Griseoviridin. Part I.

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The formula $C_{22}H_{29}O_7N_3S$ is proposed for griseoviridin, a *Streptomyces* antibiotic. Some chemical studies bearing on the nature of the functional groups in griseoviridin are described.

Two antibiotics, griseoviridin and viridogrisein, were isolated from cultures of a strain of *Streptomyces griseus*, and also from an unidentified *Streptomyces* strain, by Bartz, Standiford, Mold, Johannessen, Ryder, Maretzki, and Haskell (2nd Ann. Symp. on Antibiotics, Washington, October, 1954; Antibiotics Annual, 1954—5). Griseoviridin was reported to be a colourless, neutral, optically active compound which gives negative results with ferric chloride, in the Sakaguchi reaction, and in the Jacobs–Hoffman test for unsaturated lactones. We now describe some studies on the degradation of this material which was kindly supplied by Dr. Bartz and his colleagues.

Griseoviridin crystallises well from pyridine or methanol and retains solvent tenaciously, but the dried material gives satisfactory analyses for the formula $C_{22}H_{29}O_7N_3S$ (though the hydrogen value is not conclusively proved). The corresponding molecular weight was consistent with the values obtained by the isothermal-distillation method and by *X*-ray examination (carried out by Dr. G. W. R. Bartindale). The antibiotic does not contain thiol, methoxyl, or methylimino-groups, but about five active hydrogen atoms were detected by the Zerewitinov method. Griseoviridin gives a positive result in the chromate–nitric acid test for alcohols, but it is not a vicinal diol, for there is only slight reaction with periodic acid. No titratable groups could be detected by potentiometric titration of griseoviridin even with perchloric acid in acetic acid (Hall, *J. Amer. Chem. Soc.*, 1930, **52**, 5115; Markunas and Riddick, *Analyt. Chem.*, 1951, **23**, 337); it is, therefore, concluded that all three nitrogen atoms are present in amide-type groups, and it is also likely that amide-NH groups account for several of the observed active hydrogen atoms. The ultraviolet light absorption of griseoviridin ($\lambda_{max.}$ 2205 Å, ε 44,000) appears to indicate the presence of at least three chromophoric groups, such as $\alpha\beta$-unsaturated acid residues. Consideration of the infrared spectrum is deferred to Part II (following paper).

Acetylation of griseoviridin with acetic anhydride–pyridine yields a diacetyl derivative, $C_{26}H_{33}O_9N_3S$, which is regarded as the *OO*-diacetate, but several attempts to prepare a benzoyl derivative gave only gums. Treatment of griseoviridin with acetic anhydride–acetic acid–perchloric acid furnished a well-defined perchlorate diacetate. It therefore appears that griseoviridin contains two hydroxyl groups and, as shown in the sequel, this conclusion is supported by the isolation of diacetyl derivatives from hydrogenation and desulphurisation reactions. The potentiometric titration curve of the perchlorate diacetate showed only one break (pK_a 6·25) corresponding to two equivalents of alkali; one of these must correspond to the liberation of a weak base from its perchlorate, and the other is presumably due to a weakly acidic group such as an enolic system. This view is supported by the fact that, after treatment with hot water or alkali, the perchlorate gave a purple colour with ferric chloride. Attempts to hydrogenate griseoviridin under various conditions gave unsatisfactory results owing mainly to its insolubility in suitable solvents. In quantitative micro-hydrogenations of the acetylation products considerably more than two mols. of hydrogen were absorbed, but accurate data were not obtained because the rate of absorption declined continually, possibly as a result of the poisoning effect of the sulphur on the

catalyst. The acetylation product could, however, be hydrogenated in the presence of a large amount of palladium (on charcoal or strontium carbonate) to give a crystalline product, probably hexahydrogriseoviridin diacetate. The absorption spectrum ($\lambda_{max.}$ <2150 Å; ε 21,000) showed that at least one chromophoric group is still present in this compound.

On treatment of griseoviridin diacetate with perbenzoic acid, two mols. of per-acid were rapidly consumed, the greatly reduced intensity of light absorption ($\lambda_{infl.}$ 2100 Å; $E_{1cm}^{1\%}$ 369 in EtOH) showing that reaction had occurred at double bonds (*i.e.*, not sulphide to sulphone). Since αβ-unsaturated acid residues are not usually attacked by perbenzoic acid, reaction may well have occurred at a diene system. It is of interest that the absorption spectrum of this product was almost identical with that of hexahydrogriseoviridin diacetate.

The reaction of griseoviridin with diazomethane yielded much yellow gum, and a small quantity of a colourless, crystalline compound, $C_{23}H_{31}O_7N_3S$. This does not contain a methoxyl group or any titratable group, and its ultraviolet and infrared spectra closely resemble those of griseoviridin. In Kuhn–Roth determinations this compound gave considerably higher values than those obtained from griseoviridin itself; this may be due to *C*-methylation or to *cyclo*propane ring formation.

Alkaline hydrolysis of griseoviridin yields ammonia (1 mol.) and on acidification, hydrogen sulphide (*ca.* ½ mol.). No lead sulphide is precipitated on treatment of the alkaline hydrolysate with alkaline lead acetate solution, indicating that hydrolysis produces an acid-labile sulphur compound, and not sodium sulphide. Two or three titratable groups are generated during alkaline hydrolysis, according to the conditions, but one of these recombines in faintly acid solution within a few hours.

Acetic acid was detected in griseoviridin hydrolysates by paper chromatography. Acetyl-value determinations, however, gave very small results (*ca.* 0·2—0·5%) and it is therefore concluded that griseoviridin does not contain an *O*- or *N*-acetyl group but that some acetic acid is produced by hydrolytic degradation. Kuhn–Roth determinations disclosed the presence of one *C*-methyl group.

Hydrolysis of griseoviridin with dilute sulphuric acid gives carbon dioxide (0·74 mol.) but very little hydrogen sulphide is liberated.

Experimental

Infrared spectra were determined in " Nujol," unless otherwise stated.

Griseoviridin.—The *compound* separated from pyridine in well-defined plates which, after being dried at 120°/0·1 mm., melted at 228—230° (Found: C, 54·9 H, 5·8; O, 24·4; N, 8·7; S, 6·2; active H, 1·04, 1·11. $C_{22}H_{29}O_7N_3S$ requires C, 55·1; H, 6·1; O, 23·4; N, 8·8; S, 6·7; 5H, 1·04%; *M*, 479·5). Crystallisation from methanol gave large crystals, m. p. 160° (with effervescence), which were dried at 50°/0·1 mm. to give a *hemimethanolate* (Found: C, 54·2; H, 6·3; O, 25·1; N, 8·6; S, 6·0. $C_{22}H_{29}O_7N_3S,0{\cdot}5CH_3{\cdot}OH$ requires C, 54·5; H, 6·3; O, 24·2; N, 8·5; S, 6·5%). On drying at 80°/0·1 mm. it lost weight (2·7%; required 3·2%), giving the anhydrous compound (Found: C, 55·2; H, 6·2; O, 23·0; N, 8·6; S, 6·4%). The dried material did not contain methoxy-groups (Zeisel) and gave only very small and non-reproducible *N*-Me estimations. It showed $[\alpha]_D^{27}$ −237° (0·5% in MeOH). Light absorption: $\lambda_{max.}$ 2205 (ε 44,000), $\lambda_{infl.}$ 2775 Å (ε 1500 in EtOH). Infrared max.: 3300, 1748, 1684, 1645, 1600, 1515, 1412, 1374, 1307, 1276, 1188, 1105, 1083, 1044, 1029, 991, 957, 893, 845, 770, and 759 cm.$^{-1}$.

Molecular weight (by A. J. Durré). With dimethylformamide as solvent at 50°, the Signer method (Clark, *Ind. Eng. Chem. Anal.*, 1941, **13**, 820) gives a value of 485—490. Similar results were obtained with acetic acid as solvent, but some discoloration occurred.

Unit-cell dimensions and cell molecular weight (by G. W. R. Bartindale). Griseoviridin crystallises from methanol in two forms: form A is obtained above 30° and form B at lower temperatures. Form B loses solvent in air, to give form A (identical *X*-ray powder photographs). The cell dimensions of the monoclinic crystals of form A are: $a = 10{\cdot}72 \pm 0{\cdot}01$, $b = 9{\cdot}58 \pm 0{\cdot}03$, $c = 11{\cdot}705 \pm 0{\cdot}01$, $\beta = 93°\ 33' \pm 3'$. The density (mean of two determinations) is 1·377. The cell molecular weight is, therefore, 995·4 ± 7 units, the unit cell containing $2C_{22}H_{29}O_7N_2S + 1CH_3{\cdot}OH$. There are no systematic absences and the space group is, therefore, *P*2, *Pm*, or *P*2/*m*. Since the analytical data (Found: C, 54·0; H, 6·1%; see also above) indicate that form A is a hemimethanolate, the calculated unit-cell molecular weight would be 991·0.

Test for alcohol groups. Griseoviridin in water gave a light blue colour in 1·5—2 hr. in the chromate–nitric acid test (Fearon and Mitchell, *Analyst*, 1932, **57**, 372) for primary and secondary alcohol groups.

Treatment with periodic acid. The antibiotic (60 mg.) in methanol (10 c.c.) was treated with 1% periodic acid (10·0 c.c.). After 16 hr. excess of periodic acid was titrated (arsenite–iodine method) and it was found that only *ca.* 0·1 mol. of periodate had been consumed.

Griseoviridin diacetate. A suspension of griseoviridin (3·0 g.) in acetic anhydride (25 c.c.) and pyridine (60 c.c.) was left at room temperature for 16 hr. The resulting solution was evaporated to dryness *in vacuo*, xylene (100 c.c.) added, and the mixture re-evaporated similarly. Repeated recrystallisation of the residual solid from methanol–ether–light petroleum (b. p. 40—60°) gave needles of the *diacetate* which after drying at 100°/1 mm. sintered at *ca.* 125° and melted with decomposition at 137—140° (Found, after drying at 100°/1 mm. for 6 hr. : C, 55·4; H, 5·8; N, 7·7; Ac, 14·3, 14·7. $C_{26}H_{33}O_9N_3S$ requires C, 55·4; H, 5·9; N, 7·4; 2Ac, 15·3%). The product showed $[\alpha]_D^{27}$ −230° (0·44 in MeOH); $\lambda_{max.}$ 2180 (ε 41,800) and $\lambda_{infl.}$ 2850 Å (ε 1000 in EtOH); max. at 3378, 1745, 1686, 1650, 1600, 1504, 1372, 1285, 1244, 1209, 1195, 1119, 1065, 1027, 1001, 992, 955, 893, 845, 826, 763, and 725 cm.$^{-1}$.

Treatment of Griseoviridin with Acetic Anhydride–Perchloric Acid.—Griseoviridin (500 mg.), suspended in acetic acid (2·5 c.c.) and acetic anhydride (2·5 c.c.), was treated with 70% perchloric acid (0·2 c.c.); an exothermic reaction occurred and the solid rapidly dissolved. The mixture was kept at 50—55° for 30 min. and the colourless solid (330 mg.) which had separated was collected and washed with a little acetic acid. The *perchlorate diacetate* thus obtained decomposed above 170° without melting (Found : C, 46·6, 46·9; H, 5·2, 5·4; O, 30·8, 31·7; N, 6·3, 6·3; S, 4·7, 4·7; Cl, 5·6, 5·7; Ac, 14·4, 13·5. $C_{26}H_{33}O_9N_3S,HClO_4$ requires C, 47·0; H, 5·2; O, 31·3; N, 6·3; S, 4·8; Cl, 5·3; 2Ac, 13·0%). It showed $[\alpha]_D$ −188° (0·19% in dimethylformamide). Light absorption : $\lambda_{max.}$ 2150 (ε 32,100 in H_2O) : max. at 3663, 3413, 1751, 1672, 1618, 1590, 1513, 1422, 1408, 1339, 1304, 1236, 1176, 1104, 1058, 1035, 1013, 1004, 961, 928, 886, 858, 850, 821, 769, and 745 cm.$^{-1}$. The pK_a value (6·25; equiv., *ca.* 300) was determined by potentiometric titration in 50% methanol.

The same product (identical infrared spectrum) was obtained similarly from griseoviridin diacetate. The perchlorate in ethanol gave no colour with ferric chloride (except on long storage); it dissolved in boiling water, and the cooled solution gave an immediate purple colour with ferric chloride. A purple colour was also obtained by dissolving the solid in sodium hydroxide solution, neutralising the mixture, and adding ferric chloride.

Hydrogenation of Griseoviridin Diacetate.—The diacetate (50·5 mg.) in ethyl acetate (4 c.c.) was hydrogenated in the presence of pre-reduced palladised strontium carbonate (50 mg.; 5% Pd), 5·22 c.c. being absorbed at 20° (calc. for $C_{26}H_{33}O_9N_3S$: 2·16 c.c. per mol. of hydrogen).

In another experiment the diacetate (2·0 g.) in ethyl acetate was hydrogenated in the presence of 5% palladised strontium carbonate (two 3 g. portions) until absorption ceased. Evaporation of the filtered solution followed by repeated recrystallisation from methanol–ether and methanol gave fine needles of *hexahydrogriseoviridin diacetate*, m. p. 213—214° (Found : C, 55·2; H, 7·0; O, 24·8; N, 7·2; S, 5·5. $C_{26}H_{39}O_9N_3S$ requires C, 54·8; H, 6·9; O, 25·3; N, 7·4; S, 5·6%). Infrared max. at 3448, 3344, 3195, 1739, 1692, 1639, 1603, 1592, 1548, 1520, 1330, 1266, 1248, 1229, 1211, 1103, 1071, 1032, 991, 961, 940, 855, 840, 804, and 724 cm.$^{-1}$.

Reaction of Griseoviridin Diacetate with Perbenzoic Acid.—(*a*) The diacetate (641 mg.) in chloroform (75 c.c.) was treated with 0·35M-perbenzoic acid (25 c.c.), aliquot parts being titrated at intervals. Reaction was complete in 24 hr. at 0°, 1·96 mols. of per-acid being consumed : at room temperature 2·14 mols. were consumed and reaction was complete in 7 hr.

(*b*) Chloroformic perbenzoic acid (100 c.c.; 0·34M) was added to the diacetate (3·5 g.) in chloroform (50 c.c.). Next day excess of per-acid was destroyed by addition of citronellol (5 c.c.) and after 20 min. the solution was evaporated *in vacuo*. Repeated extraction with boiling anhydrous ether yielded a colourless amorphous solid (3·1 g.) of indefinite m. p. (135—180° with decomposition). Different runs gave material of varying analysis (C, 48—51%) and attempts to purify the product by recrystallisation failed; these difficulties may be due to reaction with traces of water. The material was, therefore, used directly for hydrolysis.

Treatment of Griseoviridin with Diazomethane.—Griseoviridin (650 mg.) in methanol (30 c.c.) was treated with excess of diazomethane and after 3 hr. the remaining diazomethane was destroyed with acetic acid. Evaporation yielded a gum from which some griseoviridin was recovered by trituration with acetone. The mother-liquors were evaporated and the yellow residue dissolved in a little methanol; this solution slowly deposited crystalline material (100 mg., m. p. 220—240°). Repeated recrystallisation from methanol yielded plates of a *compound*,

m. p. 262—264° (decomp.) (Found : C, 56·4; H, 6·0; O, 22·7; N, 8·5; S, 6·6; *C*-Me, 5·8; OMe, 0. $C_{23}H_{31}O_7N_3S$ requires C, 56·0; H, 6·3; O, 22·7; N, 8·6; S, 6·5; 2 *C*-Me, 6·1%). No ionisable groups could be detected in the range pH 3·2—12·4 by potentiometric titration in 50% aqueous dimethylacetamide. Light absorption : $\lambda_{max.}$ 2700 (ε 2800) and $\varepsilon_{infl.}$ 2210 Å (ε 46,000) in EtOH; infrared max. at 3425, 1730, 1698, 1642, 1592, 1511, 1412, 1370, 1326, 1307, 1221, 1189, 1101, 1066, 1050, 990, 843, 829, and 761 cm.$^{-1}$.

Alkaline Hydrolyses.—(*a*) Griseoviridin (125 mg.) was boiled with 0·5N-barium hydroxide solution (25 c.c.) in a stream of nitrogen; ammonia (0·92 mol.) was evolved during 50 min. In similar experiments the perbenzoic acid oxidation product and hexahydrogriseoviridin diacetate also gave ammonia (0·90 and 1·03 mol. respectively). In another experiment griseoviridin (108 mg.) was boiled with sodium hydroxide solution, and the ammonia produced was identified by reaction with phenyl *iso*thiocyanate : the derivative had m. p. and mixed m. p. 150—152°. The alkaline solution was acidified with sulphuric acid and steam-distilled, hydrogen sulphide being evolved. The distillate was basified and evaporated to dryness *in vacuo*. The resulting sodium salt was converted into the hydroxamic acid, and the latter identified as acethydroxamic acid by paper chromatography following Thomson's procedure (*Austral. J. Sci. Res.*, 1951, **4**, *B*, 180). Acetic acid is thus formed during the hydrolysis.

(*b*) A hydrolysate obtained similarly was acidified after 50 minutes' boiling and the evolved hydrogen sulphide estimated as lead sulphide. The yield corresponded to 1·86% of sulphur, *i.e.*, about a third of that present in the antibiotic. Sodium sulphide is apparently not present in the alkaline hydrolysate because only a trace of brown precipitate was formed on the addition of alkaline lead acetate solution.

(*c*) Griseoviridin (145 mg.) was refluxed for 1 hr. with 25 c.c. of 0·1N-sodium hydroxide. Titration with standard acid showed that acid groups had been generated during the hydrolysis (sap. equiv. : 182). Similar results were obtained after 3 hours' refluxing (equiv., 169) or after 1 hour's boiling with N-alkali (equiv., 180). More standard acid was then added to each hydrolysate so that the total was exactly equivalent to the alkali taken (*i.e.*, the organic acids were liberated from the sodium salts). Next day the solutions were re-titrated with alkali (phenolphthalein indicator) but less alkali was now required (equiv., 285, 295). It therefore appears that three titratable acid groups are generated by alkaline hydrolysis but one of these recombines slowly in faintly acid solution.

(*d*) A suspension of griseoviridin (*ca.* 100 mg.) in 0·1N-sodium hydroxide (25 c.c.) was shaken at room temperature until all the solid had dissolved (24 hr.) and then for a further 24 hr. Titration with acid showed that two acid groups had been liberated (sap. equiv., 308, 284, 312). Addition of the calculated quantity of acid as in (*c*) and re-titration with alkali after 24 hr. gave equiv., 470, 494. Again one of the acid groups has apparently recombined. The cold alkaline hydrolysate did not smell of ammonia.

Acidic Hydrolyses.—(*a*) When griseoviridin (56·6 mg.) was boiled with 2N-sulphuric acid (20 c.c.), carbon dioxide was liberated (0·74 mol. in 2 hr.), much tar being formed. Only a trace of hydrogen sulphide was evolved under these conditions.

(*b*) A sample (116 mg.) of griseoviridin was refluxed with 0·1N-sulphuric acid (25 c.c.) in a stream of nitrogen for 15 min. (dissolution required 5 min.). The solution remained colourless and only a trace of hydrogen sulphide was evolved; no volatile aldehyde or ketone was produced. Titration of the solution with standard alkali showed the formation of 0·88 mol. of acid.

(*c*) The solution obtained by boiling griseoviridin (58 mg.) with 5N-sulphuric acid (20 c.c.) for 1 hr. in nitrogen was steam-distilled. Acetic acid was identified in the distillate by the procedure already described.

Acetyl Value and C-Methyl Determinations (by A. J. Durré).—Wiesenberger's procedure (*Mikrochem. Mikrochim. Acta*, 1948, 33, 51) was used. Preliminary experiments with 12N-sulphuric acid for hydrolysis of glucose penta-acetate gave acetyl values in the range 5—5·5 groups. Under similar conditions glucose also gave an " acetyl value " of *ca.* 0·5 group. Satisfactory results were obtained, however, when both compounds were hydrolysed with *ca.* 7N-sulphuric acid for ½ hr. When griseoviridin was hydrolysed similarly the observed acetyl value was 0·22% ($C_{22}H_{29}O_7N_3S$ requires 1Ac, 9·0%). More prolonged hydrolysis (1—5 hr.) gave values of *ca.* 0·5%.

In view of these results Kuhn–Roth determinations were also carried out in *ca.* 7N-sulphuric acid (Found : *C*-Me, 2·9, 2·7, 2·5. $C_{22}H_{29}O_7N_3S$ requires *C*-Me, 3·1%).

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