Carbon-13 Nuclear Magnetic Resonance Assignments in the Trichothecene Mycotoxins

Robert A. Ellison* and Frank N. Kotsonis

School of Pharmacy, University of Wisconsin, Madison, Wisconsin 53706

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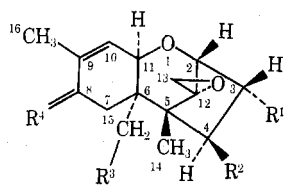
The trichothecenes (Chart I) are a family of at least 29 sesquiterpenes produced by several genera of fungi of which at least one is a notorious plant pathogen.[1] Most of these compounds are also very toxic to mammals and have been implicated in a number of epidemic disease states resulting from the ingestion of moldy foods and feeds.[2]

Chart I



1, R_1 = OAc; R_2 = OAc; R_3 = OAc; R_4 = α-OCOCH$_2$CH(CH$_3$)$_2$
2, R_1 = OH; R_2 = OAc; R_3 = OAc; R_4 = α-OCOCH$_2$CH(CH$_3$)$_2$
3, R_1 = OH; R_2 = OH; R_3 = OAc; R_4 = α-OCOCH$_2$CH(CH$_3$)$_2$
4, R_1 = OH; R_2 = OH; R_3 = OH; R_4 = α-OCOCH$_2$CH(CH$_3$)$_2$
5, R_1 = OH; R_2 = OH; R_3 = OH; R_4 = α-OH
6, R_1 = OH; R_2 = OAc; R_3 = OAc; R_4 = H$_2$
7, R_1 = H; R_2 = OCOCH=CHCH$_3$; R_3 = H; R_4 = 0
8, R_1 = H; R_2 = OCOCH=CHCH$_3$; R_3 = H; R_4 = α-H,β-7,8-epoxy

Owing to the relative complexity of the structure, particularly with regard to the variation in number and positioning of oxygen atoms, these compounds are not always readily distinguished from one another. Consequently carbon-13 magnetic resonance (^{13}C NMR) was expected to be especially valuable in assigning structures. Recently ^{13}C NMR assignments have been proposed for two somewhat different group of trichothecenes.[3a,b]

It is anticipated that functional group variation on such a relatively condensed and interconnected skeleton could make assignments based on analogy or calculation somewhat tenuous. Indeed, examination of the recorded spectra showed that the lower field oxygenated methines in particular were difficult to differentiate. To avoid this problem we relied on a series of molecules (1–6) which permitted systematic and progressive alteration of functionality so as to restrict undesired chemical shift changes. Further, we employed the Birdsall technique[4a,b] to correlate the ^{13}C NMR spectrum of T-2 toxin (2) with that of the established proton spectrum[5,6] in order to provide a secure base from which to assign the series. This approach has resulted in reassignment of several lines proposed in one of the earlier studies.[3a]

Complete assignments for compounds 1–8 are listed in Table I. Because of its availability in quantity, T-2 toxin (2) was selected for Birdsall treatment. It is a member of a five-compound series which is characterized by successive deesterification. Results of the Birdsall plot for 2 are shown in Table II. The proton resonances were obtained from intersects with an error of ±0.25 ppm using nine off-resonance proton decoupling frequencies ranging from −4900 to −6100 Hz. The accuracy of the technique could be gauged by comparing the values obtained for easily assigned carbon atoms such as C-10, C-4′, and C-5′. Positions for the isovaleryl ester of 1–4 were verified by comparison with calculated values[7] and were absent in T-2 tetraol (5). With the chemical shifts of 2 now relatively secure, it remained to examine the other compounds. This was accomplished by placing the resonances in groups according to their multiplicity.

Methyl Quartets. The chemical shift for C-16 in 2 was corroborated by comparison with calculated values[7] which agreed remarkably well (calcd, 19.03 ppm; found, 19.40 ppm). This value remained constant in the series 1–5. On the other hand, the complex influence of the skeleton on chemical shift was evidenced by the lack of agreement between similar calculated values for C-14 and those which were found (13.27 and 6.38 ppm, respectively). While this

Table I
^{13}C NMR Spectra of the Trichothecenes (δ in ppm from Me$_4$Si)

	Carbon atom							
	2	3[h]	4[h]	5	6	7	8	9
Acyl T-2 toxin[a] (1)	76.2 (d)	78.7 (d)	78.2 (d)	47.7 (s)	42.0 (s)	26.7 (t)	67.2 (d)	135.0 (s)
T-2 toxin[b] (2)	78.7 (d)	76.0 (d)	82.4 (d)	48.2 (s)	42.2 (s)	26.7 (t)	67.4 (d)	134.6 (s)
HT-2 toxin[c] (3)	78.7 (d)	79.1 (d)	79.8 (d)	47.7 (s)	41.8 (s)	26.7 (t)	68.9 (d)	134.2 (s)
T-2 triol[d] (4)	78.7 (d)	79.3 (d)	79.3 (d)	47.5 (s)	43.1 (s)	27.4 (t)	68.1 (d)	133.7 (s)
T-2 tetraol (5)	78.5 (d)	79.1 (d)	80.0 (d)	48.0 (s)	45.1 (s)	28.5 (t)	64.8 (d)	138.1 (s)
Diacetoxyscirpenol[e] (6)	79.3 (d)	76.7 (d)	83.1 (d)	49.1 (s)	44.0 (s)	20.6 (t)	27.4 (t)	140.6 (s)
Trichothecin[f] (7)	73.4 (d)	35.8 (t)	78.7 (d)	48.2 (s)	42.6 (s)	41.3 (t)	175.3 (s)	138.8 (s)
Crotocin[g] (8)	74.3 (d)	36.2 (t)	78.5 (d)	47.7 (s)	41.3 (s)	58.1 (d)	50.4 (d)	137.2 (s)

	10	11	12	13	14	15	16
Acyl T-2 toxin[a] (1)	124.0 (d)	66.3 (d)	63.6 (s)	41.4 (t)	6.1 (q)	63.6 (t)	19.4 (q)
T-2 toxin[b] (2)	124.2 (d)	66.3 (d)	64.1 (s)	46.2 (t)	6.4 (q)	63.9 (t)	19.4 (q)
HT-2 toxin[c] (3)	124.6 (d)	67.4 (d)	64.3 (s)	45.3 (t)	6.6 (q)	63.9 (t)	19.4 (q)
T-2 triol[d] (4)	125.1 (d)	66.3 (d)	64.5 (s)	45.3 (t)	6.4 (q)	61.7 (t)	19.4 (q)
T-2 tetraol (5)	121.6 (d)	67.6 (d)	64.3 (s)	45.1 (t)	6.3 (q)	61.0 (t)	19.9 (q)
Diacetoxyacirpenol[e] (6)	119.8 (d)	67.4 (d)	64.5 (s)	46.4 (t)	6.4 (q)	63.4 (t)	22.5 (q)
Trichothecin[f] (7)	137.2 (d)	69.2 (d)	65.4 (s)	46.4 (t)	4.9 (q)	14.4 (q)	17.7 (q)
Crotocin[g] (8)	122.9 (d)	69.4 (d)	65.9 (s)	46.8 (t)	6.2 (q)	15.9 (q)	21.0 (q)

[a] C-1′, 172.2 (s); C-2′, 42.4 (t); C-3′, 25.2 (d); C-4′ and C-5′, 21.7 (q); three acetate methyls, 20.3 (q); three acetate carbonyls, 170.6 (s) and 170.1 (2 s). [b] C-1′, 172.0 (s); C-2′, 42.6 (t); C-3′, 25.0 (d); C-4′ and C-5′, 21.9 (q); two acetate methyls, 20.3 (q) and 20.8 (q); two acetate carbonyls, 170.4 (s) and 169.7 (s). [c] C-1′, C-2′, C-3′, C-4′, and C-5′ same as in *b*; acetate methyl, 20.8 (q); acetate carbonyl, 170.0 (s). [d] C-1′, C-2′, C-3′, C-4′, and C-5′ same as in *b*. [e] Two acetate methyls, 20.6 (q); and two acetate carbonyls, 171.9 (s) and 172.4 (s). [f] C-1′, 166.6 (s); C-2′, 120.7 (m); C-3′, 146.8 (d); and C-4′, 14.8 (q). [g] C-1′, 166.4 (s); C-2′, 120.2 (m); C-3′, 146.5 (d) and C-4′, 14.8 (q). [h] C-3 and C-4 assignments in 1, 3, and 5 could be reversed.

Table II
Birdsall Plot Determined ^{1}H Frequencies Compared to Known ^{1}H Frequencies in T-2 Toxin[a]

Carbon atom	^{1}H frequency, δ[b]		Carbon atom	^{1}H frequency, δ	
	Known	Determined[c]		Known	Determined
C-2	3.41	3.41	C-14	0.58	0.79
C-3	3.85–4.20	4.14	C-15	3.85–4.20	3.70
C-4	5.54	5.28	C-16	1.65	1.74
C-7		2.16	C-2′	2.47	2.58
C-8	5.21	4.75	C-3′		1.62
C-10	5.72	5.82	C-4′ and C-5′	0.87	0.83
C-11	3.85–4.20	3.91			
			Acetate		
C-13	2.88, 2.84	2.58	methyls	1.94, 2.01	1.74

[a] Concentration 600 mg/ml Me_2SO-d_6. [b] δ in parts per million from Me_4Si. [c] Error estimated to be ±0.25 ppm.

might be explained by steric compression,[8] it is difficult to assign an a priori value to this effect in these molecules. As expected, the chemical shift of C-14 also varies very little throughout the series. C-15 appears as a quartet in both **7** and **8** and resonates close to the crotonyl methyl in these compounds. The assignments for each were made on the assumption that the latter would not respond to the change in functionality in ring A although they are clearly reversible.

Methylene Triplets. All of the triplets in **2** were well separated. This reflected their different environments and each was identified by the Birdsall plot. The chemical shift of C-7 varied only slightly in **1–5** but suffered an upfield shift upon loss of the C-8 oxygen in **6**.[9] A shift downfield on going from **6** to **7** is expected for the oxidation at C-8[10] and this triplet is lost in crotocin (**8**). This permits assignment of the 36.24-ppm resonance in **8** to C-3. The resonance for C-13 varies very little across the series **2–8** with the exception of the conversion **1** to **2**, which was unexpected. The chemical shift for C-15 is corroborated by the shielding of 2.2 ppm upon deacylation of **3**[9] as well as conversion to an upfield quartet in **7** and **8**.

Methine Doublets. The difficulty in clearly assigning chemical shifts to the doublets of C-3 and C-11 with the Birdsall plot derives from the overlap of the corresponding proton resonances (Table II). While this may have been resolved by proton double resonance, assignments were reached by examining the effects of structural change. Thus, the doublet at 66.30 ppm in **2** is relatively constant during alteration to the bicyclooctane moiety and is therefore assigned to C-11. Of particular importance is the retention of this doublet upon loss of the C-3 hydroxyl in **7** and **8**.

Among the more difficult assignments are those of C-2, C-3, and C-4, which occur as a group of lower field doublets. While chemical shifts could be assigned in **2** with some confidence, assignment in the remaining molecules required careful attention to progressive changes in the series **1–6**. Given that acylation results in deshielding of the oxygenated carbon and shielding of the penultimate carbon,[9] the doublets at 79.78 and 79.12 ppm in **3** were assigned to C-4 and C-3, respectively. These coalesced to a single resonance at 79.34 ppm in **4**. This confirms the doublet at 78.68 ppm in **2** as being that of C-2 since it would not be expected to be responsive to changes at C-15. The expected shielding of C-2 in the **2** to **1** transition is also evident. The assignments in **5**, where all three doublets again become visible, was more difficult but C-4 was placed at lowest field under the assumption that this atom would be more likely than C-3 to respond to altered functionality at C-8. Similar shifts to those seen in **3** but in the opposite direction allow the lines in **1** at 78.68 and 78.24 ppm to be attributed to C-3 and C-4. Owing to the closeness of the resonances for C-3 and C-4 in **1**, **3**, and **5**, however, it is clear that our assignments for these could easily be reversed. The relative position of C-2 and C-4 is maintained in both **7** and **8** where the loss of hydroxyl at C-3 was expected to have a similar influence on both carbons.

Assignment of C-8 by using only the Birdsall plot is ambiguous owing to the discrepancy between deduced and actual proton chemical shifts at C-8. Although the carbon chemical shifts of C-8 and C-11 in **2** are relatively close, the latter had previously been assigned. Consequently, the lower doublet must be C-8. Further, the shielding (3.32 ppm) of C-8 upon deacylation of **4** is reasonable. In crotocin (**8**), the C-8 doublet moved upfield to become part of the ring A epoxide. The new doublet at 58.12 ppm was assigned to C-7. While no distinction between C-7 and C-8 could be made on the basis of model calculations, the higher field resonance was assigned to C-8 on the basis of comparison with epoxycyclohexene.[11] The assignment of C-8 in **5** is also corroborated by the 3.5-ppm upfield shift in the line attributed to C-10 upon deacylation of **4** which reflects operation of the allyl acetate rule.[12] Close examination of the spectrum reveals small (0.7 ppm) changes in the chemical shifts of C-2, C-3, and C-4 on esterification of **5**. These serve to again call attention to the subtle effects of structural modification on the chemical shifts of distant centers.

The chemical shift of the upfield olefinic carbon in **8** is readily distinguished from that of C-10 by the dramatic deshielding of the latter which occurs on conversion to **7**. The lowest field doublet in **7** and **8** would clearly be expected to be the β-olefinic carbon of the ester.

Quaternary Singlets. The assignments of C-9 are straightforward. Of the remainder, the low-field singlet was attributed to C-12 owing to its relative invariance with functional group alteration. The distinction between C-5 and C-6 was made on the basis of the 1.3-ppm deshielding of the high-field singlet which accompanied deacylation of **3**. The alternate line (C-5) also shifts but to a much lesser extent and in fact is shielded which is not consistent with being β to a hydroxyl function.[9]

Partial relaxed Fourier transform (PRFT) spectra of 2 toxin were obtained by the inversion recovery method using a (−180°–t–90°–T–) sequence. The relative spin-lattice relaxation times (T_1's) of the various assigned carbons were determined by altering t (0.005 → 9.99 sec). At t = 0.005 sec all carbons except C-15 [no signal; therefore, t_1 (C-15) ln 2 ~ 0.005 sec] gave inverted signals, while at t = 1.00 sec all the carbons gave positive signals. However, at an intermediate t (0.200 sec) only the unprotonated carbons (C-5, C-6, C-9, C-12, and the ester carbonyls) and the *gem*-dimethyl carbons of the isovaleroxy side chain gave negative signals. The longer relaxation times observed for the unprotonated carbons[13] and the *gem*-dimethyl carbons[14] were to be expected if our assignments were correct.

These observations, in addition to the general trend in the relaxation times of the other carbons, corroborated our carbon assignments in T-2 toxin.

Experimental Section

All spectra were obtained with a Bruker HX-90E spectrophotometer operating at 22.624 MHz in the pulse Fourier transform mode. Field–frequency stabilization was obtained from the deuterium resonance of the solvent (Me_2SO-d_6). Proton decoupling was accomplished with a broad band modulator of Bruker design. Free induction decay (FID) data were averaged with a Nicolet 1080 computer.

In the case of spectra for the Birdsall plots, 25000 transients of T-2 toxin (600 mg/ml) were averaged before multiplication (TC = −1) of the accumulated FID.

Partially relaxed Fourier transform (PRFT) spectra of T-2 toxin (600 mg/ml) were obtained by the inversion recovery method using a $(-180°-t-90°-T-)_X$ sequence where T (= 15 sec) was greater than $5T_1$ with the FID being accumulated after the 90° pulse. Different t values were employed (0.005–9.99 sec) with X = 1000 (sweeps) before multiplication (TC = −1) of the accumulated FID.

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Registry No.—1, 21259-21-2; 2, 21259-20-1; 3, 26934-87-2; 4, 34114-98-2; 5, 34114-99-3; 6, 2270-40-8; 7, 637-96-7; 8, 21284-11-7.

References and Notes

(1) C. Booth, "The Genus Fusarium", Commonwealth Mycological Institute, Kew, Surrey, 1971.
(2) (a) J. V. Rodricks and R. M. Eppley in "Mycotoxins", I. F. H. Purchase, Ed., American Elsevier, New York, N.Y., 1974, Chapter 9; (b) E. B. Smalley and F. M. Strong, *ibid.*, Chapter 10; (c) A. Z. Joffe, *ibid.*, Chapter 11; D. M. Saito and K. Ohtsubo, *ibid.*, Chapter 12.
(3) (a) J. R. Hanson, T. Marten, and M. Siverns, *J. Chem. Soc., Perkin Trans. 1*, 1033 (1974); (b) W. Breitenstein and C. Tamm, *Helv. Chim. Acta*, **58**, 1172 (1975).
(4) (a) B. Birdsall, N. J. M. Birdsall, and J. Feeney, *J. Chem. Soc., Chem. Commun.*, 316 (1972); (b) N. J. Bach, M. E. Boaz, E. C. Kornfeld, C. J. Chang, H. G. Floss, E. W. Hagaman, and E. Wenkert, *J. Org. Chem.*, **39**, 1272 (1974).
(5) The published spectrum of **2** was taken with carbon tetrachloride as solvent.[6] Although some chemical shift changes were noted in the dimethyl sulfoxide spectrum, these could be reassigned without difficulty.
(6) (a) J. R. Bamburg, N. V. Riggs, and F. M. Strong, *Tetrahedron*, **24**, 3329 (1968); (b) B. K. Tidd, *J. Chem. Soc. C*, 218 (1967).
(7) G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists", Wiley-Interscience, New York, N.Y., 1972, p 38.
(8) Reference 7, p 43.
(9) Reference 7, p 47.
(10) J. B. Stothers, "Carbon-13 NMR Spectroscopy", Academic Press, New York, N.Y., 1972, Chapter 5, Section IIC, p 175.
(11) D. R. Paulson, F. Y. N. Tang, G. F. Moran, A. S. Murray, B. P. Pelka, and E. M. Vasquez, *J. Org. Chem.*, **40**, 184 (1975).
(12) N. J. Bach, M. E. Boaz, E. C. Kornfeld, C. J. Chang, H. G. Floss, E. W. Magaman, and E. Wenkert, *J. Org. Chem.*, **39**, 1272 (1974).
(13) Reference 7, p 7.
(14) A. Allerhand, D. Doddrell, and R. Komorowski, *J. Chem. Phys.*, **55**, 189 (1971).

Organic Reactions at Alumina Surfaces. Elimination Reactions Effected by Dehydrated Chromatographic Alumina

Gary H. Posner* and George M. Gurria

Department of Chemistry, The Johns Hopkins University, Baltimore, Maryland 21218

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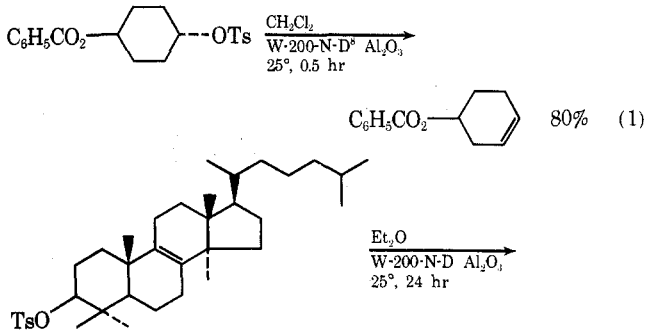
We have reported that stirring solutions of secondary *cyclic* *p*-toluenesulfonate esters over neutral, activity I, Woelm, chromatographic alumina at 25° produces olefins in high yields.[1] The simplicity and mildness of this procedure compare favorably with other methods for overall dehydration of alcohols,[2] and this method has been used recently in other laboratories to prepare some cycloalkenes.[3] Secondary *acyclic* tosylates, however, are converted by activity I alumina to 2:1 mixtures of olefins and alcohols. We reasoned that high temperature, vacuum dehydration of the alumina might form an activated alumina having some useful properties. We report here that Woelm W-200 (Brockmann activity super I),[4] neutral alumina dehydrated at 400° and 0.06 Torr for 24 hr is indeed a mild, effective, new reagent (1) for conversion of *acyclic* secondary tosylates and sulfamates to roughly 10:1 mixtures of olefins and alcohols, (2) for elimination of *cyclic* secondary tosylates to olefins even in presence of normally base and acid labile functionalities, and (3) for elimination of some rearrangement-prone tosylates and sulfamates to olefins *without any rearrangement*. The predominant mechanism of these alumina promoted reactions appears to be an anti elimination, with a syn elimination pathway occurring to some extent.

When 2-octyl tosylate or 2-octyl *N,N*-dimethylsulfamate was stirred in ether at 25° for 24 hr over neutral, dehydrated Woelm W-200 alumina, octenes and 2-octanol were formed in approximately 10:1 ratio and in 70–95% yields; the *cis*-2-octene predominated over the *trans*-2-octene and over the 1-octene.[5]

In contrast to activity I Woelm alumina, which transforms cyclohexyl tosylates to cyclohexenes (~90%) *and* cyclohexanols (~5%),[1] dehydrated W-200 alumina converts cyclohexyl tosylates to cyclohexenes without any detectable trace of cyclohexanols. Thus, 3β-cholestanyl tosylate was converted to 2-cholestene in 83% isolated yield with no cholestanol(s) being formed. This result should be compared with previous alumina-promoted reactions of 3β-tosyloxy sterols, which gave olefin and alcohol mixtures.[6] *p*-Toluenesulfonic acid elimination from *trans*-4-benzoyloxycyclohexyl tosylate illustrates the functional group selectivity of dehydrated W-200 alumina (eq 1).[7] Control experi-



ments establish that the following compounds can be recovered in 70–90% yields after being stirred over dehydrated W-200 alumina for 24 hr at 25°: 1-iodooctane, 2-iodooctane, 5-nonanone, and diethyl dodecanedioate. Thus selective elimination of *p*-toluenesulfonic acid from bifunctional tosylates containing halo, keto, and carboxylic ester groups is practical with dehydrated alumina.

Neopentyl tosylates are known to undergo facile solvolytic rearrangements, and several 3-tosyloxy-4,4-dimethyl sterols suffer 4 → 3 methyl migration when exposed to alu-