Phytotoxic Compounds Produced by *Fusarium equiseti*. Part V.[1] Transformation Products of 4β,15-Diacetoxy-3α,7α-dihydroxy-12,13-epoxytrichothec-9-en-8-one and the Structures of Nivalenol and Fusarenone

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The absolute configuration of the toxic $C_{19}H_{24}O_9$ metabolic product of *Fusarium scirpi* is established as 4β,15-diacetoxy-3α,7α-dihydroxy-12,13-epoxytrichothec-9-en-8-one (I; $R^1 = R^4 = H$, $R^2 = R^3 = Ac$) by the synthesis of a derivative from a trichothecane of known absolute configuration. Some derivatives and transformation products of the enone (I; $R^1 = R^4 = H$, $R^2 = R^3 = Ac$) are described. Nivalenol, obtained from rice infected with *Fusarium nivale*, is identical with the parent alcohol (I; $R^1 = R^2 = R^3 = R^4 = H$). Fusarenone, from the same source, is the 4β-monoacetate (I; $R^1 = R^2 = R^4 = H$, $R^3 = Ac$).

THE structure 4β,15-diacetoxy-3α,7α-dihydroxy-12,13-epoxytrichothec-9-en-8-one (I; $R^1 = R^4 = H$, $R^2 = R^3 = Ac$) (for nomenclature see ref. 2) was assigned,[3,4] mainly on spectroscopic evidence, to the toxic $C_{19}H_{24}O_9$ minor metabolic product, m.p. 135—136°, isolated[5] from culture filtrates of *Fusarium scirpi* (CMI 45490) and *Gibberella intricans* (= *F. equiseti*) (CBS, Wollenweber strain). The availability of further supplies of material has now permitted a more extensive investigation culminating in a formal proof of structure, and also of absolute configuration, by relating the compound to 4β,15-diacetoxy-12,13-epoxytrichothec-9-en-3α-ol (IV; $R^1 = R^2 = Ac$, $R^3 = OH$) (diacetoxyscirpenol).

Alkaline hydrolysis of the 4β-acetate in the latter compound is facilitated by the presence of the neighbouring *trans*-3α-hydroxy-substituent, and was shown[6] to occur considerably faster than that of the 15-acetate. In consequence, the 15-monoacetate (IV; $R^1 = Ac$, $R^2 = H$, $R^3 = OH$) was obtained,[7] in acceptable yield, on partial hydrolysis of the 4β,15-diacetate with methanolic N-ammonium hydroxide for 4 hr. at room

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[6] A. W. Dawkins, *J. Chem. Soc.* (*C*), 1966, 116.
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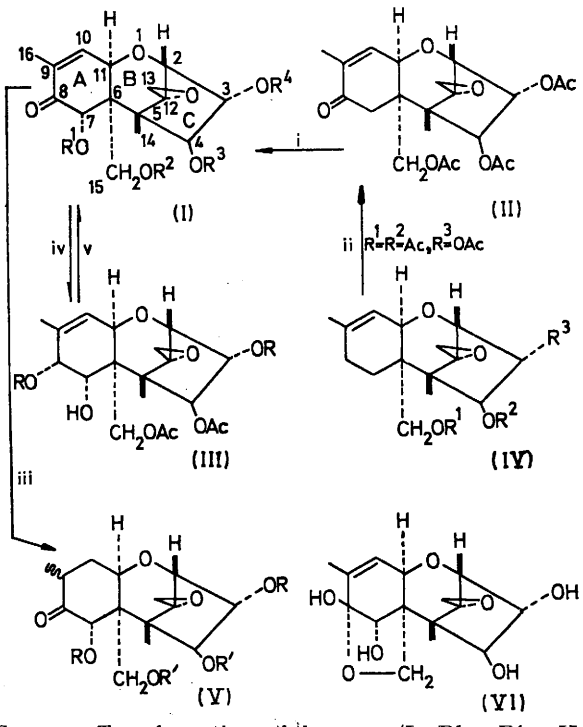
temperature. Under these conditions both ester groups were removed from the diacetate (I; $R^1 = R^4 = H$, $R^2 = R^3 = Ac$); however, with a shorter reaction time (0·5 hr.) a monoacetate was obtained, shown to be the 4β-acetate (I; $R^1 = R^2 = R^4 = H$, $R^3 = Ac$) by its n.m.r. spectrum (see Table). The greatly enhanced rate of hydrolysis of the 15-acetate residue in (I; $R^1 = R^4 = H$, $R^2 = R^3 = Ac$) is attributed to participation



SCHEME Transformations of the enone (I; $R^1 = R^4 = H$, $R^2 = R^3 = Ac$)

Reagents: i, $Pb(OAc)_4$; ii, Bu^tOCrO_3H; iii, Pd–H_2; iv, $NaBH_4$; v, MnO_2.

by the neighbouring 7α-hydroxy-substituent and provides additional evidence for the configurational assignment at this centre. The favourable proximity of the 7α- and 15-substituents may be observed on molecular models, which show that such participation is sterically impossible when the 7-substituent has the β-configuration.

Hydrolysis of both ester groups in (I; $R^1 = R^4 = H$, $R^2 = R^3 = Ac$), as just described or with 0·1N-potassium hydroxide at room temperature, furnished the $C_{15}H_{20}O_7$ tetraol (I; $R^1 = R^2 = R^3 = R^4 = H$), $\nu_{max.}$ 1675 cm.$^{-1}$, $\lambda_{max.}$ 220 nm. (ε 6500) $[\alpha]_D^{20}$ +15°, sometimes obtained in the form of the hemiacetal (VI) which showed no i.r. carbonyl absorption but underwent rapid tautomeric change in 95% ethanol to give a u.v. spectrum identical with that of the keto-form (I; $R^1 = R^2 = R^3 = R^4 = H$). Acetylation of both the diacetate (I; $R^1 = R^4 = H$, $R^2 = R^3 = Ac$) and the monoacetate (I; $R^1 = R^2 = R^4 = H$, $R^3 = Ac$) and of both open-chain and ring forms of the tetraol (I; $R^1 = R^2 = R^3 = R^4 = H$) gave the same tetra-acetate (I; $R^1 = R^2 = R^3 = R^4 = Ac$).

The enone chromophore of the diacetate (I; $R^1 = R^4 = H$, $R^2 = R^3 = Ac$) was eliminated on catalytic hydrogenation to give the dihydro-derivative (V; $R = H$, $R' = Ac$) and on reduction with sodium borohydride to give the amorphous allylic alcohol (III; $R = H$). The latter was characterised as the diacetyl derivative (III; $R = Ac$) and was reoxidised to the diacetate (I; $R^1 = R^4 = H$, $R^2 = R^3 = Ac$) by manganese dioxide. The value of $J_{7,8}$ (5 Hz) in the n.m.r. spectra of the triol (III; $R = H$) and its diacetyl-derivative is consistent only with a *cis*-orientation of the hydrogens at positions 7 and 8 and hence with an α-orientation of the 8-hydroxy-substituent. Sodium borohydride reduction of the 8-one (I; $R^1 = R^4 = H$, $R^2 = R^3 = Ac$) is therefore subject to steric control and takes place predominantly from the less crowded β-face of the molecule to give the quasi-axial 8α-alcohol. The failure of the triol (III; $R = H$) to undergo complete acetylation under normal conditions follows from steric hindrance of the 7α-hydroxy-group by an additional vicinal α-substituent. Although toluene-*p*-sulphonation of the enone (I; $R^1 = R^4 = H$, $R^2 = R^3 = Ac$) gave only a mono-derivative, judged from the n.m.r. spectrum to be the 3-toluene-*p*-sulphonate (I; $R^1 = H$, $R^2 = R^3 = Ac$, $R^4 = Ts$), acetylation gave the fully acetylated product (I; $R^1 = R^2 = R^3 = R^4 = Ac$).

All these reactions are consistent with the structure and relative configuration (I; $R^1 = R^4 = H$, $R^2 = R^3 = Ac$) deduced [4] from the n.m.r. spectrum. That this also represents the absolute configuration follows from the direct conversion of 3α,4β,15-triacetoxy-12,13-epoxytrichothec-9-en-8-one (II) into (I; $R^1 = R^2 = R^3 = R^4 = Ac$) by acetoxylation at position 7 with lead tetra-acetate in acetic acid. Diacetoxyscirpenol (IV; $R^1 = R^2 = Ac$, $R^3 = OH$) has been related [6,7] to verrucarol (IV; $R^1 = R^2 = R^3 = H$)

Chemical shifts (τ values) and coupling constants (J in Hz) for hydrogens in the enone (I; $R^1 = R^4 = H$, $R^2 = R^3 = Ac$) and related compounds

Compound	Position 2	3	4	7	8	10	11	13	14	15 [a]	16	OAc	$J_{2,3}$	$J_{3,4}$	$J_{7,8}$
(I; $R^1 = R^4 = H$, $R^2 = R^3 = Ac$) [4]	6·1(d)	5·7(dd)	4·7(d)	5·05		3·3(d) [b]	5·2(d)	6·9(s)	8·9	5·7(AB)	8·1	8·1 7·8	4·6	3	
(I; $R^1 = R^2 = R^3 = R^4 = Ac$) [4]	6·1(d)	4·8(dd)	4·2(d)	4·0		3·5(d) [b]	5·4(d)	7·1(AB) [c]	9·2	5·6(AB)	8·2	8·1 7·8	4·6	3	
(I; $R^1 = R^2 = R^4 = H$, $R^3 = Ac$) [d]	6·2(d)	5·7(dd)	4·45(d)	5·15		3·3(d) [b]	5·2(d)	6·9(AB) [c]	8·9	6·25(AB)	8·1	7·8	4·5	3·5	
(I; $R^1 = H$, $R^2 = R^3 = Ac$, $R^4 = Ts$)	6·2(d)	5·2(dd)	4·2(d)	5·25		3·5(d) [b]	5·45(d)	7·0(s)	9·1	5·8(AB)	8·15	8·1	4·7	3·3	
(III; R = H) [d]	6·3(d)	5·8(dd)	4·5(d)	6·0(d)	*ca.* 5·5	4·35(d) [e]	5·5	6·9(AB) [c]	8·85	5·6(AB)	8·1	7·95 7·85	4·5	2·5	5
(III; R = Ac) [d]	6·1(d)	4·8(dd)	4·15(d)	5·25(d)	4·5(d)	4·25(d) [e]	5·7(d)	6·9(AB) [c]	8·9	5·55(AB)	8·2	7·9	4·5	2·5	5

[a] $J_{15a\text{-}gem}$ 12. [b] $J_{10,11}$ 6. [c] $J_{13\text{-}gem}$ 4. [d] In presence of D_2O. [e] $J_{10,11}$ 5.

the absolute configuration of which is known,[8] and has been converted [7] into the enone (II) by acetylation and oxidation of the activated 8-methylene group with t-butyl chromate.

The high cytotoxicity of the diacetate (I; $R^1 = R^4 =$ H, $R^2 = R^3 =$ Ac) is diminished by hydrolysis of the ester groups; nevertheless, the parent tetraol (I; $R^1 = R^2 = R^3 = R^4 =$ H) is still appreciably toxic.[9] Recently, two compounds, nivalenol,[10] $C_{15}H_{20}O_7$, $[\alpha]_D$ +21°, and its monoacetate, fusarenone,[11] have been isolated from rice infected by a toxic strain of *F. nivale* and have been described as new mycotoxins although their structures have not been elucidated.[12] Comparison of the published physical and chemical properties of nivalenol and of its acetylation and reduction products [12] with those of the tetraol (I; $R^1 = R^2 = R^3 = R^4 =$ H) and the corresponding acetylation and reduction products, (I; $R^1 = R^2 = R^3 = R^4 =$ Ac) and (IV; R = R′ = H or Ac), respectively, indicates that the parent $C_{15}H_{20}O_7$ tetraols are identical.* Fusarenone is identical with the 4-monoacetate (I; $R^1 = R^2 = R^4 =$ H, $R^3 =$ Ac) † (comparison of i.r. spectra [11]). The lengthy procedure and relatively severe conditions used [10,11] for the isolation of nivalenol and fusarenone, in conjunction with the ready lability of the ester groups of the enone (I; $R^1 = R^4 =$ H, $R^2 = R^3 =$ Ac), suggests [13] that both compounds may be artifacts, derived by hydrolysis of the diacetate (I; $R^1 = R^4 =$ H, $R^2 = R^3 =$ Ac).

EXPERIMENTAL

M.p.s are corrected. I.r. spectra were determined for Nujol mulls and u.v. spectra and optical rotations for solutions in ethanol. N.m.r. spectra were obtained for solutions in deuteriochloroform, with tetramethylsilane as internal standard, with a Varian HA100 spectrometer. Light petroleum had b.p. 60—80°. Merck silica gel 7734 was used in chromatography.

4β,15-Diacetoxy-3α,7α-dihydroxy-12,13-epoxytrichothec-9-en-8-one (I; $R^1 = R^4 =$ H, $R^2 = R^3 =$ Ac) was isolated from culture filtrates of *F. scirpi* as previously described.[5] The *diacetyl* derivative (I; $R^1 = R^2 = R^3 = R^4 =$ Ac) prepared, by Dr. A. W. Dawkins, with acetic anydride–pyridine at room temperature for 72 hr., formed prisms, m.p. 169° (from ethanol), $[\alpha]_D^{20}$ +110° (*c* 0·1) (Found: C, 57·55; H, 5·7; Ac, 34·3. $C_{23}H_{28}O_{11}$ requires C, 57·5; H, 5·9; 4 × Ac, 35·8%), $\nu_{max.}$ 1755 and 1745 (ester C=O) and 1697 and 1670 (C=C–C=O) cm.$^{-1}$, $\lambda_{max.}$ 226 nm. (ε 8500). Tetra-acetylnivalenol [12] has m.p. 165°, and n.m.r. spectrum identical with that of (I; $R^1 = R^2 = R^3 = R^4 =$ Ac).[4].

The 3α-*toluene*-p-*sulphonate* (I; $R^1 =$ H, $R^2 = R^3 =$ Ac, $R^4 =$ Ts), prepared with toluene-*p*-sulphonyl chloride in pyridine at room temperature for 10 days, crystallised from ethyl acetate–light petroleum in needles, double m.p. 75—78° and 130—132° (Found: C, 57·2; H, 5·9. $C_{26}H_{30}O_{11}S$ requires C, 56·7; H, 5·5%), $\nu_{max.}$ 3400, 1750, 1695, and 1605 cm.$^{-1}$.

4β,15-*Diacetoxy*-3α,7α-*dihydroxy*-12,13-*epoxytrichothecan*-8-*one* (V; R = H, R′ = Ac).—The enone (I; $R^1 = R^4 =$ H, $R^2 = R^3 =$ Ac) (274 mg.) in acetic acid (10 ml.) was hydrogenated (uptake 1·0 mol.) over 10% palladium–charcoal (100 mg.). The product was twice recrystallised from ethyl acetate–light petroleum, giving the *dihydro-derivative* (V; R = H, R′ = Ac) as needles (233 mg.) m.p. 199—200° (Found: C, 57·4; H, 6·8. $C_{19}H_{26}O_9$ requires C, 57·3; H, 6·6%), $\nu_{max.}$ 3484, 3455, 1749, 1736, 1723, and 1691 cm.$^{-1}$; u.v. end absorption only: ε 2900 (205 nm.), 2300 (210), 1400 (215), 1000 (220), and 600 (230).

The *diacetyl* derivative (V; R = R′ = Ac) crystallised from ethyl acetate–light petroleum in needles, m.p. 207° (Found: C, 57·6; H, 6·3. $C_{23}H_{30}O_{11}$ requires C, 57·3; H, 6·3%), $\nu_{max.}$ 1740 cm.$^{-1}$. Tetra-acetyldihydronivalenol [12] has m.p. 205°.

Hydrogenation of the enone (I; $R^1 = R^4 =$ H, $R^2 = R^3 =$ Ac) in the presence of a reduced Adams platinum oxide catalyst (uptake 2 mol. in 3 hr.) gave, on chromatography of the crude product, a series of intractable gums.

3α,4β,7α,15-*Tetrahydroxy*-12,13-*epoxytrichothec*-9-*en*-8-*one* (I; $R^1 = R^2 = R^3 = R^4 =$ H) (with Dr. A. W. Dawkins).—The enone (I; $R^1 = R^4 =$ H, $R^2 = R^3 =$ Ac) (40 mg.) in ethanol (0·5 ml.) and 0·1N-potassium hydroxide (4 ml.) was set aside for 18 hr. at room temperature. The solution was neutralised with hydrochloric acid and continuously extracted with chloroform. The recovered solid residue crystallised from ethyl acetate in prisms (22 mg.), m.p. 190°, $[\alpha]_D^{20}$ +15° (*c* 0·1), of the *ketone* (I; $R^1 = R^2 = R^3 = R^4 =$ H) (Found: C, 57·5; H, 6·5. $C_{15}H_{20}O_7$ requires C, 57·7; H, 6·45%), $\nu_{max.}$ 3460, 3300br, 1675, 1655, 1350, 1285, 1260, 1205, 1175, 1130, 1108, 1087, 1060, 1040, 1032, 980, 960, 915, 855, 800, and 720 cm.$^{-1}$, $\lambda_{max.}$ 220 nm. (ε 6500).

Recrystallisation of the ketone (I; $R^1 = R^2 = R^3 = R^4 =$ H) from imperfectly dried ethyl acetate sometimes gave a *hydrate*, triple m.p. 138—140°, 190°, and 223—225° (decomp.) (Found: C, 54·8; H, 6·6. $C_{15}H_{20}O_7,H_2O$ requires C, 54·4; H, 6·7%), $\nu_{max.}$ 3580, 3500, 3410, 3285, 3220, 1610, 1362, 1348, 1332, 1318, 1300, 1287, 1260, 1250, 1237, 1202, 1175, 1160, 1138, 1100, 1078, 1065, 1055, 1030, 1008, 982, 960, 935, 902, 888, 858, 842, 815, 800, 758, 728, and 712 cm.$^{-1}$, which, on drying at 100°/10^{-1} mm. furnished the *hemiacetal* (VI), m.p. 223—225° (decomp.) (Found: C, 57·7; H, 6·4%), $\lambda_{max.}$ 220 nm. (ε 5800) within 15 min. of dissolution; i.r. spectrum not significantly different from that of the hydrate. Nivalenol is stated [12] to have m.p. 222—223°, $[\alpha]_D^{24}$ +21° (*c* 1·3), and the expected analytical figures for $C_{15}H_{20}O_7,H_2O$.

Acetylation of both ketone (I; $R^1 = R^2 = R^3 = R^4 =$ H) and hemiacetal (VI) forms with acetic anhydride–pyridine at room temperature for 18 hr. furnished the tetra-acetate (I; $R^1 = R^2 = R^3 = R^4 =$ Ac), m.p. 168°, identified by its i.r. spectrum.

3α,4β,7α,15-*Tetrahydroxy*-12,13-*epoxytrichothecan*-8-*one* (V; R = R′ = H).—The ketone (V; R = H, R′ = Ac)

* After this paper had been submitted, Dr. T. Tatsuno informed the author that he had, independently, reached the same conclusion.

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(20 mg.) in methanol (1 ml.) and N-sodium hydroxide (2 ml.) was set aside for 4 hr. at room temperature. The methanol was removed under reduced pressure and the aqueous residue was neutralised with dilute hydrochloric acid and continuously extracted with ethyl acetate, giving a solid, m.p. 225—235° (decomp.) (10 mg.). Recrystallisation from ethanol furnished the *ketone* (V; R = R′ = H), m.p. 242—245° (decomp.) (Found: C, 57·0; H, 6·7. $C_{15}H_{22}O_7$ requires C, 57·3; H, 7·1%), $\nu_{max.}$ 3480, 3350, and 1700 cm.$^{-1}$. The m.p. of dihydronivalenol is given [12] as 242—245°.

4β-*Acetoxy*-3α,7α,15-*trihydroxy*-12,13-*epoxytrichothec*-9-*en*-8-*one* (I; $R^1 = R^2 = R^4 = H$, R^3 = Ac).—The enone (I; $R^1 = R^4 = H$, $R^2 = R^3$ = Ac) (50 mg.) in methanol (3 ml.) and N-ammonium hydroxide (3 ml.) was set aside for 30 min. at 20°, and the methanol was then removed *in vacuo*. The residual aqueous solution was extracted with ethyl acetate, yielding a gum (36 mg.), which was chromatographed, in ether–light petroleum (1 : 1), on silica gel (2 g.; 4 × 1·2 cm.). Elution with ether (75 ml.) yielded a glassy solid (32 mg.), which gave a single spot, R_F 0·43, visible under u.v. light [t.l.c. on Merck HF_{254} silica gel in chloroform–methanol (10 : 1)], and contained no starting material (R_F 0·68). The glassy solid was subjected to short-path distillation at 170—180°/10^{-1} mm., giving the 4β-*acetate* (I; $R^1 = R^2 = R^4 = H$, R^3 = Ac) as an amorphous solid, m.p. 75—77° (Found: C, 58·1; H, 6·7. $C_{17}H_{22}O_8$ requires C, 57·6; H, 6·3%), $\nu_{max.}$ 3440br, 1720, and 1680 cm.$^{-1}$. The i.r. spectrum was identical with that of fusarenone,[11] m.p. 78—80°.

Acetylation with acetic anhydride–pyridine gave the tetra-acetate (I; $R^1 = R^2 = R^3 = R^4$ = Ac), m.p. 163—165° identified by its i.r. spectrum.

When the hydrolysis with methanolic ammonium hydroxide was allowed to proceed for 4 hr. at 25° the sole product was the tetraol (I; $R^1 = R^2 = R^3 = R^4 = H$).

4β,15-*Diacetoxy*-12,13-*epoxytrichothec*-9-*ene*-3α,7α,8α-*triol* (III; R = H).—The enone (I; $R^1 = R^4 = H$, $R^2 = R^3$ = Ac) (180 mg.) in methanol (9 ml.) at 0° was treated with sodium borohydride (100 mg.) in methanol (3 ml.) and, after the initial reaction had subsided, the mixture was kept at room temperature for 1 hr. After the addition of water and acetic acid (to pH 6), the methanol was removed *in vacuo* and the residual aqueous solution was continuously extracted with chloroform during 18 hr. Crystallisation of the extract (187 mg.) from ethanol gave a mixture of needles and plates, m.p. 50—110°, which could not be separated by further recrystallisation. Chromatography on silica gel (5 g.; 10 × 1·4 cm.) and elution with ether–methanol (99 : 1; 75 ml.) furnished a gum (110 mg.) which was subjected to short-path distillation at 150—170°/10^{-1} mm., giving the amorphous *triol* (III; R = H), m.p. 90—95° (Found: C, 57·2; H, 6·7. $C_{19}H_{26}O_9$ requires C, 57·3; H, 6·6%), $\nu_{max.}$ 3450br, 1745, 1725, 1710, and 1660 cm.$^{-1}$, u.v. end absorption only: ε 5000 (205 nm.) 1600 (210), 650 (215), and 320 (220).

The *diacetyl* derivative (III; R = Ac), prepared with acetic anhydride–pyridine at room temperature for 72 hr., was subjected to short-path distillation at 150°/10^{-3} mm. giving an amorphous solid, m.p. 78—80°, which crystallised from ethyl acetate–light petroleum in prisms, m.p. 182—184°, $[\alpha]_D^{20}$ +22° (*c* 0·05), (Found: C, 57·1; H, 6·1. $C_{23}H_{30}O_{11}$ requires C, 57·3; H, 6·3%), $\nu_{max.}$ 3590, 1740, and 1650 cm.$^{-1}$.

Oxidation of the triol (III; R = H) (10 mg.) with manganese dioxide (100 mg.) in chloroform (3 ml.) at room temperature for 7 days and recovery in the usual way [1] gave a gum (6 mg.) which crystallised from ether in prisms, m.p. 130—134°, of the enone (I; $R^1 = R^4 = H$, $R^2 = R^3$ = Ac), identified by its i.r. spectrum.

Oxidation of the Enone (II) *with Lead Tetra-acetate*.—The enone (II), m.p. 135—138°,[7] (10 mg.) and lead tetra-acetate (30 mg.) in glacial acetic acid (1 ml.) were heated at 90° for 18 hr. The cooled solution was poured into water (4 ml.) and extracted with chloroform. The organic layer was washed with sodium hydrogen carbonate and water. The recovered neutral fraction (10 mg.), in acetone, was applied as a thin streak to a plate (20 × 20 cm.) coated with Merck silica gel G_{254}. The chromatogram was developed in chloroform, air dried, developed a second time, and examined in u.v. light. Dark bands were observed at R_F 0·94, 0·87w, 0·59w, 0·40, 0·35, and 0·23 (starting material). The material from the band R_F 0·35 was extracted with acetone at room temperature and crystallised from ethyl acetate–light petroleum to give prisms (1 mg.), m.p. 167—169°, of a (+)-tetra-acetate, identical with the tetra-acetate (I; $R^1 = R^2 = R^3 = R^4$ = Ac) (i.r. spectra).

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