Phytotoxic Compounds Produced by *Fusarium equiseti*. Part 9.[1] Reactions of some 9β,10β; 12,13-Diepoxytrichothecanes

John Frederick Grove
School of Molecular Sciences, University of Sussex, Brighton, Sussex, BN1 9QJ

The 9β,10β-epoxides of a number of naturally-occurring non-macrocyclic 12,13-epoxytrichothec-9-enes have been prepared, and the possible formation of 10β,13-epoxytrichothecanes when these diepoxides are treated with nucleophilic reagents has been investigated. In the presence of a 15-oxygen substituent, as in diacetoxyscirpenol 9β,10β-epoxide, intramolecular attack with formation of a 9α,15-epoxide takes precedence over the intermolecular addition of a nucleophile. With the 8α-hydroxy compound trichothecodiol 9β,10β-epoxide, intramolecular attack also occurred giving an 8α,9α; 10β,13-diepoxytrichothecane together with known derivatives of trichothecolone. With trichodermol epoxide, the two epoxide groupings reacted independently. The significance of these results to the mechanism of action of the 12,13-epoxytrichothec-9-ene mycotoxins is discussed. An unexpected autoxidation is reported.

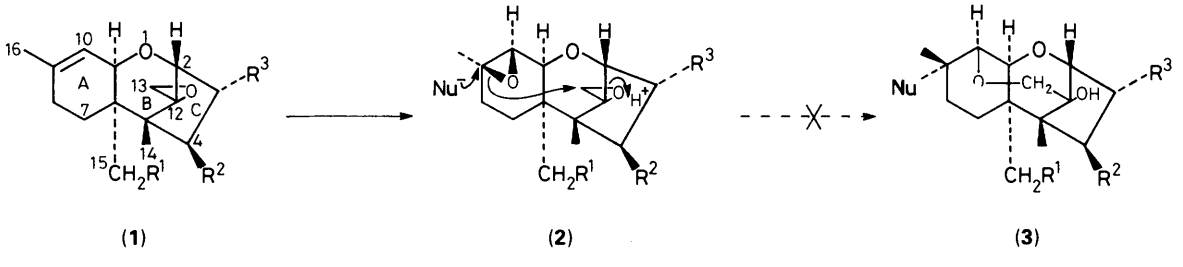
Although the biological activity of the trichothecene mycotoxins is associated with the 12,13-epoxide, other structural features, notably the presence of a 9-ene and of bulky ester functions, are important factors in the manifestation of the highest activity.[2,3] When laboratory animals are treated with diacetoxyscirpenol (**1**; $R^1 = R^2 = OAc$, $R^3 = OH$), the delay[4] between application of the mycotoxin and the production of an observable effect suggests the intervention of a metabolite. The 9β,10β-epoxide (**2**; $R^1 = R^2 = OAc$, $R^3 = OH$), presumed to arise *in vivo* by the action of a mixed function oxidase on the 9-ene, was considered for this role; and a reaction of type (**2**) ⟶ (**3**), in which the 12,13-epoxide is opened by intramolecular attack to give a 10β,13-epoxytrichothecane (**3**), was envisaged as participating in the mechanism of action of this group of trichothecene mycotoxins.

There is good n.m.r. evidence (see below) that the 9β,10β-epoxides (**2**) retain the normal trichothec-9-ene ring A conformation (**2A**).[1]* Diaxial opening of conformation (**2A**) implies attack at the least-substituted position 10, giving a 9β-hydroxy-10α-substituted product; but the alternative half-chair ring A structure (**2B**), which in trichothec-9-enes participates in the formation of 10,13-cyclotrichothecanes,[1] is conformationally ideal for the formation of a 10–O ⟶ 13–C bridge, diaxial opening giving the 9α-substituted product (**3**).

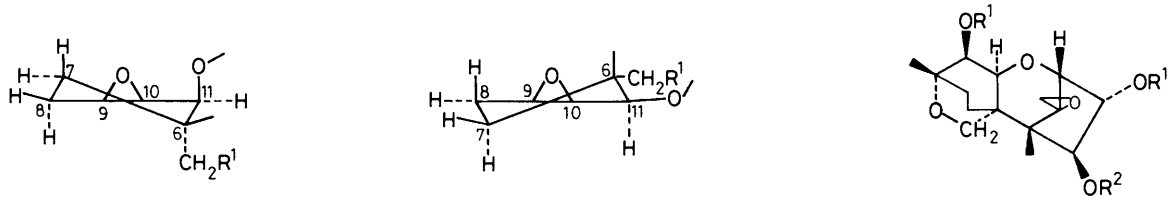
In the work described herein, no evidence has been obtained for the rearrangement (**2**) ⟶ (**3**) with diacetoxyscirpenol and its close relatives. Moreover, the 9β,10β-epoxide (**2**; $R^1 = R^2 =$ OAc, $R^3 = OH$) has recently been shown to be markedly less active than the parent 9-ene in a standard protein synthesis inhibition assay.[5] On the other hand, the 9β,10β-epoxide (**6**; R = H) of the 8α-hydroxytrichothec-9-ene trichothecodiol (**5**; R = H) (Scheme 2) readily underwent a reaction similar to the 'epoxide migration'[6] familiar to sugar chemists, with formation of the 8α,9α; 10β,13-diepoxy compound (**7**; R = H).

In the presence of an oxygen substituent at position 15, intramolecular attack on a trichothecene 9β,10β-epoxide takes precedence over the intermolecular addition of a nucleophile in both acid and basic media, and the shape of the resulting rigid all-boat [2,2,2]oxabicyclo-octane ring system (**4**) then precludes any attack on a 12,13-epoxide by a β-oxygen anion at position 10. Thus, the 9β,10β-epoxide (**2**; $R^1 = R^2 = OAc$, $R^3 = OH$)[7], obtained from diacetoxyscirpenol, gave the 9α,15; 12,13-diepoxide (**4**; $R^1 = R^2 = H$) in 1M-sodium hydroxide at room

* For the sake of clarity C-16 and 10-H are omitted from structures (**2A**) and (**2B**).



(**1**) (**2**) (**3**)



(**2A**) (**2B**) (**4**)

Table 1. ^{1}H N.m.r. resonances (δ, *J* in parentheses[a]) for the 9β,10β-epoxides and their relatives and rearrangement products.

Compound	Position												
	2	3	4	7	8	10	11	13	14	15	16	Ac	OH[b]
(**2**; $R^1 = R^2 = OAc, R^3 = OH$)	3.78d (4.9)	4.19dd (4.9, 3.0)	5.07d (3.0)	α1.40ddt (12.7, 4.5, 2.2) β1.85td (12.7, 4.8)	α1.63ddd (15.0, 12.7, 4.8) β2.0ddd (15.0, 4.8, 2)	3.17d (5.4)	4.07dd (5.4, 2.2)	3.12d AB 2.73d (3.9)	0.76s	4.15d AB 3.98d (12.5)	1.36s	2.08 2.15	1.73
(**2**; $R^1 = R^2 = R^3 = OAc$)	3.96d (4.8)	5.20dd (4.8, 3.5)	5.64d (3.5)	α1.40ddt (12.9, 4.8, 2.2) β1.82td (12.9, 4.8)	α1.60ddd (15.0, 12.7, 4.8) β1.98ddd (15.0, 4.8, 2)	3.14d (5.4)	3.95dd (5.4, 2.2)	3.12d AB 2.75d (3.9)	0.70s	4.27d AB 4.07d (12.4)	1.36s	2.09 2.12 2.15	—
(**2**; $R^1 = R^3 = H, R^2 = OH$)	3.91d (5.3)	α2.58dd (15.7, 7.6) β1.90ddd (15.7, 5.3, 3.1)	4.30dd (7.6, 3.1)	α1.13m[c] β1.91m[c]	1.72m[c]	3.04dd (5.6, 0.8)	3.52dd (5.6, 2.2)	3.15d AB 2.76d (3.9)	0.75s	0.82s	1.34s	—	1.6
(**19**)	3.75d (5.6)	α1.92dd (15.1, 5.0) β2.54ddd (15.1, 10.9, 5.6)	4.27dd (10.9, 5.0)	α1.0m[c] β1.87m[c]	1.75m[c]	3.10dd (5.6, 1.0)	4.24dd (5.6, 2.1)	3.09d AB 2.73d (3.8)	0.82s	1.08s	1.35s	—	1.9
(**19**)[d]	3.68d (5.6)	α1.70dd (14.8, 4.9) β2.28ddd (14.8, 10.7, 5.6)	3.90dd (10.7, 4.9)	α0.62ddt (12.6, 5.0, 2.1) β1.80td (12.6, 5.0)	α1.45ddd (14.7, 12.7, 5.1) β1.70ddd (14.7, 5.0, 2.1)	2.85dd (5.6, 0.9)	4.13dd (5.6, 2.1)	2.60d AB 2.33d (4.1)	0.66s	0.95 s	1.08s	—	
(**18**)	4.19dd[c] X	2.62m[c] AB	—	α1.07ddt (12.3, 4.5, 2.0) β1.86td (12.3, 4.4)	α1.72ddd (14.2, 12.3, 4.5) β1.95dddd (14.2, 4.4, 2, 0.8)	3.05dd (5.4, 0.8)	3.67dd (5.4, 2.1)	3.29d AB 2.90d (3.8)	0.77s[e]	0.74s[e]	1.35s	—	—
(**6**; R = H)	3.91d (5.3)	α2.59dd (15.7, 7.6) β1.95ddd (15.7, 5.3, 3.0)	4.33dd (7.6, 3.0)	α1.41dt (14.4, 2.2) β2.07dd (14.4, 4.9)	4.17dd (4.9, 1.0)	3.20dd (5.7, 1.1)	3.62dd (5.7, 2.2)	3.14d AB 2.76d (3.9)	0.78s	1.02s	1.45s	—	1.74
(**6**; R = Ac)	3.91d (5.2)	α2.51dd (15.5, 7.9) β2.01ddd (15.5, 5.2, 3.7)	5.56dd (7.9, 3.7)	α1.41dt (14.9, 2.0) β2.07m	5.25ddd (4.5, 2.0, 1.1)	3.23dd (5.7, 1.1)	3.73dd (5.7, 2.2)	3.16d AB 2.78d (3.9)	0.64s	1.03s	1.36s	2.08 2.10	—

Table 1 (*continued*)

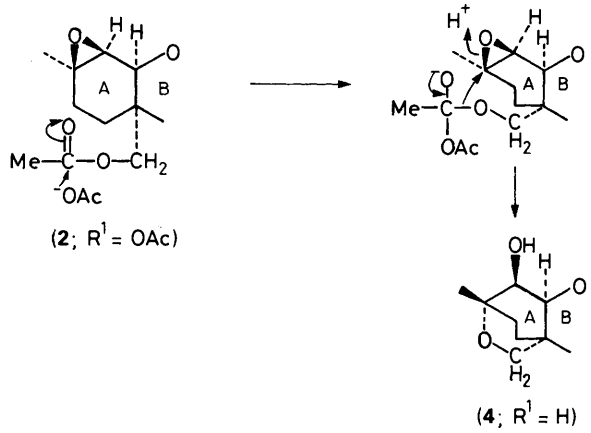
Compound	Position												
	2	3	4	7	8	10	11	13	14	15	16	Ac	OH[b]
(**4**; $R^1 = R^2 = H$)[f]	3.57d (4.6)	4.09A	4.10B		2.0m[c] 1.6m[c]	3.73dd (8.2, 0.8)	4.06dd (8.2, 2.1)	2.91d AB 2.72d (4.0)	0.61s	3.77d (9.3) 3.67dd (9.3, 2.6)	1.10s	—	
(**4**; $R^1 = H$, $R^2 = Ac$)[g]	3.84d (4.9)	4.19dd (4.9, 3.1)	4.94d (3.1)		2.0m[c] 1.6m[c]	3.89dd (8.1, 0.8)	4.22dd (8.1, 2.5)	3.04d AB 3.77d (3.9)	0.68s	3.77d (9.3) 3.73dd (9.3, 2.5)	1.20s	2.15	3.32d(3) (2.8) 2.86d(10) (1.0)
(**4**; $R^1 = R^2 = Ac$)	3.91d (4.8)	5.15dd (4.8, 3.4)	5.60d (3.4)	α1.56m[c] β1.95m[c]	α1.74m[c] β1.95m[c]	5.04dd (8.3, 1.4)	4.20dd (8.3, 2.5)	3.01d AB 2.76d (3.9)	0.58s	3.98dd (9.7, 2.7) 3.77d (9.7)	1.11s	2.07 2.10 2.16	—
(**7**; R = Ac)	4.20d (4.1)	α2.28dd (15.4, 8.0) β1.93ddd (15.4, 4.1, 2.7)	5.65dd (8.0, 2.7)	α1.82dd (16.0, 2.5) β2.79d (16.0)	3.05d (2.5)	3.77d[e] (2.5)	3.92d[e] (2.5)	3.97d AB 3.94d (13.2)	1.04s	1.27s	1.41s	2.07	2.21
(**15**; R = Ac)	5.18dd[e] (9.8, 6.0)	1.72dt (12.2, 9.8) 2.51dt (12.2, 6.1)	5.12dd[e] (9.8, 6.1)	2.78d AB 2.14d (15.0)	—	6.60dd (6, 1.5)	4.08d (6)	4.29d AB 4.26d (12.4)	1.09s	0.92s	1.84brs	2.06 2.06 2.07	—
(**16**; R = Ac)	3.98d (5.0)	α2.47dd (15.4, 7.9) β2.23dt (15.4, 4.9)	5.25dd (7.9, 4.8)	2.80ddd (13.2, 6.4, 1.4)	—	6.78dq (6.4, 1.5)	3.82dd (6.4, 1.4)	2.36ddd (13.6, 6.5) 2.21t (13.6)	0.97s	0.97s	1.84d (1.5)	2.10	1.76
(**20**; $R^1 = H$, $R^2 = OAc$, $R^3 = OH$)	5.22dd (12.0, 6.1)	2.13ddd (13.1, 6.1, 1.7) 1.88ddd (13.1, 12.0, 3.9)	4.95dd (3.9, 1.7)	α1.16m β1.75m[c]	1.75m[c]	4.91d (3.3)	4.31dd (3.3, 2.1)	4.52d AB 4.21d (12.1)	1.11s[e]	1.08s[e]	1.40s	2.04 2.08 2.12 2.20	1.92
(**17**; R = H)	7.31d (5.8)	6.29d (5.8)	—	α1.08ddt (12.9, 4,2) β1.64td (12.9, 5.3)	α1.79ddd (14.8, 12.3, 4.5) β1.90ddd (14.8, 5,2)	3.11d (4.8)	3.56dd (4.8, 1.8)	3.79dd (12.1, 4.8) 3.71dd (12.1, 8.3)	1.05s	0.83s	1.37s	—	2.73dd (8.3, 4.8)
(**17**; R = OMe)[g]	6.04s	—	—	α1.06m β1.65td (12.6, 5.3)	α1.79ddd (15.0, 12.4, 4.5) β1.90ddd (14.6, 4.4, 1.5)	3.11d (4.9)	3.64dd (4.9, 1.8)	3.77d AB 3.65d (12.1)	1.09s	0.84s	1.37s	—	2.82dd (4.0, 4.5) 3.77s OMe

[a] First-order approximations from line separations, unless stated otherwise. [b] In absence of D_2O. [c] Not first order. [d] In C_6D_6. [e] Assignments may be reversed. [f] In CD_3OD. [g] With D_2O present.

Table 2. Coupling constants (Hz) for hydrogens at positions 7 and 8 in the ^{1}H n.m.r. spectra of the 9-enes (**1**) and (**5**) and the 9β,10β-epoxides (**2**), (**6**), and (**18**).

Compound	7α, 7β	7α, 8α	7α, 8β	7β, 8α	7β, 8β	8α, 8β
(**1**; $R^1 = R^2 = R^3 = OH$)[a,b]	12.9	5.8	0.9	12.2	6.1	18.3
(**5**; R = Ac)	14.5	—	0.5	—	5.1	—
(**2**; $R^1 = R^2 = R^3 = OAc$)	12.9	4.8	2.2	12.9	4.8	15.0
(**19**)[c]	12.6	5.0	2.1	12.7	5.0	14.7
(**6**; R = Ac)	14.9	—	2.0	—	4.5	—
(**18**)	12.3	4.5	2.0	12.3	4.4	14.2

[a] In CD_3OD [b] by computer simulation [c] in C_6D_6.

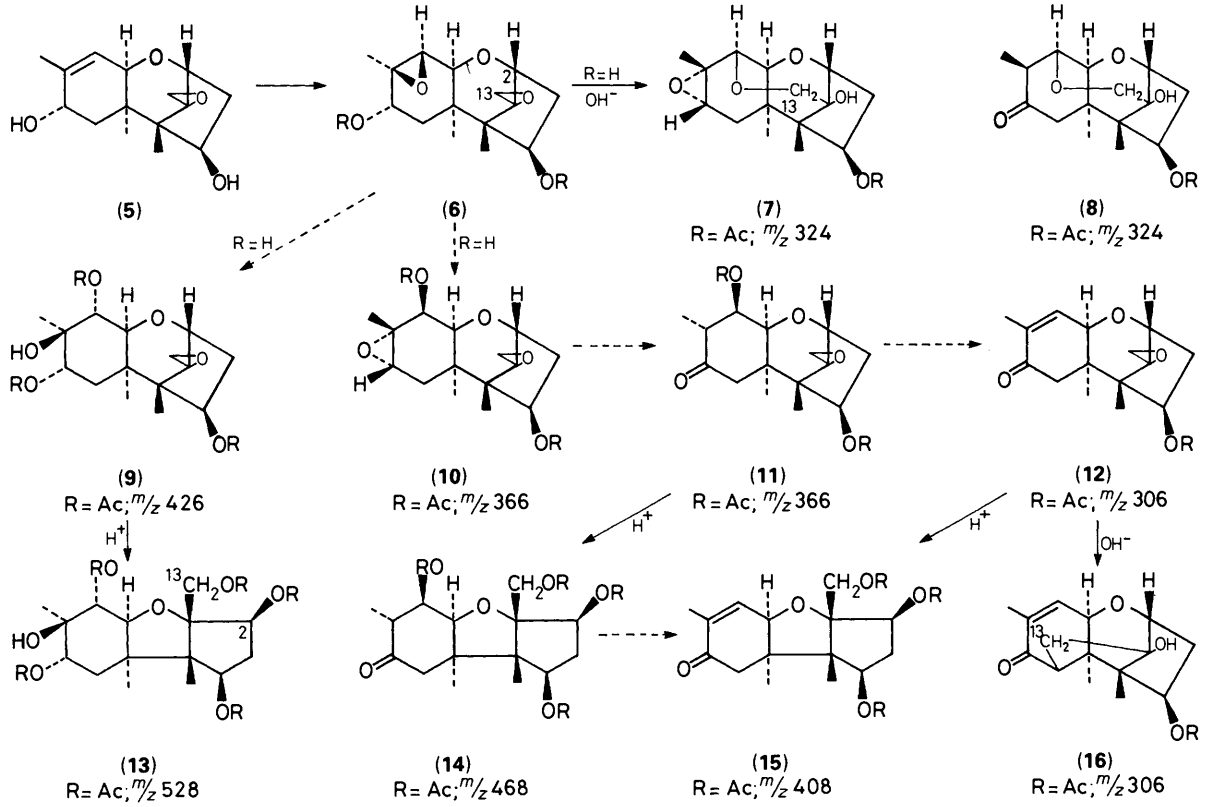


Scheme 1. Formation of the 9α,15-epoxy compound (**4**; R^1 = H).

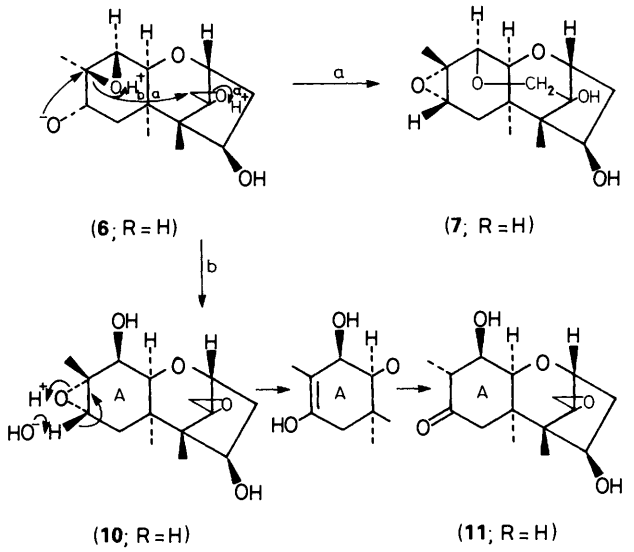
temperature and this product was also obtained when the reaction was carried out at 100 °C. When the epoxide (**2**; $R^1 = R^2$ = OAc, R^3 = OH) was heated with acetic acid at 100 °C it was converted into the 9α,15-epoxy compound (**4**; R^1 = H, R^2 = Ac) as shown in Scheme 1. This compound was also obtained as the only isolable product when the epoxide (**2**; $R^1 = R^2$ = OAc, R^3 = OH) was heated with water, pH 5, at 100 °C. Acetylation of both 9α,15-epoxides (**4**; R^1 = H, R^2 = H or Ac) yielded the same triacetate (**4**; $R^1 = R^2$ = Ac).

The ^{1}H n.m.r. spectra (see Table 1) of the 9α,15-epoxy compounds are noteworthy for a number of long range W-couplings ($J_{8\alpha,10\alpha}$ 0.8—1.4 Hz, $J_{7\alpha,11}$ 2.1—2.5 Hz, $J_{7\beta,15}$ 2.5—2.7 Hz) and these have been used to assign signals to 7-H_2 and 8-H_2 in the triacetate (**4**; $R^1 = R^2$ = Ac) and thence, by analogy, to assign 10-H and 11-H in the analogues (**4**; R^1 = H, R^2 = H and Ac). Ring A W-couplings also occur in the 9β,10β-epoxides (**2**) and (**6**) ($J_{7\alpha,11}$ 2.2 Hz[8]; $J_{8\beta,10}$ 0.8—1.1 Hz) and in the parent 9-enes (**1**) and are useful in the assignment of signals to 7α-H and 8β-H. The couplings provide firm evidence for the normal trichothecene ring A conformation (**2A**) in the 9β,10β-epoxides in solution at 25 °C. Molecular models show that W-couplings do not occur in the alternative ring A conformation (**2B**).

The 360 MHz spectra of 7-H and 8-H in trichothec-9-enes (**1**) and in their 9β,10β-epoxides (**2**) are essentially first order and the derived coupling constants ($J_{7\alpha,8\alpha}$ 4.5 Hz, $J_{7\alpha,8\beta}$ 2.0 Hz, $J_{7\beta,8\alpha}$ 12.3 Hz, $J_{7\beta,8\beta}$ 4.4 Hz) are consistent with those expected



Scheme 2. Rearrangement of trichothecodiol 9β,10β-epoxide (**6**; R = H).



Scheme 3. Formation of the products (**7**; R = H) and (**11**; R = H) from trichothecodiol 9β,10β-epoxide (**6**; R = H).

for the conformation (**2A**). The signals arising from 7-H and 8-H are most easily seen in the spectrum of trichodermone epoxide (**18**), where there is less interference in the 1—2δ region from more intense C–Me and Ac resonances, but there was excellent correspondence between the spectra for the epoxides (**18**) and (**2**; $R^1 = R^2 = R^3 = OAc$) (Table 2).

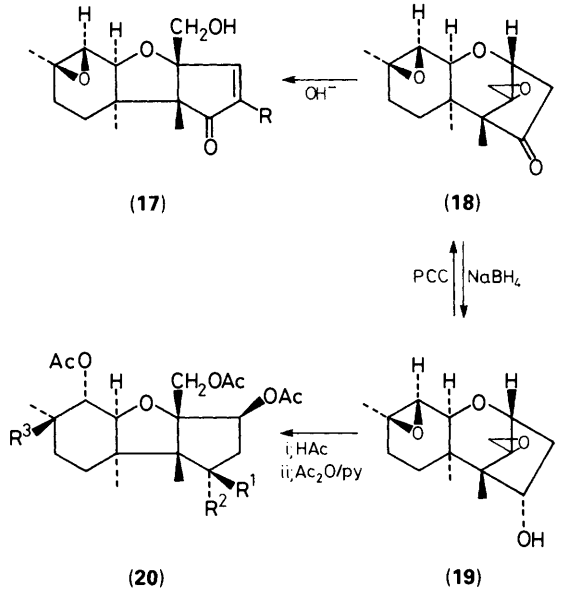
Following the discouraging results in the diacetoxyscirpenol series described above, attention was switched to the naturally-occurring trichothec-9-enes trichodermol (**1**; $R^1 = R^3 = H$, $R^2 = OH$), and its relatives, and trichothecodiol (**5**; R = H) in which there is no oxygen substituent at position 15.[9] Initially it was thought likely that the allylic 8α-hydroxy substituent in trichothecodiol might direct some epoxidation to the α-face. However, with 3-chloroperoxybenzoic acid in dichloromethane at room temperature, the 9β,10β-epoxide (**6**; R = H) was the only product.

The two diepoxides (**2**; $R^1 = R^3 = H$, $R^2 = OH$)[10]* and (**6**; R = H) presented a marked contrast in their behaviour under basic conditions. Whereas the diepoxide (**2**; $R^1 = R^3 = H$, $R^2 = OH$) was recovered unchanged after 8 h at 100 °C in 1M-sodium hydroxide, the diepoxide (**6**; R = H) was completely converted into a complex mixture of products after only 4 h. This mixture was separated into its constituents by column chromatography after acetylation under conditions where tertiary OH groups, at positions 9 and 12, are not acetylated.[9] The resulting acetates differed widely in molecular weight (see Scheme 2) and could then be detected by chemical ionisation mass spectrometry (c.i.m.s.) and separated.

The most readily obtained product [isolated as the acetate (**16**; R = Ac)] from the diepoxide (**6**; R = H) was the 7β,13-epoxy compound isotrichothecolone (**16**; R = H),[11,12] the known rearrangement product under these conditions of trichothecolone (**12**; R = H) which must be formed by dehydration of the intermediate ketol (**11**; R = H). Likewise, the major product from the action of acetic acid at 100 °C on the diepoxide (**6**; R = H) was, after acetylation, the known triacetate (**15**; R = Ac)[11] of the apotrichothecene 'trichothecolone glycol' (**15**; R = H).

The second major product [isolated as the acetate (**7**; R = Ac)] from the action of sodium hydroxide on the diepoxide (**6**; R = H) was the isomeric diepoxide (**7**; R = H) in which intramolecular attack (Scheme 3) from the axial oxygen anion at position 8 is accompanied by conformational change resulting in the formation of a 10–O ⟶ 13–C bridge as outlined above. The ^{1}H n.m.r. spectrum (Table 1) (δ 3.05, 8-H; δ 1.41, s, 16-H) was consistent with the 8α,9α-epoxide (**7**; R = Ac) and excluded the alternative 8-ketone (**8**; R = Ac). Molecular models of structure (**7**; R = Ac) show that φ7β,8β ~90°, consistent with the observation that $J_{7\beta,8} = 0$.

In addition to the rearrangement products of trichothecolone, which were characterised, the crude material (before chromatography) from the base-catalysed rearrangement contained, on mass spectroscopic evidence after acetylation, minor products of molecular weight corresponding to (**9**; R = Ac) and (**10** or **11**; R = Ac); and the crude material from the acid-catalysed rearrangement contained products of molecular weight corresponding to (**13**; R = Ac) and (**14**; R = Ac). The crude material from the action of water (pH 5) at 100 °C on the diepoxide (**6**; R = H) was very similar to that obtained with acetic acid and consisted, on mass spectroscopic evidence after acetylation, of the apotrichothecene (**15**; R = Ac) together with products of molecular weight corresponding to (**14**; R = Ac) and (**10** or **11**; R = Ac). The configuration of the substituents at positions 9 and 10 in these minor products is unproven: the tetraol (**9**; R = H) is the expected resultant of the diaxial opening of the epoxide (**6**; R = H) by an external nucleophile but it is thought unlikely that this structure would readily lose the elements of water to form a ketone since no hydrogen atom is available for *trans* diaxial elimination. The same argument vitiates the formation of a ketone from the polyol (**13**; R = H). More probably, the ketone (**11**; R = H) is derived from the 8α,9α-epoxide (**10**; R = H) according to Scheme 3 which envisages competing pathways a and b dependent on the conformation of ring A. In hypothetical 8-oxo intermediates, a 9-methyl substituent is assumed to adopt the equatorial configuration.



Scheme 4. Reactions of 4-epitrichodermol 9β,10β-epoxide (**19**) and relatives.

When trichodermol epoxide (**2**; $R^1 = R^3 = H$, $R^2 = OH$) was heated with acetic acid the product (after acetylation) was a gum of composition $C_{25}H_{36}O_{11}$ which can only be the penta-acetate (**20**; $R^1 = R^3 = OAc$, $R^2 = H$) in which opening of the 9β,10β-epoxide has occurred independently of a trichothecane ⟶ apotrichothecane rearrangement involving

* Owing to an acute shortage of material some confirmatory experiments were carried out with the 9β,10β-epoxide (**19**) of 4-epitrichodermol.[10]

the 12,13-epoxide. The epimer (**20**; R^1 = H, R^2 = R^3 = OAc) was obtained similarly from 4-epitrichodermol epoxide (**19**) and was accompanied by the tetra-acetate (**20**; R^1 = H, R^2 = OAc, R^3 = OH). Both products had an ^{1}H n.m.r. spectrum of ring C typical of the apotrichothecane ring system[1].

Likewise, when trichodermone epoxide (**18**) [obtained either directly from trichodermone or by oxidation of the epoxide (**19**) with pyridinium chlorochromate] was treated with 1M-sodium hydroxide at room temperature in an inert atmosphere, the known[13] neotrichodermone rearrangement occurred, without involvement of the 9β,10β-epoxide, to give the apotrichothec-2-en-4-one (**17**; R = H).

These results show that trichothecane 9β,10β-epoxides are readily opened by intramolecular attack but much less readily by external nucleophiles. They do not support the involvement of a 9β,10β-epoxide in the mechanism of action of the non-macrocyclic trichothec-9-ene mycotoxins.

When trichodermone epoxide was treated with sodium hydroxide in air in the presence of methanol, the product was the enone (**17**; R = OMe), assigned from the chemical shift of 2-H (δ 6.04) by comparison with the shifts of the vinylic hydrogens in 2-methoxy- (δ 6.37)[14] and 3-methoxycyclopent-2-en-1-one (δ 5.45).[15]

Experimental

M.p's were taken on a Kofler hot stage apparatus and are corrected. Identifications were confirmed by comparison of the i.r. spectra (mulls in Nujol). Unless stated otherwise, ^{1}H n.m.r. spectra were obtained at 360 MHz in $CDCl_3$ with $SiMe_4$ as internal standard. Molecular weights were taken from the mass spectra. NH_3 was used to obtain chemical ionisation mass spectra (c.i.m.s.). Negative c.i. was used for mass measurement at high resolution. In analytical t.l.c. Merck silica gel 60 F_{254} was used with chloroform–methanol (9 : 1). Spots were visualised in u.v. light or in iodine vapour or by heating after spraying with sulphuric acid–methanol, as appropriate. Merck silica gels 7 739 and 7 734 were used in preparative t.l.c. (20 × 20 cm plates: 0.1 cm layer) and in column chromatography, respectively. Unless stated otherwise, acetylations were carried out in pyridine with acetic anhydride at room temperature during 24 h. Light petroleum had b.p. 60—80 °C.

3α,4β,15-*Triacetoxy*-9β,10β; 12,13-*diepoxytrichothecane* (**2**; R^1 = R^2 = R^3 = OAc).—3α,4β,15-Triacetoxyscirpene (**1**; R^1 = R^2 = R^3 = OAc) (40 mg) and 3-chloroperoxybenzoic acid (22 mg) in dichloromethane (5 ml) were stirred at room temperature for 2 h. Recovery, after washing with aqueous sodium hydrogen carbonate, furnished a solid (43 mg) which crystallised from ethyl acetate–light petroleum in prisms, m.p. 188—190 °C of the *diepoxide* (**2**; R^1 = R^2 = R^3 = OAc). R_F 0.69 (Found: C, 59.2; H, 6.4%; $M$$NH_4^+$ 442. $C_{21}H_{28}O_9$ requires C, 59.4; H, 6.6%; M 424); $\nu_{max.}$ 1 740 cm^{-1}.

4β,15-*Diacetoxy*-9β,10β; 12,13-*diepoxytricothecan*-3α-*ol* (**2**; R^1 = R^2 = OAc, R^3 = OH).—R_F 0.55, was prepared according to ref. 7. Acetylation gave the triacetate (**2**; R^1 = R^2 = R^3 = OAc).

9β,10β; 12,13-*diepoxytrichothecan*-4β,8α-*diol* (**6**; R = H).—Trichothecodiol (**5**; R = H) (266 mg) and 3-chloroperoxybenzoic acid (220 mg) in dichloromethane (15 ml) were stirred at room temperature for 24 h. Dichloromethane (10 ml) was added, and stirring was continued for a further 24 h when the crystalline precipitate (205 mg, m.p. 208—220 °C) was collected. The filtrate was washed with iron(II) sulphate, water, and aqueous sodium hydrogen carbonate. The recovered material (78 mg) was combined with the precipitate and twice recrystallised from dichloromethane to give prisms of indefinite m.p. (m.p. 230—250 °C), R_F 0.27 of the *diepoxide* (**6**; R = H) *hydrate* (Found: C, 59.8; H, 7.4% $M$$NH_4^+$ 300. $C_{15}H_{22}O_5$·H_2O requires C, 60.0; H, 8.0% M 282) $\nu_{max.}$ 3 450 br. cm^{-1}. The *diacetate* (**6**; R = Ac) crystallised from ethyl acetate–light petroleum as prisms, m.p. 170—172 °C, R_F 0.73 (Found: C, 62.2; H, 7.3% $C_{19}H_{26}O_7$ requires C, 62.3; H, 7.2%); $\nu_{max.}$ 1 735 cm^{-1}.

9β,10β; 12,13-*Diepoxytrichothecan*-4β-ol (**2**; R^1 = R^3 = H, R^2 = OH) R_F 0.42 and its 4α-*epimer* (**19**) R_F 0.50 were prepared according to ref. 10.

9β,10β; 12,13-*Diepoxytrichothecan*-4-*one* (**18**).—(*a*) Trichodermone (20 mg) similarly furnished the *diepoxide* (**18**) as prisms (12 mg) m.p. 193—194 °C from ethyl acetate–light petroleum, R_F 0.73 (Found: C, 68.0; H, 7.7%. $C_{15}H_{20}O_4$ requires C, 68.2; H, 7.6%); $\nu_{max.}$ 1 737 cm^{-1}.

(*b*) 9β,10β; 12,13-Diepoxytrichothecan-4α-ol (**19**) (10 mg) in dichloromethane (3 mg) was stirred at room temperature for 3 h with pyridinium chlorochromate (12 mg) in the presence of anhydrous sodium acetate (5 mg) and powdered molecular sieves (3A; 20 mg). After the addition of diethyl ether (3 ml), the reaction mixture was filtred through a column of silica gel (3 × 1 cm) made up in dichloromethane. The column was washed with diethyl ether (10 ml), and recovery from the combined filtrate and washings then furnished the *diepoxide* (**18**) (9 mg).

Attempted Base Catalysed Rearrangements.—(*a*) The diepoxide (**2**; R^1 = R^2 = OAc, R^3 = OH) (37 mg) in methanol (1 ml) and 1M-sodium hydroxide (2 ml) was set aside at room temperature for 24 h. The solution was neutralised with M-hydrochloric acid and continuously extracted with chloroform for 30 h. The product (25 mg) crystallised from ethyl acetate in prisms (20 mg) m.p. 240—242 °C, R_F 0.14 of 9α,15; 12, 13-*diepoxytrichothecan*-3α,4β,10β-*triol* (**4**; R^1 = R^2 = H) (Found: C, 60.3; H, 7.3%; $M$$NH_4^+$ 316. $C_{15}H_{22}O_6$ requires C, 60.4; H, 7.4%; M 298); $\nu_{max.}$ 3 480, 3 415, and 3 350 cm^{-1}.

Acetylation gave 3α,4β,10β-*triacetoxy*-9α,15; 12,13-*diepoxytrichothecane* (**4**; R^1 = R^2 = Ac) as prisms or needles m.p. 193—194 °C, R_F 0.68, from ethyl acetate–light petroleum (Found: C, 59.1; H, 6.4%; $M$$NH_4^+$ 442. $C_{21}H_{28}O_9$ requires C, 59.4; H, 6.6%; M 424); $\nu_{max.}$ 1 754, and 1 733 cm^{-1}.

The triol (**4**; R^1 = R^2 = H) was the only product (t.l.c.) when the diepoxide (**2**; R^1 = R^2 = OAc, R^3 = OH) was heated in methanol and 1M-sodium hydroxide (1 : 2) for 3 h at 100 °C.

(*b*) The diepoxide (**6**; R = H) (10 mg) was recovered, by neutralisation and continuous extraction with chloroform, after standing 24 h at room temperature in 1M-sodium hydroxide (0.5 ml).

(*c*) The diepoxide (**6**; R = H) (25 mg) was heated at 100 °C for 4 h with 1M-sodium hydroxide (1.0 ml). The cooled solution was neutralised with hydrochloric acid and continuously extracted with chloroform for 6 h. The recovered gum (26 mg) in pyridine was acetylated with acetic anhydride during 6 days at room temperature giving a gum (27 mg) R_F 0.70, 0.55, 0.50, and 0.45. The mass spectrum (e.i.) showed a molecular ion (M^+) at m/z 366 corresponding to the species (**10** or **11**; R = Ac); and on c.i., $M$$H^+$, and $M$$NH_4^+$ ions at m/z 427, 444; 367, 384; 325, 342; and 307, 324 corresponding to the species (**9**; R = Ac), (**10** or **11**; R = Ac), (**7** or **8**; R = Ac) and (**12** or **16**; R = Ac) respectively. The acetylated gum, in benzene, was chromatographed on a silica column (2 g, 1.0 × 6 cm) made up in benzene. Elution with chloroform gave the following fractions, monitored by analytical t.l.c. (i) 10 ml, 2 mg, discarded (ii) 6 ml, 8 mg; R_F 0.7;

MH^+/MNH_4^+ at *m/z* 367, 384. (iii) 6 ml, 5 mg; R_F 0.55; MH^+/MNH_4^+ at *m/z* 325, 342. (iv) 9 ml, 6 mg; R_F 0.50; MH^+/MNH_4^+ at *m/z* 307, 324 and MNH_4^+ at *m/z* 444. (v) 50 ml, 2 mg; R_F 0.45; MH^+/MNH_4^+ at *m/z* 369, 386.

Fraction (iii) crystallised from diethyl ether in prisms (3 mg) m.p. 172—180 °C, R_F 0.55 of 4β-*acetoxy*-8α,9α; 10β,13-*diepoxytrichothecan*-12-*ol* (**7**; R = Ac) (Found: C, 62.8; H 7.6%; M^+ 324. MH^+/MNH_4^+ at *m/z* 325, 342. $C_{17}H_{24}O_6$ requires C, 63.0; H, 7.5%; *M* 324); $\nu_{max.}$ 3 471, and 1 725 cm^{-1}.

Fraction (iv) was subjected to preparative t.l.c. in chloroform–methanol (9:1) and the u.v. absorbing band R_F 0.50 was extracted with chloroform giving a gum (3 mg) which crystallised from diethyl ether in prisms (2 mg) (converted to needles at 150 °C) m.p. 177—180 °C, M^+ 306; MH^+/MNH_4^+ at *m/z* 307, 324, identified as acetylisotrichothecolone (**16**; R = Ac) by the n.m.r. spectrum and confirmed by comparison of the i.r. spectrum with that of a specimen of acetylisotrichothecolone (lit.,[11] m.p. 185—186 °C) prepared[11] from trichothecolone (**12**; R = H).

(*d*) The diepoxide (**2**; $R^1 = R^3 = H$, $R^2 = OH$) (8 mg) was recovered after heating for 8 h at 100 °C in methanol (0.2 ml) and 1M-sodium hydroxide (0.6 ml) by neutralisation and extraction with ethyl acetate. In a confirmatory experiment the diepoxide (**19**) (25 mg) was also recovered after 12 h under the same conditions.

(*e*) The diepoxide (**18**) (5 mg) in methanol (0.2 ml) and 1M-sodium hydroxide (1.0 ml) was set aside at room temperature under nitrogen for 15 h. The yellow solution was extracted with chloroform giving a gum (3 mg) which crystallised from ethyl acetate–light petroleum in prisms, m.p. 125 °C, R_F 0.56 of 9β,10β-*epoxy*-13-*hydroxyapotriochothec*-2-*en*-4-*one* (**17**; R = H) (Found: C, 68.1; H, 7.7%; MH^+ 265 M^- 264.1354 $C_{15}H_{20}O_4$ requires C, 68.2; H, 7.6%; *M* 264.1361); $\nu_{max.}$ 3 458, 3 413, and 1 713 cm^{-1}; $\lambda_{max.}$ 215 nm (Log ε 3.97).

When the reaction took place in air, albeit in a stoppered tube, the product formed rosettes of needles (3 mg). M.p. 130—131 °C, R_F 0.55 of 9β,10β-*epoxy*-13-*hydroxy*-3-*methoxyapotrichothec*-2-*en*-4-*one* (**17**; R = OMe) (Found: M^- 294.1453 $C_{16}H_{22}O_5$ requires *M* 294.1467); $\nu_{max.}$ 3 305, 1 714, and 1 635 cm^{-1}; $\lambda_{max.}$ 250 nm (Log ε 3.80).

Attempted Acid Catalysed Rearrangements.—A. With Acetic Acid.—(*a*) The diepoxide (**2**; $R^1 = R^2 = OAc$, $R^3 = OH$) (74 mg) in acetic acid (2 ml) was heated at 100 °C for 8 h. After removal of the solvent *in vacuo* (10^{-1} mmHg), the product, in benzene, was chromatographed on a column of silica gel (2 g, 1 × 6 cm) made up in benzene. Fractional elution with chloroform, monitored by t.l.c. gave (i) 30 ml, R_F 0.6, 17 mg intractable gums; followed by (ii) 40 ml, R_F 0.45, 25 mg gum, which crystallised from ethyl acetate–light petroleum in prisms (7 mg) m.p. 200–203 °C, R_F 0.14 of 4β-*acetoxy*-9α,15; 12,13-*diepoxytrichothecan*-3α,10β-*diol* (**4**; $R^1 = H$, $R^2 = Ac$) (Found: C, 58.6; H, 6.8%; MNH_4^+ 358. $C_{17}H_{24}O_7 \cdot 0.5\ H_2O$ requires C, 58.4; H, 7.2%; *M* 340); $\nu_{max.}$ 3 480, and 1 730 cm^{-1}. Acetylation gave the triacetate (**4**; $R^1 = R^2 = Ac$).

(*b*) The diepoxide (**6**; R = H) (10 mg) in acetic acid (0.5 ml) was heated at 100 °C for 5 h when no starting material remained (t.l.c.). After removal of the solvent under reduced pressure, the residue was acetylated with acetic anhydride–pyridine at room temperature during 3 days. The product was an amorphous solid (8 mg), m.p. 50—60 °C, R_F 0.73 and 0.57. The mass spectrum (e.i.) showed M^+ at *m/z* 408 attributed to structure (**15**; R = Ac); and on c.i., MH^+ and MNH_4^+ ions at *m/z* 529, 546; 469, 486; and 409, 426 corresponding to the species (**13**; R = Ac), (**14**; R = Ac), and (**15**; R = Ac) respectively. The solid was subjected to preparative t.l.c. in chloroform–methanol (9:1) and the u.v.-absorbing band, R_F 0.73, was extracted with chloroform. The amorphous solid obtained (4 mg) was identified as 2β,4β,13-triacetoxyapotrichothec-9-en-8-one (triacetyltrichothecolone glycol) (**15**; R = Ac) by comparison of the n.m.r. spectrum (Table 1) with that of a specimen prepared[11] from trichothecolone (**12**; R = H).

(*c*) The diepoxide (**2**; $R^1 = R^3 = H$, $R^2 = OH$) (2 mg) in acetic acid (0.1 ml) was heated at 100 °C for 3 h. After work-up as described in (*b*) above, the acetylated product was a gum R_F 0.70 consisting of 3β,4β,9β,10α,13-*penta-acetoxyapotrichothecane* (**20**; $R^1 = R^3 = OAc$, $R^2 = H$) (Found: MNH_4^+ 530; $[M - H]^-$ 511.2146. $C_{25}H_{36}O_{11}$ requires *M* 512. $C_{25}H_{35}O_{11}$ requires *m/z* 511.2179).

(*d*) The diepoxide (**19**) (33 mg) in acetic acid (0.3 ml) was heated at 100 °C for 4 h. After working up as described in (*b*) above, the acetylated product (49 mg) in dichloromethane was chromatographed on a column of silica gel (1.5 g, 1 × 5 cm) made up in dichloromethane and monitored by analytical t.l.c. After a forerun (90 ml), elution with dichloromethane–methanol furnished a series of gummy fractions (i) 50 ml (200:1), 13 mg R_F 0.75 (ii) 50 ml (200:1), 10 mg R_F 0.75 and 0.73 (iii) 20 ml (100:1), 2 mg R_F 0.68 and (iv) 60 ml (100:1), 7 mg R_F 0.60.

Fraction (i) consisted of 3β,4α,9β,10α,13-penta-acetoxyapotrichothecane (**20**; $R^1 = H$, $R^2 = R^3 = OAc$) (Found: MNH_4^+ 530. $C_{25}H_{36}O_{11}$ requires *M* 512); $\nu_{max.}$ OH absent, 1 740 cm^{-1}; δ_H 5.30 d (3.6) 10-H; 5.3dd (11.9, 6.3) 2-H; 4.94dd (3.7, 1.5) 4-H; 4.47, 4.22 AB (12.1) 13-H.

Fraction (iv) crystallised from diethyl ether in prisms (3 mg), m.p. 181—185 °C of 3β,4α,10α,13-*tetra-acetoxyapotrichothecan*-9β-*ol* (**20**; $R^1 = H$, $R^2 = OAc$, $R^3 = OH$) (Found: C, 58.6; H, 7.4%; MNH_4^+ 488. $C_{23}H_{34}O_{10}$ requires C, 58.7; H, 7.3%; *M* 470); $\nu_{max.}$ 3 539, 3 398br, and 1 737 cm^{-1}.

B. *With Water* (pH 5).—(*a*) The diepoxide (**2**; $R^1 = R^2 = OAc$, $R^3 = OH$) (37 mg) in chloroform (0.1 ml) and water (pH 5, 2 ml) was heated under reflux for 6 h. The cooled solution was decanted from a little tarry material and extracted with chloroform giving (i) a gum (25 mg) R_F 0.55 and 0.45. Continuous extraction of the aqueous residue with chloroform for 10 h then yielded (ii) a gum (8 mg) R_F 0.45, 0.28, 0.18, 0.13 and 0.08 which proved intractable. Fraction (i), in benzene, was chromatographed on a column of silica gel, (1.5 g, 1.0 × 5 cm) made up in benzene. Fractional elution with chloroform gave, after a forerun (15 ml), (*a*) 20 ml, 8 mg, R_F 0.55 identified as starting material. (*b*) 10 ml, 2 mg, interband and (*c*) 50 ml, R_F 0.45, 5 mg gum, which crystallised from ethyl acetate–light petroleum in prisms m.p. 200—203 °C of the diol (**4**; $R^1 = H$, $R^2 = Ac$).

(*b*) The diepoxide (**6**; R = H (22 mg) in water (1.0 ml) was heated under reflux for 2 h and the cooled solution was then continuously extracted with chloroform for 6 h giving a gum (15 mg). The gum was acetylated with acetic anhydride–pyridine at room temperature during 5 days giving a gum (19 mg) R_F 0.76 and 0.68. The mass spectrum (e.i.) showed a molecular ion at *m/z* 408, corresponding to the species (**15**; R = Ac) and on c.i., MH^+ and MNH_4^+ ions at 469, 486; 409, 426 and 367, 384; corresponding to the species (**14**; R = Ac), (**15**; R = Ac) and (**10** or **11**; R = Ac) respectively.

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