Phytotoxic Compounds Produced by *Fusarium equiseti*. Part 7.[1] Reactions and Rearrangement of the 7-Hydroxy-12,13-epoxytrichothec-9-en-8-one Skeleton

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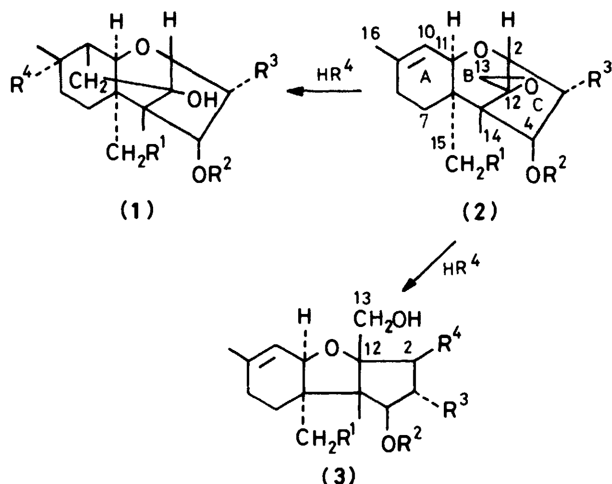
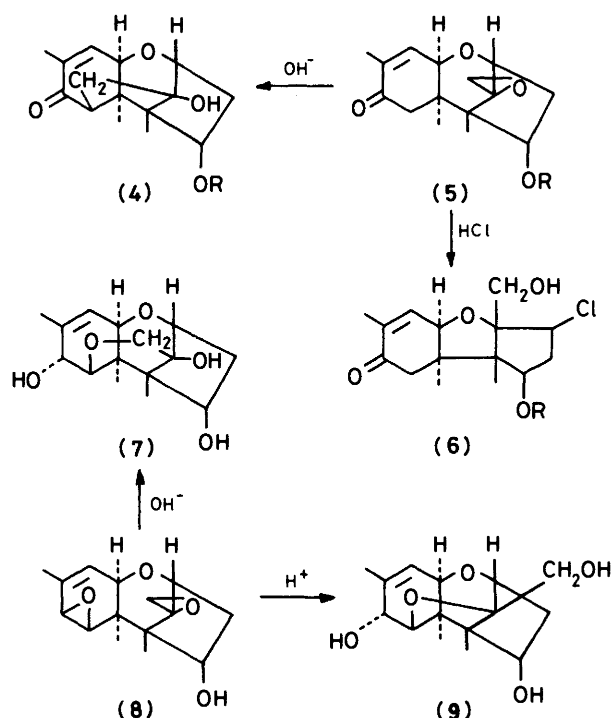
7α,15-Dihydroxy-12,13-epoxytrichothec-9-en-8-ones, *e.g.* nivalenol and vomitoxin, rearrange under mild basic conditions to the isomeric 7,13-epoxy-A-nortrichothecane-7-carboxylic acid 15-lactones. With hydrogen chloride, normal addition to the 12,13-epoxide of these compounds occurs, with retention of configuration at C-12, and the more usual rearrangement to the 2β-chloroapotrichothec-9-en-8-one skeleton is not seen. Catalytic hydrogenation of diacetylnivalenol takes place from the β-face to give the corresponding (9*R*)-trichothecan-8-one. Reliable procedures for the preparation of vomitoxin and nivalenol from their acetates are outlined.

Biological activity in the naturally occurring trichothec-9-enes is associated with the presence of a 12,13-epoxy linkage, and derivatives lacking this structural feature are inactive.[2] The 12,13-epoxide is protected from rearside nucleophilic attack by ring A and by the rigid oxabicyclo[3.2.1]octane system of rings B/C; and the stability of these trichothecenes depends on the ease of generation of ionic centres which can participate in an intramolecular attack on the epoxide, a process frequently accompanied by skeletal rearrangement. The location of these ionic centres depends on the nature of the functional groups in ring A. The simple naturally occurring trichothec-9-enes *e.g.* trichodermol (2; $R^1 = R^2 = R^3 = H$),[3] verrucarol (2; $R^1 = OH$, $R^2 = R^3 = H$),[4] and diacetoxyscirpenol (2; $R^1 = OAc$, $R^2 = Ac$, $R^3 = OH$),[5,6] are stable in basic media (apart from, where appropriate, the saponification of ester groups), but undergo acid-catalysed rearrangement to 10,13-cyclotrichothecane (1) and apotrichothecene (3) products. With the



introduction of an 8-ketone, *e.g.* in trichothecolone (5; R = H), the formation of 10,13-cyclotrichothecane products is prevented, but in strongly basic media a 7,13-cyclotrichothecene product (isotrichothecolone) (4; R = H) results from intramolecular attack on the epoxide by the carbanion generated at position 7.[7] A similar reaction occurs with the 7β,8β-epoxide crotocol (8)[8] leading to the 7,13-epoxytrichothecene (7). The isomeric 7,12-epoxytrichothecene (9) results from the action of acids on crotocol (8). This paper is concerned with the reactions in basic and acidic media of the 7α-hydroxytrichothec-9-en-8-ones



vomitoxin[9,10] and nivalenol[11] and their naturally occurring esters, 3-acetylvomitoxin[12] and 4,15-diacetylnivalenol.[13,14] These reactions have not been investigated hitherto.

Before the use of solid grain media for the production of the important mycotoxin vomitoxin (deoxynivalenol) (10; $R^1 = R^2 = R^3 = R^4 = H$)[15] was superceded by the liquid culture of *Fusarium graminearum*,[16] a useful alternative route to this compound involved the deacylation, in 0.1M-sodium hydroxide at room temperature during 18 h,[12] of the 3-acetyl derivative (10; $R^1 = R^2 = R^3 = H$, $R^4 = Ac$), which was available by liquid culture of both *F. culmorum*[12] and an organism described as *F. roseum*.[9] However, the crude vomitoxin resulting from this procedure could not be crystallised until a by-product (5—10%) had been removed by preparative t.l.c.[12] This by-product has now been identified as the isomeric A-nortrichothecane (16; $R^1 = R^2 = H$) by ^1H n.m.r. spectroscopic examination (Table 1) of the monoacetate (16; $R^1 = H$, $R^2 = Ac$). In the latter compound, rings B/C of the trichothecane nucleus were intact but the 12,13-epoxide had been opened to give a tertiary OH ($\nu_{max.}$ 3 500 cm^{-1}). In ring A the 9-ene-

Table 1. ^{1}H N.m.r. resonances (δ; *J*, from line seperations and excluding long range coupling, in parentheses) for the A-nortrichothecanes (**16**; R^1 = H and OAc, R^2 = Ac) and the chlorohydrins (**18**; R^1 = R^2 = Ac, R^2 = H and OAc)

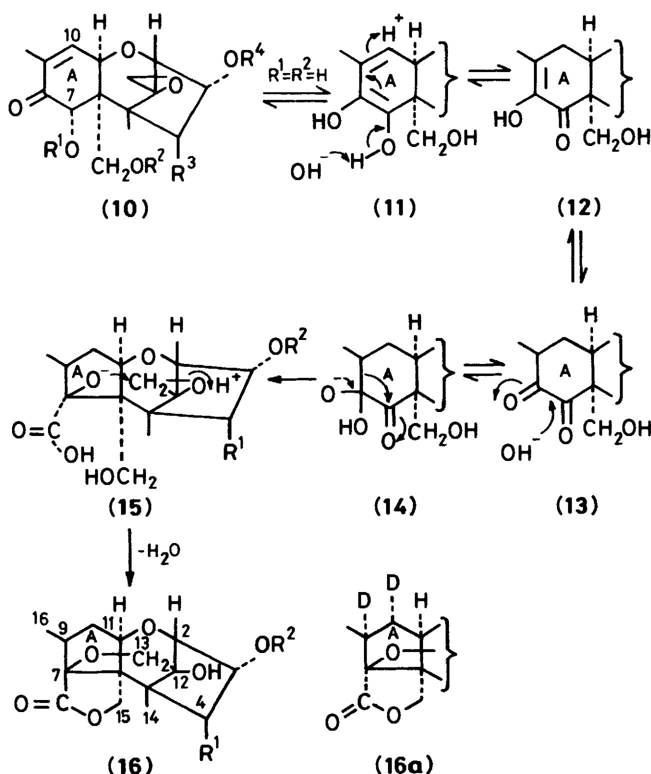
Comp.	Position 2	3	4α	4β	7	9	10α	10β	11	13	14	15	16	OH(s)	OAc(s)
(**16**; R^1 = H, R^2 = Ac)	3.97d (4.6)	5.36ddd (4.6, 4.3, 10.7)	1.70dd (4.3, 14.5)	2.29dd (14.5, 10.7)		2.55ddq (3.4, 7.4, 11.1)	2.38ddd (11.1, 5.0, 15.1)	1.75dd[a] (15.1, 3.4)	4.20d[a] (5.0)	3.98d 4.47d^{AB} (12.5)	1.19s	3.76d 4.23d^{AB} (10.3)	1.30d[a] (7.4)	1.26	2.10
(**16**; R^1 = OAc, R^2 = Ac)	4.02d (4.9)	5.40dd (4.9, 3.8)	5.13d (3.8)			2.52ddq (3.2, 7.4, 11.1)	2.37ddd (11.1, 4.8, 15.0)	1.75dd[b] (15.0, 3.2)	4.12d[b] (4.8)	3.95d 4.42d^{AB} (12.5)	1.06s	3.77d 4.58d^{AB} (10.6)	1.27d[b] (7.4)	2.20	2.10 2.13
(**17**; R^1 = R^2 = Ac)	4.06d (4.7)	5.22dd (4.7, 3.5)	5.71d (3.5)		5.75s	2.91m (6.5, 13.0)	1.98ddd (13.0, 3.1, 14.5)	2.27ddd (14.5, 6.5, 2.8)	4.33t (2.8)	2.76d 3.15d^{AB} (3.6)	0.80s	4.19d 4.52d^{AB} (12.5)	1.05d (6.5)		2.00 2.12 2.16 2.16
(**18**; R^1 = R^3 = Ac, R^2 = H)	4.28d (4.7)	5.42ddd (4.7, 10.7, 3.2)	2.12dd (3.2, 15.2)	2.43dd (15.2, 10.7)	5.73s			6.49d (5.1)[c]	4.81d (5.1)[d]	3.81d 4.54d^{AB} (10.4)	1.18s	4.24d 4.58d^{AB} (11.4)	1.87s[c,d]	2.77	1.85 2.15 2.20
(**18**; R^1 = R^3 = Ac, R^2 = OAc)	4.37d (4.7)	5.50dd (4.7, 3.2)	5.62d (3.2)		5.73s			6.50d (5.3)[c]	4.70d (5.2)[d]	3.78d 4.55d^{AB} (10.4)	1.05s	4.62$_{AB}$ (12)	1.87s[c,d]	2.90	1.86 2.14 2.17 2.21
(**19**; R = Ac)	4.42d (4.0)	5.49dd (4.1, 3.1)[e]	5.49d (3.1)[e]		5.43s	2.78m (6.5, 13.0)	*ca.* 2.1m	*ca.* 2.1m	4.32t (2.9)	3.89d 4.50d^{AB} (10.3)	1.25s	4.07d 4.96d^{AB} (12.0)	1.09d (6.4)	2.92	1.90 2.13 2.13 2.15

[a] 1.76s, 4.21s, and 1.26s in (**16a**; R^1 = H, R^2 = Ac). [b] 1.76s, 4.15s, and 1.27s in (**16a**; R^1 = OAc, R^2 = Ac). [c] $J_{10,16}$ = 1.5 Hz. [d] $J_{11,16}$ = 0.5 Hz. [e] By computer simulation.

appeared to have been reduced (CHMe at δ 1.30) and the CO group was now part of a γ-lactone ($\nu_{max.}$ 1 770 cm^{-1}). Both CH_2OR groups revealed by the n.m.r. spectrum were contained in rings, which must number five in all. These facts lead to structure (**16**) in which $\Phi hH_{10\beta.11}$ is *ca.* 90° and $J_{10\beta.11} = 0$. The values for the vicinal coupling constants $J_{9.10\beta}$ (3.4 Hz, *trans*) and $J_{9.10\alpha}$ (11.1 Hz, *cis*) in ring A, which is forced into a rigid envelope conformation with C-9 and C-10 eclipsed, are consistent only with a β-configuration for the methyl substituent (9*S*).

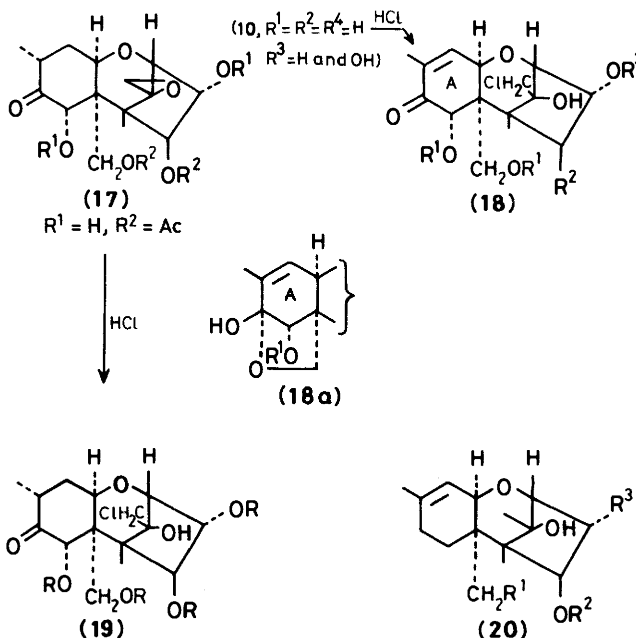
The formation of the A-nortrichothecane (**16**) is accommodated by the mechanism shown in the Scheme, in which the dienediol (**11**) is first converted into the diosphenol (**12**). Benzilic acid rearrangement of the 7,8-diketone (**13**) with attack on the more accessible C-8 then leads to the extrusion of C-8 in the sequence (**14**) ⟶ (**15**); opening of the 12,13-epoxide by the tertiary oxygen anion and lactonisation of the carboxy residue then gives structure (**16**). When the course of the reaction was followed by u.v. spectroscopy, a chromophore $\nu_{max.}$ 320 nm, attributed to the ionised dienediol species (**11**) (calc. 328 nm), increased in intensity with time up to a maximum at 5 h, and then slowly declined. After 5 h, a second chromophore $\nu_{max.}$ 294 nm, attributed to the ionised diosphenol species (**12**) (calc. 304 nm), was seen and slowly increased in intensity up to a maximum at 48 h. When the reaction was carried out in sodium deuterioxide, the ^1H n.m.r. spectrum of the acetylated product (**16a**; R^1 = H, R^2 = Ac) showed that deuterium was incorporated at positions 9 and 10α, as predicted by the Scheme. In this compound the signals from the hydrogens at positions 10β, 11, and 16 appeared as singlets (Table 1).

An analogous reaction occurred with the equally important mycotoxin nivalenol (**10**; $R^1 = R^2 = R^4$ = H, R^3 = OH), giving the A-nortrichothecane (**16**; R^1 = OH, R^2 = H) which formed a diacetate (**16**; R^1 = OAc, R^2 = Ac). Although the rate of formation and decline of the species (**11**) was not much affected, the yield of the A-nor product (**16**; R^1 = OH, R^2 = H) was greatly increased (30%) if M-sodium hydroxide was used and the reaction time was extended to 1—2 days.



Scheme. Rearrangement of the 7α,15-dihydroxy-12,13-epoxytrichothec-9-en-8-one skeleton in sodium hydroxide.

Dihydronivalenol (**17**; $R^1 = R^2$ = H) was stable in M-sodium hydroxide at room temperature[14] and when the diacetyl derivative (**17**; R^1 = H, R^2 = Ac) was set aside in this reagent for 2 days there was no u.v. spectroscopic evidence for the presence of an enediol species. The ^1H n.m.r. spectrum of the tetra-acetate (**17**; $R^1 = R^2$ = Ac) (Table 1) showed that 9-H was axial ($J_{9.10}$ 13.0 Hz) and hence the 16-Me group was equatorial (α). The same criterion served to identify 10α-H which, as expected, was the more shielded of the two hydrogens at position 10. Since epimerisation at position 9, through the enol, is most unlikely to take place in acetic acid, catalytic hydrogenation of nivalenol and its esters[14] therefore takes place from the β-face of the molecule. Hydrogenation from the β-face to give the corresponding 9α-methyl compound may be of general occurrence[4–6] in the trichothec-9-enes. Whatever the course of the reduction, the diacetate (**17**; R^1 = H, R^2 = Ac) has the wrong (*R*) configuration at position 9 for the stepwise synthesis of the A-nortrichothecane (**16**; R^1 = OAc, R^2 = Ac) by selective oxidation at C-7 followed by benzilic acid rearrangement of the resulting diketone.



Hydrolysis of the 4- and 15-acetyl groups in diacetylnivalenol (**10**; $R^1 = R^4$ = H, R^2 = Ac, R^3 = OAc), which was sometimes obtained as a hydrate, is facilitated by the neighbouring 3α- and 7α-hydroxy groups[14] and is essentially complete within 30 min, in 0.1M-sodium hydroxide at room temperature. Ring A rearrangement is not, therefore, an interfering factor in the preparation of nivalenol from its diacetate,[14] a metabolic product of *F. equiseti* in liquid culture. In the absence of an oxygen substituent at position 4, the hydrolysis of the acyl residue in 3-acetylvomitoxin (**10**; $R^1 = R^2 = R^3$ = H, R^4 = Ac) is slower, but is complete in 1.5 h. Under these conditions vomitoxin is obtained, free from the A-nortrichothecane (**16**; $R^1 = R^2$ = H), by crystallisation of the crude product, and further purification by chromatography[12] is unnecessary. Like nivalenol,[14] vomitoxin is frequently obtained as a hydrate.

Whilst concentrated hydrochloric acid at room temperature satisfactorily brings about the trichothecene (**2**)→apotrichothecene (**3**) rearrangement,[3,4] hydrogen chloride in a non-polar

solvent[5] for 15—60 min, gives a cleaner product without deacetylation[6] and, contrary to an earlier report,[17] smoothly converted trichothecin (**5**; R = COCH=CHMe-*E*) into the known 2β-chloroapotrichothecene (**6**; R = COCH=CHMe-*E*) in quantitative yield. However, although these conditions converted nivalenol to a chlorohydrin, shown below to have the structure (**18**; R^1 = R^3 = H, R^2 = OH), they failed with the di- and tetra-acetates (**10**; R^1 = R^4 = H and Ac, R^2 = Ac, R^3 = OAc) when only starting material was recovered after 7 days at room temperature or after 8 h at 63 °C. The dihydro derivative (**17**; R^1 = H, R^2 = Ac) was similarly unaffected. With concentrated hydrochloric acid both the di- and tetra-acetates (**10**; R^1 = R^4 = H and Ac, R^2 = Ac, R^3 = OAc) yielded the chlorohydrin (**18**; R^1 = R^3 = H, R^2 = OH), but it is likely that deacetylation preceded the opening of the 12,13-epoxide. The dihydro derivative (**17**; R^1 = H, R^2 = Ac) likewise gave the chlorohydrin (**19**; R = H) as indicated by the spectroscopic properties of the tetra-acetate (**19**; R = Ac). 3-Acetylvomitoxin (**10**; R^1 = R^2 = R^3 = H, R^4 = Ac) with hydrogen chloride in chloroform gave, after 24 h at room temperature, a product which appeared from the ^{1}H n.m.r. spectrum to be a mixture of the chlorohydrin (**18**; R^1 = R^2 = H, R^3 = Ac) and the hemiacetal tautomer (**18a**). Acetylation (*cf.* nivalenol[14]) gave only one product, a triacetate (**18**; R^1 = R^3 = Ac, R^2 = H). Acetylation of the nivalenol chlorohydrin (**18**; R^1 = R^3 = H, R^2 = OH) gave only a tetra-acetyl derivative (**18**; R^1 = R^3 = Ac, R^2 = OAc). Both this compound and the triacetate (**18**; R^1 = R^3 = Ac, R^2 = H) contained hydroxy groups ($\nu_{max.}$ 3 450 cm^{-1}) which must be tertiary. The coupling constants $J_{2,3\beta}$ = 4.7 Hz in the ^{1}H n.m.r. spectra were consistent with the presence of an unrearranged trichothecene skeleton.[18] Both compounds therefore result from the normal addition (as defined[19]) of hydrogen chloride to the epoxide with, perforce, retention of configuration at C-12 giving chlorohydrins of structure (**18**). Only one other trichothecene 12,13-epoxide-opening reaction occurs without molecular rearrangement, namely the fission of (**2**) with lithium aluminium hydride to give the 12-hydroxytrichothecenes (**20**).[4,5,6]

The 7α-hydroxy-12,13-epoxytrichothec-9-en-8-ones are thus remarkable for undergoing an unusual reaction in acidic conditions and a novel rearrangement in mildly basic media.

Experimental

M.p.s were taken on a Kofler hot-stage apparatus and are corrected. I.r. spectra were determined on Nujol mulls and u.v. spectra were obtained in methanol, unless otherwise stated. N.m.r. spectra were obtained at 360 MHz in $CDCl_3$ with $SiMe_4$ as internal standard. In analytical t.l.c., Merck silica gel HF_{254} was used with chloroform–methanol (9:1): spots were detected in u.v. light (trichothec-9-en-8-ones) or in iodine vapour. Preparative t.l.c. (0.1. cm layer) was carried out on plates (20 × 20 cm). NH_3 was used to obtain chemical ionisation mass spectra (c.i.m.s.). Acetylations were carried out in pyridine with acetic anhydride at room temperature during 24 h. Identifications were confirmed by comparison of the i.r. spectra. Light petroleum had b.p. 60—80 °C.

4β,15-*Diacetoxy*-3α,7α-*dihydroxy*-12,13-*epoxytrichothec*-9-*en*-8-*one*[14] (**10**; R^1 = R^4 = H, R^2 = Ac, R^3 = OAc), $\nu_{max.}$ 3 510, 3 410, 1 738, 1 718, 1 690, and 1 655w cm^{-1}. This compound was sometimes obtained from ethyl acetate as a *hydrate*, $\nu_{max.}$ 3 500, 3 535, 3 260, 1 737, 1 685, 1 657w, and 1 620w cm^{-1} (Found: C, 55.1; H, 6.4%. $C_{19}H_{24}O_9{\cdot}H_2O$ requires C, 55.1; 6.3%).

(9R)-4β,15-*Diacetoxy*-3α,7α-*dihydroxy*-12,13-*epoxytrichothecan*-8-*one* (**17**; R^1 = H, R^2 = Ac) was prepared by catalytic hydrogenation of 4,15-diacetylnivalenol (**10**; R^1 = R^4 = H, R^2 = Ac, R^3 = OAc) as described previously.[14] Acetylation gave the tetra-acetate (**17**; R^1 = R^2 = Ac).[14]

(9S)-3α,7β,12,15-*Tetrahydroxy*-7,13-*epoxy*-A-*nortrichothecane*-7α-*carboxylic Acid* 15-*Lactone* (**16**; R^1 = R^2 = H).—3-Acetylvomitoxin (**10**; R^1 = R^2 = R^3 = H, R^4 = Ac)[12] (70 mg) in methanol (1 ml) and 0.1M-sodium hydroxide (5.00 ml) was set aside at room temperature for 48 h. After potentiometric neutralisation with 0.1M-hydrochloric acid (2.80 ml, 1.06 equiv. consumed) the solution was extracted with ethyl acetate (8 × 3 ml). Trituration with ethyl acetate of the gummy product (58 mg) afforded a solid (10 mg) which after two recrystallisations from ethyl acetate gave the A-*nortrichothecane* (**16**; R^1 = R^2 = H) as an amorphous powder, m.p. > 250 °C, R_F 0.18 (Found: C, 60.8; H, 6.6. $C_{15}H_{20}O_6$ requires C, 60.8; H, 6.6%), $\nu_{max.}$ 3 440, 3 400, and 1 762 cm^{-1}. End absorption only in the u.v.

The *acetate* (**16**; R^1 = H, R^2 = Ac) crystallised from ethyl acetate–light petroleum as prisms, m.p. 210—212 °C, R_F 0.47 [Found: C, 60.3; H, 6.5%; *M* − 60 278.1160. $C_{17}H_{22}O_7$ requires C, 60.3; H, 6.5%; $C_{15}H_{18}O_5$ ($C_{17}H_{22}O_7$ − $C_2H_4O_2$) requires 278.1154]. $\nu_{max.}$ 3 500, 1 770, and 1 730 cm^{-1}.

The use of CD_3OD and 0.1M-NaOD in this reaction gave the [9,10α-$^2H_2$]-A-nortrichothecanes (**16a**; R^1 = H, R^2 = H and Ac).

(9S)-3α,4β,7β,12,15-*Pentahydroxy*-7,13-*epoxy*-A-*nortrichothecane*-7α-*carboxylic Acid* 15-*Lactone* (**16**; R^1 = OH, R^2 = H).—4,15-Diacetylnivalenol[14] (**10**; R^1 = R^4 = H, R^2 = Ac, R^3 = OAc) (200 mg) in methanol (2 ml) and M-sodium hydroxide (2 ml) was set aside at room temperature for 24 h. The solution was acidified with M-hydrochloric acid (2 ml) and extracted, first with ethyl acetate (9 × 2 ml), and then, continuously, with chloroform for 30 h. The combined products (122 mg) were recrystallised twice from ethyl acetate giving the A-*nortrichothecane* (**16**; R^1 = OH, R^2 = H) as prisms (38 mg), m.p. > 280 °C, R_F 0.06 [Found; C, 57.7; H, 6.5%; MH^+ (c.i.m.s.) 313. $C_{15}H_{20}O_7$ requires C, 57.7; H, 6.5%; *M* 312]; $\nu_{max.}$ 3 440 (br), 1 775, and 1 760 cm^{-1}. The 3,4-*diacetate* (**16**; R^1 = OAc, R^2 = Ac), crystallised from ethyl acetate–light petroleum as needles or prisms, m.p. 240—244 °C (decomp.), R_F 0.52 [Found: C, 57.9; H, 6.2%; MH^+ (c.i.m.s.) 397. $C_{19}H_{24}O_9$ requires C, 57.6; H, 6.1%; *M* 396], $\nu_{max.}$ 3 380, 1 760, and 1 739 cm^{-1}.

The use of CD_3OD and M-NaOD in this reaction gave the [9,10α-$^2H_2$]-A-nortrichothecanes (**16a**; R^1 = OH, R^2 = H) and (**16a**; R^1 = OAc, R^2 = Ac), MH^+ (c.i.m.s.) 399.

Alkaline Hydrolysis of Acetylated Trichothec-9-en-8-one Alcohols.—The compound (0.5 mmol) was dissolved in ethanol (5 ml) and the solution was made up to 20 ml with 0.1M-sodium hydroxide. Aliquots were withdrawn at intervals and titrated against 0.1M-hydrochloric acid (Table 2).

Enolisation of the 7-Hydroxytrichothec-9-en-8-one System.—The compound (0.5 μmol) was dissolved in methanol (1 ml). The solution was made up to 5 ml with sodium hydroxide (0.1M or 1M) and the u.v. spectrum was determined at intervals (*l* = 1 cm). A band $\lambda_{max.}$ 320 nm was replaced after nearly 48 h by a second band $\lambda_{max.}$ 294 nm.

Estimation of the intensity of the 294 nm chromophore was exceptionally difficult as it appeared first, after 5 h, as an inflexion on the side of the broad 320 nm band. With vomitoxin (**10**; R^1 = R^2 = R^3 = R^4 = H) in 0.1M-sodium hydroxide ε_{294} had reached a steady value of *ca.* 350 at 45 h. The figures for nivalenol (**10**; R^1 = R^2 = R^4 = H, R^3 = OH) were similar. With the dihydro compound (**12**; R^1 = H, R^2 = Ac) no significant chromophore was seen during 48 h.

Table 2. Hydrolysis of acetylated trichothec-9-en-8-one alcohols in 0.1 M sodium hydroxide

Compound	Time (min)	2	10	20	30	40	60	90
		Equivalents of alkali consumed						
(**10**; $R^1 = R^4 = H, R^2 = Ac, R^3 = OAc$)		1.18	1.56	1.94	1.97	1.98		
(**10**; $R^1 = R^2 = R^3 = H, R^4 = Ac$)			0.51		0.88		0.92	0.95

Table 3. U.v. absorption at 320 nm for alkaline solutions of 7α-hydroxytrichothec-9-en-8-ones.

Compound	NaOH	Time (h)	0	0.5	1	2	3	5	7	9	21	31	45
			ε_{320}										
(**10**; $R^1 = R^2 = R^3 = R^4 = H$)	0.1M		0	295	580	1 240	1 534	1 582	1 415	1 240	766		590
(**10**; $R^1 = R^2 = R^4 = H, R^3 = OH$)	0.1M		0	250	518	932	1 060	1 114	984	981	725		
	M		0	172	431	885	1 165	1 402	1 273		852	550	464

Preparation of Vomitoxin.—The acetate (**10**; $R^1 = R^2 = R^3 = H$, $R^4 = Ac$) (31 mg) in methanol (0.5 ml) and 0.1M-sodium hydroxide (2.00 ml) was set aside at room temperature for 1.5 h. The solution was neutralised potentiometrically with 0.1M-hydrochloric acid (0.95 equiv. alkali consumed) and extracted with ethyl acetate (4 × 1 ml). Recovery gave a gum (21 mg) which crystallised from ethyl acetate as hexagonal prisms of *vomitoxin hydrate* (**10**; $R^1 = R^2 = R^3 = R^4 = H$), m.p. 103—104 °C (decomp.) (loss of solvent) (Found: C, 57.8; H, 7.2%, *M*, 296. $C_{15}H_{20}O_6{\cdot}H_2O$ requires C, 57.3; H, 7.1%; $C_{15}H_{20}O_6$ requires *M*, 296); $\nu_{max.}$ 3 580, 3 515, 3 410, 3 150 (br), 1 700, and 1 610 cm^{-1}; $\lambda_{max.}$ 222 nm. Acetylation gave the triacetate (**10**; $R^1 = R^2 = R^4 = Ac$, $R^3 = H$),[12] m.p. 154 °C, from ethyl acetate–light petroleum.

Preparation of Nivalenol (*cf. Ref.* 14).—The acetate (**10**; $R^1 = R^4 = H$, $R^2 = Ac$, $R^3 = OAc$) (120 mg) in ethanol (3 ml) and 0.1M-sodium hydroxide (9 ml) was set aside at room temperature for 30 min. 1M-Hydrochloric acid (0.8 ml) was added and the solution was continuously extracted with chloroform for 24 h. The product (70 mg) consisted of nivalenol hydrate.[14]

Acid Catalysed Rearrangement of Trichothecin.—A stream of dry hydrogen chloride was passed for 15 min through a solution of trichothecin (5; R = COCH=CHMe-*E*) (20 mg) in dichloromethane (2 ml) at room temperature. The recovered product crystallised from benzene–light petroleum as needles (21 mg), m.p. 129—131 °C (lit.,[17] m.p. 132 °C) of the 2-chloroapotrichothecene (**6**; R = COCH=CHMe-*E*) (trichothecin chlorohydrin).

Attempted Acid Catalysed Rearrangement of Nivalenol and its Acetates and of 3-*Acetylvomitoxin.*—*With hydrogen chloride.* (i) Nivalenol (**10**; $R^1 = R^2 = R^4 = H$, $R^3 = OH$) (20 mg) in chloroform–ethanol (1:1, 10 ml) was treated with a stream of dry hydrogen chloride at room temperature for 1 h. The gummy product crystallised from ethyl acetate as prisms (15 mg), m.p. 130 °C (decomp.), $\nu_{max.}$ 3 500, 3 460, 3 355, 3 300, 1 705w, and 1 625w cm^{-1}, $\lambda_{max.}$ end absorption only, of a solvate which, after drying *in vacuo* over phosphorus pentaoxide, crystallised from ethyl acetate as prisms, m.p. 215—219 °C (decomp.) of 13-*chloro*-3α,4β,7α,12,15-*pentahydroxytrichothec*-9-*en*-8-*one* (**18**; $R^1 = R^3 = H$, $R^2 = OH$) [Found: C, 51.7; H, 6.55%; *M*, 348. $C_{15}H_{21}ClO_7$ requires C, 51.65; H, 6.1%; *M*, 348 (Cl = 35)], $\nu_{max.}$ 3 520, 3 400, 3 320, 1 730, and 1 625 cm^{-1}.

The 3α,4β,7α,15-*tetra-acetate* (**18**; $R^1 = R^3 = Ac$, $R^2 = OAc$) was obtained as an amorphous solid, m.p. 90—100 °C by sublimation at 150 °C/10^{-1} mmHg followed by precipitation from ethyl acetate–light petroleum (Found: C, 52.8; H, 5.6%. $C_{23}H_{29}ClO_{11}$ requires C, 53.4; H, 5.7%), $\nu_{max.}$ 3 470, 1 750, 1 705, and 1 640 cm^{-1}; $\lambda_{max.}$ 220 nm.

(ii) 4,15-Diacetylnivalenol (**10**; $R^1 = R^4 = H$, $R^2 = Ac$, $R^3 = OAc$) (20 mg) in chloroform (2 ml) was treated with hydrogen chloride for 2 h and the solution was set aside at room temperature for 7 days. The recovered gum showed spots at R_F 0.65 (starting material) and 0.46 on t.l.c. It was acetylated and the product was chromatographed as a column of silica gel (2 g, 10 × 1 cm) made up in benzene. Benzene–methanol (100:1, 100 ml) eluted the tetra-acetate (**10**; $R^1 = R^2 = R^4 = Ac$, $R^3 = OAc$) (15 mg).

(iii) 4,15-Diacetylnivalenol (25 mg) in chloroform (4 ml) was heated under reflux for 8 h whilst a stream of hydrogen chloride was passed through the solution. Only starting material (20 mg) was recovered.

(iv) The tetra-acetate (**10**; $R^1 = R^2 = R^4 = Ac$, $R^3 = OAc$) (30 mg) was recovered after heating under reflux for 1 h with hydrogen chloride in chloroform as described in (iii) above.

(v) The dihydro-compound (**17**; $R^1 = H$, $R^2 = Ac$) (20 mg) in chloroform (3 ml) was treated with hydrogen chloride for 1 h and the solution was set aside at room temperature for 24 h. The gummy product was subjected to preparative t.l.c. in chloroform–methanol (9:1) and the silica from 10 × 1 cm horizontal bands from the plate was separately extracted with chloroform. Starting material R_F 0.46 (6 mg), crystallised from ethyl acetate–light petroleum, was recovered from the fifth band.

(vi) 3-Acetylvomitoxin (**10**; $R^1 = R^2 = R^3 = H$, $R^4 = Ac$) (20 mg) in chloroform (4 ml) was treated as described in (v) above. The recovered product was subjected to preparative t.l.c. in chloroform–methanol (9:1). Material (12 mg) from a band R_F 0.35 crystallised from ethyl acetate–light petroleum as needles, m.p. 190—205 °C, $\nu_{max.}$ 3 500, 3 400, 1 740, 1 680w, 1 630, and 1 620, but was judged on the basis of the ^1H n.m.r. spectrum (10-H at both δ 6.52 and 5.45) to be a mixture of the enone (**18**; $R^1 = R^2 = H$, $R^3 = Ac$) and the corresponding hemiacetal (**18a**). It was acetylated and the product was subjected to preparative t.l.c. in chloroform–methanol (9:1). After two recrystallisations from ethyl acetate–light petroleum the material (6 mg) from a band R_F 0.70 formed needles, m.p. 179 °C of 3α,7α,15-*triacetoxy*-13-*chloro*-12-*hydroxytrichothec*-9-*en*-8-*one* (**18**; $R^1 = R^3 = Ac$, $R^2 = H$) [Found: C, 55.2; H, 6.0%; MH^+ (c.i.m.s.), 459. $C_{21}H_{27}O_9Cl$ requires C, 55.0; H, 5.9%; *M*(Cl = 35) 458]; $\nu_{max.}$ 3 450, 1 740, 1 715, 1 695, and 1 655w cm^{-1}; $\lambda_{max.}$ 226 nm (ε 8 000).

With concentrated hydrochloric acid. (i) 4,15-*Diacetylnivalenol* (**10**; $R^1 = R^4 = H$, $R^2 = Ac$; $R^3 = OAc$) (200 mg) in ethanol (5 ml) and concentrated hydrochloric acid (5 ml) was set aside at room temperature for 24 h. The ethanol was removed under reduced pressure, water (5 ml) was added, and the solution was extracted first with ethyl acetate and then continuously with chloroform for 24 h. The combined extracts (105 mg) crystallised from ethyl acetate in prisms m.p. 130 °C (decomp.) of the solvate of the chlorohydrin (**18**; $R^1 = R^3 = H$; $R^2 = OH$) (see above).

(ii) The tetra-acetate (**10**; $R^1 = R^2 = R^4 = Ac$, $R^3 = OAc$)

(20 mg) in ethanol (1 ml) and hydrochloric acid (3 ml) was treated as described above. Crystallisation of the product afforded the chlorohydrin (**18**; $R^1 = R^3 = H$, $R^2 = OH$) solvate (3 mg), m.p. 130 °C (decomp.).

(iii) The dihydro-compound (**17**; $R^1 = H$; $R^2 = Ac$) (30 mg) in methanol (1.5 ml) and hydrochloric acid (3 ml) was treated as in (i). The solid product (27 mg) crystallised from ethyl acetate as prisms (8 mg), m.p. 200—202 °C (decomp.) of 13-*chloro*-3α,4β,7α,12,15-*pentahydroxytrichothecan*-8-*one* (**19**; R = H) [Found: C, 51.3; H, 7.0%; MH^+ (c.i.m.s.) 351. $C_{15}H_{23}ClO_7$ requires C, 51.3; H, 6.7%; *M*, 350 (Cl = 35)] $\nu_{max.}$ 3 550, 3 490, 3 390, 3 320, and 1 730 cm^{-1}).

The 3α,4β,7α,15-*tetra-acetate* (**19**; R = Ac) was obtained as an amorphous solid, m.p. *ca.* 90 °C [Found: MH^+ (c.i.m.s.) 519 $C_{23}H_{31}ClO_{11}$ requires *M* 518] $\nu_{max.}$ 3 460 and 1 740br. cm^{-1}.

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References

1 Part 6. J. F. Grove, *J. Chem. Soc. C*, 1970, 378.
2 J. F. Grove and P. H. Mortimer, *Biochem. Pharmacol.*, 1969, **18**, 1473.
3 W. O. Godtfredson and S. Vangedal, *Acta Chem. Scand.*, 1965, **19**, 1088.
4 J. Gutzwiller, R. Mauli, H. P. Sigg, and C. Tamm, *Helv. Chim. Acta*, 1964, **47**, 2234.
5 A. W. Dawkins, *J. Chem. Soc. C*, 1966, 116.
6 H. P. Sigg, R. Mauli, E. Flury, and D. Hauser, *Helv. Chim. Acta*, 1965, **48**, 962.
7 J. Gutzwiller, C. Tamm, and H. P. Sigg, *Tetrahedron Lett.*, 1965, 4495.
8 J. Gyimesi and A. Melera, *Tetrahedron Lett.*, 1967, 1665.
9 T. Yoshizawa and N. Morooka, *Agric. Biol. Chem.*, 1973, **37**, 2933.
10 R. F. Vesonder, A. Ciegler, and A. H. Jensen, *Appl. Microbiol.*, 1973, **26**, 1008.
11 T. Tatsumo, *Cancer Res.*, 1968, **28**, 2393.
12 M. M. Blight and J. F. Grove, *J. Chem. Soc., Perkin Trans. 1*, 1974, 1691.
13 P. W. Brian, A. W. Dawkins, J. F. Grove, H. G. Hemming, D. Lowe, and G. L. F. Norris, *J. Exptl. Bot.*, 1961, **12**, 1; B. K. Tidd, *J. Chem. Soc. C*, 1967, 218.
14 J. F. Grove, *J. Chem. Soc. C*, 1970, 375.
15 R. F. Vesonder, J. J. Ellis, W. F. Kwolek, and D. J. DeMarini, *Appl. Env. Microbiol.*, 1982, **43**, 967.
16 J. D. Miller, A. Taylor, and R. Greenhalgh, *Can. J. Microbiol.*, 1983, **29**, 1171.
17 G. G. Freeman, J. E. Gill, and W. S. Waring, *J. Chem. Soc.*, 1959, 1105.
18 J. F. Grove, unpublished results.
19 R. E. Parker and N. S. Isaacs, *Chem. Rev.*, 1959, **59**, 737.

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