569. *Griseoviridin. Part III.* Degradation to 10-Aminodecanoic Acid, and Other Reactions.*

By D. E. Ames and R. E. Bowman.

Octahydrodethiogriseoviridin diacetate is hydrogenated in hot hydrochloric acid to give 10-aminodecanoic acid and is reductively hydrolysed by the Clemmensen method to a 10-amino-*x*-hydroxydecanoic acid. Other reactions of griseoviridin are also described and a partial structure is suggested.

In previous work [1] we reported that acid hydrolysis of octahydrodethiogriseoviridin diacetate yields two moles of α-amino-acid : α-alanine was separated but no other amino-acid could be isolated. The formation of two mols. of alanine has now been established by treating the hydrolysate with ninhydrin and estimating the acetaldehyde produced. Both α-amino-acid groups liberated on hydrolysis are therefore accounted for and, since three ninhydrin-sensitive components can be detected by paper chromatography, the other two must be formed from the third nitrogen function during hydrolysis. These other two components appeared to be unstable on liberation from their hydrochlorides : this observation has now been confirmed by paper chromatography of the hydrolysate in butanol–ammonia, only the alanine spot then being detected with ninhydrin. A possible explanation of this instability is the presence of a keto-group in the other two amino-compounds so that self-condensation occurs on neutralisation. Attempts to avoid this difficulty by carrying out the hydrolysis under reducing conditions were therefore made. First, octahydrodethiogriseoviridin diacetate in warm hydrochloric acid was hydrogenated over a platinum catalyst. After completion of hydrolysis by refluxing, the mixture was examined by paper chromatography in butanol–ammonia; in addition to alanine, four well-defined spots could be detected with ninhydrin. Treatment with anion-exchange resin showed that two of these products were amines and two were amino-acids. 10-Aminodecanoic acid was isolated by counter-current distribution and was readily identified by the absence of a *C*-methyl group.

Clemmensen reduction also yielded several stable ninhydrin-sensitive products. One of these, which was not isolated, was indistinguishable from 10-aminodecanoic acid in paper chromatography. Another component, $C_{10}H_{21}O_3N$, is regarded as a 10-amino-*x*-hydroxydecanoic acid, since it must have been produced from the same fragment of the molecule as 10-aminodecanoic acid. The hydroxy- and amino-groups are not vicinal as the substance is not oxidised by periodic acid; moreover it is a rather weak acid (pK_a' 5·2; cf. 10-aminodecanoic acid, pK_a' 5·0) and is therefore not an α-hydroxy-acid.

These results imply that the hydroxyl group of the amino-hydroxy-acid residue is readily converted into the chloro-group in hydrochloric acid so that reduction then leads to 10-aminodecanoic acid. This reaction probably explains the anomalous analysis obtained for a crystalline "hydrochloride" from griseoviridin and concentrated hydrochloric acid (Part II) since some covalently-bound chlorine could well be present in an impurity. To

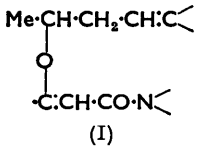
* Part II, *J.*, 1955, 4264.

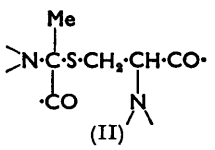
[1] Ames and Bowman, *J.*, 1955, 4264.

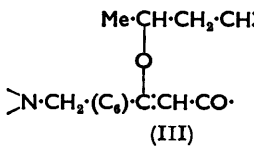
prepare a similar salt and avoid this difficulty, griseoviridin in dioxan was treated with perchloric acid. A perchlorate, $C_{22}H_{29}O_7N_3S,HClO_4$, was obtained in good yield; as in the formation of the " hydrochloride," there is no overall addition of water during the reaction. Like griseoviridin, the salt furnishes nearly one mol. each of ammonia and α-amino-acid on acid hydrolysis. In potentiometric titration the perchlorate showed pK_a' 6·9 (equiv., 400); back-titration then gave pK_a' values of 6·5 (equiv., 450) and 2·1 and a further titration with alkali gave pK_a' 2·5 and 5·9 (equiv., 650). These results are evidently due to further reactions or hydrolysis during titration, but there are apparently two titratable groups corresponding to the pK_a' value of *ca.* 6. The perchlorate shows an ultraviolet absorption maximum at 218 mμ in water and at *ca.* 270 mμ in neutral or alkaline solution (ethyl acetoacetate has maxima at 244 and 277 mμ, respectively).[2] This evidence is considered to indicate the presence of a β-oxo-acyl group (presumably β-oxo-amide) in the perchlorate and also in the " hydrochloride."

Further examination of the acid hydrolysis of octahydrodethiogriseoviridin diacetate disclosed that only traces of carbon dioxide were evolved (whereas griseoviridin gives *ca.* 0·74 mole). Octahydrodethiogriseoviridin diacetate dissolves readily in cold concentrated hydrochloric acid but the solution does not give any colour with ferric chloride; similar treatment of griseoviridin, however, produces a deep red-purple colour. These observations are consistent with the suggested presence of a potential β-oxo-group in griseoviridin, absent in octahydrodethiogriseoviridin diacetate. Previous evidence indicated the presence of the system Me·CH(O–)·CH_2·CH:C in griseoviridin, the oxygen atom being part of an acid-labile group. The latter group being assumed to be the source of the postulated ketone, this system may be extended to (I) which would also account for the following observations: First, the intensity of light absorption at 220 mμ falls sharply when griseoviridin is treated with hydrochloric acid; this could be attributed to cleavage of the conjugated enol ether to a β-oxo-amide. Secondly, the potential β-oxo-acid system would account for the formation of carbon dioxide (and a little acetic acid) from griseoviridin but not from the desulphurisation product.

The presence of two alanine residues in octahydrodethiogriseoviridin diacetate enables the proposed sulphur-containing moiety of griseoviridin to be extended to (II). The groupings (I) and (II) contain 14 of the 22 carbon atoms and must therefore include part of the 10-aminodecanoic acid residue, necessarily in the C(O–):CH·CO group, as in structure (III). Only one carbon atom is left, which must be placed as an acyl group, if a diacyl-


(I)


(II)


(III)

amino-group is present, as the spectroscopic evidence suggests. The two hydroxyl groups must then be present in the chain of six carbon atoms which also contains one, or possibly two, double bonds.

If these suggested groupings are correct, octahydrodethiogriseoviridin diacetate will contain a β-alkoxy-group in a saturated acid residue, and acid hydrolysis should lead to an unsaturated acid by elimination of the β-alkoxy-group. The following reactions might explain the formation, in the Clemmensen process, of a *saturated* amino-acid bearing only *one* hydroxyl group:

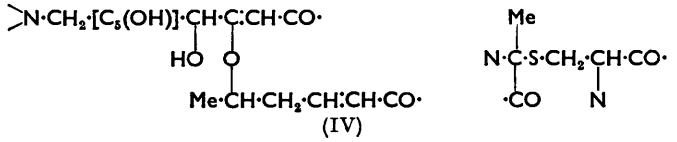
·CH(OH)·CH(OR)·CH_2·CO· ⟶ ·CH(OH)·CH:CH·CO_2H ⟶ ·CO·$[CH_2]_2$·CO_2H ⟶ ·$[CH_2]_3$·CO_2H

An analogy is provided by the work of Fittig and his collaborators [3] who found that α-, β-, and δ-hydroxy-γ-valerolactone each gave lævulic acid on vigorous acid hydrolysis.

Although alternative explanations of the evidence at present available might be possible, the partial structure (IV) is tentatively proposed for griseoviridin. Such a structure

[2] Geissman, *J. Amer. Chem. Soc.*, 1953, **75**, 4008.
[3] Fitting and Schaak, *Annalen*, 1898, **299**, 45; Fittig and Lepere, *ibid.*, 1904, **334**, 92.

containing two amide and one diacylamine links would be tricyclic but it should be emphasised that the number of double bonds and rings cannot be stated with certainty as the number of hydrogen atoms in griseoviridin, and especially in the desulphurisation products, may differ by ±2 from the values given.



(IV)

Experimental

The solvent systems used for paper chromatography and counter-current distribution were: butan-1-ol–water–acetic acid, 4 : 5 : 1 by volume (System A); and butan-1-ol–water–ammonia solution (*d* 0·88), 6 : 5 : 1 (System B).

Hydrolysis of Octahydrodethiogriseoviridin Diacetate.—Estimation of alanine. The diacetate (0·2065 g.) was refluxed for 7 hr. with concentrated hydrochloric acid (10 c.c.). After the solution had been evaporated to dryness under reduced pressure, water (50 c.c.), citric acid (5 g.), sodium citrate (5 g.), and ninhydrin (0·5 g.) were added, and the solution distilled into 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid. The yield of acetaldehyde derivative was 0·1525 g. (1·78 mol.). In a similar experiment, acetaldehyde was estimated as the dimedone derivative, m. p. 139—140°, the yield being 1·21 mol.

Carbon dioxide evolution. The diacetate (60·8 mg.) was refluxed with 2N-sulphuric acid (25 c.c.) in a slow stream of nitrogen; carbon dioxide evolved was estimated by absorption in barium hydroxide solution but the amount was very small (0·06 mol. in 1 hr.).

Hydrogenation–Hydrolysis of Octahydrodethiogriseoviridin Diacetate.—The acetate (1·0 g.) was dissolved in concentrated hydrochloric acid (10 c.c.) and, after addition of water (10 c.c.), the solution was hydrogenated in the presence of platinum oxide (0·5 g.) at room temperature for 5 hr. and then at about 50° for 5 hr. The filtered solution was refluxed for 7 hr. with more hydrochloric acid (20 c.c.) and evaporated under reduced pressure. The residue in acetic acid (20 c.c.) was then shaken in hydrogen with platinum oxide (0·5 g.) for 3 hr. (if this step was omitted the product obtained was impure apparently owing to contamination with covalent chloro-compounds). After the filtered solution had been evaporated under reduced pressure, water (30 c.c.) was added and the mixture was re-evaporated. The residue was subjected to counter-current distribution in solvent system B (20 × 15 c.c. of upper phase and 13 × 10 c.c. of lower phase). Examination of the fractions by paper chromatography (same solvent) showed that the upper phases 5—20 contained only one amino-acid (R_F 0·6). These phases were combined and evaporated; recrystallisation of the residue from methanol furnished 10-amino-decanoic acid, m. p. and mixed m. p. 183—186° (Found: C, 63·7; H, 11·5; N, 7·5; *C*-Me, nil. Calc. for $C_{10}H_{21}O_2N$: C, 64·1; H, 11·3; N, 7·5%). The infrared spectrum was also identical with that of an authentic sample (prepared by hydrogenation of 9-cyanononanoic acid); the two samples gave identical spots on paper chromatograms in both solvent systems A(R_F 0·9) and B.

Reduction–Hydrolysis of Octahydrodethiogriseoviridin Diacetate.—Zinc wool (20 g.) was shaken for 7 min. with mercuric chloride (3 g.) in 0·5N-hydrochloric acid (100 c.c.) and washed three times with water. The diacetate (3·0 g.) was dissolved in concentrated hydrochloric acid (75 c.c.) and added to the zinc under water (75 c.c.). After the solution had been refluxed for 3 hr., more amalgamated zinc (5 g.) was added, and the mixture refluxed for a further 3 hr. The remaining zinc was removed and washed with water (250 c.c.), the combined filtrates being applied to Amberlite resin IR 120 (H). The column was washed with 0·5N-hydrochloric acid (100 c.c.) and water (250 c.c.); zinc salts and most of the alanine were not retained on the column under these conditions. Elution with 3N-ammonia solution and evaporation afforded a gum which was subjected to counter-current distribution in solvent system A (13 × 10 c.c. lower phase and 24 × 15 c.c. upper phase). The fractions were examined by paper chromatography with the same solvent system; the butanol fractions 16—20 contained only a component, R_F 0·6. Evaporation of these fractions and recrystallisation of the residue from ethanol gave 10-*amino*-x-*hydroxydecanoic acid* as prisms, m. p. 189—191° (Found: C, 58·8; H, 10·6; N, 6·6. $C_{10}H_{21}O_3N$ requires C, 59·1; H, 10·4; N, 6·9%). In paper chromatograms with solvent system B, the acid had R_F 0·3 but counter-current distribution in this medium did not

give an efficient separation. The compound did not undergo hydrogenation in acetic acid in the presence of platinum. The optical rotation of the sample was too small to be determined on the material available.

Reaction of Griseoviridin with Perchloric Acid (with D. D. EVANS).—A filtered solution of griseoviridin (1·0 g.) in boiling dioxan (50 c.c.) was cooled to room temperature, and perchloric acid (0·7 c.c.; 72%) was added; separation of an oil soon began. After addition of dioxan (50 c.c.), the mixture was left overnight; the crystalline yellow mass was filtered off and washed with a little dioxan. Most of the solid dissolved on being boiled with methanol (150 c.c.), and the filtered solution was concentrated to *ca.* 50 c.c. Long needles of a *perchlorate* slowly separated at 0°; it decomposed rapidly at *ca.* 200° without melting (Found: C, 46·0; H, 4·8; N, 7·0; Cl, 5·9. $C_{22}H_{29}O_7N_3S,HClO_4$ requires C, 45·6; H, 5·1; N, 7·2; Cl, 5·5%). Ultraviolet absorption spectra: in water, $\lambda_{max.}$ 214 mμ (ε, 28,200); at pH 6·8, $\lambda_{max.}$ 218 and 270 mμ (ε, 25,700 and 21,200); and in 0·1N-sodium hydroxide, $\lambda_{max.}$ 223 and 271 mμ (ε, 27,600 and 20,100). The infrared spectrum (KBr disc) showed maxima at 3540, 3470, 3320, 3090, 3000, 2910, 2355, 2015, 1715, 1660, 1612, 1579, and 1510 cm.$^{-1}$.

Hydrolysis of the Perchlorate (with A. J. DURRÉ).—The perchlorate was hydrolysed by boiling 6N-hydrochloric acid during 12 hr. After evaporation of the hydrolysate under reduced pressure, the residue was dissolved in water and aliquot portions used to estimate nitrogen present as ammonium salt and as α-amino-acid [Found: N (as NH_3), 1·82; N (as α-amino-acid), 1·99. $C_{22}H_{29}O_7N_3S,HClO_4$ requires N, 2·40% per group]. As in the case of griseoviridin tar formation occurred during hydrolysis.

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RESEARCH DEPARTMENT,
PARKE, DAVIS & CO. LTD., HOUNSLOW, MIDDLESEX. [*Received, March 23rd,* 1956.]