the solvent was removed under vacuum and the remaining material was subjected to column chromatography to isolate the products.

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Conformational Effects in Trichothecenes: Structures of 15-Hydroxy C4 and C8 Ketones

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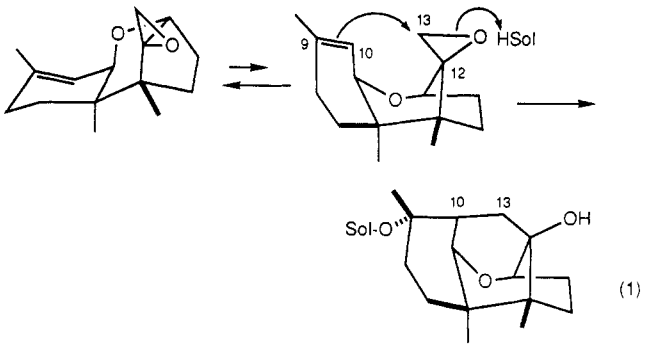
The trichothecene complex of antibiotics constitutes an important group of mycotoxins whose biological activities have attracted a good deal of attention because of their potency and diversity.[1] Interest remains high both in the synthesis[2] and chemical reactivities[3] of the trichothecenes. The chemical reactivity of trichothecenes is particularly rich because of their ability to undergo a variety of intramolecular rearrangements.[1a,3,4]

The course of these rearrangements is strongly influenced by the conformation of this ring system. In particular, the rate of the trichothecene-10,13-cyclotrichothecene rearrangement (eq 1) is increased tremendously when the tetrahydropyranyl B ring assumes the boat form.[5] Although the B ring has a large preference



(1)

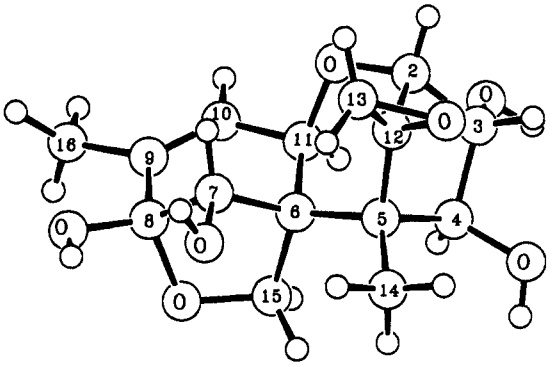


Figure 1. Ball and stick diagram for **1a**. The drawing was labeled and a laser printer file prepared by the PLOTMD[9] program.

for the chair form in typical trichothecenes, this preference can be strongly influenced by bonding and nonbonding interactions in this ring system. Herein, we report the structures of the hemiketals of trichothecenes derived from the intramolecular addition of the C-15 hydroxyl group with the C-8 carbonyl group of nivalenol (**1**, NIV) and with the C-4 carbonyl group of 15-hydroxyscirpene-4,8-dione (**2**). The introduction of the 8,15- and 4,15-hemiketal linkages in these compounds has a marked effect on the conformational mobility of the trichothecene ring system.

Nivalenol (**1**, NIV) is one of the more common naturally occurring trichothecenes and is produced by several species of *Fusarium* associated with grains found worldwide, but especially in Japan.[6] The spectral data[7] for NIV are entirely consistent with the structure illustrated in **1**. It therefore was somewhat unexpected that a single-crystal X-ray diffraction analysis of a single crystal of NIV showed that NIV had crystallized from MeOH–H_2O in the hemiketal form **1a** (Figure 1). The preference for **1a** in the crystalline form may be due either to a kinetic effect of the crystallization of **1a** over that of **1** or to a lower crystal lattice energy for **1a**, compared to that of **1**.

Since none of the published NMR data suggested that NIV in solution exists in any form other than **1**, we have scanned the ^{13}C NMR spectrum of NIV to see if there is any indication of the presence of **1a**, but we found NIV to have limited solubility, and therefore it is not suitable for the detection of minor amounts of **1a**. The only solvent we found in which NIV was sufficiently soluble was DMSO-d_6. In this solvent, NIV exists as a mixture of 82% **1** and 18% **1a** as shown by integrating the ^{13}C signals whose chemical shifts for C-8 and C-10 in **1** are δ 200.0 and 138.0, respectively, and in **1a** are δ 103.8 and 120.7, respectively.

Since NIV is too insoluble to determine this equilibrium in other solvents, we studied the equilibrium for deoxynivalenol (DON, **3**) a mycotoxin which is more commonly encountered than NIV, especially in North America.[1b] In the present study, ^{13}C NMR spectra were recorded in DMSO-d_6, $(CD_3)_2CO$, and CD_3OD, and these data along with the published values obtained in $CDCl_3$[8] are given in Table I. The two signals which are most diagnostic for

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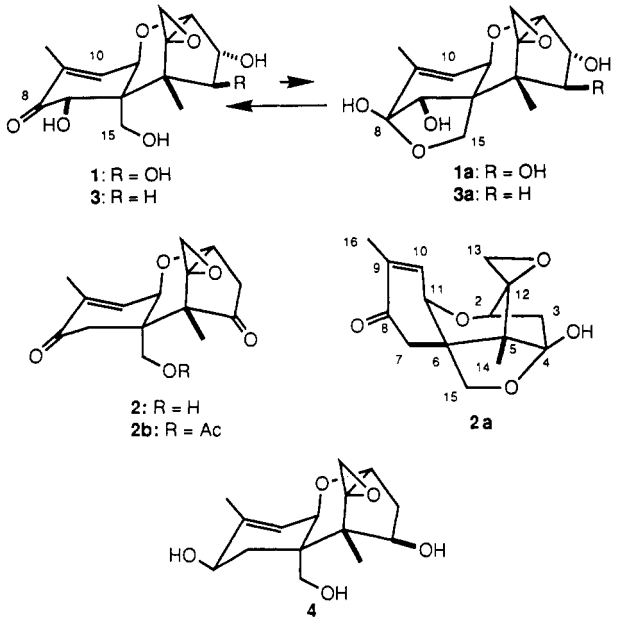

the presence of the hemiketal are those of carbons 8 and 10. The chemical shifts of these carbons in the keto forms **3** are ca. 200 and 140 ppm, respectively, compared to ca. 100 and 120 ppm, respectively, in the hemiketal form **3a**. To determine the relative amounts of these two forms in the three solvents, signals corresponding to the same carbon in both forms were integrated where these resonances could be identified and where they were sufficiently resolved to permit integration. It was thus determined that DON exists in the hemiketal form to the following extent: ca. 10% in DMSO-d_6 and ca. 6% in $(CD_3)CO$. No evidence of hemiketal formation was found in CD_3OD.

Whiting and Edward[10] found that the degree of intramolecular cyclization of hydroxy ketones to hemiketals did not correlate well with solvent polarity though there was a pronounced preference for the open forms in water. For DON, we could detect none of the form **3a** in CD_3OD, which is the most water-like of the solvents studied. MM2 calculations[11] gave an energy difference of ca. 2.5 kcal/mol between **1** and **1a** and **3** and **3a**, with the open forms favored in both cases.

In order to determine the preference for the C-15 hydroxyl to form a hemiketal with C-8 or C-4, 15-hydroxyscirpene-4,8-dione (**2**) was synthesized from 8β-hydroxyverrucarol (**4**) by treatment with activated manganese dioxide. Manganese dioxide normally is selective in its oxidation of allylic alcohols but was reported to also selectively oxidize verrucarol to the corresponding C-4 ketone without oxidation at C-15.[12] A proton NMR spectrum of the first-formed product **2a** of this oxidation exhibited the typical AB signals for the 12,13-epoxide group (ca. δ 3), the C-7 protons (ca. δ 2.5), and the C-15 protons (ca. δ 4). The C-4 proton was absent, and the C-3 proton showed a coupling pattern consistent with the absence of a proton at C-4. Although the C-10 vinyl proton had moved downfield (δ 6.60) as expected, it had a significantly lower $J_{10,11}$ coupling constant (2.5 Hz versus the usual 4–5 Hz). The initial ^{1}H NMR spectrum showed ~5% of another compound **2**, and upon standing in $CDCl_3$ solution, this minor compound increased in concentration until after about 2 h an equilibrium mixture of **2a** and **2** (2:1) had formed. The minor compound exhibited resonances consistent with those expected for diketone **2**: δ 6.46 (1 H, dd, $J_{10,11}$ = 5 Hz and $J_{10,16}$ = 1.3 Hz, H-10), 4.40 (1 H, d, $J_{10,11}$ = 5 Hz, H-11), and 4.20 (1 H, d, $J_{2,3\beta}$ = 4 Hz, H-2). Although the ^{13}C NMR spectrum of this mixture was complex, there was a unique signal at δ 115.2 that can be assigned to the hemiketal C-4 in **2a**. Acetylation of this mixture gave a single monoacetate whose ^{1}H NMR spectrum was that expected for **2b**.

The structure of **2a** was established unambiguously by single-crystal X-ray diffraction analysis. The most notable feature of this structure is that the tetrahydropyranyl ring (B ring) assumes a boat conformation (Figure 2). Molecular mechanics calculations (MM2)[11] show that when C-4 and C-15 are linked by an ether bridge, the boat conformation of the B ring is favored by ca. 2 kcal/mol over the chair conformation. Since it is the boat conformation that is required in order for trichothecenes to undergo the 10,13-cyclotrichothecene rearrangement reaction,[3] these results suggest that the rate of this rearrangement may be greatly accelerated by suitable structural modifications of the trichothecenes.[5]

Table I. ^{13}C Chemical Shifts of DON (3)[a]

position	$CDCl_3$[7b]	CD_3OD[b]	Me_2CO-d_6	DMSO-d_6
2	80.6	82.3	81.6	80.2
3	68.6	69.7	69.3	67.8
4	43.0	44.7	44.4	43.7
5	46.0	47.1	46.5	45.4
6	52.1	53.6	53.0	51.8
7	70.2	71.5	70.6	69.3
8	202.3	201.8	200.7 (104.7)[c]	200.2 (104.0)[c]
9	135.7	137.0	135.6	134.8
10	138.5	139.5	139.7 (122.0)[c]	138.3 (121.0)[c]
11	74.4	75.9	75.4	74.5
12	65.7	66.9	66.5	66.0
13	47.2	48.1	47.5	46.7
14	13.9	14.8	14.5	14.5
15	61.4	61.9	61.5	60.1
16	14.9	15.4	15.2	15.1

[a] Referenced to TMS at 0 ppm; reported for keto form **3** only. [b] Hemiketal form not observed. [c] Chemical shift values for hemiketal form **3a**.

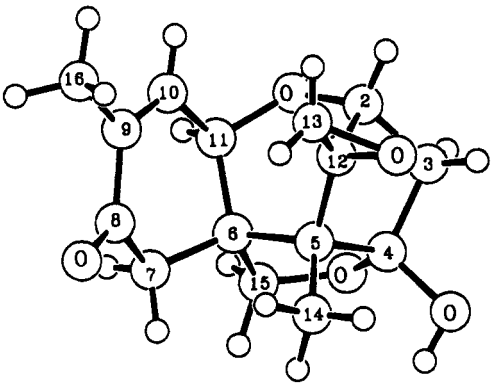

Figure 2. Ball and stick diagram for **2a**. The drawing was labeled and a laser printer file prepared by the PLOTMD[9] program.

Experimental Section

Melting points are uncorrected. NMR spectra were recorded at 200 or 400 MHZ (^{1}H) and 50 or 100 MHz (^{13}C). Proton spectral assignments were made using single-frequency decoupling experiments and comparison to literature data. Carbon-13 assignments were made using INEPT or APT analyses and comparison of chemical shift data to those in the literature. Thin-layer chromatography (TLC) was performed on glass-supported silica gel 60 F254 (0.2 mm). Visualization was accomplished by spraying

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with vanillin/sulfuric acid. A Chromatotron (Harrison Research Laboratories) Model 7924 was used for preparative TLC with the plates prepared according to the instruction manual supplied by the manufacturer in thicknesses of 1, 2, or 4 mm using E. Merck 60 F254 silica gel on circular glass disks. DON and NIV were purchased from Research Foods Limited, Downsview, Ontario, Canada.

4,15-Hemiketal Form of 15-Hydroxytrichothec-9-ene-4,8-dione (2a). A mixture of 100 mg (0.36 mmol) of 8β-hydroxyverrucarol[13] and 800 mg of activated manganese dioxide[13] in 10 mL of acetone was stirred at reflux for 1 h. The reaction mixture was allowed to cool and filtered, and after the residue was washed with several small portions of acetone, the combined filtrates were concentrated. The residue was purified by preparative TLC (Chromatotron: silica, 2% methanol in dichloromethane) followed by crystallization from acetone to yield 65 mg (65%) of white crystalline **2a**: mp 170–172 °C; IR ($CHCl_3$) 3560, 1735, 1670, 1350 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75 (3 H, s, H-14), 1.78 (3 H, dd, J = 2.5, 2.0 Hz, H-16), 2.20 (1 H, dd, J = 16 and 4 Hz, H-3β), 2.59 and 2.39 (1 H each, AB, J = 16 Hz, H-7), 2.52 and 2.90 (1 H each, AB, J = 4 Hz, H-13), 2.31 (1 H, d, J = 16 Hz, H-3α), 3.78 and 3.95 (1 H each, AB, J = 14 Hz, H-15), 3.92 (1 H, d, J = 4 Hz, H-2), 4.55 (1 H, dd, J = 2.5, 2.0 Hz, H-11), 6.60 (1 H, dq, J = 2.5, 2.5 Hz, H-10); high-resolution mass spectrum (EI, 70 eV) calcd for $C_{15}H_{18}O_5$ (M^+) 278.1199, found 278.1154.

15-Acetoxytrichothec-9-ene-4,8-dione (2b). A 1:1 mixture of acetic anhydride and pyridine (0.1 mL each) was added to 10 mg of a 2:1 mixture of **2a** and **2**, and the resulting solution was left at room temperature overnight. Solvents were evaporated under a stream of nitrogen to yield **2b** as a glass, which was purified by preparative TLC (20% EtOAc/hexane): IR (CH_2Cl_2) 1745, 1685, 1370, 1055 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.80 (3 H, s, H-14), 1.82 (3 H, dd, J = 1.3, 1.0 Hz, H-16), 1.95 (3 H, s, acetate CH_3), 2.05 and 2.93 (1 H each, AB, J = 14 Hz, H-7), 2.45 (1 H, dd, J = 16, 2 Hz, H-3β), 2.90 (1 H, d, J = 16 Hz, H-3α), 2.70 and 3.25 (1 H each, AB, J = 4 Hz, H-13), 3.88 and 4.12 (1 H each, AB, J = 11.6 Hz, H-15), 4.18 (2 H, m, H-2 and H-11), 6.45 (1 H, dd, J = 4, 1.3 Hz, H-10); ^{13}C NMR ($CDCl_3$) 75.8 (C-2), 37.1 (C-3), 211.8 (C-4), 54.1 (C-5), 47.8 (C-6), 41.5 (C-7), 195.8 (C-8), 139.2 (C-9), 136.3 (C-10), 67.6 (C-11), 64.7 (C-12), 49.5 (C-13), 5.4 (C-14), 63.6 (C-15), 15.5 (C-16), 20.4 (CH_3COO), and 169.9 (CH_3COO).

X-ray crystallographic analysis of 1a: $C_{15}H_{12}O_5H_2O$, FW = 330.3, 0.18 × 0.24 × 0.32 mm colorless crystal, Nicolet R3m diffractometer, λ(Cu Kα) = 1.541 84 Å (incident beam graphite monochromator), orthorhombic space group $P2_12_12_1$, a = 6.730 (1) Å, b = 14.143 (3) Å, c = 15.482 (2) Å, V = 1473.7 (4) Å^3, Z = 4, ρ = 1.49 g cm^{-3}, μ = 9.8 cm^{-1}, $F(000)$ = 704, T = 295 K. Lattice parameters from 25 reflections in the range of $27 \le 2\theta \le 76°$, data collection range of $-7 \le h \le 2$, $0 \le k \le 16$, $0 \le l \le 18$, max sin θ/λ = 0.59 Å^{-1}, 2θ–θ scan with variable speed of θ = 8–30 deg min^{-1}, 2θ scan range = $[2\theta(K_{\alpha 1}) - 1.0]$ to $[2\theta(K_{\alpha 2}) + 1.0]$, 3 standard intensities monitored every 60 data, maximum change in standard intensities of 2.6%, 1495 unique reflections, 1399 with $F_o \ge 3\sigma(F_o)$, R_{sym} for equivalent reactions = 0.01. All calculations done with the SHELXTL[15] package on a CRAY-X/MP computer, structure solved with direct methods, refinement by full-matrix least-squares, $\sum w(|F_o| - |F_c|)^2$ minimized with $w[2(F_o) + 0.00025F_o^2]$, carbon and oxygen parameters refined with anisotropic parameters, hydrogen atoms with isotropic terms, secondary isotropic extinction parameter = 0.0035 (5), final R, wtd R and error-of-fit values of 0.033, 0.038, 1.43, minimum and maximum in final difference map of −0.17 and 0.18 e Å^{-3}.

X-ray crystallographic analysis of 2a: $C_{15}H_{12}O_5$, FW = 272.3, 0.2 × 0.3 × 0.34 mm colorless crystal, Enraf-Nonius CAD4 diffractometer, λ(Mo Kα) = 0.710 69 Å (incident beam graphite monochromator), monoclinic space group $P2_1$, a = 7.545 (1) Å, b = 8.210 (2) Å, c = 10.820 (2) Å, β = 108.74 (1)°, V = 6304.7 (4) Å^3, z = 2, ρ = 1.42 g cm^{-3}, μ = 0.71 cm^{-1}, $F(000)$ = 284, T = 293 K. Latice parameters from 25 reflections in the range of $12 \le \theta \le 26°$, data collection range of $0 \le h < 8$, $0 < k < -9$, $-11 < l < 12$, $2 < \theta \le 50°$, 2θ–θ scan with variable speed of θ = 8.14–1.81 deg min^{-1}, θ scan range = 1.5(0.96 + 0.34 tan θ)°, background values obtained from the lower and the upper sixths of scan range, 5 standard intensities monitored every 1 h of crystal X-ray exposure, maximum and average change in standard intensities of 3.6 and 1.4%, 1330 total reflections, 1295 without standards, 1112 with $I_o \gg 3\sigma(I_o)$, R_{sym} for equivalent reflections = 0.004. All calculations done with the TEXRAY[16] package on a DEC Microvax II computer, structure solved with the direct methods link MITHRIL,[17] refinement by full-matrix least-squares, $\sum w(|F_o| - |F_c|)^2$ minimized with $w = [2(F_o) + 0.009F_o^2]$, carbon and oxygen parameters refined with anisotropic parameters = 0.00002 (4), final R, wtd and error-of-fit values of 0.030, 0.036, 1.82 minimum and maximum in final difference map of −0.13 and 0.16 e Å^{-3}.

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Registry No. **1**, 23282-20-4; **1a**, 126640-47-9; **2**, 126503-40-0; **2a**, 126541-26-2; **2b**, 126541-27-3; **3**, 51481-10-8; **3a**, 126503-41-1; **4**, 63783-99-3.

Supplementary Material Available: ^1H and ^{13}C NMR spectra of **2b** and atomic coordinates, equivalent isotropic displacement parameters, bond lengths, and bond angles from the X-ray crystallographic analyses of **1a** and **2a** (9 pages). Ordering information is given on any current masthead page.

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Solvolysis of 2-Aryl-2-propyl Benzoates. Validity of the Assumption of Constant *p*-Nitrobenzoate/Benzoate Rate Ratios

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The Hammett correlation analysis, modified by using σ^+ constants,[1] $\log (k_X/k_H) = \sigma^+\rho$,[2] has long been a useful probe for elucidating the mechanism of solvolytic reactions.[3,4] In carrying out such an analysis sometimes an estimation of rate constants is necessary if the reactivity of a substrate is too high or too low to measure directly. An assumption of a constant rate ratio for substrates containing two different leaving groups is generally applied to the structurally related systems. Thus, a factor of 20.8[5] was used to multiply the rate constant for a benzoate ester to obtain that for the corresponding *p*-nitrobenzoate in 80% acetone.[6] A decade ago this assumption was chal-

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