.37; mp 168–170 °C; $[\alpha]^{21}$ = –6.67° (*c* 0.15, CHCl$_3$); MS *m/z* 306.145 (M – AcOH), calcd for C$_{19}$H$_{26}$O$_7$ 306.147; ^{1}H NMR δ 0.83 (3 H, CH$_3$-14), 1.85 (3 H, CH$_3$-16, $J_{16,10}$ = 1.1 Hz), 1.90–2.30 (4 H, H-7, m), 2.03 (3 H, Ac-CH$_3$), 2.11 (3 H, Ac-CH$_3$), 2.89, 3.08 (2 H, H-13, J_{AB} = 4.0 Hz), 3.73 (1 H, H-2, $J_{2,3}$ = 4.6 Hz), 4.14 (2 H, H-15, J_{AB} = 2.7 Hz), 4.14 (2 H, H-11, $J_{11,10}$ = 5.8 Hz), 5.15 (1 H, H-3, $J_{2,3}$ = 5.0, $J_{3,4}$ = 10.0 Hz), 5.58 (1 H, H-10, $J_{10,11}$ = 5.7, $J_{10,16}$ = 1.1 Hz).

Fraction A. The original fraction A (Greenhalgh et al., 1984b) was subjected to medium-pressure liquid chromatography on a column (60 cm × 2.5 cm, i.d.) of silica gel (LiChroprep Si 60, 25–40 μm, 220 g) on a FMI laboratory pump (Model RPSY; Fluid Metering Inc.).

The oil (4.08 g) was dissolved in a minimum amount of 10% ethyl acetate/hexane and placed on the column. The column was then eluted with the following solvents: fractions 1–69, 10% ethyl acetate/hexane, ca. 1.3 L;

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fractions 70–142, 20% ethyl acetate/hexane, ca. 1.3 L; fractions 143–460, 30% ethyl acetate/hexane, 6 L; fractions 460–690, concentration of ethyl acetate gradually increased to 100%. The fractions collected (651) were reduced to 52 samples by combining appropriate fractions, and the solvent was removed.

Sample 11. The material (47.1 mg) appeared to be a single compound, R_f 0.71. It was recrystallized from ethyl acetate and hexane to give white prisms, II (20 mg): mp 243–243.5 °C; $[\alpha]^{21}$ = +120° (*c* 0.10, CHCl$_3$); MS *m/z* 250.160 (M$^+$), calcd for C$_{15}$H$_{22}$O$_3$ 250.157; ^{1}H NMR δ 0.63 (3 H, CH$_3$-15), 1.13 (3 H, CH$_3$-14), 1.43 (2 H, H-7, $J_{7,8}$ = 2.3, 5.4, J_{AB} = 13.3 Hz, m), 1.64 (3 H, CH$_3$-16), 1.74 (1 H, H-4, J_{AB} = 13.8, J = 7.9, 11.7 Hz), 1.8–1.9 (2 H, H-8, m unresolved), 1.96 (1 H, H-4, J_{AB} = 13.4, $J_{4,3}$ = 3.2, 10.2 Hz), 2.26 (1 H, H-3, $J_{3,4}$ = 7.9, 10.0, J_{AB} = 19.9 Hz), 2.56 (1 H, H-3, $J_{3,4}$ = 3.0, 11.8, J_{AB} = 19.9 Hz), 3.42, 4.18 (2 H, H-13, J_{AB} = 11.2 Hz), 3.94 (1 H, H-11), 5.18 (1 H, H-10).

Sample 13. The oil (167 mg) was further purified. After elution with chloroform (500 mL), the solvent was changed to 5% methanol in chloroform. This resulted in the separation of two compounds. One, R_f 0.67 (12 mg), was recrystallized from ethyl acetate/petroleum ether, III: mp 116–119 °C; $[\alpha]^{24}$ = −16.5° (*c* 0.12, CHCl$_3$); MS *m/z* 236.178 (M$^+$), calcd for C$_{15}$H$_{24}$O$_2$ 236.178; ^{1}H NMR δ 0.88 (3 H, CH$_3$-13), 0.95 (3 H, CH$_3$-14), 0.98 (3 H, CH$_3$-15), 1.01 (3 H, CH$_3$-12), 1.24 (1 H, H-7, $J_{7,8}$ = 4.8 Hz), 1.38–1.49 (6 H, H-3, H-4, H-5, m), 1.71 (1 H, 8-OH), 1.82 (1 H, H-10β, J_{AB} = 18.5, $J_{10\beta,8}$ = 1.8, $J_{10\beta,1}$ = 1.2 Hz), 2.33 (1 H, H-1), 2.59 (1 H, H-10α, J_{AB} = 18.5 Hz, 4.03 (1 H, H-8).

The second compound, IV (R_f 0.69), was recrystallized from ether/petroleum ether (8.6 mg): mp 75.5–78.5 °C; $[\alpha]^{24}$ = +7.97° (*c* 0.12, CHCl$_3$); MS *m/z* 290.149 (M$^+$), calcd for C$_{19}$H$_{26}$O$_6$ 290.152; ^{1}H NMR δ 0.81 (3 H, s, CH$_3$-14), 1.70 (3 H, CH$_3$-16, $J_{16,10}$ = 1.3 Hz), 2.03 (3 H, CH$_3$-3 Ac), 2.10 (3 H, CH$_3$-15 Ac), 1.78–2.19 (4 H, H-7, H-8, unresolved m), ca. 2.0 (2 H, H-4, m), 2.84, 3.08 (2 H, H-13, J_{AB} = 4.0 Hz), 3.74 (1 H, H-2, $J_{2,3}$ = 4.6 Hz), 3.82, 4.06 (2 H, H-15, J_{AB} = 12.2 Hz), 4.00 (1 H, H-11, $J_{11,10}$ = 5.6 Hz), 5.15 (1 H, H-3, $J_{3,2}$ = 4.6, $J_{3,4}$ = 9.6, 7.4 Hz), 5.45 (1 H, H-10, $J_{10,11}$ = 5.4, $J_{10,16}$ = 1.3 Hz). The data indicate this compound to be calonectrin (Gardner et al., 1972).

Sample 16. The material (112 mg) was eluted with chloroform (500 mL) and then 2% methanol in chloroform. The fractions collected were of varying purity. Further recrystallization from ether/petroleum ether yielded white needles, V (7.2 mg): R_f 0.63; mp 117–118.5 °C; $[\alpha]^{21}$ = +51.0° (*c* 0.98, CHCl$_3$); MS *m/z* 380.148, calcd for C$_{19}$H$_{24}$O$_8$ 380.147; ^{1}H NMR δ 1.09 (3 H, CH$_3$-14), 1.88 (3 H, CH$_3$-Ac), 1.89 (3 H, H-16, $J_{16,10}$ = 0.8 Hz), 2.13 (3 H, CH$_3$-Ac), 2.14, 2.28 (2 H, H-4, $J_{4,3}$ = 4.7, 10.9, J_{AB} = 15.3 Hz), 3.10, 3.15 (2 H, H-13, J_{AB} = 4.3 Hz), 3.76 (1 H, 7-OH, $J_{OH,7}$ = 2.0 Hz), 3.91 (1 H, H-2, $J_{2,3}$ = 4.5 Hz), 4.22, 4.28 (2 H, H-15, J_{AB} = 12.0 Hz), 4.70 (1 H, H-11, $J_{11,10}$ = 5.8 Hz), 4.81 (1 H, H-7, $J_{7,OH}$ = 2.0 Hz), 5.22 (1 H, H-3, $J_{3,2}$ = 4.6, $J_{3,4}$ = 10.9, 4.6 Hz, m), 6.57 (1 H, H-10, $J_{10,11}$ = 5.8, $J_{10,16}$ = 0.8 Hz). The MS and NMR data are identical with that of 3,15-diacetyldeoxynivalenol (Yoshizawa et al., 1978).

Sample 24. It was purified by chromatography, on elution initially with chloroform (500 mL) and then 2% methanol/chloroform (200 mL). Some solid material was recrystallized from ether/petroleum ether to give a crystalline solid, VI (9.5 mg): mp 154–155.5 °C; $[\alpha]^{22}$ = −10.6° (*c* 0.095, CHCl$_3$); MS *m/z* 308.163 (M$^+$), calcd for C$_{17}$H$_{24}$H$_5$ 308.162; ^{1}H NMR δ 0.82 (3 H, CH$_3$-15), 1.04 (3 H, CH$_3$-14), 1.09 (1 H, 7-OH, J = 6.3 Hz), 1.70 (3 H, CH$_3$-16), 2.10 (3 H, Ac-CH$_3$), 1.9, 2.2 (2 H, H-8, $J_{8,7}$ = 5.9, J_{AB} = 17.2 Hz), 2.06 (2 H, H-4), 3.08, 3.13 (2 H, H-13, J_{AB} = 4.4 Hz), 3.76

(1 H, H-2, $J_{2,3}$ = 4.5 Hz), 4.10 (1 H, H-11, $J_{11,10}$ = 5.5 Hz), 4.46 (1 H, H-7, $J_{7,8}$ = 5.9, 10.1, $J_{7,10}$ = 6.3 Hz), 5.16 (1 H, H-3, $J_{3,4}$ = 9.2, 6.6, $J_{3,2}$ = 4.5 Hz), 5.40 (1 H, H-10, $J_{10,11}$ = 5.45, $J_{10,16}$ = 1.2 Hz).

Sample 25. The oil (356 mg) was first eluted on a Chromatotron with chloroform (500 mL), followed by 5% methanol in chloroform (250 mL). Appropriate fractions were combined to give five samples of varying purity. One sample was further purified by Prep TLC (2-mm plates) with 7% methanol in chloroform as the developing solvent system. Two bands were eluted from the plate with ethyl acetate and recrystallized from ethyl acetate/hexane. The first material, VII (1.5 mg), had R_f 0.50: mp 174–175 °C; $[\alpha]^{23}$ = −14.3° (*c* 0.14, CHCl$_3$); MW *m/z* 306.148 (M − AcOH), calcd for C$_{17}$H$_{22}$O$_5$ 306.147; ^{1}H NMR δ 1.14 (3 H, CH$_3$-14), 1.41 (1 H, d, 7-OH), 1.72 (3 H, CH$_3$-16), 1.97–2.18 (2 H, H-4, m, $J_{4,3}$ = 9.8 Hz), 1.95, 2.41 (2 H, H-8, $J_{7,8}$ = 5.9, $J_{A,B}$ = 17.6 Hz), 2.08 (3 H, Ac-CH$_3$), 2.09 (3 H, Ac-CH$_3$), 3.09, 3.23 (2 H, H-13, J_{AB} = 4.4 Hz), 3.78 (1 H, H-2, $J_{2,3}$ = 4.4 Hz), 4.11 (1 H, H-11, $J_{11,10}$ = 5.4 Hz), 4.05, 4.31 (2 H, H-15, J_{AB} = 12.2 Hz), 4.55 (1 H, H-7, $J_{7,8}$ = 5.9, 11.0 Hz), 5.17 (1 H, H-3, $J_{2,3}$ = 4.5, $J_{3,4}$ = 10.0, 8.0 Hz), 5.38 (1 H, H-10, $J_{10,11}$ = 5.4, $J_{10,16}$ = 1.3 Hz).

The second material, VIII (9.3 mg), had R_f 0.44: mp 153–155.5 °C, $[\alpha]^{21}$ = −18.2° (*c* 0.11, CHCl$_3$); MW *m/z* 308.166 (M$^+$), calcd for C$_{17}$H$_{24}$O$_5$ 308.162; ^{1}H NMR δ 0.77 (3 H, CH$_3$-14), 0.98 (3 H, CH$_3$-15), 1.69, 2.21 (2 H, H-7, J_{AB} = 14.3, $J_{7,8}$ = 1.4, 5.9 Hz), 1.86 (3 H, CH$_3$-16), 2.07–2.11 (2 H, H-4, $J_{4,3}$ = 9.6, 5.8 Hz), 2.12 (3 H, CH$_3$-Ac), 2.87, 3.07 (2 H, H-13, J_{AB} = 4.0 Hz), 3.72 (1 H, H-2, $J_{2,3}$ = 4.6 Hz), 4.04 (1 H, H-11, $J_{11,10}$ = 5.7 Hz), 4.11 (1 H, H-8, $J_{8,7}$ = 5.9, 1.4 Hz), 5.16 (1 H, H-3, $J_{2,3}$ = 4.6, $J_{3,4}$ = 5.5, 9.8 Hz), 5.59 (1 H, H-10, $J_{10,11}$ = 5.9 Hz).

RESULTS AND DISCUSSION

Compounds VI and VIII, and VII and I, are isomeric and are hydroxylated derivatives of isotrichodermin and calonectrin, respectively. Comparison of the ^{1}H NMR spectra of VI and VIII with that of isotrichodermin differed principally in the region 4.0–5.0 ppm, which shows no resonances for isotrichodermin. However, DHCAL with hydroxyl groups at C-7 and C-8 exhibits two resonances (4.50, 3.99 ppm) in this region, which were assigned to H-7 and H-8, respectively (Greenhalgh et al., 1984a). The ^{1}H NMR of VI has a multiplet resonance at 4.46 ppm, suggesting it to be the 7-OH isomer. A ^{1}H homonuclear correlation spectrum revealed an AB system (1.9–2.2 ppm), which showed long-range coupling to the CH$_3$-16 and H-10. This indicates the AB system to be the C-8 methylene group, thereby providing further proof for the structural assignment involving a C-7 hydroxyl: H-7 was also coupled to the AB system. This confirms the structure of VI as 3-acetoxy-7-hydroxy-12,13-epoxytrichothech-9-ene (7-OH-ITD). The isomer VIII has a resonance at 4.11 ppm, which by comparison with the spectrum of DHCAL indicates it is the 8-hydroxy analogue, 3-acetoxy-8-hydroxy-12,13-epoxytrichothec-9-ene (8-OHITD).

Both compounds, VII and I, show the presence of two acetoxy moieties in their ^{1}H NMR species as in calonectrin. Again, comparison of the region 4.00–5.00 ppm with that of DHCAL indicates that VII is the 7-hydroxy isomer (3,15-diacetoxy-7-hydroxy-12,13-epoxytrichothec-9-ene) and I is the 8-hydroxy isomer (3,15-diacetoxy-8-hydroxy-12,13-epoxytrichothec-9-ene, based on resonances at 4.55 and 4.15 ppm, respectively. These assignments also correlate with the chemical shifts of the AB system of H-13 protons, which for the 7-OH isomers VI and VII are 3.10 and 3.16 ppm, respectively, while for both 8-OH isomers, VIII and I, they appear at 2.97 ppm. Final proof of the

Table I. ¹³C NMR Chemical Shift Assignments of Trichothecene Derivatives of Isotrichodermin and Calonectrin

carbon no.	isotri- chodermin[a]	7-OHITD (VI)	8-OHITD (VIII)	DHCAL[a]	CAL (IV)	7-OHCAL (VII)	8-OHCAL (I)
2	78.2	79.0	78.1	78.5	77.9	78.9	78.1
3	71.7	71.4	71.5	71.0	71.1	71.1[b]	71.1
4	38.6	39.5	38.1	41.3	39.1	39.6	39.1
5	45.3	44.6	45.4	46.0	45.2	46.1	45.3
6	40.2	46.2	39.6	46.2	42.8	46.4	42.3
7	24.4	74.7	33.6	69.7	20.9	71.0[b]	30.0
8	28.2	41.4	68.0	70.4	28.0	41.7	67.8
9	139.5	138.0	138.8	139.3	140.3	139.4	139.5
10	119.4	119.6	122.1	129.1	118.9	119.0	121.6
11	71.5	69.0	71.3	70.2	68.0	70.0	67.0
12	65.2	64.9	65.2	64.9	64.9	64.3	64.9
13	48.4	47.7	48.5	47.8	48.2	47.8	48.6
14	11.0	10.2	11.0	15.0	11.9	15.9	11.7
15	15.9	15.0	19.0	63.3	63.5	64.4	65.0
16	23.1	22.4	20.4	20.3	23.0	22.4	20.3
C=O (Ac)	170.6	170.6	170.6	170.0/170.4	170.4/170.7	170.0/170.1	170.5/170.4
CH₃ (Ac)	20.9	20.9	20.9	21.1/20.4	20.4/20.8	21.0/20.8	20.8/20.9

[a] From Greenhalgh et al. (1984b) (for comparison). [b] Assignments may be reversed.

(a)

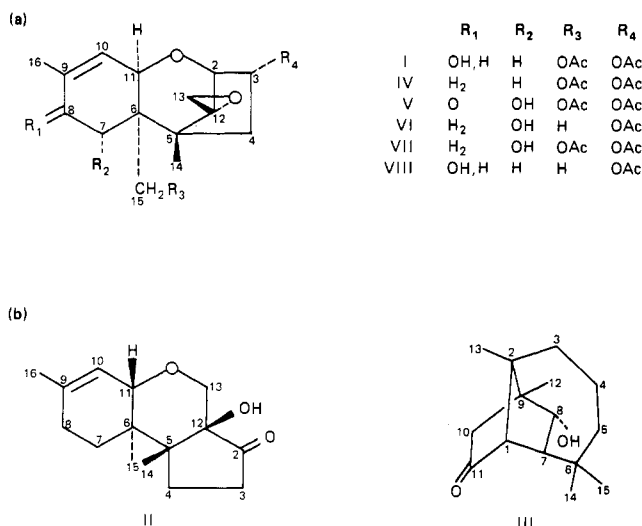|	R₁	R₂	R₃	R₄
I	OH,H	H	OAc	OAc
IV	H₂	H	OAc	OAc
V	O	OH	OAc	OAc
VI	H₂	OH	H	OAc
VII	H₂	OH	OAc	OAc
VIII	OH,H	H	H	OAc

(b)

II   III

Figure 1. Structures of the secondary metabolites isolated from *F. roseum* (ATCC 28114): (a) trichothecenes; (b) sambucoin (II) and culmorone (III).

assignments was obtained from the ¹³C NMR chemical shifts (Table I). The ¹³C chemical shifts for the C-7 resonances of the two 7-OH isomers VI and VII (74.7 and 71.1 ppm, respectively) are at lower field than those of the two 8-hydroxylated compounds, VIII and I (68.0 and 67.8 ppm, respectively). In addition, the C-10 resonance for the 8-OH isomers occur at lower field than those of the 7-OH isomers.

Compound II was thought to be sambucoin, recently isolated from *Fusarium sambucinum* although a large discrepancy existed with the reported melting points, 205–210 °C (Mohr et al., 1984). A ¹H homonuclear correlation spectrum (Figure 2) supports the identity of II as sambucoin and confirms the assignments of Mohr et al. (1984), except for clarification of H-3, H-4 and the H-7, H-8 AB systems, as well as the CH₃-14 and CH₃-15 protons, the latter assignment based on long-range coupling between H-7α and CH₃-15. The ¹³C chemical shifts and assignments are also consistent with those reported.

The ¹H NMR of compound III indicated that it is not a trichothecene but is structurally similar to culmorin. The ¹³C spectrum shows a keto group (216.5 ppm) and only one CHOH resonance of culmorin; otherwise, the spectra are identical. The keto group was assigned to position C-11 based on the homonuclear relay 2D NMR (Blackwell, 1985).
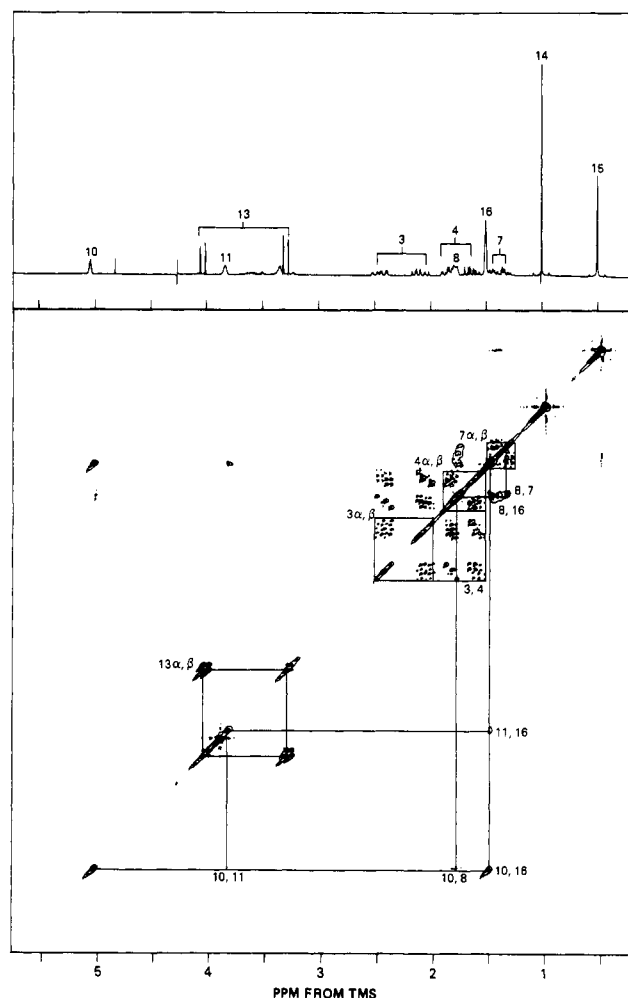

Figure 2. 250-MHz ¹H homonuclear correlation NMR spectrum (COSY-45) of sambucoin in CDCl₃. The 16K 1D spectrum is shown above the plot, and resonances are labeled according to the numbering system in Figure 1.

Barton and Werstiuk (1968) have reported the synthesis of two hydroxy ketones from culmorin and established their stereochemistry. The properties of the compound isolated agreed with those of the 11-keto isomer of culmorone (lit. mp 116–118 °C). This is the first time that it has been isolated as a natural product. Culmorin and most likely culmorone are thought to be mevalonate derived (Hanson and Nyfeler, 1976). The structures of the

secondary metabolites isolated from *F. roseum* (ATCC 28114) are shown in Figure 1.

The ^{13}C NMR chemical shift assignments of the trichothecene derivatives isolated are presented in Table I. Assignments are based on comparison to those of isotrichodermin and DHCAL previously isolated (Greenhalgh et al., 1984b) and supported by multiplicity determination by DEPT spectra. Subsequent isolation of quantities of DHCAL has enabled the unambiguous assignment of the resonance by heteronuclear shift-correlated 2D NMR. The greater sensitivity of ^1H chemical shifts and J_{HH} data to substitution in the trichothecene ring by hydroxyl groups was used to assign the ^{13}C resonances, which occur within a small chemical shift range (ca. 1.5 ppm). This resulted in corrections to the assignments of C-2, C-7, C-8, and C-11 of DHCAL to those previously reported (compound V; Greenhalgh et al., 1984a). A $^1H/^1H$ homonuclear correlated spectrum of DHCAL emphasizing long-range coupling was in agreement with the assignment of an α conformation to both the 7- and 8-hydroxyl moieties (Greenhalgh et al., 1985b).

Table I shows that hydroxy substitution of the trichothecene molecule at C-7, C-8, and C-15, the latter acetylated, has little effect on the ^{13}C chemical shifts of the B- and C-ring carbon atoms but a profound effect in ring A. A hydroxyl group at C-7 produces a 4-6 ppm downfield shift of the C-6 resonance, while a hydroxyl at C-8 produces a upfield shift of ca. 3 ppm on C-16 together with a small downfield shift on C-10. In the case of DHCAL, in which both C-7 and C-8 are substituted with hydroxyl groups, a marked downfield shift of C-10 is observed. By comparison in the ^{13}C spectrum of ADON, which has a keto group at C-8, the C-10 resonance occurred at 138 ppm. This suggests that the C-10 chemical shift may be a sensitive indicator of the extent and type of substitution in the A ring.

Machido and Nozoe (1972) isolated small amounts of trichodiene, trichodiol, and 12,13-epoxytrichothec-9-ene from *Trichothecium roseum* and suggested that they were biogenetic intermediates of trichothecin. This implies that the 4,8-dihydroxy-12,13-epoxytrichothec-9-ene, which they also isolated, resulted from oxidation of 12,13-epoxytrichothec-9-ene with either C-4 or C-8 as the primary oxidation sites. In the case of *F. roseum* and *F. culmorum*, (Greenhalgh et al., 1985b) all the trichothecene metabolites isolated have an oxygenated moiety at C-3, indicating this to be the primary oxidation site for these isolates, followed by secondary oxidation resulting in various degrees of oxidation at the C-7, C-8, and C-15 positions. The presence of different enzyme systems in fungi, which effect specific oxidation, could account for the diverse oxidation patterns of trichothecene metabolites isolated from different fungal species.

Both sambucoin and sambucinol have been reported to be formed by *Fusarium sambucinum* and *F. culmorum* in stirred fermentor conditions. In the case of *F. roseum* in still culture conditions, only sambucoin was isolated to-

gether with traces of apotrichothecenes (Greenhalgh et al. 1985b). None of these compounds, however, provided further information that would permit differentiation between the two biosynthetic pathways proposed for sambucoin or sambucinol by Mohr et al. (1984).

In summary, *F. roseum* and *F. culmorum* produce a variety of trichothecene metabolites related to the parent polyols isotrichodermin, calonectrin, and deoxynivalenol, suggesting that primary oxidation occurred at the 3-position. It is further suggested that the oxidative systems present in a particular isolate control primary oxidation and the type of trichothecene (i.e., 3-, 4-, or 3,4-oxygenated) produced, thus differentiating between fungal species. Some metabolites isolated such as the deacylcalonectrins are suspected to be degradation products resulting from the isolation procedure used rather than true secondary metabolites.

LITERATURE CITED

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