Tetrahedron Vol 45, No 8, pp 2373 to 2383, 1989
Printed in Great Britain

0040-4020/89 $3 00 + 00
Pergamon Press plc

THE NMR SPECTRA OF TRICHOTHECENES AND RELATED FUNGAL METABOLITES

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(*Received in South Africa* 28 *September* 1988)

INTRODUCTION

The first trichothecene, trichothecin was isolated in 1948 by Freeman and Morrison[1] At that time, the sophisticated spectroscopic techniques used today for the characterization of compounds were not available This is especially true of nuclear magnetic resonance spectroscopy (NMR), which only became a commercial reality in the late 1950s The data produced at 60 and later 100 MHz for proton (^{1}H) resonance were limited, comprising mainly of chemical shifts and some coupling constants With the advent in the 1970s of Fourier Transform NMR and superconducting magnets, which enable fields of up to 600 MHz to be achieved, NMR is now the principal technique employed for the determination of the structure of trichothecenes, including their stereochemistry and conformation

During the past two decades, the trichothecenes have emerged as important mycotoxins due to their toxic effects on animals and humans Further interest was stimulated in part, by the heavy contamination of cereal crops in North America with 4-deoxynivalenol in the 1980s and the resulting health hazard[2]

The trichothecenes are mevalonate-derived sesquiterpenes, which have in common a tricyclic ring system. They differ in the degree of oxidation at the ring carbon atoms, C-3, C-4, C-7, C-8 and C-15 and the extent and type of esterification of the hydroxyl moieties. Including the acylated derivatives, there are over 90 compounds in this class In addition,there are a growing number of trichothecene-related compounds which are derived from the common intermediate trichodiene, e g. sambucinol, sambucoin and several apotrichothecene derivatives The structures of these compounds are shown in Figure 1

NMR spectral assignments of proton chemical shifts and coupling constants in the older literature were based on single frequency off-resonance proton decoupling. This technique together with spin-lattice relaxation time measurements and biosynthetic enrichment studies were used to assign the spectra of trichodermin[3], trichodermol[4], and trichothecin[5] The newer techniques for establishing carbon resonance assignments include Distortionless Enhanced Polarization Transfer (DEPT) and ^{1}H/^{13}C heteronuclear correlation spectra (HETCOR) Similarly, for proton assignments, 2D or ^{1}H/^{1}H homonuclear correlated spectra (COSY) are now used as well as nuclear Overhauser effect (NOE) difference spectra Although the ^{1}H and ^{13}C resonances for

nearly all of the trichothecenes have been assigned, not all have been confirmed Caution should therefore be exercised in their use especially for data in the earlier literature

The use of NOE difference spectra has become routine for the determination of the stereochemistry of trichothecenes Corley *et al* [6] were able to confirm the stereochemistry of the α- and β-epimers of 8-hydroxysambucoin by determining the NOE effects between the 14- and 15-methyl groups and neighbouring protons

An application of ^{13}C NMR for the characterization of new structures was demonstrated in the case of sambucinol by Mohr *et al.*[7] Its ^{1}H spectrum showed the absence of a resonance for H-11 However, the ^{13}C spectrum showed an unassigned resonance at 108 ppm Ketals are known to have a resonance in this region and thus it was concluded that sambucinol had a ketal moiety at C-11 Sambucinol and sambucoin represent the first of the trichothecene-related compounds that were isolated Several of the 2D NMR techniques were used recently to determine the structure of sambucinic acid[8]

^{13}C NMR was also used to study the biosynthesis of trichothecolone by *Trichothecium roseum* [4] [2-^{13}C]Mevalonic acid was incorporated in the trichothecenes with enrichment at C-4, C-8 and C-14, indicating the folding pattern of farnesyl pyrophosphate Similarly, the incorporation of [1-^{13}C] and [2-^{13}C]acetate in deoxynivalenol by *Fusarium graminearum* showed ^{13}C coupling between C-5 and C-12, and C-6 and C-15, which confirmed the 1,2-methyl and 1,5-hydride shifts involved in the formation of the trichodiene intermediate[9]

This article examines the use of various ^{1}H and ^{13}C NMR techniques for the characterization of trichothecenes and trichothecene-related compounds (Figure 1), excluding the macrocyclic trichothecenes, which have been well documented by Jarvis *et al* [10] It also discusses the spectral data with a view to correlating the assignments of resonances with specific carbons or protons in the trichothecene molecule

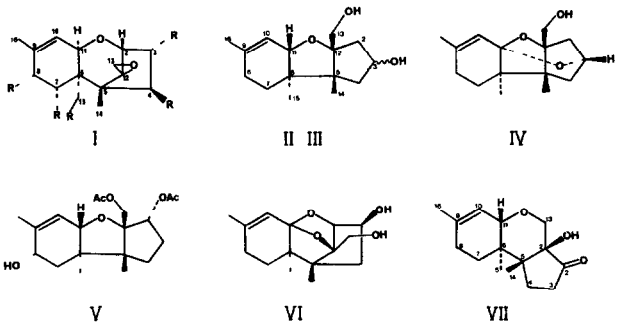

Figure 1 Structures of trichothecene and related compounds, I = trichothecenes, II = 3α,13- dihydroxyapotrichothecene, III = 3β,13-dihydroxyapotrichothecene, IV = 3α,11α-oxy-13- hydroxyapotrichothecene, V = 8-hydroxy-2,13-diacetoxyapotrichothecene, VI = sambucinol, VII = sambucoin

Proton NMR

The structural similarity of the trichothecenes leads to characteristic resonances in their ^{1}H NMR spectra The most obvious being the AB quartet for the 13-methylene protons(2 8-3 2 ppm, J= 4 0 Hz) The 14- and 15-methyl groups give sharp singlet resonances in the range 0 75-1.5 ppm, while the 16-methyl appears as a broad singlet(1.7-1.9 ppm) The resonance of the H-2 protons occurs around 3 6 ppm and a broad signal near 5 5 ppm was assigned to H-10 [11]

Collections of the ^{1}H chemical shift data for trichothecenes have been reported by Bamburg and Strong[12], Cox and Cole[13], Tamm and Mori[14] and Savard et al.[11] and for ^{13}C resonances by Cox and Cole[13,15], Tamm and Mori[14], and more recently by Avent et al[16] In addition, data pertaining to individual trichothecenes can be found in the literature.

The proton chemical shifts of the trichothecenes are summarized in Figure 2 This data is based in part on an earlier study[11] and also on rearranged products[17,18] Although such data is mainly used for identification purposes, it can also provide information about the configuration and conformation of the molecules The absolute configuration of trichothecenes, however, cannot be determined by NMR but the spectra do give the relative configuration of each asymmetric centre in the molecule

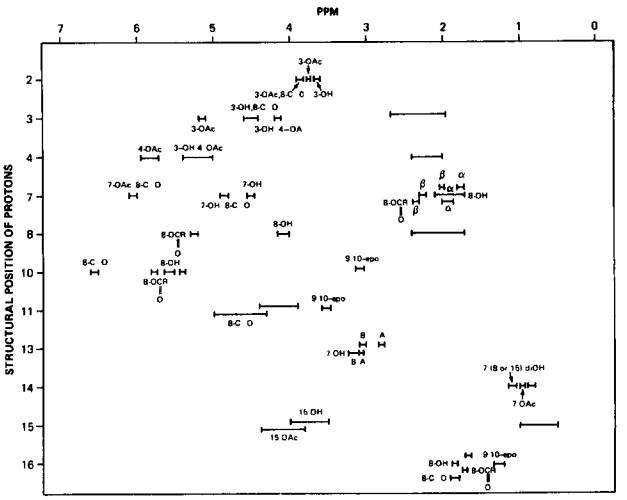

Figure 2 Characteristic ^{1}H chemical shifts for trichothecenes

In the A-ring of trichothecenes when C-7 has two methylenic protons, one is often found to couple with H-11, while the other exhibits some small coupling to the 15-methyl or one of the 15-CH_2OR protons These long-range couplings are only possible if the conformation of the A-ring allows the protons coupling to adopt a "W" conformation relative to each other Hence, the H-7 coupling with H-11 must have an α- and the one coupling to H-15, a ß-configuration Even though these small couplings are not visible for all trichothecenes, one may assume that the conclusions apply to all trichothecenes since all are obtained through the same biosynthetic pathway and thus cyclize with the same configuration

The established configuration of the C-7 protons can be used to determine that of the protons at C-8 When C-8 is hydroxylated, the values of $J_{7,8}$ reveal that all the naturally occurring C-8 hydroxylated trichothecenes have an α-configuration, with the one exception, 8ß-hydroxytrichothecene[19]

Although the $J_{8,10}$ value could be used to define the configuration at C-8, this has been

found to be impractical Whereas an 8α-hydroxylated trichothecene shows H-10 as a doublet of quartets ($J_{8,10}$=0 Hz, $J_{10,11}$=5 5 Hz, $J_{10,16}$=1 6 Hz), an 8β-hydroxylated trichothecene shows H-10 as a doublet of pentets ($J_{8,10}$=$J_{10,16}$=1 6 Hz) However, acylation of an 8α-OH moiety changes the conformation of the A-ring sufficiently for $J_{8,10}$ to be equal to $J_{10,16}$ and the resonance for H-10 also becomes a doublet of pentets.

In the case of the C-ring of trichothecenes, its rigidity dictates that only two dihedral angles, i.e φ= 0° or 120°, are possible between a C-3 substituent and a C-4 substituent. All known 3,4-oxygenated trichothecenes e.g. nivalenol, T-2, DAS, etc, have a $J_{3,4}$ of about 3 Hz and are therefore trans-substituted

The conformation of the trichothecenes is also very important for understanding their biological and chemical properties The preferred conformation has been shown to have the A-ring in a half-chair and the B-ring in a chair conformation [17,18,20,21] NMR evidence for this includes the couplings observed between H-7α/ H-11 and H-7β/H-15, and the effect of a 7α-OH group on the chemical shift of the 13-methylene protons(Fig 2), which are only possible if the B-ring is in the chair conformation.

Of the characteristic resonances, several are also common to the trichothecene-related compounds, such as the apotrichothecenes (APO) **II-IV**, the sambucinols (SAM) **V** and sambucoin (SAC) **VI**

The trichothecenes may be hydroxylated at C-15, unlike the trichothecene-related APO and SAM derivatives isolated to date The latter compounds exhibit a 15-methyl singlet around 0 9 ppm In all cases, the 16-methyl appears as a broad singlet due to coupling with protons at positions C-8, C-10 and C-11

The most important difference between the trichothecenes and the apotrichothecenes lies in the configuration at C-11 In all the APO compounds isolated, H-11 has the β-configuration Trichothecenes and APO compounds derived from the rearrangement of trichothecenes[18,22,23] possess an alpha H-11 This indicates that the APOs isolated from crude fungal extracts are in fact naturally occurring metabolites and are not derived by the rearrangement of trichothecenes, and also, that they are formed by a different oxidized trichodiene intermediate than the trichothecenes

In the spectra of the apotrichothecenes, the multiplets for H-10 and H-11 have the same chemical shift as the trichothecenes but a smaller coupling constant, i e $J_{10,11}$ = 1 7 Hz as opposed to 5 Hz, because of the reversed configuration at C-11. In sambucoin, the oxygen bridge is between C-13 and C-11 as opposed to C-12 and C-11 as in the apotrichothecenes, restoring the 6-membered character of the B-ring but with a reverse configuration at C-11 The resonance of H-10 is moved upfield to 5 18 ppm but retains the small J of about 1 Hz.For those compounds with a ketal moiety at C-11, **IV** and **VI**, the H-10 resonance is only slightly shifted to 5.4 ppm but is still a broad singlet due to long range coupling to CH_3-16 and H-8.

Both the 3α-OH, **II** and the 3β-OH, **III** epimers of apotrichothecene have been isolated[24] Although their proton spectra are similar, it is possible to differentiate between them The

singlet for the 15-methyl, which was identified by its long-range coupling to H-7β, appears at 0 82 ppm for the 3β-OH and at 0.99 ppm for the 3α-OH epimer. Similarly, the 14-methyl appears at 1 07 and 0 92 ppm for the 3β-OH and the 3α-OH epimers respectively.

Another feature of the proton NMR spectrum of the apotrichothecenes is the large AB character of the 13-methylene protons for the 3α-OH epimer as compared to that of 3β-OH epimer This difference may be due to hydrogen-bonding between the 13-OH and the 3α-OH in one case, and between the 13-OH and the ether oxygen in the other, thus creating different environments for each of the H-13 protons. Coupling between H-2β and H-4β is also only observed for the 3α-OH epimer A more detailed analysis of the proton NMR spectra of apotrichothecenes has been reported[24]

Another naturally occurring trichothecene-related compound is 3α,11α-oxy,13-hydroxyapotrichothec-9-ene **IV**) This compound could be produced from 3α-OH APO (**II**) under mild acidic conditions but not from the 3β-OH epimer (**III**) One characteristic of this compound is the extreme rigidity of the caged B- and C- rings, which locks the H-2α and H-4α into a W conformation yielding a relatively large coupling constant of 2 0 Hz. However, this compound still shows the characteristic trichothecene-like resonances for the 14-, 15-, and 16-methyls, 13-methylene protons and the collapsed H-10 resonance typical of the apotrichothecenes The hydroxylated derivative of **II**, 8-OH-2,13-diacetoxyapotrichothec-9-ene (**V**) has been recently characterized[25], lending further credence to the hypothesis that the apotrichothecene derivatives are natural products

Table 1 250 MHz ^{1}H NMR Data for Trichothecene-related compounds (chemical shifts, (J in Hz))

	II	III	IV	V	VI	VII
2α	1 74 (12 2,2 2)	2 23 (14 9,6 3)	2 13 (11 5,2 0)	5 07 (12 1,6 5)		
2β	2 60 (12 2,6 2,1 7)	2 10 (14 9,4 1)	1 44 (11 5,3 8)		3 90	
3α		4 51 (6 3,5 8,6 3, 4 1)		1 85 (m)	4 22 (8 1,4 3)	2 26 (19 9,10 0 7 9)
3β	4 28 (10 2,6 2,2 2 1 3)		4 51 (3 4,1 8,1 8)	2 07 (m)		2 56 (19 9,11 8,3 0)
4α	2 13 (12 9,10 1)	2 45 (14 6,6 4)	2 46 (13 5,2 0,3 4)	2 17 (13.1,6 8)	2 57 (15 1,8 1)	1 74 (13 8,11 7,7 9)
4β	1 67 (12 9,1 7,1 3)	1 38 (14 6,5 8,1 3)	1 16 (13 5,3 0)	1 24 (13 1,6 8)	1 48 (15 1,4 3,0 9)	1 96 (13 4,10 2,3 2)
7α	1 37 (13 1,5 6,2 7)	1 36 (13 5)	1 34 (12 9,6 6,1 6)	1 63 (14 2)	1 34 (13 0,5 3)	1 43 (13 3,5 4,2 3)
7β	1 54 (13 1,9 8)	1 57 (13 5)	1 86 (12 9,5 6,4 4,1)	1 94 (14 2,7 5)	1 93 (13 0,1 7,0 5)	1 56 (13 3)
8α	1 93 (m)	1 99 (m)	1 80		1 80	1 8-1 9
8β	2 03 (m)	1 99 (m)	2 03	4 08 (7 5)	2 02	1 8-1 9
10	5 52 (1 6)	5 50 (1 6)	5 35 (3 3,1 6)	5 68 (1 1)	5 42	5 18
11	4 15 (2 5)	4 11		4 05		3 94
13A	3 18 (11 4)	3 56 (10 9)	3 66 (12 1)	3 82 (11 8)	4 03 (11 2)	3 42 (11 2)
13B	3 73 (11 4)	3 78 (10 9)	3 79 (12 1)	4 39 (11 8)	4 10 (11 2)	4 18 (11 2)
14	0 92	1 07	1 04	0 97	1 04	1 13
15	0 99	0 82	0 90 (0 7)	1 06	0 81 (0 5)	0 63
16	1 63 (2 4,1 2)	1 63	1 67	1 77	1 73	1 64

Sambucinol (**VI**) and sambucoin (**VII**) were first isolated in 1984[7] The ^{1}H NMR spectra of these compounds are described in Table 1 Some unusual characteristics of the sambucinol spectrum, apart from those mentioned previously, are the lack of coupling between H-2 and H-3 and the long-range coupling between H-2 and H-4β Both are indications that the 3-OH has a β-orientation similar to the minor epimer of dihydroxyapotrichothecene The long range coupling between the 15-methyl and H-7β seen in the COSY spectrum also allows

differentiation between the 14- and 15-methyl singlets. The spectrum of sambucoin is also characterized by the large chemical shift difference (0.76 ppm) between the two branches of the CH_2-13 AB system

CARBON 13 NMR

Carbon-13 spectral data provide complementary information to the proton spectrum for determining the structure of these sesquiterpenes While not as informative on long range interactions, i.e. greater than 2-3 bonds, the resolution of the spectrum provides an easily interpreted count of the number of carbon atoms Also when combined with the use of DEPT, it details the multiplicity of the carbon atoms in the molecule and permits identification of the quaternary centres Since the effect of a substituent in the molecule on the chemical shift is largely restricted to the carbon atom to which it is attached, the comparison of related spectra reveals structural elements of compounds even though their proton spectra may be similar In addition, the ^{13}C-spectrum provides unambiguous structural information for the highly oxygenated trichothecenes that may give poor proton spectra Although carbon-13 spectra appear to be sensitive to the configuration of a substituent,(cf compounds **II** and **III**, Figure 5), the relative configuration cannot be determined a priori from the spectrum For this, it is necessary to analyze the NOE effects in the proton spectrum. The consistency of chemical shifts within a series of related compounds is, however, a confirmation of the configuration Such data revealed that hydroxyl groups in trichothecenes are almost always in the α-configuration at positions C-3, C-7 and C-8 but β at C-4

The resonance assignments for some 30 trichothecenes, and the trichothecene-related compounds (**III** to **VII**) are summarized in Table 2. They were largely determined by HETCOR experiments, the proton spectra having been previously assigned When the amount of material was insufficient, comparison to unambiguously assigned spectra and DEPT spectra were used

The HETCOR spectrum of one of the apotrichothecene derivatives (**V**) is shown in Figure 3 Analysis of the COSY spectrum gives the assignments of the two hydroxyl protons at positions 8 and 2, as well as the two methylene AB systems at positions 3 and 4. Long range coupling discriminates between the three methyl resonances at 14, 15 and 16. The HETCOR fixes the assignments of C-14 and C-16, which are adjacent in the carbon spectrum and discriminates between C-3 and C-4 and between C-2, C-8 and C-11. It also shows the exact chemical shift of the protons at positions 3 and 4 which lie under the acetate methyl groups

Table 2 62 8 MHz ^{13}C NMR data for trichothecenes (I) and related compounds (II to VII)

Carbon#	Chemical Shift (ppm from TMS) I	II	III	IV	V	VI	VII
2	78-81	43 5	44 4	42 2	75 7	88.2	216 0
3	68-71 (C4,R=H) 78-81 (C4,R=OH)	72 8	73.4	76 6	31 8	72 5	34 3
4	84-85 (R=OH) 39-41 (R=H)	44 5	45 7	42 7	32 6	45 1	26.8
5	44-49	52 6	54.9	56 0	55 7	51 0	46 4
6	40-44 (C7&8,R=H) 46-48 (C7or8,R=O,OH) 51-52 (C7&8,R=O,OH)	44 4	45.1	47.6	43 9	47 7	37.6
7	27-30 (R=H)	27 7	27.4	28.3	38 5	29 7	27 5
8	71-74 (R=OH) 30-37 (C7,R=H,C8,R=O,OH)	29 6	29 1	28 2	68 8	28 9	27 4
9	136-140	135 5	135 2	141 7	136 1	146 0	135 4
10	137-139 (C8,R=O) 120-124 (C15,R=H) 118-120 (C15,R=H)	121 4	121 1	119 5	122 7	116 3	122 7
11	67-71	81 4	81 1	105 5	82 3	108 1	75.6
12	64-65 5	92 6	95 1	88 7	92 4	93 5	74 6
13	47-48	63 4	65 0	65 3	64 4	59 4	66 4
14	6-8 (C4,R=OH) 10-15 (C4,R=H)	19 4	20 0	14 7	19 9	16 2	14 1
15	61-65 (R=OH) 14-18 (R=H)	17 8	16 2	13 9	16.5	14 9	15 8
16	20-22 (C8,R=H,OH) 15-18 (C8,R=O)	22 5	22 5	22 9	19 5	22 6	22 4

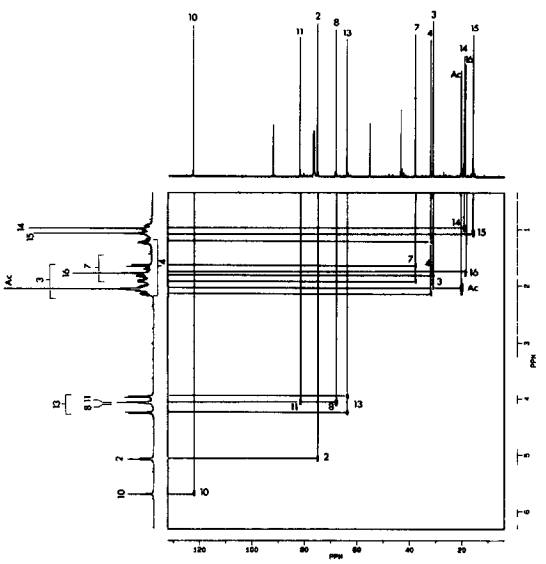

Figure 3 $^{13}C/^{1}H$ correlation spectrum (125/500 MHz) of 8-hydroxy,-2,13-diacetoxyapotrichothecene (V) Single pulse spectra are shown as projections

The assignment of the quaternary carbons in trichothecenes is relatively straightforward The two atoms, C-12 at 65 ppm and C-9 at 135-140 ppm being attached to oxygen and in a double bond respectively, are readily identified Differentiating between C-5 and C-6 resonances can be a problem and assignments have been made on the basis of long range $^{1}H/^{13}C$ coupling to H-4 or H-7 and H-11[26] More recently, selective heteronuclear NOE effects were used to assign these quaternary carbons[15] However, knowledge of the correct assignments for deoxynivalenol and its derivatives, as well as that of the biosynthetic pathway[9] permitted the discrimination between C-5 and C-6 on the basis of isotopic enrichment from specifically labelled acetate for all other trichothecenes The C-5 resonance received enrichment from the [1-^{13}C] acetate and showed $^{13}C/^{13}C$ coupling to C-12, while C-6 was enriched from [2-^{13}C]acetate and showed coupling to C-15. This has also been used to confirm the assignment of the 14- and 15-methyl resonances when a

correlation experiment was not possible (Figure 5).

The $^{1}H/^{13}C$ correlation experiment can also be used to resolve overlapping multiplets in the proton spectrum For example, in the proton spectrum of T-2 toxin, the AB system of the methylene protons at C-7 can be resolved from the methylene and methine groups of the side-chain, although all these protons lie under the acetate methyl singlets(2 0 ppm). The intensity of the epoxide methylene resonances in correlation and multiplicity spectra tends to be strongly reduced due to the large $J_{C,H}$ value of 175 Hz and may be easily missed. For this reason, experiments using the DEPT pulse sequence are to be preferred over those employing INEPT or J-sorting, which are more sensitive to experimental delays

Even HETCOR experiments may lead to erroneous assignments if the proton chemical shifts are not well-defined This resulted in an incorrect assignment for C-7 and C-11 of deoxynivalenol [26], for which H-11 and H-7 overlap (4 80 and 4 83 ppm, respectively) This assignment was corrected by Avent *et al* [16] A 500 MHz proton correlation spectrum of 3-acetyldeoxynivalenol (H-7 at 4 79 ppm, H-11 at 4 66 ppm) has confirmed this result, thus assigning C-11 to the resonance at 70 2 ppm and C-7 at 74.5 ppm

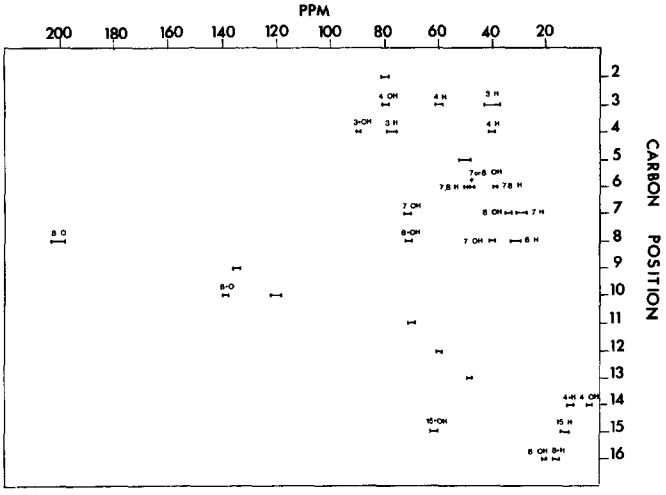

Figure 4 Characteristic ^{13}C chemical shifts for trichothecenes

The structural similarity of the trichothecenes leads to characteristic ^{13}C resonances and some comparative features between them and the trichothecene-related compounds (II - VI) are apparent(Table 2) Since substitution effects on chemical shifts are direct, the effect of changes are highly localized The C-2 resonance occurs at lower field (80 ppm) than the other ring hydroxy methine carbons when it is part of the six membered B-ring Ring strain, as in sambucinol, will shift the resonance to lower field (88 ppm)

Oxygenation of both C-3 and C-4 results in the C-4 chemical shift occurring at higher field, followed by C-2 and C-3 in all cases In general, the C-5 resonance occurs at about 45 ppm and is generally independent of substitution at C-4 When the B-ring is five-membered

as in compounds II-VI, then the C-5 resonance generally shifts downfield to 53-55 ppm In comparison, the C-6 resonance is very sensitive to substitution at positions C-7 and C-8, especially if C-15 is hydroxylated A downfield shift of approximately 2 ppm is introduced on sequential substitution at these positions. The C-7 and C-8 resonances are straightforward, with the exception that the magnitude of the effect of an C-8 hydroxyl moiety on C-7 is less than observed for C-4 when C-3 is hydroxylated As a result, the chemical shift of C-4 is always at about 40 ppm and C-7 at 35 ppm under these conditions

The C-11 to C-13 resonances are very insensitive to structural changes, and indeed the resonance at 48 ±0 5 ppm assigned to C-13 is as specific an indicator of trichothecenes in the carbon spectrum as the epoxide AB system is in the proton spectrum. The C-9 and especially C-10 positions are sensitive to substitution at C-8 and C-15. A ketone at C-8 causes a 15 ppm downfield shift of C-10, due to the conjugation introduced into the ring. In general, acetylation of the tertiary alcohols has a minimal effect on the C-13 chemical shift, while a small effect (1-2 ppm) is seen on acetylation of the primary hydroxyl at C-15.

An additional indicator of substitution at specific positions was observed in the methyl resonances The 16-methyl is relatively insensitive to structural changes unless a ketone is present at C-8, causing a large upfield shift (5-7 ppm) induced by conjugation The 14-methyl resonance is also an indicator of substitution at C-4 Hydroxylation at C-4 causes this resonance to shift upfield to 6-8 ppm The C-13 chemical shift data for the trichothecenes are summarized in Figure 4

Comparison between the trichothecenes and the trichothecene-related compounds (II-VI) reflects the change of the B- ring from a six to a five-membered ring. The lack of the epoxide changes the C-13 chemical shift from 48 to 63-65 ppm and that of C-12 from 65 to 92 ppm A downfield shift of 10-12 ppm is also observed for C-11, and both C-9 and C-10 reflect the absence of substitution in the "A" ring The shifts of C-5 and C-6 are reversed, with C-5 always at lower field Other substitution effects in the rings remained the same The spectra of the 3α-OH and 3B-OH epimers of APO, which have been enriched from [2-^{13}C]acetate are shown in Figure 5 The effects of configuration are clearly visible in the ^{13}C spectrum, with the chemical shift differences maximized at the chiral centre at C-3 The ^{13}C/^{13}C coupling, which assists in the determination of assignments of positions C-15 and C-6, and C-5 and C-12 is also shown in Figure 5

In summary, both the proton and the carbon chemical shift data provide characteristic resonances for the trichothecenes and trichothecene-related compounds The stereochemical data, i e configuration and conformation of both types of compounds is readily derived from the proton spectra using techniques such as COSY and NOE difference spectra. The carbon spectra more readily yield information on the functionality of the carbon atoms in the molecule Correlation of NMR data with structure facilitates the rapid assignment of the structures of new trichothecenes and trichothecene-related compounds

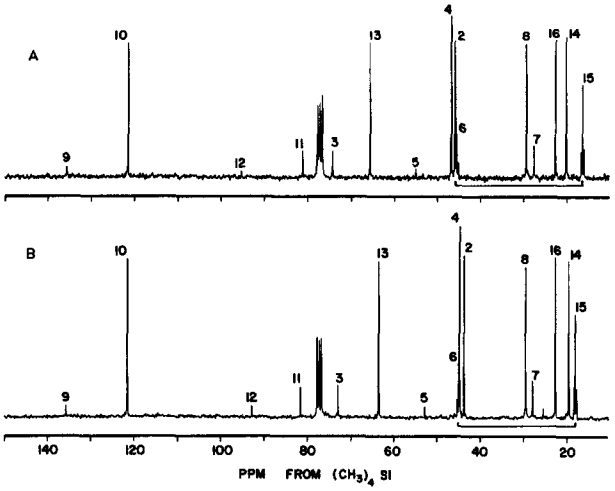

Figure 5 62 8 MHz ^{13}C NMR spectra of (A) 3β-OH and B) 3α-OH epimers of 3,13-dihydroxyepotrichothecene enriched from [2-^{13}C] acetate

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PRC Publication No 1144