Carbon-13 and Proton Nuclear Magnetic Resonance Spectral Assignments of Deoxynivalenol and Other Mycotoxins from *Fusarium graminearum*

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Precise ^1H and ^{13}C NMR spectral assignments of deoxynivalenol, 3- and 15-acetyldeoxynivalenol, deacetoxyscirpenol, T-2 toxin, and zearalenone have been made in conjunction with an NMR study of mycotoxin biosynthesis in *Fusarium graminearum*. ^1H spectral resolution at 250 MHz was sufficient to assign most resonances from the complete set of $^1H/^1H$ coupling constants. The ^{13}C NMR spectra were assigned by heteronuclear shift correlation 2D NMR. A ^{13}C spin-echo multiplicity determining sequence was used in assignment of the quaternary carbon atoms and to complement the correlation data, specifically where ^1H resonances were not resolved. The 2D spectrum permitted individual ^1H resonance assignments of the macrocyclic ring of zearalenone as well as resolving those of positions 7 and 8 of diacetoxyscirpenol, and positions 7, 18, and 19 of T-2 toxin. The correlation spectra provided the unambiguous chemical shift assignments that render facile the assignment of ^{13}C spectra of related toxins, aided by multiplicity determination.

The trichothecenes are a family of fungal secondary metabolites produced by several genera of Fungi Imperfecti. They occur as natural contaminants of cereal crops and are responsible for a variety of toxic responses in animals and man (Rodricks et al., 1977; Tamm and Breitenstein, 1980).

In recent years, concern has centered on deoxynivalenol (DON, vomitoxin), a trichothecene produced in the field by *Fusarium graminearum* (Morooka et al., 1978; Yoshizawa et al., 1978; Pathre and Mirocha, 1979; Vesonder et al., 1973). Isolates of this fungus can also produce other trichothecenes including diacetoxyscirpenol (DAS), T-2 toxin, and acetylated derivatives of DON, as well as zearalenone and butenolide (Miller et al., 1983). The chemical structures and the carbon atom numbering for some of these compounds are shown in Figure 1.

With a view to developing methods for the control of mycotoxin formation in the field by a knowledge of the potential steps for inhibition, a biosynthetic study was initiated with ^{13}C NMR. As a prerequisite to this study, it was necessary to verify the ^1H and ^{13}C spectral assignments of DON and other potential metabolites, by techniques based on shift correlation or spin coupling relationships rather than empirical rules based on chemical shift.

Previously reported ^{13}C NMR assignments of the trichothecenes were obtained at relatively low field (25 MHz or less) (Cole and Cox, 1981) by employing off-resonance decoupling and SFORD (Cole et al., 1981; Hanson et al., 1974). These techniques can produce anomalous results when the signal to noise ratio is poor and resonances are closely spaced. Disagreement has resulted regarding the ^{13}C assignments of DAS and T-2 toxin (Hanson et al., 1974; Breitenstein and Tamm, 1975) and zearalenone (Mirocha and Pathre, 1979; Cole and Cox, 1981). To date, the most definitive study of trichothecenes was based on the Birdsall plot method, correlating the ^1H and ^{13}C chemical shift for T-2 toxin (Ellison and Kontsonis, 1976).

An NMR strategy was used in this study to unambiguously assign both ^{13}C and ^1H spectra for the compounds shown in Figure 1, as well as the 3- and 15-monoacetyl derivatives of DON. ^{13}C spectra were assigned by heteronuclear shift correlation 2D NMR and correct ^1H assignments. A multiplicity-determining ^{13}C spin echo (Brown et al., 1981) was used in the assignment of quaternary carbons and to confirm the 2D correlation, specifically where ^1H resonances were not resolved. These techniques have the advantages of requiring relatively small (milligram) amounts of material, and the spin-echo technique can be applied to the crude fungal extracts obtained from biosynthetic studies.

MATERIALS AND METHODS

Chemicals. T-2 toxin, DAS, and zearalenone were purchased from Myco-lab Co., Chesterfield, MO, and used without further purification. DON was isolated from infected corn and purified in the laboratory (mp 151–153 °C) (Greenhalgh, 1982). 3-Acetyldeoxynivalenol (3-AcDON, mp 184–186 °C) and 15-acetyldoxynivalenol (15-AcDON, mp 142–145 °C) were isolated from fermentor growths of *Fursarium roseum* and *F. graminearum*, respectively. All toxins were prepared as 10–100 mg/mL solutions in $CDCl_3$ in 5 mm o.d. NMR tubes.

NMR Spectroscopy. ^1H and ^{13}C spectra were recorded at 250 and 62.8 MHz, respectively, on a Bruker WM 250 NMR spectrometer. ^1H spectra were acquired with 16K data points, a 2200-Hz spectral window, 90° (6 μs) pulses, and a 10-s repetition rate. Resolution was enhanced by Gaussian line shape transformation. A 5-mm fixed frequency probe (with a 90° pulse of 12 μs) was employed for all ^{13}C spectra. Chemical shifts are referenced to $CDCl_3$ at 77.0 and 7.24 ppm, respectively, for ^{13}C and ^1H and are reported as ppm relative to tetramethylsilane (Me_4Si; TMS in the figures).

The multiplicities of ^{13}C spectral resonances were determined by the spin-echo sequence of Brown et al. (1981), with echo delays based on an average J_{CH} = 125 Hz. Four 16K spectra were recorded for each experiment; the combination of 4 $(1/2J)$, 8 $(1/J)$, and 2 $(1/4J)$ minus 6 $(3/4J)$ ms echo delay spectra was sufficient to determine the multiplicities of all spectral resonances.

Heteronuclear shift correlation 2D NMR spectra (Freeman and Morris, 1978; Hall et al., 1980) were recorded at 360-MHz ^1H and 90.5-MHz ^{13}C on a Bruker WM 360 spectrometer. The pulse phases were cycled according to Bax and Morris (1981) to permit quadrature detection in the ^1H domain. Sweep widths in the ^{13}C and ^1H dimensions were 5200 and 1200 Hz, respectively, with 4K data points in the ^{13}C dimension. Incremented ^{13}C spectra (256) of 64 scans each, corresponding to an evolution time (t_1)

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Table I. 250-MHz ^1H Chemical Shift Assignments and Coupling Constants[a] for Epoxytrichothecene Skeleton

proton position	T-2	DAS	DON	3-AcDON	15-AcDON
2	3.60 ($J_{2,3}$ = 4.9)	3.61 ($J_{2,3}$ = 4.9)	3.62 ($J_{2,3}$ = 4.5)	3.87 ($J_{2,3}$ = 4.5)	3.60 ($J_{2,3}$ = 4.4)
3	4.10 ($J_{3,2}$ = 4.9; $J_{3,4}$ = 2.8)	4.10 ($J_{3,2}$ = 4.9; $J_{3,4}$ = 2.9)	4.53 ($J_{3,4}$ = 10.7, 4.5; $J_{3,2}$ = 4.5)	5.18 ($J_{3,4}$ = 11.2, 4.5; $J_{3,2}$ = 4.5)	4.50 ($J_{4,3}$ = 10.6, 4.5; $J_{2,3}$ = 4.4)
4	5.29 ($J_{4,3}$ = 2.8)	5.11 ($J_{4,3}$ = 2.9)	2.21 (J_{AB} = 14.8) 2.07 ($J_{4,3}$ = 10.7, 4.5)	2.35 (J_{AB} = 15.1) 2.12 ($J_{4,3}$ = 11.2, 4.5)	2.20 (J_{AB} = 14.8) 2.07 ($J_{4,3}$ = 10.6, 4.5)
7	2.31 (J_{AB} = 15.3) 1.82 ($J_{7,8}$ = 5.7)	2.00 (J_{AB} = 11.0) 1.67	4.83 ($J_{7,OH}$ = 2.0) 3.82 (OH)	4.79 ($J_{7,OH}$ = 2.1) 3.81 (OH)	4.81 ($J_{7,OH}$ = 1.9) 3.76 (OH)
8	5.20 ($J_{8,7}$ = 5.7)	1.93 (m)			
10	5.72 ($J_{10,11}$ = 5.9)	5.45 ($J_{10,11}$ = 5.3)	6.61 ($J_{10,11}$ = 5.9; $J_{10,16}$ = 1.5)	6.56 ($J_{10,11}$ = 5.9; $J_{11,16}$ = 1.5)	6.58 ($J_{10,16}$ = 1.3; $J_{10,11}$ = 5.8)
11	4.27 ($J_{11,10}$ = 5.9)	4.02 ($J_{11,10}$ = 5.3)	4.80 ($J_{11,10}$ = 5.9)	4.66 ($J_{11,10}$ = 5.9)	4.87 ($J_{10,11}$ = 5.8)
13	2.98 (J_{AB} = 3.9) 2.72	2.99 (J_{AB} = 4.9) 2.70	3.15 (J_{AB} = 4.3) 3.07	3.14 (J_{AB} = 4.3) 3.08	3.11 (J_{AB} = 4.2) 3.06
14	0.71 (s)	0.74 (s)	1.13 (s)	1.12 (s)	1.04 (s)
15	4.00 (J_{AB} = 12.6) 4.22	4.10 (J_{AB} = 12.3) 3.90	3.89 (J_{AB} = 11.7) 3.73	3.83 (J_{AB} = 11.7) 3.73	4.21 (s)
16	1.67 (s)	1.65 (s)	1.86 ($J_{16,10}$ = 1.5)	1.85 ($J_{16,10}$ = 1.5)	1.86
18	2.10				
19	2.0 (m)				
20, 21	0.86 ($J_{20,19}$ = 2.0) 0.89 ($J_{21,19}$ = 2.0)				
CH_3 (Ac)	1.96, 2.06 (s)	1.98, 2.07 (s)		2.10 (s)	1.85 (s)

[a] Chemical shifts (ppm from Me_4Si); coupling constants (Hz).

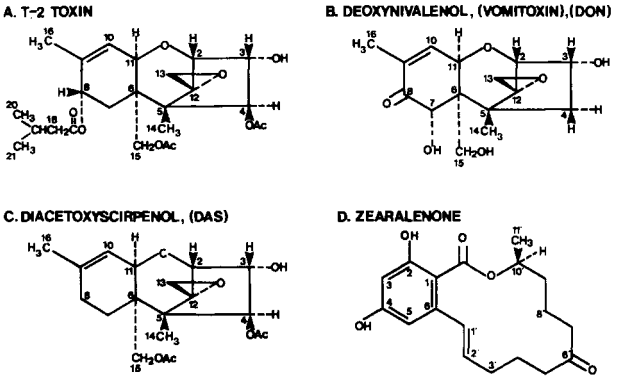

Figure 1. Chemical structures of mycotoxins of *F. graminearum*.

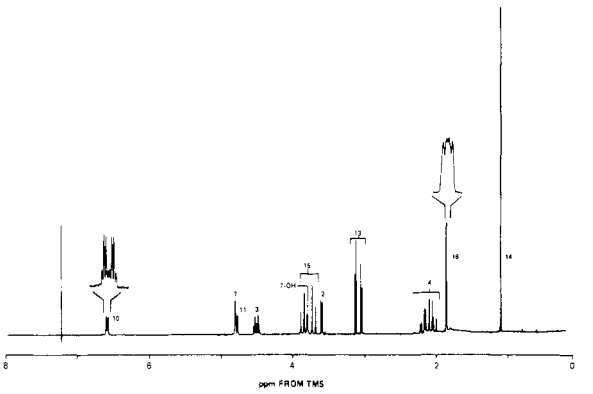

Figure 2. Spectral assignments of 250-MHz ^1H spectrum of DON.

increase of 200 μs, were acquired with an 8-μs (90°) ^{13}C and a 27-μs (90°) ^1H pulse width and a 2-s repetition rate. Free induction decays in both dimensions were Gaussian weighted, and zero filling was used to give a final data matrix of 8192 × 512 data points. Spectra were displayed in the absolute value mode. Contour plots proved to be the most useful for determining C–H connectivity, while individual spectral slices at single ^{13}C frequencies (proton slices) were convenient for the resolution of overlapped proton resonances.

RESULTS

Trichothecenes. *^1H Assignments.* The ^1H spectra (250 MHz) of the pure trichothecenes are essentially first order and can be assigned from relative peak intensities and $^1H/^1H$ coupling constants; those for DON together with the structural assignments are given in Figure 2. The methylene protons of the epoxide moiety (C-13) show an AB system centered at 3.11 ppm (J = 4 Hz), which is characteristic of all trichothecenes (Bamburg and Strong, 1971; Mirocha et al., 1977). Similarly, the C-15 methylene protons exhibit AB character due to nonequivalence. The methyl protons of C-14 and C-16 are readily distinguished by the long-range coupling of the former to the C-10 proton (J = 1.5 Hz) and a very small coupling (J = 1 Hz) to the C-11 proton.

The C-7 OH proton of DON appears to be less labile than the other two OH protons (C-3 and C-15), possibly due to hydrogen bonding with the carbonyl oxygen at position 8 (Greenhalgh et al., 1984a). Fine coupling (J = 2.1 Hz) between the OH and the methine proton at C-7 was observed in $CDCl_3$ but not MeOH-d_4, making it diagnostic for this position.

The ^1H assignments for DAS, T-2, DON, and its acetylated derivatives aree summarized in Table I. The ^1H spectrum of 15-AcDON has been previously reported (Miller et al., 1983) and is included here for comparison. The epoxide AB systems in DON and 3-AcDON appear to have somewhat less AB character than those of DAS and T-2. While the epoxide J_{AB} is characteristic for the epoxytrichothecene skeleton, the separation of the AB doublet and chemical shift are sensitive to the nature of other substituents in the molecule, particularly at positions 3, 4, 7, and 8. Acetylation of the 3-OH group in DON produces a downfield shift in the ABXY system of positions 2, 3, and 4 and a slight upfield shift for protons on positions 10 and 11.

Previous ^1H spectral assignments of the trichothecenes have been made (Cole and Cox, 1981; Yoshizawa and Morooka, 1973; Bamburg and Strong, 1971), based principally on chemical shift arguments, since much of the specific coupling information is obscured at low field (100 MHz or less). Due to spectral overlap, assignments are often not complete, although most ^1H spectra of trichothecenes appear first order at 250 MHz or greater (Pohland et al., 1982). The proton spectral assignments of DAS and T-2 in Table I are based on the $^1H/^{13}C$ 2D correlation

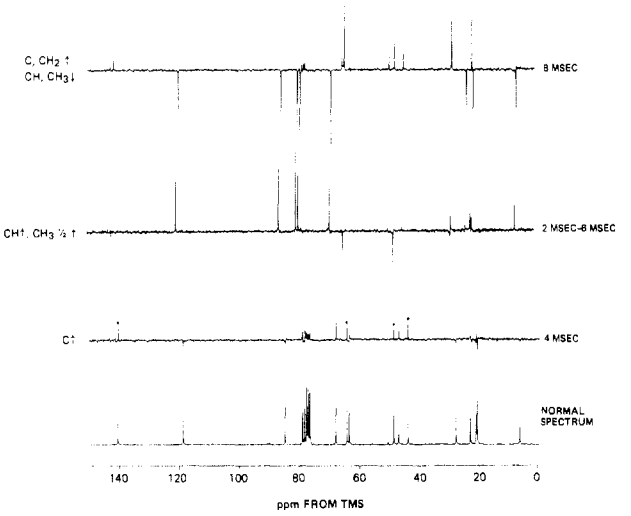

Figure 3. 62.8-MHz ^{13}C spin-echo multiplicity sorting spectra of DAS ($90°-\tau_{DO}-180°-\tau_{BB}$-FID). The echo delays are indicated on the right of each spectrum. Quaternary carbons are indicated (●) in the 4-ms echo delay spectrum.

spectra, which resolve overlapping ^1H resonances. In DAS, for example, the AB character of the C-7 protons is resolved from the AB multiplet of the C-8 protons, even though these resonances are obscured in the 1D spectrum by those of the two acetyl methyl groups. Similarly, in the 2D correlation spectrum of T-2 toxin, resonances due to the C-7, C-18, and C-19 protons are resolved from under those of the acetyl methyl groups. In addition, the 2D spectrum of T-2 toxin showed that the C-18 protons were equivalent, whereas the C-19 proton resonance was broadened by coupling to both protons of C-18 and those of the terminal methyl groups (C-20 and C-21), although resolution was not sufficient to provide specific coupling information at these positions.

Multiplicity Sorting. Modulation of the ^{13}C spin echo by proton noise decoupling (Brown et al., 1981; Patt and Shoolery, 1982; Le Coq and Lallemand, 1981) is only one of several techniques available for multiplicity determination. Polarization transfer techniques [e.g., INEPT (Burum and Ernst, 1980; Morris and Freeman, 1979) and DEPT (Bendall et al., 1981; Doddrell et al., 1982)] suppress quaternary carbons, since parameters are adjusted for optimum transfer of magnetization between directly bonded carbon–proton pairs. The spin-echo method simultaneously determines multiplicities and chemical shifts of *all* carbon resonances and is therefore suitable to the elucidation of structures of secondary metabolites, which contain several quaternary carbons.

The multiplicity sorting experiment for DAS is shown in Figure 3. The $1/J$ spectrum (8-ms echo delay) sorts resonances with even multiplicity (positive) from those with odd multiplicity (negative). In particular, this display facilitates the determination of the multiplicities of the adjacent resonances at 20.8 and 21.1 ppm, assigned to the acetate methyl and C-7, respectively.

Quaternary carbons are sorted from methylene carbons by the $1/2J$ spectrum (4-ms echo delay). Only resonances due to quaternary carbons remain at full intensity. The intensities of the methylene resonances are considerably reduced (not nulled, due to variation in J_{CH} across the spectrum) and are distinguished from those of the quaternary carbons by comparison to the $1/J$ spectrum. The resonance at 67 ppm appears positive in the $1/2J$ spectrum, but its identification as a quaternary carbon is negated by the fact that it appears negative in the $1/J$ spectrum.

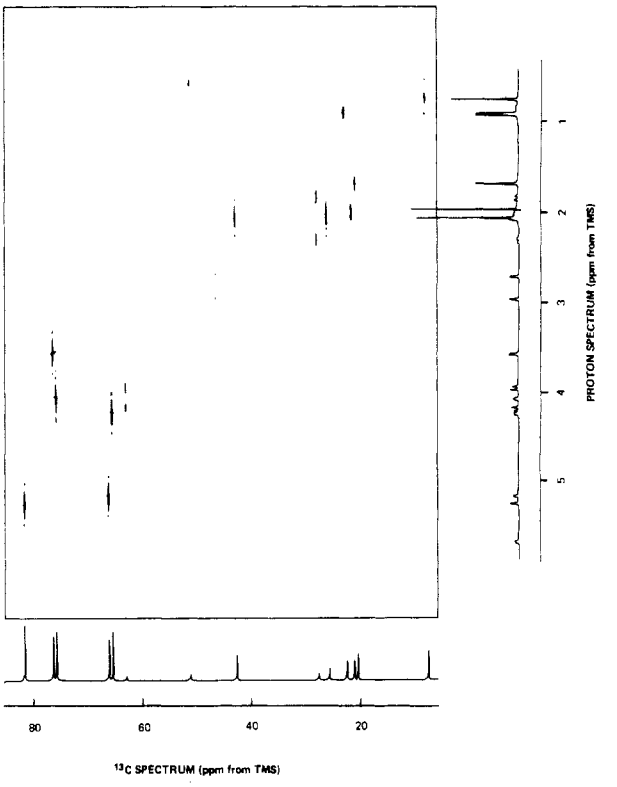

Figure 4. Contour plot of the $^1H/^{13}C$ correlation spectrum of T-2 toxin. The ^{13}C projection is displayed at the bottom of the figure and the normal 1D ^1H spectrum along the ^1H axis.

Subtraction of the $1/4J$ (2 ms) and $3/4J$ (6 ms) spectra effectively nulls the quaternary resonances and permits the sorting of methine and methyl resonances on the basis of relative intensity. The methine resonances appear positive with full intensity, while those due to methyl groups are positive with half intensity.

Comparison of the three spectra of Figure 3 enables the multiplicities of all DAS resonances to be determined. The multiplicity data alone are sufficient to assign resonances at 118 and 140 ppm to C-10 and C-9, respectively. This enables the sweep width required in the ^{13}C dimension of the correlation spectrum to be reduced to the upfield portion of the spectrum (5–80 ppm).

$^1H/^{13}C$ 2D Correlation Spectra. The 2D chemical shift correlation spectrum of T-2 toxin is displayed as a contour plot in Figure 4. Quaternary carbons are absent from the ^{13}C projection. Additional peaks appear that are artifacts due to folding and data processing, and thus a comparison between the ^{13}C projection and the 1D ^{13}C spectrum is necessary to determine the authenticity of the resonances. The resonance due to the epoxide methylene group (C-13) that occurs at 3.0 ppm in the ^1H direction produces only a very weak signal. The J_{CH} value of this resonance (175 Hz) is sufficiently higher than the optimized value of 140 Hz to render polarization transfer inefficient. For the same reason, the methylene and methyl resonances (at 0–40 ppm in the ^{13}C direction, J = 126–130 Hz) are reduced in intensity compared to the methine resonances downfield of 60 ppm (J = 140–150 Hz).

Where the proton spectrum is not resolved, a 2D correlation spectrum is insufficient to assign both ^{13}C and ^1H resonances, and additional information may be obtained from multiplicity data. The 2D plot resolves the ^1H spectrum of T-2 in the region of 2 ppm (Figure 4). The AB proton system (centered at 2.0 ppm) correlates with a ^{13}C resonance at 27.7 ppm, which then can be assigned to C-7. The assignment of the two acetyl methyl groups

Table II. 62.8-MHz ^{13}C Chemical Shift Assignment for Epoxytrichothecene Skeleton

carbon no.	^{13}C chemical shift[a] T-2	DAS	DON	3-AcDON	15-AcDON
2	78.7	78.9	80.6	79.1	80.7
3	78.2	78.2	68.6	71.3	68.9
4	84.3	84.6	43.0	40.5	43.3
5	48.3	48.7	46.0	45.9	46.4
6	42.9	43.8	52.1	52.0	51.4
7	27.7	21.1	70.2	70.2	70.1
8	68.0	27.8	202.3	199.8	199.4
9	136.3	140.4	135.7	135.8	135.5
10	123.8	118.6	138.5	138.4	138.6
11	67.3	67.9	74.4	74.5	73.5
12	64.2	64.3	65.7	65.1	65.4
13	47.0	47.0	47.2	47.5	47.3
14	6.6	6.6	13.9	14.1	13.8
15	64.5	63.5	61.4	62.2	62.2
16	20.1	23.0	14.9	15.2	15.3
17	172.7				
18	43.4				
19	25.6				
20, 21	22.2				
CH_3 (Ac)	20.8	20.8		20.9	
C=O	170.2	170.6		170.3	
(Ac)	172.5	172.5			

[a] ppm from Me_4Si.

through correlation to the resonance at 20.8 ppm is confirmed by multiplicity determination. The two additional proton resonances correlate with ^{13}C resonances at 43.4 and 25.6 ppm, which prove to be due to a methine and methylene group and are assigned to C-19 and C-18, respectively. The assignment of the other ^{13}C resonances (60–90 ppm) is straightforward, since the ^1H spectrum is well resolved.

Similarly, the 2D correlation spectrum of DAS resolved the protons of C-7 and C-8 from those of the acetate and C-16 methyl groups (see Table I). However, the multiplicities of the two ^{13}C resonances at 21.1 and 27.7 ppm, which are correlated to these protons, are the same. The lower field resonance was assigned to C-8 on the basis of the chemical shift effect of the adjacent double bond. The observation that the protons correlated to C-8 are equivalent while those of C-7 are an AB system is consistent with this assignment.

The correlation experiment for DON led to the assignment of the methyl resonances that occur at 13.9 and 14.9 ppm to C-14 and C-16, respectively, through correlation to ^1H resonances at 1.1 and 1.9 ppm. Resolution was not sufficient, however, to resolve the methine resonances of C-7 and C-11, which are adjacent in both ^{13}C and ^1H spectra. A second 2D spectrum with a much smaller ^{13}C spectral window enabled a correlation to be made between the known ^1H assignments for position 7 (4.83 ppm) and position 11 (4.80 ppm) and the ^{13}C resonances at 70.2 and 74.4 ppm, respectively. The quaternary positions, C-5 and C-6, were distinguished through long-range coupling, giving rise to weak cross peaks at the C-5 chemical shift (46.0 ppm) and the C-4 protons at 2.1 ppm and between the C-6 chemical shift (52.1 ppm) and the C-7 or C-11 protons.

The ^{13}C assignments for DAS, DON, and T-2 obtained by these two techniques are summarized in Table II. Spectra of the acyl derivatives of DON were assigned easily from a $1/J$ spin-echo spectrum. Acetylation at the 3-position affects the chemical shifts of positions 2, 3, and 4 sufficiently that both DON and 3-AcDON can be identified in ^{13}C spectra of crude fungal extracts (Blackwell et al., 1984). The ^{13}C spectral assignments of T-2 and DAS

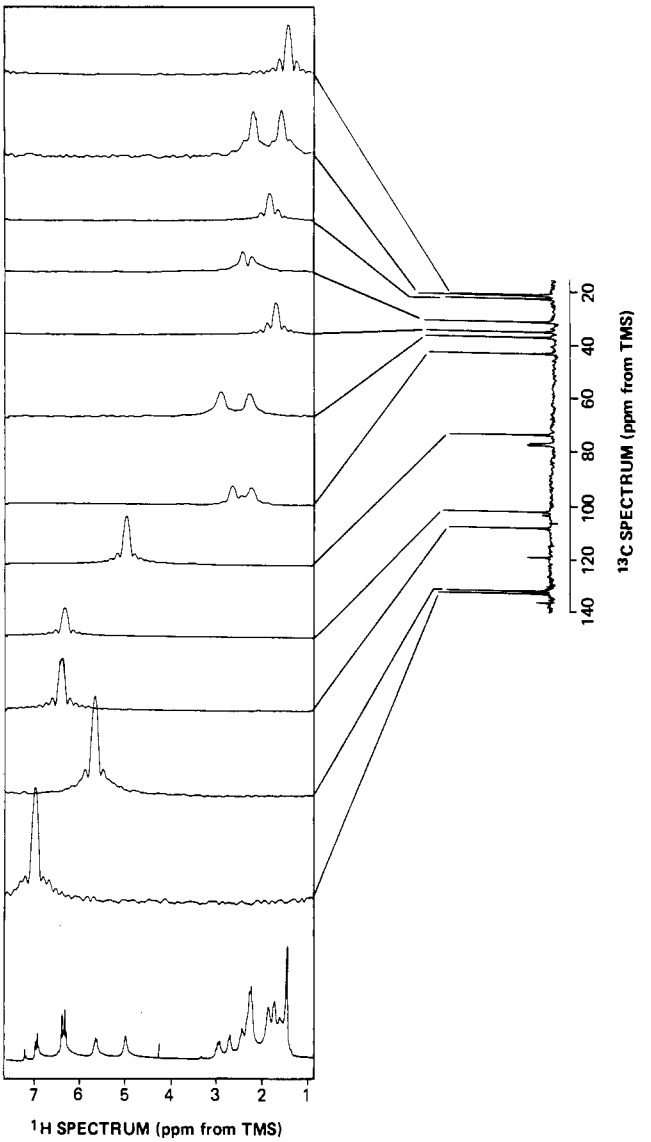

Figure 5. Proton slices from the $^1H/^{13}C$ correlation spectrum of zearalenone. Each ^1H slice is taken at the indicated ^{13}C frequency. The 1D ^1H spectrum is shown on the same scale as the individual slices.

agree with those of Cole and Cox (1981) and Ellison and Kontsonis (1976), confirming that assignments for C-2 and -4 and C-3 and -11 (Hanson et al., 1974) are reversed. Similarly, the assignments for DON clarify those in a recently published spectrum (Yoshizawa, 1983) and indicate that C-7 and -11 are reversed. These assignments are consistent with those of related compounds fusaron-X and trichothecin reported by Cole and Cox (1981) but indicate that assignments for C-2 and -4 are reversed in the spectrum of trichothecin (Dockerill et al., 1978), which was corrected to be in accord with ^{13}C biosynthetic incorporation data.

Zearalenone. The proton slices corresponding to individual ^{13}C resonances in the 2D correlation spectrum of zearalenone (Figure 5) resolve the individual ^1H resonances corresponding to each position of the macrocyclic ring. C-8′ and C-9′ were assigned to the only equivalent methylene protons in the spectrum. All other methylene protons of the macrocyclic ring are AB systems.

The 2D correlation spectrum of zearalenone even when complimented with multiplicity determination was insufficient to distinguish between the methylene carbons of the macrocyclic ring. A combination of homonuclear ^1H coupling data (at 400 MHz), the correlation spectrum,

Table III. ^{13}C (62.8 MHz) and ^{1}H (250 MHz) Chemical Shift Assignment for Zearalenone

carbon no.	^{13}C chemical shift[a]	^{1}H chemical shift[a]
1	103.7	
2	161.2	
3	108.7	6.42 ($J_{3,5}$ = 2.3 Hz)
4	165.5	
5	102.6	6.36 ($J_{5,3}$ = 2.3 Hz)
6	144.0	
1′	133.3	6.95 ($J_{1',2'}$ = 15.3 Hz)
2′	132.3	5.62 ($J_{2',4'}$ = 15.3 Hz, $J_{2',3'}$ = 10.3, 3.6 Hz)
3′	30.9	2.30, 2.21
4′	20.9	2.10, 1.46
5′	36.7	2.84, 2.15
6′	212.8	
7′	42.9	2.60, 2.10
8′	22.1	1.70
9′	34.7	1.59
10′	73.4	4.94 ($J_{10',11}$ = 6.1 Hz, $J_{10',9'}$ = 5.8 Hz)
11′	20.6	1.32 ($J_{11',10'}$ = 6.1 Hz)
12′	171.6	

[a] ppm from Me_4Si.

and the pattern of isotopic enrichment observed in the biosynthesis of zearalenone from specifically labeled acetate (Blackwell et al., 1984) was used to assign both ^{13}C and ^{1}H spectra of this molecule (Table III). The $^{1}H/^{1}H$ coupling pattern was confirmed independently by a COSY spectrum. All ^{13}C assignments were confirmed by $^{13}C/^{13}C$ couplings observed in the spectrum of zearalenone biosynthetically enriched from [1,2-^{13}C]acetate. This assignment resolves the discrepancies between two previously published ^{13}C assignments of zearalenone (Mirocha and Pathre, 1979; Cole and Cox 1981), where resonances for positions 1 and 6, 3 and 5, and 2 and 4 are reversed and those of the macrocyclic ring unclear. Differences between the present assignment and that of Mirocha and Pathre (1979) (i.e., reversal of positions 7′ and 5′, 4′ and 8′, 5 and 3, and 2 and 4) are such that it does not negate the coupling pattern that they observed in zearalenone enriched from [1,2-^{13}C]acetate. This is because the coupling constants (J_{CC}) for these systems [C-6′ and C-7′ (J_{CC} = 40 Hz), C-4′ and C-5′ (J_{CC} = 35 Hz), and C-8′ and C-9′ (J_{CC} = 36 Hz)] are similar. Low ^{13}C incorporation, spectral overlap, and insufficient spectral digitization can lead to errors of several hertz in the measurement of J_{CC} values, and it is therefore necessary to have additional methods (i.e., ^{13}C–^{1}H correlations) to determine resonance assignments.

DISCUSSION

The NMR strategy presented herein for the assignment of spectra of fungal secondary metabolites is only one of many possible combinations of NMR pulse experiments that are being developed for use in structure determination and spectral assignment (Hall et al., 1980; Stinson et al., 1982; Rinaldi and Salomon, 1983).

Due to the accumulation and processing time involved with the S/N available, the 2D correlation experiment is limited to the analysis of purified compounds available in sufficient quantities. It is not, therefore, generally suited to the study of secondary metabolites, where sample size maybe a limiting factor. However, the unambiguous chemical shift information that it provides can be used to identify related mycotoxin metabolites from 1D spectra. The 2D spectrum does not provide complete ^{13}C information when the ^{1}H spectrum is insufficiently resolved, and interpretation must take into account the intensity dependence on J_{CH}. The simple spin–echo technique has proven useful in the analysis of ^{13}C NMR spectra of crude fungal extracts from *F. graminearum*. Spectral interpretation is relatively insensitive to variations in J_{CH} across the spectrum, even though the values of J for the trichothecenes range from 125 up to 175 Hz. Since to null a multiplet generally requires a more rigourous matching of individual J_{CH} to the echo delay, the $1/J$ spectrum proves to be the most useful. For a series of compounds, the combination of chemical shift, NOE, and odd vs. even multiplicity information present in this single spectrum is often sufficient to completely assign the spectrum. New metabolites isolated from these extracts may be characterized from the simultaneous ^{13}C chemical shift and multiplicity data provided by the $1/J$ echo delay spectrum, in combination with GC–MS (Greenhalgh et al., 1984b; Blackwell et al., 1984). The application of this and other NMR techniques to biosynthetic studies of fungal secondary metabolites is in progress.

Registry No. DON, 51481-10-8; 3-AcDON, 50722-38-8; 15-AcDON, 88337-96-6; DAS, 2270-40-8; T-2 toxin, 21259-20-1; zearalenone, 17924-92-4.

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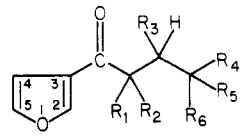
Synthesis and Analysis of Various 3-Furyl Ketones from *Perilla frutescens*

John E. Garst* and Benjamin J. Wilson

Synthesis of 3-cyanofuran from 3-bromofuran and CuCN in *N*-methylpyrrolidone or equilibration of 3-furoic acid with 1,3-dicyanobenzene gave equally good yields. The latter procedure also afforded 2-cyanofuran from 2-furoic acid, but both 3-methyl- and 5-methyl-2-furoic acid gave only methylfuran decarboxylation products. Reaction of either 2- or 3-cyanofuran with isoamylmagnesium bromide gave moderate yields of the respective isoamyl 2- or 3-furyl ketones after imine hydrolysis. 1-(3-Furyl)-2-(phenylselenenyl)-4-methylpentan-1-one, formed quantitatively by reaction of isoamyl 3-furyl ketone with phenylselenenyl chloride, gave excellent yields of isoegomaketone upon H_2O_2 oxidation. Isoegomaketone was selectively deconjugated to egomaketone by successive treatments with potassium *tert*-butoxide in dry *tert*-butyl alcohol and dilute HOAc. The isoegomaketone methanol Michael adduct 1-(3-furyl)-3-methoxy-4-methylpentan-1-one, prepared by stirring with suspended Na_2CO_3 in methanol, was found to be a likely artifact of methanol extraction rather than a true constituent of *Perilla frutescens* as previously reported; the other three 3-furyl ketones have been identified and quantitated.

Acute bovine pulmonary toxicity, caused by ingestion of moldy sweet potatoes containing 4-ipomeanol (1), led



1, $R_1 = R_2 = R_3 = R_4 = H$; $R_5 = CH_3$; $R_6 = OH$
2, $R_1 = R_2 = R_3 = R_4 = H$; $R_5 = R_6 = CH_3$
3, $R_1 = R_2 = H$; $R_3 = R_4$ = olefin; $R_5 = R_6 = CH_3$
4, $R_1 = R_4 = H$; $R_2 = R_3$ = olefin; $R_5 = R_6 = CH_3$
5, $R_1 = R_2 = R_6 = H$; $R_3 = OCH_3$; $R_4 = R_5 = CH_3$
6, R_1 = Br; $R_2 = R_3 = R_4 = H$; $R_5 = R_6 = CH_3$
7, R_1 = PhSe; $R_2 = R_3 = R_4 = H$; $R_5 = R_6 = CH_3$

to structure–toxicity studies of numerous 3-substituted furans in mice (Garst and Wilson, 1981). Those studies revealed a 0.99 correlation coefficient between acute murine toxicity and the 5-furan ^{13}C NMR resonance shift for divergent 3-substituted furans. A good linear relationship was also discovered between acute toxicity and an HPLC measure of the octanol–water partition coefficient of methyl through hexyl 3-furyl ketones. These studies had previously identified perilla ketone (2) as a potent and lung-selective pulmonary toxicant in mice (Wilson et al., 1977, 1978). The widespread mint plant *Perilla frutescens* is a major cause of bovine respiratory toxicity (Wilson et al., 1977, 1978) and is a natural source of 2, egomaketone (3), isoegomaketone (4), and the methyl ether 5 (Ueda and Fujita, 1962; Ito, 1964; Ina and Suzuki, 1971). Since use of the plant as a condiment in Japanese restaurants presents human health questions and existing procedures afforded unsatisfactory quantities of these agents for toxicity evaluation in large animals, a facile, moderate-scale synthesis of these agents was developed from 3-furoic acid.

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MATERIALS AND METHODS

Chemical analysis was performed with a Hewlett-Packard 1084B HPLC and a 10 cm long, 10-μm particle size, RP-8 column from Brownlee Laboratories (Santa Clara, CA). The ultraviolet absorbance signal at 204 nm was integrated for quantitation. Elution was achieved by using 2 mL min^{-1} 37% MeOH–H_2O with a column temperature of 35 °C. Typical elution times are 3.99 min for 5, 4.55 min for 3, and 5.59 min for 4. Perilla ketone elutes near 8 min, making this procedure superior to the following method used earlier for screening of ethereal *P. frutescens* extracts.

Plant analyses were performed with a Waters HPLC and utilized two C-18 Bondapak reverse-phase columns in series and 50% MeOH–H_2O as the elution solvent. Quantitation was achieved by comparing the refractive index signals of plant and authentic materials.

Boiling and melting points (Thomas-Hoover apparatus) are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 257 spectrophotometer, and ^1H NMR spectra were recorded on a JOEL Model JNM-MH-100 or

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