Tetrahedron Letters,Vol.27,No.9,pp 1019-1022,1986 0040-4039/86 $3.00 + .00
Printed in Great Britain 

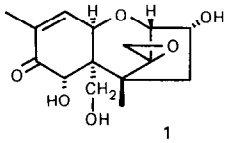
# ALKALINE DEGRADATION OF THE MYCOTOXIN 4-DEOXYNIVALENOL

J. Christopher Young*[a], Barbara A. Blackwell [a] and John W. ApSimon[b]

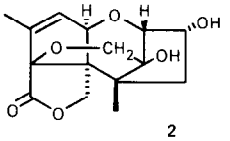
[a] Chemistry & Biology Research Institute, Agriculture Canada, Ottawa, Ontario K1A 0C6
[b] Ottawa-Carleton Chemistry Institute, Carleton University, Ottawa, Ontario, Canada K1S 5B6

Abstract: Treatment of the Fusarium trichothecene mycotoxin 4-deoxynivalenol (1) (DON) with aqueous NaOH at 75°C gave a mixture of three isomeric degradation products designated norDON-A (3a), norDON-B (4a) and norDON-C (5a). Structures and a mechanism for the formation of these products are proposed.

Young[1] recently reported that the Fusarium trichothecene mycotoxin 4-deoxynivalenol (1) (DON, vomitoxin, 3α,7α,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one) rearranges rapidly under mild alkaline conditions to give at least four products, one of which appeared to be the lactone (2) observed by Grove[2]. In this paper, we describe the characterization of and propose a mechanism for the formation of the other three new compounds.



1



2

DON (1) was treated with 0.1 M aqueous NaOH at 75°C for 1 hr and the reaction mixture neutralized and worked up to give a mixture of norDON-A (3a), norDON-B (4a) and norDON-C (5a). For detailed spectral characterization[3], these compounds were converted to their more stable acetates and purified by preparative silica gel thin layer chromatography. Trifluroacetate (TFA) derivatives were also prepared.

The MS of norDON-A (3a) showed a molecular ion at m/z 266, which corresponded to a loss of $CH_2O$ from DON (1). The acetate (3b) and TFA (3c) derivatives gave molecular ions at m/z 392 and 554, respectively, and with appropriate resonances in the $^1H$ and $^{13}C$ NMR spectra indicated that norDON-A (3a) contained three hydroxyl groups. Congruence of UV spectra for norDON-A (3a) (λmax 277 nm) and for $Ac_3$norDON-A (3b) (λmax 247 nm) with those of isoDON (6a) and $Ac_3$isoDON (6b)[4] implied that the A ring chromophores were the same in both sets of compounds. A comparison of the NMR spectra for 3b and 6b (Tables 1 and 2) as well as IR spectra also revealed similarities in the A ring, notably the absence of 7,8 protons and the presence of a methylene group at C-10 indicated with long range coupling to the C-16 methyl. The coupling pattern for the C-2, 3 and 4 protons indicate that the C ring was undisturbed. Major differences are the absence of the C-15 carbon (and its associated protons) and the

disappearance in the $^{1}H$ spectrum of the typical AB (J=4 Hz) system of the epoxide unit. The $^{13}C$ chemical shift for C-12 was upfield at 49.4 ppm from the corresponding position in 6b (65.1 ppm) while the C-13 methylene was downfield (57.8 ppm as compared to 50.0 ppm); the data suggests opening of the epoxide ring. The presence of observable coupling (1.4 Hz) between the C-2 and C-4 positions in the $^{1}H$ spectrum, which is normally not seen in $^{1}H$ spectra of most trichothecenes, is consistent with the steric effects induced by the presence of the ring between positions C-5, 6 and 12. Taken together, the data support structure 3b for $Ac_3$norDON-A and thus 3a for norDON-A.

The MS of norDON-B (4a) also showed a molecular ion at m/z 266. Molecular ions at m/z 434 and 650 for the acetate (4b) and TFA (4c) derivatives, respectively, coupled with the presence of four acetate methyl groups in the 'H NMR spectrum were indicative of four hydroxyl groups. Rapid reaction of norDON-B (4a) with aqueous sodium periodate[5] showed that at least two of the hydroxyls were vicinal. For the tetraacetate 4b, only ester carbonyls (1740 $cm^{-1}$) were observed in the IR and the strongly conjugated enone band was absent in the UV. The presence of an aromatic system was suggested by absorptions at 1595 $cm^{-1}$ in the IR and weak bands at 274 and 279 nm in the UV[6] and aromaticity was further supported by NMR data (Tables 1 and 2) for this compound. Two adjacent aromatic protons were indicated by a pair of doublets (J=7.6 Hz) at 6.71 and 6.79 ppm. The $^{13}C$ chemical shifts in the 119-150 ppm region were indicative of a single substituted benzene system. Assignments made with the aid of empirical additivity rules for the calculation of $^{13}C$ chemical shifts in substituted benzenes[7] were in good agreement (ave. deviation 2.1 ppm) with those observed. Other features of the $^{1}H$ NMR spectrum included the presence of one aromatic and one aliphatic methyl group, an oxygenated methylene and a CH(OAc)-CH(OAc)-$CH_2$ system. The remaining $^{13}C$ resonances were rationalized after comparison with the corresponding spectra for the Fusarium metabolites apotrichothecene (7)[8] and sambucinol (8)[9,10] (cf. Table 2). Excellent agreement was observed for the C ring C-3, 4, 5 and 12 resonances as well as for the C-13 methylenes and C-14 methyls. On the basis of this evidence, structure 4b was assigned to $Ac_4$norDON-B and thereby 4a to norDON-B.

Observation of a molecular ion at m/z 266 for norDON-C (5a) confirms that this rearrangement product is isomeric with the other two. A trihydroxy structure was initially indicated by molecular ions at m/z 394 and 554 for the acetate (5b) and TFA derivatives (5c), respectively, as well as by the $^{1}H$ (Table 1) and $^{13}C$ (Table 2) NMR data for the acetate. In addition, a weak IR band at 3580 $cm^{-1}$ was suggestive of a single free hydroxyl group in the acetate derivative. Vicinal hydroxyls were indicated in norDON-C upon rapid reaction with aqueous sodium periodate. The IR and UV spectra for 5b and 4b were virtually identical suggesting similarities in the A ring portions of the molecules. An aromatic system was also indicated by both $^{1}H$ and $^{13}C$ NMR resonances (Tables 1 and 2), with two aromatic protons and six aromatic carbons clearly discernable; empirical $^{13}C$ shift calculations again gave very good agreement (average deviation 2.5 ppm) between calculated and observed. Other similarities between the proton and carbon resonances for 5b and 4b were noted, especially for the C-2 to C-4 portions of the molecules. The remaining NMR resonances were rationalized after comparison with those of the Fusarium metabolite sambucoin (9)[9,10] (cf. Table 2). Excellent agreement was observed for atoms about the B and C ring junctions, particularly for the C-5, 12, 13 and 14

resonances. Structure 5b, with the carbon skeleton of sambucoin (minus C-15) for $Ac_3$norDON-C is consistent with these spectral data; structure 5a for norDON-C follows. The formation of a tri- rather than tetraacetate may be due to steric hindrance about the C-12 hydroxyl. Mohr et al.[10] reported that sambucoin (9), with a tertiary hydroxyl, was not acetylated under conditions used in this study and Grove[2] observed that the tertiary hydroxyl in lactone 2 was unreactive to acetic anhydride and pyridine.

A proposed mechanism for the rearrangements of DON and isoDON to the observed compounds is shown in the scheme. Kinetic data[1] suggested that DON is converted relatively slowly to isoDON, which then undergoes rapid degradation. This degradation involved loss of C-15 as formaldehyde[11] via a retroaldol rearrangement; the enolate anion then proceeds simultaneously to norDON-A (3a) via one route and norDON-B (4a) and norDON-C (5a) via another.

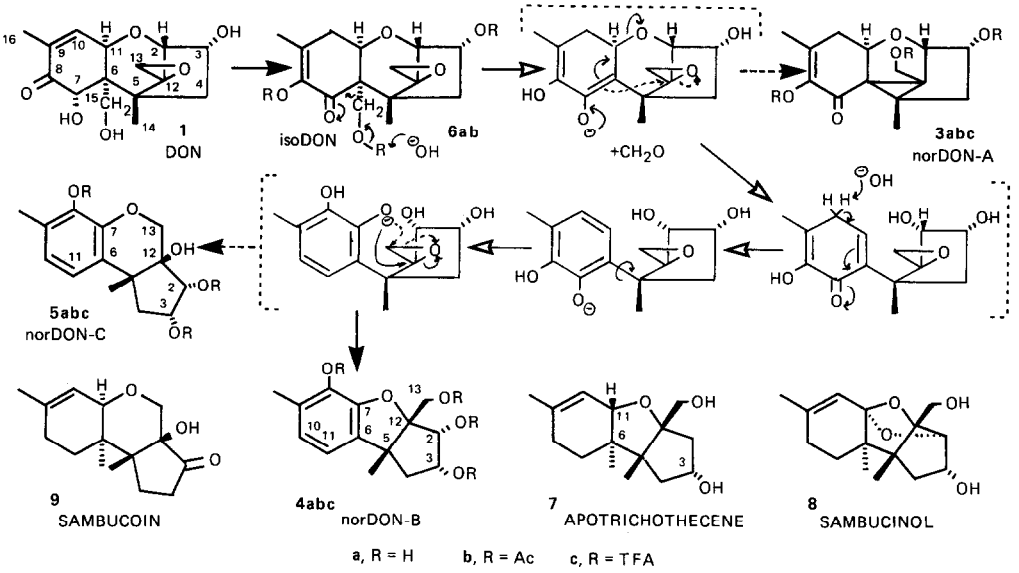



Table 1. $^1$H NMR Spectra of Acetylated DON Rearrangement Products (Chemical Shifts, $\delta$)

| Hydrogen no. | $Ac_3$norDON-A (3b) | $Ac_4$norDON-B (4b) | $Ac_3$norDON-C (5b) |
|---|---|---|---|
| 2 | 4.65 (dd,1H,$J_{2,4}$=1.4,$J_{2,3}$=3.4 Hz) | 5.20 (d,1H,$J_{2,3}$=4.2 Hz) | 5.39 (m,1H) |
| 3 | 4.74 (m,1H,$J_{3,2}$=3.4,$J_{3,4}$=9.0,9.1 Hz) | 5.27 (m,1H) | 5.38 (m,1H) |
| 4 | 1.86, 2.34 (m,2H,$J_{4,2}$=1.4, $J_{4,3}$=9.0,9.1,$J_{AB}$=15.4 Hz) | 2.0, 2.27 (m,2H) | 2.2, 2.63 (m,2H, $J_{4,2}$=1.2,$J_{4,3}$=7.5, $J_{AB}$=15.0 Hz) |
| 10 | 2.59, 2.79 (ABXY, $J_{AB}$=16.6, $J_{10,11}$=7.1,9.0,$J_{10,16}$=1.4 Hz) | 6.71 (d,1H,$J_{10,11}$=7.6 Hz) | 6.81(d,1H,$J_{10,11}$=8.1 Hz) |
| 11 | 4.58 (dd,1H,$J_{11,10}$=7.1,9.0 Hz) | 6.79 (d,1H,$J_{11,10}$=7.6 Hz) | 6.97 (d,1H,$J_{11,10}$=8.1 Hz) |
| 13 | 4.44, 4.56 (AB,2H,J=12.2 Hz) | 4.19, 4.32 (AB,2H,J=12.0 Hz) | 4.08,4.18(AB,2H,J=1.8 Hz) |
| 14 | 1.45 (s,3H) | 1.42 (s,1H) | 1.35 (s,1H) |
| 16 | 1.86 (d,3H,$J_{16,10}$=1.3 Hz) | 2.11 (s,3H) | 2.12 (s,3H) |
| Ac | 2.01, 2.09, 2.20(s,3x3H) | 1.67, 2.02, 2.08, 2.30 (s,4x3H) | 1.93, 2.05, 2.31(3,3x3H) |

Table 2. $^{13}C$ NMR Spectra of Acetylated DON Rearrangement Products (Chemical Shifts, $\delta$)

| Carbon no. | 3b | 6b | 4b | 7 | 8 | 5b | 9 |
|---|---|---|---|---|---|---|---|
| 2 | 86.0 | ( 78.9)[4] | 73.7 | | (88.2)[9] | 77.4 | |
| 3 | 76.5 | ( 71.1) | 72.2 | (72.1)[8] | (72.5) | 67.9 | |
| 4 | 36.2 | ( 41.0) | 44.1 | (45.4) | (45.1) | 40.9 | |
| 5 | 43.4 | ( 45.6) | 52.6 | (45.1) | (51.0) | 46.9 | (46.9)[9] |
| 6 | 51.5 | ( 54.5) | 130.8 | | | 129.3 | |
| 7 | 185.5 | (190.0) | 149.6 | | | 144.5 | |
| 8 | 137.7 | (141.1) | 135.8 | | | 137.9 | |
| 9 | 143.2 | (141.9) | 133.0 | | | 129.8 | |
| 10 | 34.9 | ( 35.0) | 119.1 | | | 123.6 | |
| 11 | 75.9 | ( 70.7) | 122.9 | | | 124.4 | |
| 12 | 49.4 | ( 65.1) | 94.8 | (95.1) | (93.5) | 76.6 | (74.6) |
| 13 | 57.8 | ( 50.0) | 62.7 | (65.0) | (59.4) | 68.8 | (66.4) |
| 14 | 13.3 | ( 13.9) | 15.6 | (16.2) | (16.2) | 15.5 | (14.1) |
| 16 | 17.7 | ( 17.6) | 23.0 | | | 24.5 | |
| Ac $CH_3$ | 20.1, 20.7, 20.8 | | 20.2, 20.3 20.5 20.6 | | | 20.3, 20.3, 20.5 | |
| Ac C=O | 168.5, 170.7, 170.8 | | 168.1, 170.4, 170.5, 170.8 | | | 168.8, 169.8, 170.3 | |

( ) Selected $^{13}C$ NMR shifts for comparison.

References and Notes:


1. J.C. Young, Submitted to J. Agric. Food Chem. (1985).
2. J.F. Grove, J. Chem. Soc. Perkin Trans. I 1731 (1985).
3a. Electron impact gas chromatography-mass spectrometry (GC-MS) on a Finnigan MAT 312 or 4500-B spectrometer and $^{1}H$ and $^{13}C$ NMR spectrometry on a Bruker 250 MHz spectrometer under conditions described by B.A. Blackwell, R. Greenhalgh, A.D. Bain, J. Agric. Food Chem. 32, 1078 (1984). Chemical shift assignments were confirmed by $^{1}H$ homonuclear correlation (COSY) spectra and by $^{13}C$ DEPT spectra.

b. Spectral data:
3a MS m/z 266 ($M^+$, 19%), 95 (100).
3b $R_f$ EtOAc-hexane (1:1) 0.37; IR (KBr) 1780sh, 1740, 1670 $cm^{-1}$; UV (MeOH) 247 nm; MS 392 ($M^+$,3), 43 (100); $^{1}H$ NMR (Table 1); $^{13}C$ NMR (Table 2).
3c MS 554 ($M^+$, 100).
4a MS 266 ($M^+$, 100).
4b $R_f$ 0.59; IR 1740, 1595 $cm^{-1}$; UV 230 (ε1550), 274 (ε1700), 279 nm (ε1720); MS 434 ($M^+$, 7), 392 (100); $^{1}H$ NMR (Table 1); $^{13}C$ NMR (Table 2).
4c MS 650 ($M^+$,100).
5a MS 266 ($M^+$, 80), 175 (100).
5b $R_f$ 0.53; IR 3580w, 1740, 1595 $cm^{-1}$; UV 230 (ε1650), 273 (ε1250), 278 nm (ε1290); MS 392 ($M^+$, 16), 350 (100); $^{1}H$ NMR (Table 1); $^{13}C$ NMR (Table 2).
5c MS 554 ($M^+$, 100).
Compounds 3b, 4b and 5b gave satisfactory high resolution MS results for the molecular ion.

4. R. Greenhalgh, J. Gilbert, R.R. King, B.A. Blackwell, J.R. Startin, M.J. Shepherd, J. Agric. Food Chem. 32, 1416 (1984).
5. R.R. King, R. Greenhalgh, B.A. Blackwell, J. Agric. Food Chem. 32, 72 (1984).
6. UV absorption at 273 (ε2420) and 279 nm (ε2270) for 1-acetoxy-2-methoxy-4-propenylbenzene. J.G. Grasselli, Atlas of Spectral Data and Physical Constants for Organic Compounds, CRC Press, Boca Raton FL, p B-245 (1973).
7. F.W. Wehrli, T. Wirthlin, Interpretation of Carbon-13 NMR Spectra, Heyden, London, pp 45-47 (1978).
8. B.A. Blackwell, Chemistry & Biology Research Insitute, Agriculture Canada, Ottawa, Ontario, unpublished data (1985).
9. From Blackwell[8]; Chemical shift assignments as in Mohr et al[10].
10. P. Mohr, C. Tamm, W. Zurcher, M. Zehnder, Helv. Chim. Acta 67, 406 (1984). $^{13}C$ NMR spectra for 8 and 9 were reported using a different spectrometer and solvent.
11. R. Greenhalgh, B.A. Blackwell, J.R.J. Paré, J.D. Miller, D. Levandier, R.-M. Meier, A. Taylor, J.W. ApSimon in Proceedings of Sixth International Symposium on Mycotoxins and Phycotoxins, Pretoria, South Africa, 22-25 July 1985, P. Steyn Ed., Elsevier, March 1986. The loss of $CH_2O$ has also been postulated to explain the M-30 ion in the MS of isoDON (6a).


Chemistry and Biology Research Institute Contribution No. 1586.

(Received in USA 7 November 1985)