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REGIOSELECTIVE REACTIONS WITH DERIVATIVES OF THE TRICHOTHECENE MYCOTOXINS, NIVALENOL AND VOMITOXIN[1]

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ABSTRACT.—The structure of the ketol acetate **8** obtained by Swern oxidation of 4,15-diacetoxyscirpenol [**1**] was confirmed. Trichothecene 3,4-ketol acetates underwent epimerization and/or hydrolysis in solution in the presence of Si gel. Regioselective oxidation of 4,15-diacetylnivalenol [**6**] and 15-acetylvomitoxin [**7**] to the corresponding 3-ones **12** and **18** is described. 4-Bromination of the 3-one **18** was accompanied by normal opening of the 12,13-epoxide to give the bromohydrin **22**, without skeletal rearrangement. 3-Ones in this series readily formed enol acetates. Regioselective alkali metal hydride reduction of the 3-ones was also stereoselective.

For some years the trichothecene mycotoxins most readily produced by fermentation have been the 9-enes, 4,15-diacetoxyscirpenol [**1**] and T-2 toxin [**3**], but new sources of derivatives of the important 7α-hydroxytrichothec-9-en-8-ones, nivalenol [**5**] (2) and vomitoxin (4-deoxynivalenol) (3), have recently been described, and have made these compounds more accessible. The 3-one obtained (4) from diacetoxyscirpenol [**1**] by Swern oxidation (5) showed enhanced activity against P-388 murine lymphocytic leukemia. This paper records the preparation, properties, and chemical reactions of hitherto unknown 3-ones in the nivalenol and vomitoxin series. They were required for evaluation of their biological activity.

RESULTS AND DISCUSSION

Because of the suspected instability of trichothecene 3,4-ketol acetates (4,6), the structure of the 3-one from 4,15-diacetoxyscirpenol [**1**], initially obtained in low yield by the action of chromic oxide in HOAc (7), was first confirmed as the 4β-acetate **8** by suitable ^{1}H nOe experiments. This ketol acetate underwent epimerization and/or hydrolysis in solution in the presence of Si gel, or in $NaHCO_3$, giving mixtures of 3,4-ketols. With the ketol **9**, the equilibrium in $CDCl_3$ favored the 4β-ol **10**, which, after 7 days at room temperature, was the only molecular species present; but when isolated from a solution containing MeOH, and examined immediately, ^{1}H-nmr spectroscopy in $CDCl_3$ showed about 10% of the 4α-ol **11** to be present.

This preliminary work with the model compound **8** reinforced the recommendation (4), unsupported by experimental evidence, that trichothecene 3,4-ketol acetates be isolated by crystallization rather than by chromatography. ^{1}H-Nmr nOe experiments showed that the product thus obtained from regioselective Swern oxidation of 4,15-diacetylnivalenol [**6**] (8) was the 4β-acetate **12**. In marked contrast to the results above, the mixture [**14**] of α- and β-hydroxy epimers obtained by treatment of a solution of **12** with Si gel in the presence of MeOH, was in the ratio 3:1 when analyzed by nmr spectroscopy in $CDCl_3$. There was no evidence for the presence of the 4,3-ketol species **20**, which would result from a ketoacyloxy exchange (9,10). Pure 4β-ol **15** was obtained, fortuitously, on one occasion, by heating **12** in C_6H_6. Although the major product from cc of the acetate **12** was the ketol mixture [**14**], some 4α-acetate [**13**] was also produced.

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[1]Part 11 in the series "Phytotoxic Compounds Produced by *Fusarium equiseti*." For Part 10, see Grove (1).

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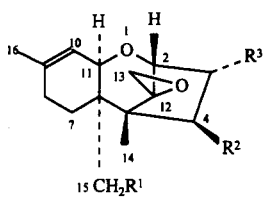
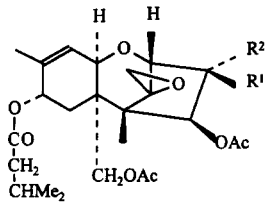
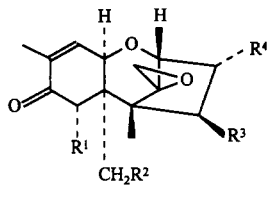


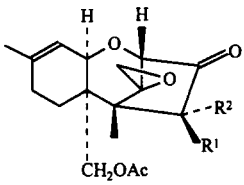
	R^1	R^2	R^3
1	OAc	OAc	OH
2	H	OH	H



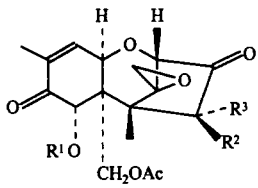
	R^1	R^2
3	H	OH
4	=O	



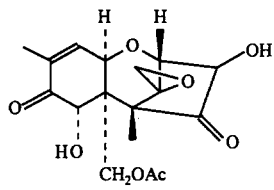
	R^1	R^2	R^3	R^4
5	OH	OH	OH	OH
6	OH	OAc	OAc	OH
7	OH	OAc	H	OH



	R^1	R^2
8	OAc	H
9	H, OH	
10	OH	H
11	H	OH



	R^1	R^2	R^3
12	H	OAc	H
13	H	H	OAc
14	H	H, OH	
15	H	OH	H
16	H	H	OH
17	Ac	OAc	H
18	H	H	H
19	H	Br	H



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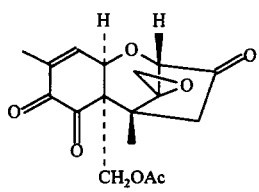

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While nOes provide a reliable method for determining the configuration of trichothecene 3,4-ketols and their esters, the ^{1}H-nmr spectra (Table 1) of the pairs of epimers show features consistent with those observed with the simple 9-enes trichodermol [**2**] and 4-epitrichodermol (11) and their derivatives (12). As expected from examination of molecular models, H-11 is the more deshielded in the 4α-substituted compound, and H-14 is the more deshielded in the 4β-substituted epimer.

In the absence of oxygenation at position 4, and after protection of the OH at position 15 (3), regioselective oxidation of 15-acetylvomitoxin [**7**] to the 3-one **18** by the Swern method, or with pyridinium chlorochromate, presented no difficulty. With pyridinium chlorochromate the best yields were obtained with 1.5 equivalents of the reagent (13) in the presence of NaOAc (14) and powdered 3Å molecular sieves (15). The crude product from the Swern method sometimes contained the trione **21**. The ^{1}H-nmr spectrum of the 3-one **18** showed long-range coupling between H-2 and H-4β.

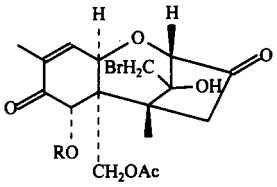
The biological activity of trichothec-9-enes is sometimes enhanced by the introduction of halogen (4). The major product (40%) from bromination of the 3-one **18** with phenyltrimethylammonium tribromide was shown by ^{1}H-nmr nOe experiments to be a 4β-bromoketone [**19**]. Despite the presence of excess anhydrous K_2CO_3 in the reaction, a liberated HBr attacked the 12,13-epoxide of unreacted **18**, giving, as a minor product (8%), the bromohydrin [**22**], which results from normal opening of the epoxide without skeletal rearrangement. The acetate **23** of the bromohydrin contained a tertiary hydroxyl group (ν max 3480 cm^{-1}). Only a few examples of normal halogenohydrin formation from trichothecene 12,13-epoxides have been recorded (16,17).

With Ac_2O/pyridine at room temperature, conditions normally used for the

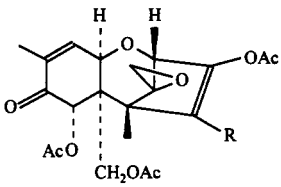
TABLE 1. ^{1}H-Nmr Spectral Data (δ, J in parentheses) for Compounds **8**, **10**, **11–13**, **15–19**, and **22–26**.[a]

Compound	Position										
	2	4	7	10	11	13	14	15	16	Ac	OH[b]
8	3.52 s	5.97 s	ca. 2.2[c]	5.49 d (5.8)[d]	4.11 d (5.8)	2.95 d AB 3.16 d (3.9)	0.88 s	4.13 d AB 4.21 d (12.4)	1.75 s[d]	2.13 2.15	—
10	3.52 s	4.30 s	ca. 2.1[c]	5.43 d (5.7)[d]	3.86 d (6.0)	2.95 d AB 3.15 d (3.5)	0.99 s	4.04 d AB 4.13 d (12.4)	1.73 s[d]	2.08	2.63
11	3.65 s	4.30 s	ca. 2.1[c]	5.45 d	4.18 d	2.71 d AB 3.02 d (4.0)	0.81 s	3.87 d AB 4.19 d (12.5)	1.73 s	2.07	
12	3.67 s	5.94 s	4.99 d (1.7)	6.55 d (5.9)[d]	4.61 d	3.20 d AB 3.27 d (4.2)	1.16 s	4.02 d AB 4.59 d (12.6)	1.93 s[d]	2.05 2.18	3.84[d] (1.7)
13	3.53 s	5.61 s	4.84 d (2.0)	6.62 d (5.9)[d]	4.98 d (5.9)	3.08 s	0.99 s	4.18 d AB 4.54 d (12.5)	1.92 s[d]	1.95 2.22	3.82[d] (2.0)
15	3.66 s	4.30 d (6.5)	4.96 d (1.8)	6.50 d (5.9)[d]	4.40 d (5.9)	3.18 d AB 3.25 d (4.1)	1.28 s	4.21 d AB 4.44 d (12.4)	1.92 s[d]	1.94	2.34 (C-4) (6.5) 3.89 (C-7) (1.8)
15[e]	3.61 s	4.25 s	4.85 s	6.55 d (5.9)[d]	4.82 d (5.9)	2.93 d AB 2.95 d (4.5)	1.02 s	4.25 d AB 4.36 d (12.2)	1.84 s[d]	1.88	3.30 m
16	3.78 s	4.26 s	4.85 s	6.59 d (5.9)[d]	4.85 d (5.9)	3.03 s	1.09 s	4.25 d AB 4.34 d (12.2)	1.91 s[d]	2.10	3.87 m
17	3.66 s	6.04 s[f]	6.06 s[f]	6.53 d	4.66 d (5.8)	2.76 d AB 3.09 d (3.6)	1.00 s	4.18 d AB 4.56 d (12.6)	1.91 s	1.96 2.10 2.22	—
18	3.55 s[g]	α2.93 d AB β2.35 d (19.4)[g]	4.94 d (1.8)	6.52 d (5.9)[d]	4.52 d (5.9)[h]	3.25 d AB 3.36 d (4.1)	1.30 s	4.19 d AB 4.31 d (12.2)	1.92 s[d,h]	1.94	3.83[d] (1.8)
19	3.75 s	4.95 s	4.97 d (1.8)	6.52 d (5.9)[d]	4.51 d (5.9)[h]	3.15 d AB 3.23 d (4.2)	1.46 s	4.11 d AB 4.40 d (12.5)	1.93 s[d,h]	2.00	3.95 d (1.8)
22	3.77 s	2.60 d AB 2.70 d (19.0)	4.66 d (1.6)	6.45 d (5.3)[d]	4.47 d (5.3)	3.98 d AB 4.50 d (9.9)	1.55 s	4.20 d AB 4.41 d (11.8)	1.91 s[d]	1.84	2.80 (C-12) 3.91 (C-7) (1.8)
23	3.79 s	2.61 d AB 2.68 d (19.3)	5.94 s	6.45 d (5.3)[d]	4.49 d (5.3)	3.87 d AB 4.53 d (9.8)	1.35 s	4.15 d AB 4.57 d (11.7)	1.88 s[d]	1.85 2.23	2.80 s
24	4.14 s	6.01 s[f]	5.96 s[f]	6.54 d (5.3)	4.69 d (5.3)	2.99 d AB 3.22 d (3.1)	1.07 s	4.26 d AB 4.31 d (12.0)	1.87 s	1.98 2.22 2.23	—
25	4.32 s	—	5.93 s	6.55 d (5.5)[d]	4.81 d (5.5)	3.08 d AB 3.25 d (3.1)	1.08 s	4.37 d AB 4.46 d (12.0)	1.87 s[d]	1.86 2.22 2.29	—
26[i]	3.91 s	5.38 d (5.9)	4.81 d (1.5)	6.56 d (5.9)[d]	4.35 d (5.9)	3.05 d AB 3.07 d (4.1)	1.34 s	4.05 d AB 4.37 d (12.3)	1.91 s[d]	1.97	2.50 (C-3) 3.87 (C-7) (1.5)

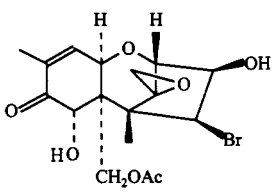
[a]First order approximations from line separations. Long-range couplings are excluded.
[b]In absence of D_2O.
[c]Not first order.
[d]$J_{10,16}$=1.5 Hz.
[e]In CD_3OD.
[f]Assignments may be reversed.
[g]$J_{2,4\beta}$=0.9 Hz.
[h]$J_{11,16}$=0.7 Hz.
[i]Also H-3, 4.34 d (5.9).



R
22 H
23 Ac



R
24 H
25 Br



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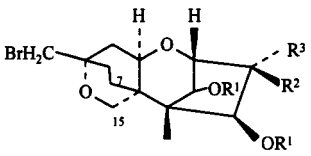
27 R^1=CH_2CH=CH_2, R^2,R^3=O
28 R^1=CH_2CH=CH_2, R^2=OH, R^3=H
29 R^1=R^2=H, R^3=OH



acetylation of trichothecene primary and secondary alcoholic hydroxyl groups, the 3-ones **18** and **19** formed the enol acetates **24** and **25**. These 3-enol acetates were readily recognized from their uv spectra, even in the presence of the 9-en-8-one chromophore. 3-Enol acetates were not formed under these conditions by 4β-acetoxy-3-ones.

Reduction of trichothecene 3- and 8-ones with alkali metal hydrides is usually stereoselective (18), but regioselective reduction of 3,8-diones had not previously been attempted. The 3α-ols **6** and **7** were the only isolable products from reduction of the 3-ones **12** and **18**, respectively, with 1 equivalent of $NaBH_4$ in *i*-PrOH. The reductions were both regioselective and stereoselective; but the yields from small-scale experiments were poor, though comparable with the yields of 4,15-diacetoxyscirpenol [**1**] and T-2 toxin [**3**] from similar reductions of the 3-ones **8** and **4**, respectively (6,19). The only isolable product from reduction of the bromoketone [**19**] under the same conditions was shown by the nmr spectrum (H-2 singlet, $J_{2,3\alpha}$=0, $\phi_{2,3\alpha}$=90°) to be the 3β-ol **26**. The course of this regio- and stereo-selective reduction, from the α-face of ring C, is presumably determined by the bulky 4β-bromo substituent. $NaBH_4$ reduction of the synthetic analogue **27** gave the 3β-ol **28**, but when the allyloxy protecting groups were removed, the 3α-ol **29** was obtained (20).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were taken on a Kofler hot-stage apparatus and are corrected. Ir spectra were obtained on mulls in Nujol and uv spectra from solutions in MeOH. Unless stated otherwise, ^{1}H-nmr spectra for the target compounds (Table 1) were recorded at 360 MHz or 500 MHz in $CDCl_3$ with $SiMe_4$ as internal standard. Mol wts were taken from the mass spectra. NH_3 was used to obtain cims. In analytical tlc, Merck Si gel 60 F_{254} was used with, unless otherwise stated, $CHCl_3$-MeOH (9:1) as eluent. Merck Si gel 7734, made up in C_6H_6, was used for cc. Light petroleum ether had bp 60–80°. Acetylations were carried out at room temperature in pyridine with Ac_2O for the time specified. Identifications were confirmed by comparison of the ir spectra. 15-Acetylvomitoxin [**7**] was dried *in vacuo* at 80° and exhibited mp 136–138°, R_f 0.55 in $CHCl_3$-MeOH (9:1) (3). It was noted that all 12,13-epoxytrichothecenes are hazardous and toxic, and therefore suitable safety precautions were taken.

OXIDATION OF 15-ACETYLVOMITOXIN [**7**]. A. WITH PYRIDINIUM CHLOROCHROMATE.—15-Acetylvomitoxin (204 mg) in CH_2Cl_2 (20 ml) at room temperature was stirred with pyridinium chlorochromate (200 mg, 1.54 equivalents) for 8 h in the presence of anhydrous NaOAc (90 mg) and powdered 3 Å molecular sieves (400 mg). The reaction mixture was diluted with Et_2O (20 ml) and filtered through a short column (2×1 cm) of Si gel to remove the tar. The column was washed with Et_2O (40 ml) and the filtrate and washings were combined. Recovery afforded a gum (211 mg) which was dissolved in C_6H_6 and chromatographed on Si gel (6 g, 16 ×1 cm). Elution with C_6H_6/MeOH (200.1), monitored by analytical tlc, gave gummy

fractions (a) 50 ml, R_f 0.75, 169 mg, 83%, and (b) 75 ml, R_f 0.55, 33 mg, 17%, consisting of starting material. Crystallization of fraction (a) from C_6H_6/light petroleum ether furnished prisms of **18**.

15-Acetoxy-12,13-epoxy-7α-hydroxytrichothec-9-ene-3,8-dione [**18**].—Mp 124°, R_f 0.75; *anal.*, found C, 60.7, H, 6.0%, $C_{17}H_{20}O_7$ requires C, 60.7, H, 6.0%; ir ν max 3430 (OH), 1762, 1739, 1694 (CO), and 1662 (C=C) cm^{-1}; uv λ max 225 nm (ε 8400); cims *m/z* $[M+NH_4]^+$, 354.

3,7α,15-Triacetoxy-12,13-epoxytrichotheca-3,9-dien-8-one [**24**].—The 3-one [**18**] (60 mg) was acetylated for 4 days giving the amorphous triacetate [**24**]: mp 50–60° (60 mg), R_f 0.80; *anal.*, found C, 60.1, H, 5.8%, $C_{21}H_{24}O_9$ requires C, 60.0, H, 5.7%; ir, ν max 1780, 1748, 1702 (CO), and 1625 (C=C) cm^{-1}; uv λ max 220 nm (ε 11560).

OXIDATION OF 15-ACETYLVOMITOXIN [**7**].—B. WITH DIMETHYL SULPHOXIDE.—To DMSO (49 μl) in CH_2Cl_2 (5 ml) at $-65\pm3°$ was added, during 10 min, with magnetic stirring, trifluoroacetic anhydride (52 μl, freshly distilled) in CH_2Cl_2 (1 ml). After a further 10 min, 15-acetylvomitoxin (**7**; 92 mg) in CH_2Cl_2 (1 ml) was added (10 min), and stirring was continued for a further 30 min. After the addition (10 min) of Et_3N (95 μl) in CH_2Cl_2 (1 ml), the reaction mixture was allowed to warm up to room temperature (40 min) and was washed at 0° with 1 M HCl followed by $NaHCO_3$ and H_2O. Recovery gave a gum (90 mg, R_f 0.75, 98%) which solidified on standing and gave the 3-one [**18**] on crystallization from C_6H_6/light petroleum ether.

Yields from this reaction were variable, in the range of 90–98%: on some occasions the crude product contained starting material (R_f 0.55), and on others an unstable substance, assumed to be the trione [**21**] on the basis of the chemical shift (δ_H 6.95) assigned to H-10 (21). Both impurities were readily removed by cc.

15-Acetoxy-4β-bromo-12,13-epoxy-7α-hydroxytrichothec-9-ene-3,8-dione [**19**].—The 3-one **18** (167 mg) in THF (2 ml) was heated under reflux with phenyltrimethylammonium tribromide (97%, 200 mg) in the presence of finely powdered anhydrous K_2CO_3 (100 mg) for 4 h. After most of the solvent had been removed in a stream of N_2, H_2O was added, and the resulting emulsion was extracted with $CHCl_3$. Trituration of the recovered gum with EtOAc afforded a solid, mp 205–215° (dec) (73 mg), the ir spectrum of which was indistinguishable from that of the pure material. Crystallization of the crude product from EtOAc gave felted needles, mp 222–225° (dec), of the bromodione **19**, R_f 0.73; *anal.* found C, 49.2; H, 4.6%, $C_{17}H_{19}O_7Br$ requires C, 49.2, H, 4.6% (Br=79); ir ν max 3400 (OH), 1767, 1735, 1690 (C=O), and 1665 (C=C) cm^{-1}; uv λ max 226 nm (ε 9800); eims, *m/z* M^+ 414. Irradiation of H-4 caused an 8% nOe enhancement of H-11 and a 5% enhancement of the downfield H-15 signal (see Table 1). Irradiation of H-2 did not enhance H-4, but caused a 2% enhancement of the upfield H-13 signal.

The gummy residue (147 mg) recovered from the mother liquors from the EtOAc trituration was dissolved in C_6H_6 and chromatographed on Si gel (5 g, 15×1 cm). Elution (5 ml fractions) with C_6H_6/MeOH, monitored by tlc, afforded (a) 200:1, 30 ml, R_f 0.73, 87 mg, giving the bromodione **19** (8 mg), and (b) 100:1, 40 ml, R_f 0.71, 31 mg, giving a solid mp 168–175° on trituration with Et_2O.

The total yield of the bromodione **19** amounted to 81 mg (39%).

The solid from fraction (b) crystallized from EtOAc/light petroleum ether in prisms (**22**, 16 mg).

15-Acetoxy-13-bromo-7α,12-dihydroxytrichothec-9-ene-3,8-dione [**22**].—Mp 170–175°; R_f 0.70; *anal.*, found C, 48.8, H, 5.1%, $C_{17}H_{21}O_7Br$ requires C, 48.9, H, 5.1% (Br=79); ir ν max 3537, 3508, 3408 br (OH), 1754, 1732, 1690 (C=O), and 1665 (C=C) cm^{-1}; uv λ max 223 nm (ε 8400); cims *m/z* $[M+NH_4]^+$, 434.

Acetylation for 18 h gave the amorphous acetate **23**: mp 70–80°; *anal.*, found C, 50.0, H, 5.5%, $C_{19}H_{23}O_8Br$ requires C, 49.7, H, 5.1%; ir ν max 3480 br (OH), 1755, 1740, 1695 (C=O), and 1650 (C=C) cm^{-1}.

3,7α,15-Triacetoxy-4-bromo-12,13-epoxytrichotheca-3,9-dien-8-one [**25**].—The bromodione **19** (10 mg) was acetylated for 4 days giving the amorphous triacetate **25**: mp 65–70°, R_f 0.76; *anal.*, found C, 50.8, H, 4.8%, $C_{21}H_{23}O_9Br$ requires C, 50.5, H, 4.6%; ir ν max 1783, 1750, 1703 (C=O), and 1645 (C=C) cm^{-1}; uv λ max 222 nm (ε 11320).

4β,15-Diacetoxy-12,13-epoxytrichothec-9-en-3-one [**8**].—Mp 160°; R_f 0.69; cims *m/z* $[M+NH_4]^+$ 382, calcd for $C_{19}H_{24}O_7$, M^+ 364, was prepared as described (4). Irradiation of H-4 caused a 7% enhancement of the overlapping upfield H-15 and/or H-11 signals at δ 4.13. Irradiation of H-2 had no effect on H-4, but caused a 5% enhancement of the downfield H-13 signal. The 3-one **8** decomposed on attempted prep. tlc, and on cc on Si gel, with loss of the H-4 signal at δ 6. The 3-one **8** partly decomposed on shaking a $CHCl_3$ solution with $NaHCO_3$ and did not form an enol acetate when subjected to the standard acetylation conditions (4 days).

15-Acetoxy-12,13-epoxy-4β-hydroxytrichothec-9-en-3-one [**10**].—The diacetate **8** (5 mg) in CH_2Cl_2 (2 ml) was stirred at room temperature with Si gel (100 mg). After 24 h, MeOH (0.2 ml) was added, when

filtration and recovery, after storage in $CDCl_3$ (see below), gave a gum (5 mg), R_f 0.55. This was kept *in vacuo* at room temperature for 7 days and afforded a 4β-ol [**10**] hydrate: mp 105–110°; *anal.*, found C, 59.5, H, 6.9% $C_{17}H_{22}O_6 \cdot H_2O$ requires C, 60.0, H, 7.1%; ir ν max 3400 br (OH), 1765, 1743 (C=O), 1675 (C=C) cm^{-1}. Irradiation of H-4 gave a 5% nOe enhancement of H-11 and a 6% enhancement of the downfield H-15 signal (for ^{1}H-nmr spectrum, see Table 1).

When the gummy product was examined by nmr spectroscopy in $CDCl_3$ immediately after recovery it contained about 10% of a 4α-ol [**11**] (for ^{1}H-nmr spectrum, see Table 1) which epimerized $CDCl_3$ over the course of 7 days to give the pure 4β-ol **10**.

4β,15-Diacetoxy-12,13-epoxy-7α-hydroxytrichothec-9-ene-3,8-dione [**12**].—4,15-Diacetylnivalenol [**6**] (108 mg) in CH_2Cl_2 (1 ml) was oxidized by the Swern procedure, as described above for 15-acetylvomitoxin [**7**], but, on working up, washing with $NaHCO_3$ was omitted. The gummy product (95 mg) solidified on trituration with Et_2O. Two recrystallizations from Et_2O afforded the dione **12** as prisms: mp 182–185°, R_f 0.67; *anal.*, found C, 57.7, H, 5.6%, $C_{19}H_{22}O_9$ requires C, 57.9, H, 5.6%; ir ν max 3465 (OH), 1770, 1743, 1680 (C=O), 1655 (C=C) cm^{-1}; uv λ max 224 nm (ε 8860). Irradiation of H-4 had no effect on H-2 but gave a 22% nOe enhancement of the (overlapping) signals due to H-11 and H-15 at δ 4.6. The dione was not recovered from a $CHCl_3$ solution after shaking with $NaHCO_3$. Crystallization from MeOH afforded a solvate, mp ca. 90°.

The amorphous acetate **17** exhibited: mp 140–145° (dec); *anal.*, found C, 58.0, H, 5, 5%, $C_{21}H_{24}O_{10}$ requires C, 57.8; H, 5.5%; ir ν max 1750, 1702 cm^{-1}.

HYDROLYSIS AND EPIMERIZATION OF THE 4β-ACETOXYDIONE **12**.—A. In one preparation of the dione **12** by the Swern procedure (see above) the trituration with Et_2O was omitted and an attempt was made to crystallize the gummy product directly from C_6H_6. Moist air was not excluded. Compound **15** was obtained.

15-Acetoxy-12,13-epoxy-4β,7α-dihydroxytrichothec-9-ene-3,8-dione [**15**] *hydrate*.—Mp 125–130° (dec); R_f 0.53; *anal.*, found C, 55.0, H, 6.0%, $C_{17}H_{20}O_8 \cdot H_2O$ requires C, 55.1, H, 6.0%; ir ν max 3440 (OH), 1730, 1687 (C=O), 1660 (C=C) cm^{-1}; cims *m/z* $[M+NH_4]^+$ 370. During 6 h it did not epimerize in CD_3OD. For a solution in CD_3OD, irradiation of H-4 gave a 16% nOe enhancement of H-11 and a 4% enhancement of the downfield H-15 signal. Irradiation of H-2 did not enhance H-4, but caused a 4% enhancement of H-7 and a 3% enhancement of the downfield H-13 signal.

B. The dione **12** (60 mg) in C_6H_6-MeOH (200:1) was chromatographed on Si gel (2 g, 6×1 cm). Elution with C_6H_6/MeOH, monitored by tlc, gave the following gummy fractions: (a) 200:1, 40 ml, R_f 0.65, 12 mg; (b) 100:1, 25 ml, R_f 0.65–0.50, 11 mg; (c) 50:1, 50 ml, R_f 0.50, 29 mg, which furnished the 4β-ol **15** mp 120–125° (dec) (2 mg) on trituration with $CHCl_3$. Crystallization of fraction (a) from EtOAc/light petroleum ether gave needles mp 90–100° (dec) (gas evolution) of a solvate, ir ν max 3530, 3430 (OH), 1740, 1688 (C=O), 1663 (C=C) cm^{-1} of the 4α-acetoxydione **13**, characterized by its nmr spectrum (Table 1).

C. The dione **12** in CH_2Cl_2 (2 ml) was stirred at room temperature with Si gel (100 mg) for 18 h. After the addition of MeOH (0.2 ml), the adsorbent was filtered off. Recovery afforded a gum (5 mg); cims *m/z* $[M+NH_4]^+$ 370 (calcd for $C_{17}H_{20}O_8$, M^+ 352). Immediate investigation by ^{1}H-nmr spectroscopy in $CDCl_3$ showed the gum to consist of the 4β-ol **15** (25%) together with the 4α-epimer **16** (75%) (Table 1).

REDUCTION OF THE 3-ONE [**12**].—To the 3-one (20 mg) in *i*-PrOH (6 ml) at room temperature was added, with stirring, $NaBH_4$ (0.8 ml of a solution containing 5.0 mg in 8 ml *i*-PrOH). After 30 min the solvent was removed at 40° under reduced pressure. The residue was dissolved in H_2O and the solution, at 0°, was neutralized with 0.1 M HCl and extracted with $CHCl_3$. ^{1}H-Nmr spectroscopy of the gummy product (20 mg) showed that the 3-one had been reduced (no H-4 singlet at δ 6) but the 8-one had been retained (H-10 at δ 6.6). The product in C_6H_6-MeOH (200:1) was chromatographed on Si gel (2 g, 6×1 cm). Fractional elution (2 ml fractions) with C_6H_6-MeOH (200:1), monitored by tlc, gave (a) 10 ml, 1 mg, R_f 0.65 and (b) 26 ml, 8 mg, R_f 0.62. Crystallization of fraction (b) from EtOAc/light petroleum ether gave prisms (5 mg) of diacetylnivalenol [**6**].

REDUCTION OF THE 3-ONE [**8**].—The 3-one (20 mg) was reduced with $NaBH_4$ in *i*-PrOH and the reaction mixture was worked up as described above. The gummy product (20 mg), in C_6H_6, was chromatographed on Si gel (2 g, 6×1 cm). Fractional elution (2 ml fractions) with C_6H_6-MeOH (200:1), monitored by tlc in $CHCl_3$-MeOH (98:2), gave (a) 50 ml, 5 mg, R_f 0.45, intractable, and (b) 80 ml, 8 mg, R_f 0.41, which crystallized from EtOAc/light petroleum ether in prisms (4 mg) mp 158–161° of 4,15-diacetoxyscirpenol [**1**].

REDUCTION OF THE 3-ONE [**18**].—The 3-one (20 mg) was reduced as described above. The gummy product (20 mg) R_f 0.53, in $CHCl_3$, was passed down a short column of Si gel (1 g) and the recovered foam (18 mg) crystallized from EtOAc, giving 15-acetylvomitoxin [**7**] (6 mg), mp 130°.

REDUCTION OF THE 3-ONE [**19**].—To the 3-one (15 mg), suspended in *i*-PrOH (5 ml) at room temperature, was added, with rapid stirring, $NaBH_4$ (1.0 ml of a solution containing 4.0 mg in 10 ml *i*-PrOH). After 4 h the reaction mixture was worked up as described above, giving a gum (12 mg). Tlc of the gum indicated that the reduction was regiospecific, with the 8-one unaffected (spots seen in uv light: no additional spots seen on exposure to iodine vapor). Tlc-monitored cc with C_6H_6-MeOH (200:1) on Si gel gave (a) 6 ml, 2 mg, R_f 0.70–0.60; (b) 10 ml, 4 mg, R_f 0.60; (c) 16 ml, 1 mg, R_f 0.49. Fractions (a) and (c) were intractable. Fraction b, after drying *in vacuo*, afforded compound **26**.

15-Acetoxy-4β-bromo-3β,7a-dihydroxy-12,13-epoxytrichothec-9-en-8-one [**26**].—Foam; *anal.* found C, 49.0, H, 5.0%, $C_{17}H_{21}O_7Br$, requires C, 48.9, 5.0%; ir ν max 3430 br (OH), 1740, 1685 (C=O) cm^{-1}; uv λ max 221 nm.

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