Phytotoxic Compounds Produced by *Fusarium equiseti*. Part 8.[1] Acid Catalysed Rearrangement of 12,13-Epoxytrichothec-9-enes

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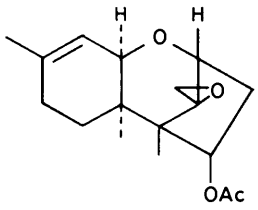
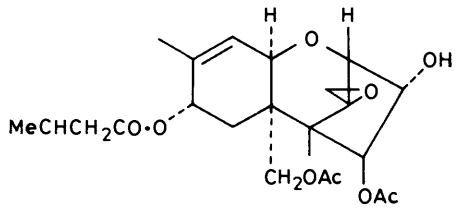
The acid catalysed rearrangement of 12,13-epoxytrichothec-9-enes to give biologically inactive 10,13-cyclotrichothecane or apotrichothec-9-ene products depends on the substitution pattern of both rings A and C. The former rearrangement is adversely affected by 8α-substitution and by the presence of a 4,15-macrolide ring; the latter rearrangement by 7α- and by 3α-substitution. Compared with the trichothecene, the greater flexibility of the apotrichothecene skeleton is reflected in the range of values for the ring C vicinal coupling constants in the ^{1}H n.m.r. spectra.

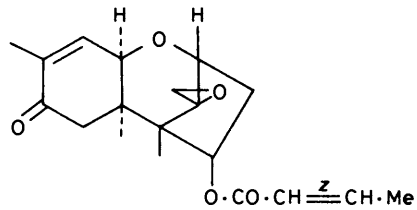
The non-macrocyclic 12,13-expoxytrichothecene mycotoxins fall readily into three groups: (I) simple ring C substituted 9-enes, *e.g.* trichodermin (**2**; $R^1 = R^3 = H$, $R^2 = OAc$)[2]; (II) 9-enes, as in group I, but with additional substituents at positions 7 and/or 8 in ring A, *e.g.* crotocin (**9**; R = COCH=CHMe)[3] and T_2-toxin (**6**; $R^1 = Me_2CHCH_2CO$, $R^2 = H$, $R^3 = OAc$, $R^4 = Ac$, $R^5 = OH$);[4] and (III) 9-en-8-ones, *e.g.* trichothecin (**14**; $R^1 = R^2 = R^4 = H$, $R^3 = OCOCH=CHMe$).[5] This group can have substituents at position 7 as well as in ring C.



Trichodermin



T_2-Toxin



Trichothecin

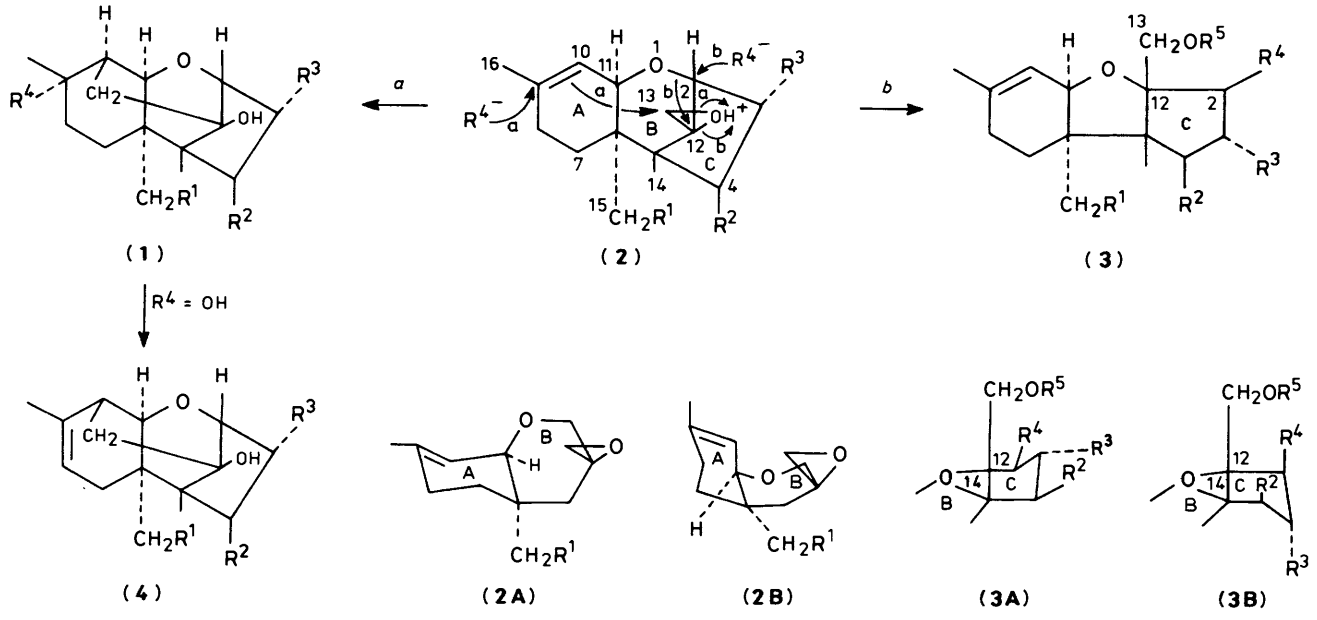
The strains of *Fusarium equiseti* Corda (Sacc.) and *F. scirpi* (= *F. equiseti*) used in the early work[6] were remarkable in that they produced, on glucose–ammonium nitrate (GAN) medium, an example of each of the three groups, *i.e.* the major metabolite diacetoxyscirpenol (**2**; $R^1 = R^2 = OAc$, $R^3 = OH$)[7–10] and the minor metabolites (**6**; $R^1 = R^4 = Ac$, $R^2 = R^5 = OH$, $R^3 = OAc$),[11] and (**14**; $R^1 = R^4 = OH$, $R^2 = R^3 = OAc$).[7,12,13] The latter subsequently became known as diacetylnivalenol.[13]

The structural features necessary for biological activity in the naturally occurring trichothec-9-enes have been outlined previously.[1,14] 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. In general, in acid media, protonation of the epoxide is followed by intramolecular rearrangement and the reaction is concluded by the addition of a nucleophile at a more accessible centre. These reactions, leading to the destruction of the epoxide and loss of biological activity,[14] are of importance in assessing potential methods for detoxification of the trichothecene mycotoxins. Additionally, a reaction of this kind is presumably involved in the alkylation step responsible for biological activity.

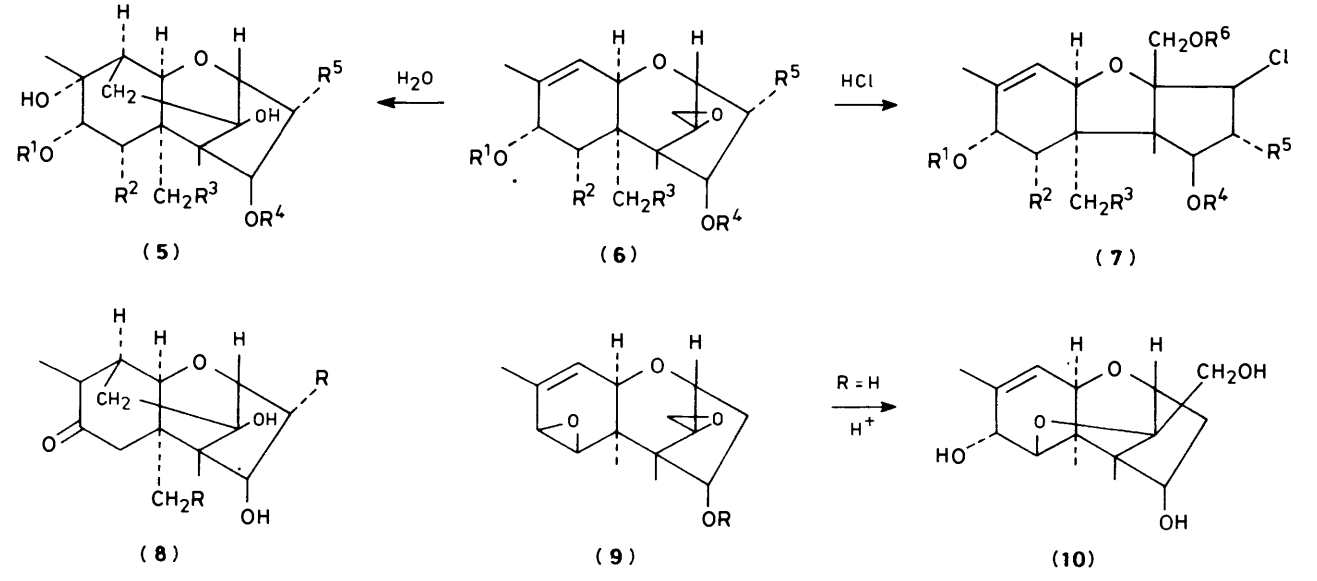
With trichothecenes of group I, *e.g.* trichodermol (**2**; $R^1 = R^3 = H$, $R^2 = OH$)[2] and verrucarol (**2**; $R^1 = R^2 = OH$, $R^3 = H$)[15] two such intramolecular rearrangements (Scheme 1) leading (*a*) to 10,13-cyclotrichothecane products (**1**) and (**4**) (8-ene) and (*b*) to the apotrichothec-9-ene skeleton (**3**) proceed readily, in good yield, under relatively mild conditions. The 7α-hydroxytrichothec-9-en-8-ones of group III are exceptional in that, unlike trichothec-9-en-8-ones substituted only in ring C, they do not rearrange to the apotrichothecene skeleton under the same conditions.[1] This paper is concerned with the behaviour of trichothecenes of group II under reaction conditions which are effective in bringing about rearrangement of group I trichothecenes. The work was initiated following the discovery that these conditions failed to bring about rearrangement of the *F. equiseti* metabolite (**6**; $R^1 = R^4 = Ac$, $R^2 = R^5 = OH$, $R^3 = OAc$).

The Trichothecene→10,13-Cyclotrichothecane Rearrangement.—Although *X*-ray crystallographic[16] and n.m.r.[12] studies have shown that the trichothec-9-ene skeleton adopts the half-chair conformation (**2A**) in the solid state and in non-polar solvents, the alternative half-chair conformation (**2B**) of the *cis*-fused A/B rings facilitates attack (*a*, Scheme 1) by the π-electron system of the 9-ene on the protonated epoxide giving the 10,13-cyclotrichothecene skeleton. The ease with which this conformational change occurs should be influenced by the nature of the substituents on ring A, but not by ring C substitution. On boiling with water (pH 5) for 6 h (adopted as the standard conditions), verrucarol (**2**; $R^1 = R^2 = OH$, $R^3 = H$), like the diacetyl derivative (**2**; $R^1 = R^2 = OAc$, $R^3 = H$),[15] gave a 10,13-cyclotrichothecane product (**1**; $R^1 = R^2 = R^4 = OH$, $R^3 = H$). As expected, the introduction of a 3α-hydroxy substituent had no effect on the rearrangement and scirpentriol (**2**; $R^1 = R^2 = R^3 = OH$) and diacetoxyscirpenol (**2**; $R^1 = R^2 = OAc$, $R^3 = OH$) gave 10,13-cyclotrichothecanes (**1**; $R^1 = R^2 = R^3 = R^4 = OH$) and (**1**; $R^1 = R^2 = OAc$, $R^3 = R^4 = OH$), respectively.

Acetylation of both products gave the triacetate (**1**; $R^1 = R^2 = R^3 = OAc$, $R^4 = OH$), previously obtained[10] under these conditions from the triacetate (**2**; $R^1 = R^2 = R^3 = OAc$). Calonectrin (**2**; $R^1 = R^3 = OAc$, $R^2 = H$) likewise[17] gives the 10,13-cyclotrichothecane (**1**; $R^1 = R^2 = OAc$, $R^3 = H$, $R^4 = OH$). A complete assignment of the signals in the ^{1}H n.m.r.



Scheme 1. Acid catalysed rearrangements of ring C substituted 12,13-epoxytrichothec-9-enes.



Scheme 2. Acid catalysed rearrangement of 12,13-epoxytrichothecenes substituted in both rings A and C.

spectrum of the 10,13-cyclotrichothecane (**1**; $R^1 = R^2 = R^3 =$ OAc, $R^4 =$ OH) was possible (Table). The coupling constants for the hydrogens at position 13 ($J_{gem} = 14.0$, $J_{10,13\alpha} = 11.8$, $J_{10,13\beta} = 5.5$ Hz) were consistent with the new 6-membered ring in a boat conformation with $\varphi_{10,13\alpha} = 0°$.

The apotrichothecene structure (**3**; $R^1 = R^2 = R^3 = R^4 =$ OAc, $R^5 =$ H) was assigned[8] to a $C_{23}H_{32}O_{10}$ tetra-acetate obtained from an attempted reduction of diacetoxyscirpenol with zinc and acetic acid. Reinvestigation of this substance has shown it to be the known[10] 10,13-cyclotrichothecane (**1**; $R^1 = R^2 = R^3 = R^4 =$ OAc) which shows dimorphism, as does the analogue (**1**; $R^1 = R^2 = R^3 =$ OAc, $R^4 =$ OH).

15-Acetoxylation, as in diacetylverrucarol (**2**; $R^1 = R^2 =$ OAc, $R^3 =$ H) and diacetoxyscirpenol (**2**; $R^1 = R^2 =$ OAc, $R^3 =$ OH), had no effect on the trichothecene→10,13-cyclotrichothecane rearrangement, but the 4,15-macrocycle verrucarin A (**2**; $R^1R^2 =$ OCOCHOHCHMeCH$_2$CH$_2$OCOCH-^{E}=CHCH^Z=CHCOO, $R^3 =$ H) was recovered unchanged when subjected to the standard conditions. 7β- and 8β-Substitution, both of which would sterically inhibit the rearrangement, are unknown amongst the naturally occurring trichothecenes with the exception of crotocin (**9**; R = COCH^Z=CHMe)[3] and some macrocyclic derivatives[18] of the verrucarins. In acidic media the more accessible 7β,8β-epoxide of crotocol (**9**; R = H) is attacked first[3] to give the 7,12-epoxytrichothec-9-ene (**10**).

Neither an 8α-hydroxy substituent nor 7α,8α-dihydroxy substitution completely inhibited the reaction, and 10,13-cyclotrichothecane products were obtained from the trichothecenes (**6**; $R^1 = R^2 = R^3 = R^4 = R^5 =$ H) (trichothecodiol[19]) and the triol (**6**; $R^1 =$ H, $R^2 = R^5 =$ OH, $R^3 =$ OAc, $R^4 =$ Ac),[13,20] but in both cases some starting material was recovered and a reaction time of 9—15 h was necessary to obtain a reasonable

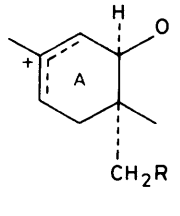
yield of product. However, the products were more complex than those from group I trichothecenes. The pentaol (**5**; R^1 = H, R^2 = R^5 = OH, R^3 = OAc, R^4 = Ac), from the triol (**6**; R^1 = H, R^2 = R^5 = OH, R^3 = OAc, R^4 = Ac), was accompanied by the 9β-hydroxy epimer, detected in the n.m.r. spectrum by signals at δ1.03 (14-H), 1.32 (16-H), and 3.51 (11-H), but not isolated. The product from trichothecodiol was intractable, but from physical data appeared to be a mixture of the tetraol (**5**; R^1 = R^2 = R^3 = R^4 = R^5 = H) and its 9-epimer with the 8-ketone (**8**; R = H). The formation of the last compound is analogous to that of the 8-enes (**4**) from the group I trichothecenes. The formation of a mixture of the polyol (**5**; R^1 = R^2 = R^4 = H, R^3 = R^5 = OH), its 9-epimer, and the 8-ketone (**8**; R = OH) from the tetraol (**6**; R^1 = R^2 = R^4 = H, R^3 = R^5 = OH) (T_2-tetraol) has recently been reported,[21] albeit under more severe conditions (121 °C, 15 lb in^{-2}, 7 h).

Esterification of an 8α-hydroxy substituent was decisive in preventing the rearrangement and T_2-toxin (**6**; R^1 = Me_2CHCH_2CO, R^2 = H, R^3 = OAc, R^4 = Ac, R^5 = OH) (in agreement with a recent statement[21]) and the diacetate (**6**; R^1 = R^4 = Ac, R^2 = R^3 = R^5 = H) were recovered unchanged, as were the *F. equiseti* metabolite (**6**; R^1 = R^4 = Ac, R^2 = R^5 = OH, R^3 = OAc) and the penta-acetate (**6**; R^1 = R^4 = Ac, R^2 = R^3 = R^5 = OAc). Although acetylation of the triol (**6**; R^1 = H, R^2 = R^5 = OH, R^3 = OAc, R^4 = Ac) under the normal conditions was incomplete,[11] giving the tetra-acetate (**6**; R^1 = R^4 = Ac, R^2 = OH, R^3 = R^5 = OAc), a longer reaction time gave the penta-acetate (**6**; R^1 = R^4 = Ac, R^2 = R^3 = R^5 = OAc) (*cf.*[20]).

These results show that Group II trichothec-9-enes with 8α-OR substituents do not readily undergo rearrangement to 10,13-cyclotrichothecanes. This is to be expected from the reduced nucleophilicity of the 9-ene π-system and a tendency to form the allylic cation (**11**). Steric factors also contribute. Although the 8α-substituent is quasi-equatorial in conformation (**2B**), there is steric hindrance of the approach of a nucleophile to the α-face of ring A. Finally, the adverse effect of a bulky OR group on the energetics of the conformational change (**2A**→**2B**) also contributes. This last factor must be the main reason for the failure of the rearrangement in the presence of a 4,15 macrolide ring.

The Trichothecene→Apotrichothecene Rearrangement.—In strongly acidic media the 12,13-epoxytrichothec-9-ene→10,13-cyclotrichothecane rearrangement is prevented, presumably by protonation of the 9-ene, and is replaced by attack from the ring B oxygen bridge (*b*, Scheme 1) to give the apotrichothec-9-ene skeleton (**3**). *X*-Ray studies[16] show that in 12,13-epoxytrichothec-9-ene (**2**) the O(1)–C(2) and C(12)–O bonds are approximately coplanar and antiparallel. The new primary alcohol group at position 13 is readily acetylated and products with the apotrichothecane skeleton are thus easily distinguished from the products of reactions giving tertiary alcohols at position 12.[1] Whilst 10M-hydrochloric acid at room temperature (24 h) was originally used[2,15] to bring about the apotrichothecene rearrangement, hydrogen chloride in a non-polar solvent (15 min)[8] gives a cleaner product and has, in general, been used in the present work. Using this method, the known chloroapotrichothecene (**3**; R^1 = R^2 = OH, R^3 = R^5 = H, R^4 = Cl)[15] was prepared from verrucarol (**2**; R^1 = R^2 = OH, R^3 = H), and the chloroapotrichothecenes (**3**; R^1 = R^2 = OAc, R^3 = R^5 = H, R^4 = Cl) and (**3**; R^1R^2 = OCOCHOH-CHMeCH_2CH_2OCOCH^E=CHCH^Z=CHCOO, R^3 = R^5 = H, R^4 = Cl) were obtained from diacetylverrucarol and verruccarin A, respectively. The structure of the macrolide chloroapotrichothecene was confirmed by the formation of a diacetate. Esterification of the hydroxy groups of verrucarol, and the presence of the 4,15-macrolide ring, thus had no effect on this rearrangement.

Although treatment with hydrogen chloride for 15 min was sufficient to bring about the rearrangement of scirpentriol (**2**; R^1 = R^2 = R^3 = OH) and diacetoxyscirpenol (**2**; R^1 = R^2 = OAc, R^3 = OH)[8] to the corresponding chloroapo-compounds (**3**; R^1 = R^2 = R^3 = OH, R^4 = Cl, R^5 = H) and (**3**; R^1 = R^2 = OAc, R^3 = OH, R^4 = Cl, R^5 = H), a longer reaction time (20—24 h) was required for the triacetate (**2**; R^1 = R^2 = R^3 = OAc). Acetylation of the product (**3**; R^1 = R^2 = R^3 = OAc, R^4 = Cl, R^5 = H) gave the known[10] tetra-acetate (**3**; R^1 = R^2 = R^3 = OAc, R^4 = Cl, R^5 = Ac). The reaction failed altogether with the toluene-*p*-sulphonate [**2**; R^1 = R^2 = OAc, R^3 = $OSO_2C_6H_4Me$-*p*] and starting material was recovered after 24 h treatment at room temperature both with hydrogen chloride in chloroform and with 10M-hydrochloric acid in ethanol. The presence of a 9-ene is not essential and the trichothecanes (**12**; R = H and Ac) were converted into the chloroapotrichothecanes (**13**; R^1 = H and Ac, R^2 = Cl, R^3 = H) although a longer reaction time was required for the triacetate (**12**; R = Ac). Although the rearrangement proceeded smoothly with simple 12,13-epoxytrichothec-9-en-8-ones, *e.g.* trichothecin (**14**; R^1 = R^2 = R^4 = H, R^3 = OCOCH^Z=CHMe)[1] and acetyltrichothecolone (**14**; R^1 = R^2 = R^4 = H,



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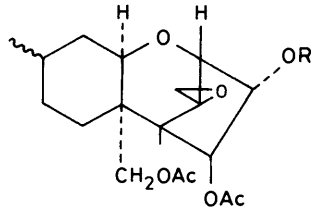

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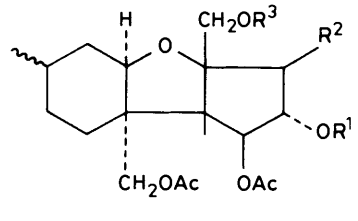

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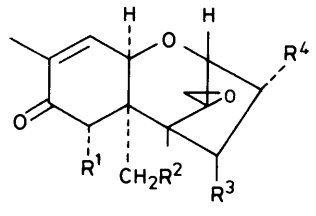

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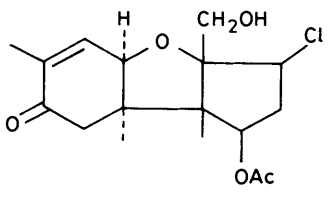

(15)

R^3 = OAc), it failed with the triacetate (**14**; R^1 = H, R^2 = R^3 = R^4 = OAc) and with the 7α-hydroxytrichothec-9-en-8-ones (**14**; R^1 = R^2 = R^4 = OH, R^3 = H and OH) studied in Part 7.[1]

The 8α-hydroxytrichothecene trichothecodiol (**6**; R^1 = R^2 = R^3 = R^4 = R^5 = H) and its diacetate (**6**; R^1 = R^4 = Ac, R^2 = R^3 = R^5 = H) were smoothly converted into the corresponding apotrichothecenes (**7**; R^1 = R^4 = H and Ac, R^2 = R^3 = R^5 = R^6 = H), acetylation of which gave the triacetate (**7**; R^1 = R^4 = R^6 = Ac, R^2 = R^5 = H). With T_2-toxin (**6**; R^1 = Me_2CHCH_2CO, R^2 = H, R^3 = OAc, R^4 = Ac, R^5 = OH) a 24 h reaction time was required for the formation of a product, shown to be the apotrichothecene (**7**; R^1 = Me_2CHCH_2CO, R^2 = R^6 = H, R^3 = OAc, R^4 = Ac, R^5 = OH) by the preparation and ^{1}H n.m.r. examination of the diacetate (**7**; R^1 = Me_2CHCH_2CO, R^2 = H, R^3 = R^5 = OAc, R^4 = R^6 = Ac). The reaction failed (starting material recovered after 24 h) with the *F. equiseti* metabolite (**6**; R^1 = R^4 = Ac, R^2 = R^5 = OH, R^3 = OAc) and with the closely related 7α,8α-disubstituted compounds (**6**; R^1 = H, R^2 = R^5 = OH, R^3 = OAc, R^4 = Ac) and (**6**; R^1 = R^4 = Ac, R^2 = R^3 = R^5 = OAc).

From these results it can be seen that the trichothecene →apotrichothecene rearrangement is adversely affected by the presence of bulky substituents at positions 3α and 7α; this despite the potential relief, offered by the rearrangement, of the non-bonded interactions[12] between 11-H and a 3α-OR group and between a 7α-OR group and C-14, which are present in the substituted trichothecene skeleton. The ease with which the trichothecene→apotrichothecene rearrangement occurs will depend on a favourable geometry for the transition state. The non-bonded interactions outlined above may influence this geometry unfavourably by causing small changes in the conformation of ring A, and, consequently, in the conformation of the ring B oxygen bridge.

In the course of this work, conditions intermediate in severity between hydrogen chloride in an organic solvent and water (pH 5) at 100 °C were also investigated, but the reactions were complicated by the partial deacylation of acylated mycotoxins. Diacetoxyscirpenol (**2**; R^1 = R^2 = OAc, R^3 = OH) was recovered after 7 days at room temperature in 0.1M-hydrochloric acid, but was converted into the 10,13-cyclotrichothecane (**1**; R^1 = R^2 = R^3 = R^4 = OH) on boiling for 1 h. Under similar conditions 3-acetylvomitoxin (**14**; R^1 = R^2 = OH, R^3 = H, R^4 = OAc) underwent partial deacylation without rearrangement, but the trichothecane (**12**; R = Ac), which cannot give a 10,13-cyclo-product, furnished the apotrichothecane (**13**; R^1 = Ac, R^2 = OH, R^3 = H) characterised as the diacetyl derivative (**13**; R^1 = R^3 = Ac, R^2 = OAc).

The chloroapotrichothecene (**3**; R^1 = R^2 = OAc, R^3 = OH, R^4 = Cl, R^5 = H) obtained[8] from diacetoxyscirpenol was also obtained,[10] with loss of an acetyl residue, by the action of 10M-hydrochloric acid on the triacetate (**2**; R^1 = R^2 = R^3 = OAc), but was assigned[10] the structure (**3**; R^1 = R^3 = OAc, R^2 = OH, R^4 = Cl, R^5 = H) since the ^{1}H n.m.r. spectrum (60 MHz) contained a triplet* (J = 4.5 Hz) at δ 5.36, attributed to a C*H*OAc group at position 3. The spectrum (100 MHz) of the chloroapotrichothecane (**13**; R^1 = R^3 = H, R^2 = Cl) also showed what appeared to be a triplet at δ 5.38 with J = 4.5 Hz. However, re-examination of this spectrum at 360 MHz (Table) revealed a second order ABX spectrum with $\Delta\delta_{2,3}$ = 2.6 Hz, $J_{2,3}$ = 11.0 Hz, $J_{2,4}$ = 0.3 Hz, and $J_{3,4}$ = 9.3 Hz. It is clear that the multiple resonance at δ 5.3 in this compound should be assigned to 4-H and that the 60 MHz spectrum of the apotrichothecene (**3**; R^1 = R^2 = OAc, R^3 = OH, R^4 = Cl, R^5 = H) was misinterpreted.[10] The bis toluene-*p*-sulphonate of this compound gave a first order n.m.r. spectrum (60 MHz) (Table) in which 2-H, 3-H, and 4-H could be assigned with confidence from the chemical shifts. The apo-compounds (**3**; R^1 = R^2 = R^3 = OAc, R^4 = Cl, R^5 = Ac) and (**13**; R^1 = Ac, R^2 = Cl, R^3 = H) likewise gave spectra capable of first-order interpretation. Irradiation of the signal at δ 1.35 (14-H) in the spectrum of the latter compound showed a n.O.e. with 3-H, confirming that this hydrogen is β-orientated and that no configurational change has occurred by intramolecular acyl migration during the rearrangement.

Conformation of Ring C *in the Apotrichothecene Skeleton.*—Contrary to the situation in the rigid ring C of the trichothecanes where the *cis* and *trans* vicinal coupling constants are 8 and 2—5 Hz respectively[12,15] and are in good agreement with those found in camphane,[22] the *trans* vicinal coupling constants (Table) in ring C of the more flexible non-macrocyclic 2β-chloro-13-hydroxyapotrichothecanes are 9—11 Hz and are greater than the *cis* vicinal coupling constants (6 Hz). This was elegantly demonstrated (Table) for the apotrichothec-9-en-8-one (**15**) [derived from acetyltrichothecolone (**14**; R^1 = R^2 = R^4 = H, R^3 = OAc)] in which both hydrogens at position 3 were seen in the n.m.r. spectrum at δ 1.99 and 2.60 and 3β-H (δ 1.99) was identified by the n.O.e. on irradiation of 14-H. The values for $J_{2,3\beta}$ and $J_{2,3\alpha}$ were 11.5 and 6.1 Hz, respectively.

Steric overcrowding of the heavily substituted β-face of ring C in these apotrichothecanes could be relieved by adoption of the envelope conformation (**3A**) in which C-3 lies above the plane of the remaining four carbon atoms and the dihedral angles $\varphi_{2\alpha,3\beta}$ and $\varphi_{3\beta,4\alpha}$ can approach 180°. In an attempt to further test this hypothesis, the apotrichothecene (**3**; R^1 = R^3 = OAc, R^2 = R^5 = H, R^4 = Cl), which has no 4β-substituent and hence a less crowded β-face, was prepared from calonectrin (**2**; R^1 = R^3 = OAc, R^2 = H). Although $J_{2\alpha,3\beta}$ decreased from 11 to 10 Hz (Table), there was no significant change in the values of the ring C vicinal coupling constants compared with the analogue (**3**; R^1 = R^2 = R^3 = OAc, R^4 = Cl, R^5 = Ac). Among derivatives of the non-macrocyclic trichothecenes, ring C coupling constants therefore have some diagnostic value for the 2β-chloroapotrichothecane skeleton (*cf.* Ref. 1). However, derivatives of the macrocyclic 12,13-epoxytrichothecenes do not conform to this pattern. In the ^{1}H n.m.r. spectrum (Table) of the apotrichothecene (**3**; R^1R^2 = $OCOCHOHCHMeCH_2CH_2$-$OCOCH\overset{E}{=}CHCH\overset{Z}{=}CHCOO$, R^3 = R^5 = H, R^4 = Cl), derived from verrucarin A, 2-H and 4-H appeared as doublets (J 5.1 and 6.3 Hz respectively) and 3α-H showed only the 16.1 Hz *gem* coupling. This result is explicable only in terms of the envelope conformation (**3B**) in which C-3 lies below the plane of ring C giving $\varphi_{2\alpha,3\beta}$ = $\varphi_{3\beta,4\alpha}$ = 90° and $J_{2\alpha,3\beta}$ = $J_{3\beta,4\alpha}$ = 0. This conformation, which increases the non-bonded interaction of the ring C β-substituents, must result from constraints associated with the presence of the macrolide ring and is presumably associated with the relief of the known non-bonded interactions in that ring. Thus, the greater flexibility of the apotrichothecene skeleton, compared with trichothecene, gives rise to a wide range of values, 0—11 Hz, for the ring C *trans* vicinal coupling constants.

The main conclusion to be drawn from this investigation is that naturally occurring non-macrocyclic trichothecene mycotoxins with esterified hydroxy substituents at positions 3α, 7α, or 8α undergo intramolecular rearrangement to biologically inactive products less readily than their simpler, less highly substituted, relatives. 3α-Acetoxy compounds are becoming increasingly common among the known naturally occurring trichothecenes and a number of these derivatives has recently been recorded[23] as metabolic products of *F graminearum*.

* Reported[10] as a double doublet, J = 4.5 Hz.

Table. ^{1}H N.m.r. resonances (δ, *J* in parentheses[a]) for compounds (**1**; $R^1 = R^2 = R^3 = OAc$, $R^4 = OH$) and (**3**; $R^1 = R^2 = R^3 = OAc$, $R^4 = Cl$, $R^5 = Ac$) and their relatives

Position	2	3α	3β	4	7	8	10	11	13	14	15	16	Ac	OH
Compd.														
(**6**; $R^1 = R^4 = Ac$, $R^2 = R^3 = R^5 = OAc$)	3.89d (4.8)		5.16dd (4.7, 3.3)	6.04d (3.4)	5.58d (5.1)	5.83d (5.1)	5.80d (5.8)	4.51d (5.8)	2.63d 3.07d^{AB} (3.4)	0.87s	4.42d 4.59d^{AB} (12.8)	1.73s	2.01 2.09 2.12(2) 2.17	
(**6**; $R^1 = R^4 = Ac$, $R^2 = R^3 = R^5 = H$)	3.81d (5.2)	2.55dd (15.5, 7.8)	2.00m	5.54dd (7.8, 3.6)		5.25dd (5.1, 0.5)	5.67d (5.4)	3.71d (5.4)	2.85d 3.11d^{AB} (3.8)	1.04s	0.71s	1.74s	2.07 2.08	
(**1**; $R^1 = R^2 = R^3 = OAc$, $R^4 = OH$)	4.15d (4.3)		5.00dd (4.3, 1.9)	5.44d (1.9)	*b*	*b*	2.20m	3.70d (3.7)	1.42dd (14.0, 5.5) 1.89dd (14.0. 11.8)	1.04s	3.88d 4.61d^{AB} (11.7)	1.23s	2.12 2.13 2.15	1.65 2.05
(**5**; $R^1 = H$, $R^2 = R^5 = OH$, $R^3 = OAc$, $R^4 = Ac$)[c]	3.81dd (4.2)		4.22dd (4.2, 1.9)	5.22d (1.9)	4.0m	4.0m	2.38m	3.77d (3.7)	1.66dd (14.1, 5.6) 1.93dd (14.1, 12.8)	1.01s	4.55^{AB} (11.8)	1.27s	2.10 2.11	
(**13**; $R^1 = R^3 = H$, $R^2 = Cl$)	4.03A (11.0)[d]		4.05B (11.0, 9.3)[d]	5.32X (9.3)[d]				3.87m	3.87d 3.91d^{AB} (12.5)	1.29s	3.82d 4.37d^{AB} (12.5)	0.88d (6.5)	2.04 2.10	3.10
(**13**; $R^1 = Ac$, $R^2 = Cl$, $R^3 = H$)	4.18d (11.1)		5.32dd (11.1, 9.4)	5.45d (9.4)				3.88m	3.91d 3.96d^{AB} (12.5)	1.35s	3.74d 4.41d^{AB} (12.5)	0.88d (6.5)	2.00 2.04 2.07	
(**3**; $R^1 = R^2 = R^3 = OAc$, $R^4 = Cl$, $R^5 = Ac$)	4.17d (11.1)		5.37dd (11.1, 9.3)	5.49d (9.3)			5.54d (5.2)[e]	3.91d (5.2)	4.15d 4.22d^{AB} (12.3)	1.31s	3.72d 4.41d^{AB} (12.5)	1.74s	2.03 2.04 2.09 2.10	
(**3**; $R^1 = R^2 = OAc$, $R^3 = OTs$, $R^4 = Cl$, $R^5 = Ts$)[f]	3.95d (11.5)		4.85dd (11.5, 9.0)	5.60d (9.0)			5.45d (5)	3.75d (5)	3.95d 4.12d^{AB} (11.0)	1.35s	3.70d 4.25d^{AB} (13.0)	1.70s	2.00 2.05	
(**3**; $R^1 = R^3 = OAc$, $R^2 = R^5 = H$, $R^4 = Cl$)	4.18d (10.2)		5.12dt (10.3, 6.2)	α1.87dd (12.6, 10.5) β2.03dd (12.6, 6.2)			5.53d (5.0)[e]	*ca.* 3.84	3.84^{AB} (12.0)	1.38s	3.83d 4.06d^{AB} (12.0)	1.73s	2.07 2.11	1.64
(**3**; R^1R^2 = OCOCHOHCHMeCH$_2$CH$_2$OCO CH=^{E}CHCH=^{Z}CHCOO, $R^3 = R^5 = H$, $R^4 = Cl$)[h]	4.45d (5.1)	2.50ddd (5.1, 6.5, 16.1)	2.35d (16.1)	5.88d (6.5)	1.95m[g]	1.79m[g]	5.50d (4.9)[e]	3.71d (4.9)	3.86d 4.05d^{AB} (11.9)	1.09s	4.27d 4.57d^{AB} (12.4)	1.74s		1.60 2.50
(**15**)	4.17dd (11.5, 6.1)	2.60m (6.1, 5.7, 12)	1.99m (11.5, 10.4, 12)	5.09dd (5.7, 10.2)	2.12d 2.92d^{AB} (15.5)		6.56d (5.3)[e]	4.04d (5.1)	3.84d 4.01d^{AB} (12.7)	1.18s	0.90s	1.84s	2.08	
(**7**; $R^1 = R^4 = Ac$, $R^2 = R^3 = R^5 = R^6 = H$)	4.15dd (10.8, 6.0)	2.56m (6.0, 5.8, 12)	2.0m	5.15dd (5.8, 9.4)	2.0m	5.22dd (5.4, 0.5)	5.78d (5.4)[e]	3.86d (5.6)	4.28^{AB} (12.0)	1.10s	0.95s	1.76s	2.06 2.07 2.08	
(**7**; $R^1 = Me_2CHCH_2CO$, $R^2 = H$, $R^3 = R^5 = OAc$, $R^4 = R^6 = Ac$)[i]	4.16d (11.0)		5.33dd (11.0, 9.3)	5.43d (9.3)		5.27dd (5.0, 1.0)	5.78d (5.1)[e]	4.00d (5.5)	4,17d 4.36d^{ABg} (12.1)	1.32s	3.90d 4.28d^{ABg} (12.3)	1.76s	1.99 2.00 2.08 2.11	

[a] First-order approximations from line separations, unless stated otherwise. Long-range couplings are excluded. [b] The axial 7α- and 8β-hydrogens were seen as a 12-line multiplet at δ 1.65 with $J_{gem} = J_{aa} = 14.0$ and J_{ae} 4.5 Hz. The equatorial 7β- and 8α-hydrogens were located at δ 1.40 and 2.05 as broadened doublets, J_{ee} 2—3 Hz. [c] In CD_3OD–D_2O. [d] By computer simulation $\Delta\delta_{2,3} = 2.6$ Hz, $J_{2,3}$ 11.0, $J_{2,4}$ 0.3, $J_{3,4}$ 9.3 Hz. [e] $J_{10,16}$ 1.0—1.5 Hz. [f] At 60 MHz. [g] Assignments may be reversed. [h] Also 4.47d, *J* 2.4 Hz, 2′-H; 2.50dq, 3′-H; 0.89d, *J* 6.8 Hz, CH*Me*; 4.1dt, 4.6dt, *J* 11.5, 4.0 Hz, 5′-H_2; 6.05d, *J* 15.7 H z, 5″-H; 8.45dd, *J* 15.7, 11.5 Hz, 4″-H; 6.76t, *J* 11.2 Hz, 3″-H; 6.10d, *J* 11.0 Hz, 2″-H. [i] Also Me_2CH at δ 0.95 *J* 6.5 Hz.

Experimental

M.p.s were taken on a Kofler hot stage apparatus and are corrected. I.r. spectra were determined for 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 (Cl = 35). NH_3 was used to obtain chemical ionisation mass spectra (c.i.m.s.). In analytical t.l.c., Merck silica gel 60 F_{254} was used with chloroform–methanol (9:1) unless stated otherwise: spots were detected in u.v. light (9-en-8-ones) or I_2 vapour (9-enes). Merck silica gels 7 739 and 7 734 were used in preparative t.l.c. (0.1 cm layer) and in column chromatography, respectively. Unless stated otherwise, acetylations were carried out in pyridine with acetic anhydride at room temperature over 24 h. All identifications were confirmed by comparison of the i.r. spectra. Light petroleum had b.p. 60—80 °C.

3α,4β,15-*Triacetoxy*-10,13-*cyclotrichothecane*-9α,12-*diol* (**1**; $R^1 = R^2 = R^3 = OAc$, $R^4 = OH$)[10].—This compound crystallised from ethyl acetate–light petroleum as prisms, m.p. 164 °C (Found: C, 59.2; H, 7.1. $C_{21}H_{30}O_9$ requires C, 59.1; H, 7.1%). The melt reset and remelted at 177—178 °C on seeding with an authentic specimen of the form m.p. 175—176 °C obtained[10] from diethyl ether–pentane. The i.r. spectra, $\nu_{max.}$ 3 440, 3 360, 1 747, and 1 710 cm^{-1}, of the two crystalline forms were identical.

3α,4β,9α,15-*Tetra-acetoxy*-10,13-*cyclotrichothecane*-12-*ol* (**1**; $R^1 = R^2 = R^3 = OAc$, $R^4 = OAc$).—This compound, prepared according to a literature method,[10] crystallised from ethyl acetate as prisms, m.p. 215 °C (lit.,[10] m.p. 196—197 °C). It was identical with the tetra-acetate (m.p. 213—215 °C) obtained[8] by the attempted reduction of diacetoxyscirpenol with zinc and acetic acid.

4β,15-*Diacetoxy*-2β-*chloroapotrichothec*-9-*ene*-3α,13-*diol* (**3**; $R^1 = R^2 = OAc$, $R^3 = OH$, $R^4 = Cl$, $R^5 = H$).—This compound, m.p. 167—168 °C, obtained from diacetoxyscirpenol with hydrogen chloride in chloroform,[8] was identical with the diacetoxychlorodiol, m.p. 162—165 °C, obtained[10] from the triacetate (**2**; $R^1 = R^2 = R^3 = OAc$) with concentrated hydrochloric acid in ethanol. Acetylation gave the tetra-acetate (**3**; $R^1 = R^2 = R^3 = OAc$, $R^4 = Cl$, $R^5 = Ac$) (see below).

Treatment of the diol (**3**; $R^1 = R^2 = OAc$, $R^3 = OH$, $R^4 = Cl$, $R^5 = H$) (40 mg) with toluene-*p*-sulphonyl chloride in pyridine at room temperature over 6 days furnished a glass (68 mg) which was subjected to preparative t.l.c. in chloroform–methanol (98:2). Two bands were obtained; R_F 0.65, a glass (50 mg) consisting of the *bis-toluene*-p-*sulphonate* (**3**; $R^1 = R^2 = OAc$, $R^3 = OSO_2C_6H_4Me$-*p*, $R^4 = Cl$, $R^5 = SO_2C_6H_4Me$-*p*) (Found: C, 55.3; H, 5.6%; *M*, 710. $C_{33}H_{39}ClO_{11}S_2$ requires C, 55.7; H, 5.5%; *M*, 710]; $\nu_{max.}$ OH absent, 1 740 cm^{-1}; and R_F 0.20, a glass (9 mg) consisting of the 13-*toluene*-p-*sulphonate* (**3**; $R^1 = R^2 = OAc$, $R^3 = OH$, $R^4 = Cl$, $R^5 = SO_2C_6H_4Me$-*p*) (Found: C, 56.7; H, 6.4. $C_{26}H_{33}ClO_9S$ requires C, 56.1; H, 6.0%); $\nu_{max.}$ 3 400br and 1 740 cm^{-1}.

12,13-*Epoxytrichothec*-9-*ene*-3α,4β,15-*triol* (*Scirpentriol*) (**2**; $R^1 = R^2 = R^3 = OH$).—This compound crystallised from ethyl acetate in two different forms: m.p. 193 °C,[6] $\nu_{max.}$ 3 460, 3 420, and 3 370 cm^{-1}; and m.p. 167 °C, $\nu_{max.}$ 3 560 and 3 300br. cm^{-1}. The form of m.p. 167 °C sometimes reset and remelted at 193 °C.

12,13-*Epoxytrichothec*-9-*ene*-4β,8α-*diol* (*Trichothecodiol*) (**6**; $R^1 = R^2 = R^3 = R^4 = R^5 = H$).—This was prepared according to literature procedure,[24] and was obtained from chloroform in both high m.p. (186—188 °C; lit.,[19] 190—191 °C), $\nu_{max.}$ 3 490 and 3 420 cm^{-1}; and low m.p. (155 °C; lit.,[24] 155 °C), $\nu_{max.}$ 3 400 cm^{-1} forms, R_F 0.25. The i.r. spectra of the two forms were identical in the region 1 800—600 cm^{-1}.

The *diacetate* (**6**; $R^1 = R^4 = Ac$, $R^2 = R^3 = R^5 = H$) was a gum, R_F 0.72 [Found: C, 65.1; H, 7.5%; MH^+ (c.i.m.s.), 351. $C_{19}H_{22}O_6$ requires C, 65.1; H, 7.5%; *M*, 350]; $\nu_{max.}$ 1 735 and 1 650w cm^{-1}.

3α,4β,7α,8α,15-*Penta-acetoxy*-12,13-*epoxytrichothec*-9-*ene* (**6**; $R^1 = R^4 = Ac$, $R^2 = R^3 = R^5 = OAc$).—This was obtained as an amorphous solid, m.p. *ca.* 90 °C (*cf.* ref. 20), R_F 0.75 [Found: C, 57.0; H, 6.4%; MNH_4^+ (c.i.m.s.), 542. Calc. for $C_{25}H_{32}O_{12}$: C, 57.2; H, 6.2%; *M*, 524] when acetylation of the triol (**6**; $R^1 = H$, $R^2 = R^5 = OH$, $R^3 = OAc$, $R^4 = Ac$)[13] was carried out for 7 days at room temperature.

Attempted Acid Catalysed Rearrangements.—A. *With water* (pH 5). The compound (20 mg) in chloroform (0.5 ml) or ethanol (0.5 ml) and water (2.0 ml) was heated under reflux for 6 h.

(i) *Verrucarol* (**2**; $R^1 = R^2 = OH$, $R^3 = H$). Continuous extraction (24 h) with chloroform furnished a gum (17 mg) which crystallised from ethyl acetate as large prisms (10 mg), m.p. 197—199 °C, R_F 0.08 of 10,13-*cyclotrichothecane*-4β,-9α,12,15-*tetraol* (**1**; $R^1 = R^2 = R^4 = OH$, $R^3 = H$) (Found: C, 63.4; H, 8.4%. $C_{15}H_{24}O_5$ requires C, 63.4; H, 8.5%); $\nu_{max.}$ 3 290br cm^{-1}.

Acetylation gave the diacetate (**1**; $R^1 = R^2 = OAc$, $R^3 = H$, $R^4 = OH$) as needles, m.p. 256 °C (from diethyl ether) (lit.,[15] m.p. 247—249 °C), $\nu_{max.}$ 3 525, 3 440, 1 730, and 1 692 cm^{-1}.

(ii) *Verrucarin A* (**2**; R^1R^2 = OCOCHOHCHMeCH$_2$CH$_2$-OCOCH=CHCH=CHCOO, $R^3 = H$) [in ethanol (1 ml)]. Starting material (15 mg) was recovered by extraction with ethyl acetate.

(iii) *Scirpentriol* (**2**; $R^1 = R^2 = R^3 = OH$). Continuous extraction (24 h) with chloroform furnished prisms (16 mg), m.p. 160—205 °C (from ethyl acetate) of 10,13-*cyclotrichothecane*-3α,-4β,9α,12,15-*pentaol* (**1**; $R^1 = R^2 = R^3 = R^4 = OH$) (Found: C, 60.4; H, 7.9%. $C_{15}H_{24}O_6$ requires C, 60.0; H, 8.05%), $\nu_{max.}$ 3 540, 3 480, 3 410, 3 380, and 3 260 cm^{-1}.

Acetylation gave the triacetate (**1**; $R^1 = R^2 = R^3 = OAc$, $R^4 = OH$) (see above).

(iv) *Diacetoxyscirpenol* (**2**; $R^1 = R^2 = OAc$, $R^3 = OH$). Extraction with ethyl acetate gave an oil which crystallised from ethyl acetate to give felted needles (13 mg), m.p. 280 °C, of 4β,15-*diacetoxy*-10,13-*cyclotrichothecane*-3α,9α,12-*triol* (**1**; $R^1 = R^3 = OAc$, $R^3 = R^4 = OH$) (Found: C, 59.55; H, 7.6%. $C_{19}H_{28}O_8$ requires C, 59.4; H, 7.3%); $\nu_{max.}$ 3 560, 3 540, 3 435, 1 735, and 1 695 cm^{-1}.

Acetylation gave the triacetate (**1**; $R^1 = R^2 = R^3 = OAc$, $R^4 = OH$) (see above).

(v) *Trichothecodiol* (**6**; $R^1 = R^2 = R^3 = R^4 = R^5 = H$). Starting material R_F 0.25 (4 mg) was recovered by extraction with chloroform. Continuous extraction with chloroform (10 h) then gave an amorphous product (9 mg), m.p. 70—80 °C, $\nu_{max.}$ 3 460br and 1 710 cm^{-1}, R_F 0.20 and 0.05. When the reaction time was increased to 9 h, no starting material was recovered and the yield of product was increased to 15 mg. The product was intractable, but was considered to be a mixture of 4β,-12-dihydroxy-10,13-cyclotrichothecan-8-one (**8**; R = H), $\nu_{max.}$ 1 710 cm^{-1} with 10,13-cyclotrichothecane-4β,8α,9α,12-tetraol (**5**; $R^1 = R^2 = R^3 = R^4 = R^5 = H$) (Found: *M*, 284. $C_{15}H_{24}O_5$ requires *M*, 284) and its 9-epimer. The components of R_F 0.20 and 0.05 were separated by preparative t.l.c., but acetylation of the amorphous products gave gums, R_F 0.65, $\nu_{max.}$ 3 450, 1 735br cm^{-1} which could not be purified.

(vi) The triol (**6**; $R^1 = H$, $R^2 = R^5 = OH$, $R^3 = OAc$, $R^4 = Ac$) (12 mg) was recovered by extraction with ethyl acetate.

Continuous extraction (24 h) with chloroform then furnished a gum which crystallised from ethyl acetate as prisms (3 mg), m.p. 255—265 °C (decomp.) of 4β,15-*diacetoxy*-10,13-*cyclotrichothecane*-3α,7α,8α,9α,12-*pentaol hydrate* (**5**; R^1 = H, R^2 = R^5 = OH, R^3 = OAc, R^4 = Ac) (Found: C, 53.1; H, 6.7%; MNH_4^+ (c.i.m.s.), 434. $C_{19}H_{28}O_{10}$·H_2O requires C, 52.8; H, 6.5%; *M*, 416]; $\nu_{max.}$ 3 570, 3 510, 3 410br, 1 710, and 1 650w cm^{-1}. When the reaction time was extended to 15 h, no starting material was recovered and the yield of crystalline pentaol hydrate was increased to 6 mg.

The triacetyl derivative (**5**; R^1 = R^4 = Ac, R^2 = R^3 = R^5 = OAc), isolated by trituration with light petroleum, was an amorphous solid, m.p. 100—110 °C, $\nu_{max.}$ 3 450br and 1 735 cm^{-1} [Found: MH^+ (c.i.m.s.), 543. $C_{25}H_{34}O_{13}$ requires *M*, 542].

(vii) *T_2-Toxin* (**6**; R^1 = Me_2CHCH_2CO, R^2 = H, R^3 = OAc, R^4 = Ac, R^5 = OH). Starting material (17 mg) was recovered by extraction with ethyl acetate. Diacetyltrichothecodiol (**6**; R^1 = R^4 = Ac, R^2 = R^3 = R^5 = H) (17 mg), the *F. equiseti* metabolite (**6**; R^1 = R^4 = Ac, R^2 = R^5 = OH, R^3 = OAc) (15 mg), and the penta-acetate (**6**; R^1 = R^4 = Ac, R^2 = R^3 = R^5 = OAc) (17 mg) were recovered by extraction with chloroform. The trichothec-9-en-8-ones trichothecolone (**14**; R^1 = R^2 = R^4 = H, R^3 = OH), nivalenol (**14**; R^1 = R^2 = R^3 = R^4 = OH), diacetylnivalenol (**14**; R^1 = R^4 = OH, R^2 = R^3 = OAc), and 3-acetylvomitoxin (**14**; R^1 = R^2 = OH, R^3 = H, R^4 = OAc)[25] were also unaffected, as was the trichothecane (**12**; R = Ac).

B. *With hydrogen chloride.* The compound (20 mg) in chloroform (2 ml) or dichloromethane (2 ml) was treated at room temperature with a stream of dry hydrogen chloride during (*a*) 15 min or (*b*) 2 h, after which treatment the solution was set aside for 18—24 h. After removal of the solvent under reduced pressure, the product was, unless stated otherwise, crystallised from ethyl acetate–light petroleum.

(i) Verrucarol (**2**; R^1 = R^2 = OH, R^3 = H) gave (*a*) the apotrichothecene (**3**; R^1 = R^2 = OH, R^3 = R^5 = H, R^4 = Cl) (12 mg), m.p. 150 °C (lit.,[15] m.p. 151—153 °C). Acetylation gave prisms, m.p. 140 °C (lit.,[15] oil) from ethyl acetate–light petroleum, of 4β,13,15-*triacetoxy*-2β-*chloroapotrichothec*-9-*ene* (**3**; R^1 = R^2 = OAc, R^3 = H, R^4 = Cl, R^5 = Ac) (Found: C, 58.7; H, 7.0. $C_{21}H_{29}ClO_7$ requires C, 58.8; H, 6.8%); $\nu_{max.}$ 1 740 cm^{-1}; R_F 0.73 (chloroform–methanol, 98:2).

(ii) Diacetylverrucarol (**2**; R^1 = R^2 = OAc, R^3 = H), R_F 0.70 (chloroform–methanol, 98:2) gave (*a*) needles, m.p. 129 °C (15 mg) of 4β,15-*diacetoxy*-2β-*chloroapotrichothec*-9-*ene* (**3**; R^1 = R^2 = OAc, R^3 = R^5 = H, R^4 = Cl) (Found: C, 59.2; H, 7.1. $C_{19}H_{27}ClO_6$ requires, C, 59.0; 7.0%); $\nu_{max.}$ 3 410 and 1 740 cm^{-1}; R_F 0.64 (chloroform–methanol, 98:2). Acetylation gave the triacetate (**3**; R^1 = R^2 = OAc, R^3 = H, R^4 = Cl, R^5 = Ac).

(iii) Verrucarin A (**2**; R^1R^2 = $OCOCHOHCHMeCH_2$-$CH_2OCOCH\overset{E}{=}CHCH\overset{Z}{=}CHCOO$, R^3 = H), R_F 0.71 gave (*a*) the *apotrichothecene* (**3**; R^1R^2 = $OCOCHOHCHMeCH_2CH_2O$-$COCH\overset{E}{=}CHCH\overset{Z}{=}CHCOO$; R^3 = R^5 = H, R^4 = Cl) as prisms (15 mg) m.p. 152—158 °C, from ethyl acetate–diethyl ether (Found: C, 59.2; H, 6.3. $C_{27}H_{35}ClO_9C_4H_8O_2$ requires C, 59.4; H, 6.9%); $\nu_{max.}$ 3 580, 3 500, 3 400br, 1 740, 1 725, 1 708, 1 652, 1 628, and 1 582 cm^{-1}.

The *diacetyl* derivative (**3**; R^1R^2 = $OCOCHOAcCHMe$-$CH_2CH_2OCOCH\overset{E}{=}CHCH\overset{Z}{=}CHCOO$, R^2 = H, R^4 = Cl, R^5 = Ac) was amorphous, m.p. 95—100 °C [Found: MH^+, 623 (c.i.m.s.). $C_{31}H_{39}ClO_{11}$ requires *M*, 622]; $\nu_{max.}$ OH absent, 1 740, 1 715, 1 630, and 1 590 cm^{-1}.

(iv) *Scirpentriol* (**2**; R^1 = R^2 = R^3 = OH). The hygroscopic amorphous product m.p. 80—90 °C from (*a*) was dried *in vacuo* at room temperature over phosphorus pentaoxide and was twice precipitated from ethyl acetate by the addition of diethyl ether. 2β-*Chloroapotrichothec*-9-*ene*-3α,4β,13,15-*tetraol* (**3**; R^1 = R^2 = R^3 = OH, R^4 = Cl, R^5 = H) (5 mg) was obtained as an amorphous solid, m.p. 140 °C (Found: C, 55.9; H, 6.8. $C_{15}H_{23}ClO_5$ requires C, 56.5; H, 7.3%); $\nu_{max.}$ 3 360br and 1 650w cm^{-1}. Acetylation gave the tetra-acetate (**3**; R^1 = R^2 = R^3 = OAc, R^4 = Cl, R^5 = Ac), m.p. 100—103 °C (lit.,[10] m.p. 105—107 °C).

(v) Triacetoxyscirpene (**2**; R^1 = R^2 = R^3 = OAc) was recovered from (*a*). The product from (*b*) crystallised as prisms (10 mg) m.p. 179—180 °C, R_F 0.80, of 3α,4β,15-*triacetoxy*-2β-*chloroapotrichothec*-9-*en*-13-*ol* (**3**; R^1 = R^2 = R^3 = OAc, R^4 = Cl, R^5 = H) (Found: C, 56.5; H, 6.3. $C_{21}H_{29}ClO_8$ requires C, 56.7; H, 6.6%); $\nu_{max.}$ 3 420, 1 740, 1 725, and 1 671w cm^{-1}. Acetylation gave the tetra-acetate (**3**; R^1 = R^2 = R^3 = OAc, R^4 = Cl, R^5 = Ac) (see above).

(vi) The toluene-p-sulphonate (**2**; R^1 = R^2 = OAc, R^3 = $OSO_2C_6H_4Me$-*p*) was recovered from (*b*).

(vii) The diacetate (**12**; R = H), when subjected to (*a*), furnished prisms (7 mg), m.p. 155—157 °C, R_F 0.55, of 4β,-15-*diacetoxy*-2β-*chloroapotrichothecane*-3α,13-*diol* (**13**; R^1 = R^3 = H, R^2 = Cl) (Found: C, 56.4; H, 7.2. $C_{19}H_{29}ClO_7$ requires C, 56.4; H, 7.2%); $\nu_{max.}$ 3 480, 3 405, 1 740, and 1 720 cm^{-1}. Acetylation gave 3α,4β,13,15-*tetra-acetoxy*-2β-*chloroapotrichothecane* (**13**; R^1 = R^3 = Ac, R^2 = Cl) which crystallised from ethyl acetate–light petroleum as prisms, m.p. 135—136 °C (Found: C, 56.3; H, 6.9. $C_{23}H_{33}ClO_9$ requires C, 56.5; H, 6.8%); $\nu_{max.}$ 1 740 cm^{-1}.

(viii) The triacetate (**12**; R = Ac) was recovered from (*a*). Reaction conditions (*b*) furnished 3α,4β,15-*triacetoxy*-2β-*chloroapotrichothecan*-13-*ol* (**13**; R^1 = Ac, R^2 = Cl, R^3 = H) as prisms (8 mg), m.p. 155—157 °C, R_F 0.84 (Found: C, 56.0; H, 6.9. $C_{21}H_{31}ClO_8$ requires C, 56.4; H, 7.0%); $\nu_{max.}$ 3 455, 1 750, 1 745, and 1 725 cm^{-1}. Acetylation gave the tetra-acetate (**13**; R^1 = R^3 = Ac, R^2 = Cl).

(ix) The 8-oxo compound (**14**; R^1 = H, R^2 = R^3 = R^4 = OAc) was recovered from (*b*).

(x) Calonectrin (**2**; R^1 = R^3 = OAc, R^2 = H), R_F 0.75, furnished, from (*b*), 3α,15-*diacetoxy*-2β-*chloroapotrichothec*-9-*en*-13-*ol* (**3**; R^1 = R^3 = OAc, R^2 ≠ R^5 = H, R^4 = Cl) as a gum, R_F 0.66 [Found: MH^+ 387 (c.i.m.s.), *M* − 15, 371.1230, and *M* − 60, 326.1276. $C_{19}H_{27}ClO_6$ requires *M*, 386. $C_{18}H_{24}$-ClO_6^+ and $C_{17}H_{23}ClO_4^+$ require *m/z* 371.1261 and 326.1285, respectively]; $\nu_{max.}$ 3 500, 1 740, and 1 615w cm^{-1}. The acetate was a gum, $\nu_{max.}$ OH absent, 1 740 cm^{-1}.

(xi) Acetyltrichothecolone (**14**; R^1 = R^2 = R^4 = H, R^3 = OAc) gave, from (*a*), 4β-*acetoxy*-2β-*chloro*-13-*hydroxyapotrichothec*-9-*en*-8-*one* (**15**) as needles (13 mg), m.p. 157—158 °C (Found: C, 59.5; H, 6.5%. $C_{17}H_{23}ClO_5$ requires C, 59.6; H, 6.8%); $\nu_{max.}$ 3 415, 1 740, and 1 678 cm^{-1}.

(xii) Trichothecodiol (**6**; R^1 = R^2 = R^3 = R^4 = R^5 = H) gave, from (*a*), 2β-*chloroapotrichothec*-9-*ene*-4β,8α,13-*triol* (**7**; R^1 = R^2 = R^3 = R^4 = R^5 = R^6 = H) as prisms (11 mg), m.p. 166—167 °C, R_F 0.14 (Found: C, 59.3; H, 7.6. $C_{15}H_{23}ClO_4$ requires C, 59.5; H, 7.6%); $\nu_{max.}$ 3 440 and 1 662w cm^{-1}.

The *triacetate* (**7**; R^1 = R^4 = R^6 = Ac, R^2 = R^3 = R^5 = H) was an amorphous solid, m.p. 55—60 °C, R_F 0.74 [Found: C, 58.1; H, 6.7%; MNH_4^+ (c.i.m.s.), 446. $C_{21}H_{29}ClO_7$ requires C, 58.8; H, 6.8%; *M*, 428]; $\nu_{max.}$ OH absent, 1 740 cm^{-1}.

(xiii) Diacetyltrichothecodiol (**6**; R^1 = R^4 = Ac, R^2 = R^3 = R^5 = H) gave, from (*a*), 4β,8α-diacetoxy-2β-chloroapotrichothec-9-en-13-ol (**7**; R^1 = R^4 = Ac, R^2 = R^3 = R^5 = R^6 = H) as a gum (19 mg), R_F 0.68 [Found: MH^+ (c.i.m.s.), 387. $C_{19}H_{27}ClO_6$ requires *M*, 386]; $\nu_{max.}$ 3 440br and 1 740 cm^{-1}. Acetylation gave the triacetate (**7**; R^1 = R^4 = R^6 = Ac, R^2 = R^3 = R^5 = H) (above).

(xiv) T_2-toxin (**6**; R^1 = Me_2CHCH_2CO; R^2 = H, R^3 = OAc, R^4 = Ac, R^5 = OH) was recovered from (*a*). The product from (*b*) formed felted needles (12 mg), m.p. 238—

239 °C of 4β,15-*diacetoxy*-2β-*chloro*-8α-(3-*methylbutyryloxy*)-*apotrichothec*-9-*ene*-3α,13-*diol* (**7**; R^1 = Me_2CHCH_2CO, R^2 = R^6 = H, R^3 = OAc, R^4 = Ac, R^5 = OH) (Found: C, 57.1; H, 7.2%; *M*, 502. $C_{24}H_{35}ClO_9$ requires C, 57.3; H, 7.0%; *M*, 502]; $\nu_{max.}$ 3 460, 3 360, 1 730, and 1 710 cm^{-1}.

The 3α,13-diacetyl derivative (**7**; R^1 = Me_2CHCH_2CO, R^2 = H, R^3 = R^5 = OAc, R^4 = R^6 = Ac) was an amorphous solid, m.p. 57—63 °C, R_F 0.76, $\nu_{max.}$ OH absent, 1 740 cm^{-1} [Found: MNH_4^+ (c.i.m.s.), 604. $C_{28}H_{39}ClO_{11}$ requires *M*, 586].

(xv) The *F. equiseti* metabolite (**6**; R^1 = R^4 = Ac, R^2 = R^5 = OH, R^3 = OAc)[11], the triol (**6**; R^1 = H, R^2 = R^5 = OH, R^3 = OAc, R^4 = Ac),[13] and the penta-acetate (**6**; R^1 = R^4 = Ac, R^2 = R^3 = R^5 = OAc) were recovered after treatment (*b*).

C. *With* 0.1M-*hydrochloric acid.* (i) The triacetate (**12**; R = Ac) (50 mg) in methanol (0.5 ml) and 0.1M-hydrochloric acid (3 ml) was heated under reflux for 2 h. The gum (37 mg) obtained by extraction with ethyl acetate was acetylated and a portion (30 mg) of the gummy product (38 mg) was subjected to preparative t.l.c. Recovery of the material from a band R_F 0.69 furnished 2β,3α,4β,13,15-penta-acetoxyapotrichothecane (**13**; R^1 = R^3 = Ac, R^2 = OAc) as a gum (14 mg), $\nu_{max.}$ OH absent, 1 750 and 1 740 cm^{-1} [Found: MH^+ (c.i.m.s.), 513. $C_{25}H_{36}O_{11}$ requires *M*, 512].

(ii) 3-Acetylvomitoxin (**14**; R^1 = R^2 = OH, R^3 = H, R^4 = OAc) (14 mg) in methanol (0.1 ml) and 0.1M-hydrochloric acid (2 ml) was heated under reflux for 3 h. The product (8 mg) obtained by extraction with ethyl acetate was subjected to preparative t.l.c. Material (4 mg) from a band R_F 0.50 was identified as starting material. Material (3 mg) from a band R_F 0.19 crystallised from ethyl acetate as hexagonal prisms of vomitoxin hydrate.[1]

(iii) Diacetoxyscirpenol (**2**; R^1 = R^2 = OAc, R^3 = OH) (0.5 g) in ethanol (1 ml) and 0.1M-hydrochloric acid (20 ml) was heated under reflux for 1 h and the aqueous solution was continuously extracted with chloroform for 15 h. Crystallisation of the product (377 mg) from ethyl acetate gave the pentaol (**1**; R^1 = R^2 = R^3 = R^4 = OH) (30 mg). Silica gel (2 g) was added to the mother liquor and the solid, after evaporation of the solvent, was added to the top of a column of silica gel (12 g, 25 × 1.2 cm). After intractable gums (60 mg) had been eluted with diethyl ether (200 ml) and diethyl ether–methanol (49:1, 200 ml), diethyl ether–methanol (20:1, 150 ml) eluted the pentaol (**1**; R^1 = R^2 = R^3 = R^4 = OH) (56 mg).

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