*Griseoviridin. Part II.**

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Experiments on the desulphurisation, acid hydrolysis, and ozonolysis of griseoviridin and its derivatives are described and partial structures are proposed for two groupings in the molecule.

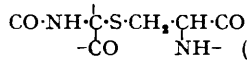
IT was shown in Part I * that hydrogenation of acetylated griseoviridin in presence of palladium catalysts fails to attack at least one of the chromophoric systems present. Desulphurisation of griseoviridin with Raney nickel, however, yielded a small amount of a crystalline product, probably $C_{22}H_{39}O_7N_3$, which exhibited no selective light absorption above 2100 Å; like griseoviridin it contained no ionisable groups. Similarly desulphurisation of griseoviridin diacetate gave, in higher yield, a crystalline product, which appears to be a diacetyl derivative of the compound obtained from griseoviridin itself. These products are provisionally named octahydrodethiogriseoviridin and its diacetate. The evidence previously obtained together with the fact that no oxygen atoms are removed during desulphurisation, indicate that griseoviridin probably contains a cyclic sulphide group. No ammonia was liberated on alkaline hydrolysis of the desulphurisation product. Hydrolysis with hydrochloric acid furnished less than one mol. of D-α-alanine but, although two α-amino-acid groups were shown to be present (Van Slyke method), no other amino-acid could be isolated.

Similar hydrolytic experiments with griseoviridin showed that only one α-amino-acid group was liberated; cystine and a much smaller amount of serine were detected by paper chromatography. The product obtained from griseoviridin diacetate and perbenzoic acid also gave cystine on hydrolysis, the amino-acid being identified by paper chromatography; this result confirms that the sulphide group has not been oxidised.

Treatment of a griseoviridin acid-hydrolysate with Brady's reagent gave a small quantity of a dinitrophenylhydrazone, probably $C_{14}H_{14}O_7N_4$, which did not contain any acidic groups. Presumably the high oxygen content is due to hydroxyl or free carbonyl groups. The absorption ($\lambda_{max.}$ 3560 Å) showed that there is no unsaturated system in conjunction with the dinitrophenylhydrazone residue. Attempts to prepare a dinitrophenylhydrazone from griseoviridin have so far been unsuccessful and so it appears that this ketone arises from a masked carbonyl group in the antibiotic.

Reduction of griseoviridin with red phosphorus and hydriodic acid gave D-cystine and α-alanine as the only recognisable products.

On the basis of the above evidence it is suggested that griseoviridin contains the system



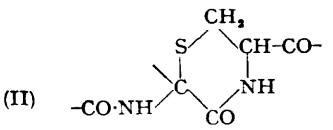
(I)

which is hydrolysed by acid to give mainly ammonia, a keto-acid, and cysteine. This would account for a number of observations. First, ammonia is obtained from griseoviridin but not from the desulphurisation product on alkaline hydrolysis. Secondly, the formation of some serine and hydrogen sulphide would be expected if cleavage first occurs to a minor extent at the S–CH_2 bond during acid hydrolysis. Thirdly, in the alkaline hydrolysis of griseoviridin the initial attack would be expected to occur at the S–CH_2 bond, rather than at the acetal-type group ·NH·C·S·, and to lead to the formation of ammonia and thioketone; this would explain why hydrogen sulphide is liberated on acidification although little or no sodium sulphide is present in the hydrolysate (see Part I). Fourthly, the desulphurisation products would then contain two α-amino-acid residues compared to the one of griseoviridin, as observed. Finally, this is consistent with the fact that griseoviridin contains one *C*-methyl group whereas octahydrodethiogriseoviridin contains two such groups.

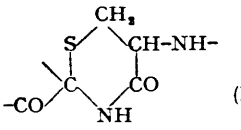
If the system (I) formulated above is indeed present in griseoviridin it must be in the form of a ring, probably (II) or (III), for fission of the molecule does not occur on desulphurisation.

* Part I, preceding paper.

Griseoviridin, although neutral and practically insoluble in water, dissolves rapidly in cold concentrated hydrochloric acid. The light absorption shown by the solution ($\lambda_{max.}$ 2160 Å, $E^{1\%}_{1cm.}$ *ca.* 500) is much less intense than that of griseoviridin in organic solvents, indicating that a chromophore has been destroyed by hydrolysis. A well-defined,



(II)



(III)

crystalline hydrochloride, isolated from the hydrolysate, could be recrystallised but decomposed without melting. Analytical data on three different specimens were concordant but corresponded to the composition, $C_{22}H_{29}O_7N_3S,1{\cdot}09HCl$. Potentiometric titration with alkali disclosed the presence of two titratable groups (pK_a 6·5), one of which is evidently the amine hydrochloride. On back-titration, however, only one titratable group, of similar pK_a value, could be detected (the titration of this single group was then reproduced on re-titration). Clearly one of the functional groups, probably the amine, has been irreversibly removed by neutralisation. Catalytic hydrogenation of the hydrochloride furnished a small amount of a crystalline product of uncertain formula. With alkali and *p*-phenylphenacyl bromide the hydrochloride gave a derivative, $C_{35}H_{37}O_8N_3S$. Neither of these compounds contained any titratable groups.

Ozonolysis studies on griseoviridin and various derivatives have been carried out, the diacetate being used first owing to its greater solubility. Distillation of the reaction mixture with very dilute sulphuric acid, water, or sodium carbonate solution yielded crotonaldehyde, isolated as the dinitrophenylhydrazone. The residual solution also gave a precipitate with Brady's reagent but no crystalline product could be isolated. Crotonaldehyde was obtained in high yield, even when the solution was thoroughly saturated with ozone, and therefore appears to be an artifact. When the solution after treatment with ozone was immediately added to Brady's reagent and the precipitate examined by paper chromatography, no crotonaldehyde or aldol derivative could be detected.

Griseoviridin also gave crotonaldehyde when treated similarly or when subjected to alkaline hydrolysis followed by ozonolysis. The crystalline hydrochloride again gave crotonaldehyde but, in this case, paper chromatography indicated the presence of the crotonaldehyde, aldol, and acetaldehyde derivatives in the product. This evidence is regarded as an indication of the presence of the system $CH_3{\cdot}CH(OH){\cdot}CH_2{\cdot}CH{:}C<$ in the hydrochloride and of the group $CH_3{\cdot}CH(O-){\cdot}CH_2{\cdot}CH{:}C<$ in griseoviridin. The oxygen atom in this system must be part of an acid-labile grouping such as an enol-ether or acetal. Hexahydrogriseoviridin diacetate gave no crotonaldehyde on ozonolysis followed by acid hydrolysis, and therefore the double bond in the above system is one of those reduced during the hydrogenation.

The epoxy-acetate (Part I) also yielded crotonaldehyde on ozonolysis and hydrolysis. It was unexpectedly found that crotonaldehyde was produced by acid hydrolysis of the epoxy-acetate; glyoxal was also identified in this hydrolysate. The grouping $CH_3{\cdot}CH(O-){\cdot}CH_2{\cdot}CH{:}N{\cdot}CO-$ would provide a possible explanation of the hydrolysis of the epoxy-acetate but the following evidence leads us to eliminate this. Since griseoviridin is non-basic the nitrogen atom would then be that evolved as ammonia on hydrolysis. Hydrogenation of the acetate, however, has been shown above to involve reduction of the double bond in the potential crotonaldehyde residue and this is incompatible with the observed liberation of ammonia on hydrolysis of hexahydrogriseoviridin diacetate. It therefore appears that another explanation of these observations must be sought and only the presence of the system $CH_3{\cdot}CH(\cdot O-){\cdot}CH_2CH{:}C<$ in griseoviridin can be proposed now.

Finally there are the infrared spectra of griseoviridin and its derivatives. In the Table the bands in the region 3500—1500 cm.$^{-1}$ are grouped arbitrarily for convenience. All the compounds show a band (B) in the range 1730—1761 cm.$^{-1}$ which in the acetylated derivatives must be due, at least in part, to the acetoxy-groups. If griseoviridin itself

contained a difficultly hydrolysed acetoxy-group this would account for all the bands (B) but the results of acetyl-value determinations (Part I) and the following considerations lead us to regard this as unlikely. First, griseoviridin and also derivatives 4, 6, 7, 8, and 9 do not show intense absorption bands near 1250 cm.$^{-1}$, a characteristic frequency of acetates. Secondly, there are too few oxygen atoms present to account for an acetoxy-group in addition to the other groups already indicated (this consideration applies equally to a lactone group). Other possible assignments for the bands (B) include ketone (in a 5-membered ring), diacylimide or hydantoin, and β-lactam. Of these the diacylimide is regarded as the most satisfactory assignment since all the bands (B) fall within the expected range (1718—1779 cm.$^{-1}$) for the 4-carbonyl group (Randall, Fowler, Fuson, and Dangl, " Infra-red Determination of Organic Structures," Van Nostrand, 1949, p. 20). Further, all the compounds except 5 show a band (C) in the range (1656—1712 cm.$^{-1}$) characteristic of the 2-carbonyl group of diacylimides (*op. cit.*). The compounds all exhibit at least one band (D) in the range 1600—1669 cm.$^{-1}$, and another (E) at 1500—1565 cm.$^{-1}$ as expected for amide groups.

Infrared absorption spectra in 3500—1500 *cm.*$^{-1}$ *region.*

	A	B	C	D	E
1. Griseoviridin	3300	1748	1684	1645	1515
				1600	
Griseoviridin *	3425	1739	1681	1597	—
2. Griseoviridin diacetate	3378	1745	1686	1650	1504
				1600	
3. Hexahydrogriseoviridin diacetate	3448	1739	1692	1639	1548
	3344			1603	1520
	3195			1592	
4. Octahydrodethiogriseoviridin	3460	1761	1669	1650	1567
	3367				1548
	3155				
5. Diacetate of no. 4	3390	1739	—	1647	1562
6. Hydrochloride	3378	1748	1667	1616	1585
					1520
7. Hydrogenation product from no. 6	3521	1739	1675	1592	1527
	3185				1511
8. *p*-Phenylphenacyl derivative of no. 6	3425	1739	1701	1645	1504
			1675	1631	
				1610	
9. Product from diazomethane and griseoviridin	3425	1730	1698	1642	1511
				1592	
10. Epoxy-acetate	3472	1751	1684	1605	1517
11. Perchlorate diacetate	3663	1751	1672	1618	1513
	3413			1590	

* Saturated solution in tetrahydrofuran. Others in " Nujol " mulls.

Experimental

Desulphurisation of Griseoviridin.—Griseoviridin (3·75 g.) in ethanol was hydrogenated in the presence of Raney nickel (several portions added at intervals). The filtered solution was concentrated to 150 c.c., refluxed for 3 hr. with more catalyst, filtered again, and evaporated to dryness *in vacuo*. Repeated recrystallisation of the residue from methanol–ethyl methyl ketone gave fine needles of *octahydrodethiogriseoviridin*, m. p. 190—192°, $[\alpha]_D^{20}$ −32° (0·5% in MeOH) (Found : C, 57·4, 57·7; H, 8·4, 8·7; O, 24·9; N, 9·1, 9·1; S, 0; *C*-Me, 5·3; Ac, 0. $C_{22}H_{39}O_7N_3$ requires C, 57·8; H, 8·6; O, 24·5; N, 9·2; 2*C*-Me, 6·6%). The yield of crude material, m. p. 160—168°, was 0·68 g. Potentiometric titration in 50% aqueous methanol revealed the absence of titratable groups in the range, pH 2—11. Infrared max. at 3460, 3367, 3155, 1761, 1669, 1650, 1567, 1548, 1353, 1337, 1259, 1226, 1177, 1133, 1115, 1089, 1055, 1048, 1015, 980, 971, 958, 935, 926, 902, 877, 855, and 830 cm.$^{-1}$.

Desulphurisation of Griseoviridin Diacetate.—Raney nickel catalyst, prepared at 0—10° from the alloy (200 g.), was washed until neutral in the usual manner and then left overnight with water (1 l.) containing acetic acid (10 c.c.). The catalyst was finally washed with water, ethanol (95%; 3 × 1 l.) and ethyl acetate (3 × 600 c.c.).

Griseoviridin diacetate (7·0 g.) in ethyl acetate was hydrogenated at a palladised strontium carbonate catalyst (5% Pd); the filtered solution was diluted to 1·5 l. with ethyl acetate and

refluxed for 2·5 hr. with the Raney nickel. The hot solution was filtered and concentrated to small volume at atmospheric pressure. After the mass had been left to crystallise, it was dried *in vacuo* to give 5·1 g. of material which softened at 140° and melted at 175—180°. Repeated recrystallisation from ethyl acetate–light petroleum (b. p. 60—80°) containing a little acetic anhydride gave *octahydrodethiogriseoviridin diacetate* as needles, m. p. 213—215°, $[\alpha]_D^{27}$ −34° (0·16% in MeOH) (Found : C, 57·6, 58·1, 58·1; H, 7·6, 7·8, 7·7; O, 27·0, 26·5, 26·4; N, 7·9, 7·7, 7·7; S, 0; Ac, 14·6. $C_{26}H_{43}O_9N_3$ requires C, 57·7; H, 8·0; O, 26·6; N, 7·8; 2Ac, 15·9%). No titratable groups were detected by potentiometric titration. No ultraviolet max. above 2100 Å (ε 3100 at 2100 Å); infrared max. at 3268, 3204, 3112, 2536, 1735, 1640, 1557, 1305, 1268, 1235, 1213, 1176, 1158, 1132, 1115, 1060, 1046, 1028, 979, 958, and 935 cm.$^{-1}$.

The same product (m. p. and mixed m.p.) was obtained by similar desulphurisation of the acetate without preliminary hydrogenation.

Examination of Octahydrodethiogriseoviridin Diacetate.—(a) *Alkaline hydrolysis.* No ammonia was evolved when the compound (81 mg.) was refluxed with N-sodium hydroxide (25 c.c.) in a slow stream of nitrogen, the solution remaining colourless. Attempts to isolate recognisable or crystalline products from the hydrolysate were unsuccessful.

(b) *Acid hydrolysis.* (i) (With A. J. Durré.) The compound was boiled with 6N-hydrochloric acid for 20·5 hr. and α-amino-acid groups estimated by the method of Van Slyke, MacFadyen, and Hamilton (*J. Biol. Chem.*, 1941, **141**, 671) (2·08 groups found).

(ii) The substance (0·5 g.) was refluxed with 6N-hydrochloric acid (20 c.c.) for 15 hr. and the solution evaporated *in vacuo*; the residue in water gave no precipitate with Brady's reagent. Treatment of the solution with ninhydrin (200 mg.) and citrate buffer mixture gave volatile aldehyde, isolated as the 2 : 4-dinitrophenylhydrazone. The derivative was identified as that of acetaldehyde by paper chromatography [upper phase of methanol–light petroleum (b. p. 100—120°)]; recrystallisation gave acetaldehyde 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 155—157°.

(iii) Hydrolysis of the diacetate (1 g.) was carried out similarly and the residue, on evaporation, taken up in water and examined by paper chromatography with butanol–water–acetic acid (4 : 5 : 1), the spots being detected with ninhydrin. Three spots (R_F 0·3, 0·6, and 0·8) of approximately equal intensity were obtained, the first corresponding to alanine. The hydrolysate was subjected to counter-current extraction with the same solvent mixture (13 × 10 c.c. of lower phase, 24 × 15 c.c. of upper phase). The three ninhydrin-sensitive components were well resolved but the other two showed a spectacular loss in intensity compared with the alanine; presumably those components are unstable when liberated from the hydrochlorides.

In another experiment, the diacetate (2·7 g.) was refluxed with concentrated hydrochloric acid (40 c.c.) for 20 hr. and the dark brown solution diluted with water (250 c.c.), filtered (charcoal), and applied to a column of Amberlite resin IR-120(H). After the column had been washed with water, the bases were eluted with 6N-ammonia until the eluate no longer gave a ninhydrin reaction. Evaporation yielded a gum (1·2 g.) containing some solid; this mixture was subjected to counter-current extraction as before. The first eight lower phases contained almost all the alanine (R_F 0·30), 0·22 g. being obtained be evaporation. Recrystallisation from aqueous ethanol (charcoal) gave D-α-alanine, $[\alpha]_D^{19}$ −13·4° (0·825 g. in 2·0 c.c. of N-HCl) (Found : C, 39·9; H, 7·9. Calc. for $C_3H_7O_2N$: C, 40·4; H, 7·9%). The identification was confirmed by degradation with ninhydrin to acetaldehyde (m. p. and mixed m. p. of dinitrophenylhydrazone). Repeated attempts to isolate crystalline products from the other fractions from the counter-current extraction were unsuccessful.

Acid Hydrolysis of Griseoviridin.—(*a*) (With A. J. Durré.) Griseoviridin was hydrolysed with 6N-hydrochloric acid, and α-amino-acid groups were estimated by the method of Van Slyke, MacFadyen and Hamilton (*loc. cit.*) and liberated ammonia by that of Conway (" Microdiffusion Analysis and Volumetric Error," Crosby Lockwood, 3rd. edn., 1950). Complete hydrolysis appeared to require *ca.* 22 hours' boiling; 0·87 α-amino-acid group was produced and 0·78 mol. of ammonia liberated, but considerable formation of tar also occurred. When griseoviridin was refluxed with 2N-sulphuric acid until dissolution was complete (20 min.), only 0·02 α-amino-acid group was produced.

(*b*) The hydrolysate obtained by refluxing griseoviridin (0·5 g.) with 6N-hydrochloric acid for 3 hr. was evaporated *in vacuo* and the residue taken up in water (100 c.c.). After addition of citric acid–sodium citrate buffer and ninhydrin (100 mg.), the solution was distilled but the distillate gave no precipitate with Brady's reagent, indicating the absence of alanine in the hydrolysate.

(*c*) A similar hydrolysate was filtered (charcoal) and treated with 2 : 4-dinitrophenylhydrazine

in 2N-hydrochloric acid. A brown solid separated slowly and after a week it was collected (centrifuge). Repeated recrystallisation from methanol (charcoal) furnished a small quantity of a *dinitrophenylhydrazone*, which formed orange needles, m. p. 220—222° (slight decomp.) (Found : C, 47·6; H, 3·7; N, 16·1. $C_{14}H_{14}O_7N_4$ requires C, 48·0; H, 4·0; N, 16·0%). No acidic groups could be detected by potentiometric titration. Light absorption : $\lambda_{max.}$ 3560 ($E^{1\%}_{1\,cm.}$ 730), $\lambda_{infl.}$ 2500 Å ($E^{1\%}_{1\,cm.}$ 350); max. at 3135, 3080, 1687, 1679, 1615, 1594, 1414, 1348, 1303, 1289, 1258, 1215, 1202, 1166, 1158, 1137, 1109, 1088, 1057, 1044, 959, 945, 922, 918, 854, 832, 774, and 765 cm.$^{-1}$.

(*d*) Griseoviridin (100 mg.) was dissolved in cold concentrated hydrochloric acid (5 c.c.) and, after addition of water (5 c.c.), the solution was refluxed for 6 hr. (bath 150°). The dark brown hydrolysate was filtered (charcoal) and examined by paper chromatography. On application of Boissonas's two-dimensional method (*Helv. Chim. Acta*, 1950, **33**, 1966) with phenol–water (7 : 3) and then propan-1-ol–water (7 : 3) two well-defined spots were obtained. The first, near the origin, corresponded to cystine while the second (R_F 0·24 and 0·33 for the two solvent mixtures) corresponded to serine when authentic amples were run simultaneously. On a one-dimensional paper chromatogram (butan-1-ol–water–acetic acid, 4 : 5 : 1, upper phase) the hydrolysate gave a bluish-brown streak on development with ninhydrin but three purple spots were observed : (i) R_F 0·37, unidentified; (ii) R_F 0·16, serine; and (iii) R_F 0·06, cystine. In both chromatograms the cystine spot was very much more intense than that of serine; in the second chromatogram the third spot was also much less intense than that of cystine. To ensure that the serine did not arise from the hydrolysis of cysteine or cystine a mixture of these was similarly treated with hydrochloric acid but no serine could be detected by paper chromatography.

Reduction of Griseoviridin with Phosphorus–Hydriodic Acid.—A mixture of griseoviridin (2·0 g.), red phosphorus (0·5 g.), and hydriodic acid (30 c.c.; *d* 1·7) was refluxed (bath 150°) for 5 hr. and filtered, the solid being washed with water. The combined solutions were concentrated to small volume under reduced pressure and the residual gum was taken up in water and applied to a column of Amberlite ion-exchange resin IRA-400(OH). The column was washed with water until the effluent was neutral. Elution of the column with 10% acetic acid (1 l.) and evaporation of the eluate furnished a gum which partially crystallised. Trituration with water yielded cystine (90 mg.) which was recrystallised from water (Found : C, 30·0; H, 5·3; N, 11·1. Calc. for $C_6H_{12}O_4N_2S_2$: C, 30·0; H, 5·0; N, 11·7%). It was identified by paper chromatography (butan-1-ol–water–acetic acid, 4 : 5 : 1) and by the identity of the infrared spectrum with that of an authentic sample; it also gave a purple colour with sodium cyanide followed by sodium nitroprusside solutions. The optical rotation ($[\alpha]_D^{22\cdot5}$ +218° in N-HCl) showed that it was the D-isomer.

The mother-liquor from which cystine had been separated was evaporated to dryness and subjected to counter-current distribution with butan-1-ol–water–acetic acid (4 : 5 : 1; 13 × 10 c.c. of lower phase and 36 × 15 c.c. of upper phase). Cystine (R_F 0·09 in same solvent mixture) was present in the first five lower phases, and alanine (R_F 0·24) in the remainder. Alanine (0·2 g.), isolated by evaporation and crystallisation of the residue from aqueous alcohol, was identified by its infrared spectrum; this sample had been almost completely racemised ($[\alpha]_D^{25}$ −0·6° in N-HCl).

Reaction of Griseoviridin with Cold Concentrated Hydrochloric Acid.—(*a*) (With Miss E. M. Tanner.) Griseoviridin dissolves very rapidly in cold concentrated hydrochloric acid. Portions of the solution were diluted with water, after a given time, for light absorption measurements :

Freshly prepared solution	$\lambda_{max.}$ 2160 Å,	$E^{1\%}_{1\,cm.}$	588;	$\lambda_{infl.}$	2850 Å;	$E^{1\%}_{1\,cm.}$	50
After 20 min.	,, 2120	,,	545	,,	2850	,,	39
After 2 days	,, 2160	,,	497	,,	—	,,	—

(*b*) Griseoviridin (3·0 g.) was dissolved in concentrated hydrochloric acid (30 c.c.), and the colourless solution evaporated to dryness *in vacuo* (bath 20—30°). On addition of acetone (20 c.c.) and a few drops of methanol crystallisation began and, after 30 minutes' shaking, the colourless crystals (2·1 g.) were separated and washed with a small volume of acetone. Recrystallisation from ether–methanol furnished well-defined needles of a *hydrochloride* which decomposed without melting below 300°. Inserted in a bath preheated to 150°, however, it melted with much decomposition at *ca.* 180°. Analytical results on different batches of this material were reproducible, and unchanged by further recrystallisation, but the Cl : N : S ratio indicated the presence of more than 1 mol. of hydrogen chloride (Found, on material dried at

20°/ or 50°/0·5 mm. : C, 51·5, 51·2, 51·2; H, 5·9, 5·6, 5·6; O, 21·4; N, 8·0, 8·1; Cl, 7·4, 7·4; S, 5·9, 5·7. $C_{22}H_{29}O_7N_3S,1{\cdot}09HCl$ requires C, 5·09; H, 5·8; O, 21·6; N, 8·1; Cl, 7·4; S, 6·2%).

(*c*) (With Miss E. M. TANNER.) In potentiometric titration of the hydrochloride in water two mols. of alkali were required (equiv., 270. Calc. for $C_{22}H_{29}O_7N_3S,1{\cdot}09HCl$: 519) but on back-titration of this solution only one mol. of acid is consumed (equiv., 540). Subsequent re-titration also requires only one mol. of alkali (equiv., 540). Neutralisation to pH 7 suffices to effect this change.

Hydrogenation of Hydrochloride.—Palladised charcoal (3 g., 10% Pd) was pre-reduced under 95% ethanol (70 c.c.), and the hydrochloride (1·0 g.) added. Absorption of hydrogen ceased when about 3 mols. had been taken up in 4 hr. The filtered solution was evaporated *in vacuo*; the residual glass dissolved in methanol and, on being left at 0° overnight, the solution deposited crystals (0·15 g.), m. p. 232—236°. The pure product separated from methanol in fine, prismatic needles, m. p. 247—248° (Found : C, 55·6; H, 6·9; O, 22·0; N, 8·8; S, 6·4. $C_{23}H_{35}O_7N_3S$ requires C, 55·5; H, 7·1; O, 22·5; N, 8·5; S, 6·4. $C_{21}H_{33}O_6N_3S$ requires C, 55·3; H, 7·3; O, 21·1; N, 9·2; S, 7·0%). Although the analytical sample was dried at 50° in a high vacuum, the substance might possibly contain methanol of crystallisation. No titratable groups were detected by potentiometric titration. Light absorption : no maximum above 2150 Å ($E^{1\%}_{1\,cm.}$ 380 at 2150 Å); max. at 3521, 1739, 1675, 1592, 1527, 1511, 1359, 1328, 1196, 1104, 1053, 979, 909, 860, 840, 766, and 725 $cm.^{-1}$.

Condensation of Hydrochloride with p-*Phenylphenacyl Bromide.*—A suspension of the hydrochloride (0·5 g.) in 95% ethanol was titrated with sodium hydroxide solution (2·0 c.c. of N required) with phenolphthalein as indicator. *p*-Phenylphenacyl bromide (0·30 g.) was added and the solution refluxed for 30 min. After 48 hr. a hard crystalline mass had separated from the solution. Recrystallisation from chloroform–ethanol furnished fine needles of a *derivative*, m. p. 288—289° (Found : C, 63·3; H, 5·5; O, 20·1; N, 6·3; S, 4·4. $C_{35}H_{37}O_8N_3S$ requires C, 63·7; H, 5·7; O, 19·4; N, 6·4; S, 4·8%).

Ozonolysis of Griseoviridin Diacetate.—(*a*) The diacetate (0·35 g.) in glacial acetic acid (25 c.c.) was saturated with ozonised oxygen, and excess of ozone then swept away by a stream of oxygen. The solution was poured into water (100 c.c.) containing sulphuric acid (0·2 c.c. of 2N) and distilled. Treatment of the distillate with Brady's reagent gave crotonaldehyde 2 : 4-dinitrophenylhydrazone (75% yield), fine red needles (from methanol), m. p. and mixed m. p. 184—186° (Found : C, 47·6; H, 4·1; O, 25·0; N, 22·9. Calc. for $C_{10}H_{10}O_4N_4$: C, 48·0; H, 4·0; O, 25·6; N, 22·4%). The identity of this product was confirmed by comparison of the infrared and ultraviolet absorption spectra and by paper chromatography [methanol–light petroleum (b. p. 100—120°)], both samples giving a single spot, of R_F 0·50.

Treatment of the distillation residue with Brady's reagent gave a yellow precipitate (0·23 g.) but this decomposed without melting and attempts to isolate a crystalline product were unsuccessful.

(*b*) In another experiment, the acetic acid solution was ozonised and then treated with zinc dust. After 10 min., the filtered solution was treated with Brady's reagent; the resulting yellow precipitate softened above 50° but gradually decomposed at 150—230° without completely melting. When examined by paper chromatography, as in (*a*), this material showed R_F <0·07 indicating the absence of the derivatives of both crotonaldehyde and aldol.

Ozonolysis of Griseoviridin.—When griseoviridin in acetic acid was ozonised the results were similar to those obtained with the acetate. No crotonaldehyde or aldol dinitrophenylhydrazone could be detected when the solution was treated directly with Brady's reagent but the crotonaldehyde derivative was obtained in 80% yield after boiling with water. When the ozonised solution was basified with sodium carbonate solution and distilled, crotonaldehyde dinitrophenylhydrazone was isolated in 56% yield.

Miscellaneous Ozonolysis.—(*a*) The crystalline hydrochloride (80 mg.) in water (15 c.c.) was treated with ozonised oxygen for 25 min. and the solution poured immediately into Brady's reagent. The precipitate, examined by paper chromatography as before, contained the derivatives of crotonaldehyde and, in smaller amounts, acetaldehyde (R_F 0·39) and aldol (R_F 0·10). Similar results were obtained by ozonolysis of the hydrochloride dissolved in 0·1N-sodium hydroxide.

(*b*) Hexahydrogriseoviridin diacetate, ozonised in acetic acid, gave no crotonaldehyde even on distillation of the mixture with dilute sulphuric acid.

(*c*) The epoxy-acetate (Part I) was ozonised similarly; no crotonaldehyde could be obtained by treating the solution with Brady's reagent until after it had been boiled with water.

(*d*) Griseoviridin (200 mg.) was boiled with 0·1N-sodium hydroxide solution for 30 min. and

the resulting solution treated with ozonised oxygen for 30 min. Steam-distillation and addition of Brady's reagent to the distillate yielded a crystalline dinitrophenylhydrazone. Spots corresponding to the crotonaldehyde and acetaldehyde derivatives were obtained by paper chromatography, the latter spot being much the less intense.

Hydrolysis of Griseoviridin Epoxy-acetate.—(*a*) The crude epoxide (50 mg.; Part I) was distilled with 0·02N-sulphuric acid (100 c.c.) and the distillate treated with Brady's reagent. The precipitated dinitrophenylhydrazone was homogeneous and was identified as the crotonaldehyde derivative by paper chromatography (cf. above). A similar experiment on griseoviridin gave no aldehyde in the distillate.

(*b*) The epoxide (320 mg.) was refluxed for 4 hr. with 6N-hydrochloric acid (15 c.c.). After addition of water (65 c.c.), the solution was distilled (30 c.c. removed) and the residue treated with Brady's reagent. A dark red solid which had separated after 20 hr. was collected (centrifuge) and recrystallised by addition of ethanol to a filtered (charcoal) solution in pyridine. The product was identified as glyoxal bisdinitrophenylhydrazone by comparison of the infrared spectrum with that of an authentic sample. It also showed the expected ultraviolet light absorption ($\lambda_{max.}$ 4600 Å) and gave a deep blue colour with aqueous-methanolic sodium hydroxide.

(*c*) In a similar experiment the hydrolysate was examined by two-dimensional paper chromatography (7 : 3 phenol–water, followed by 7 : 3 propan-1-ol–water). Development with ninhydrin revealed an intense spot near the origin corresponding to cystine but there were no other spots of comparable intensity. Paper chromatography, with butanol–water–acetic acid (4 : 5 : 1), gave two spots, of R_F 0·12 (faint, serine) and R_F 0·05 (intense, cystine) identical with those given by a griseoviridin hydrolysate run simultaneously.

Permanganate Oxidation of Griseoviridin.—A hydrolysate prepared by refluxing griseoviridin (350 mg.) with 0·1N-sodium hydroxide (20 c.c.) for 25 min. was cooled at 30—40° while barium permanganate (1·8 g.) in water (40 c.c.) was added during 10 min. After 15 min. excess of permanganate was destroyed by addition of sodium nitrite and the filtered solution was concentrated to small volume. The distillate gave a small quantity of precipitate with Brady's reagent, and this was examined by paper chromatography [methanol–light petroleum (b. p. 100—120°)]. Three spots were obtained (R_F 0·49, 0·30, and 0·28) corresponding to the crotonaldehyde, acetaldehyde, and formaldehyde derivatives. Another spot (intense blue with alkali spray) did not move from the origin.

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