696. *Viridin. Part I. Isolation and Characterisation*

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Viridin, $C_{20}H_{16}O_6$, an antifungal metabolite of *Gliocladium virens* is an alkali-labile, pentacyclic ketol containing two double bonds in addition to a benzene ring. Some reduction products of viridin and its acetyl derivative are described. Alkaline hydrolysis of viridin gives formic acid; benzene-1,2,3,4-tetracarboxylic acid is obtained on vigorous oxidation. The transformation of viridin into a stereoisomer, β-viridin is discussed.

VIRIDIN, $C_{20}H_{16}O_6$, m. p. 245° (decomp.), $[\alpha]_D^{19}$ −224°, an antifungal metabolite of *Gliocladium virens*,* was first described in 1945.[1] A revised molecular formula, $C_{19}H_{16}O_6$, suggested later [2] is inconsistent with the results of further transformations of viridin, of repeated analyses of carefully dried samples of the compound, and of an *X*-ray crystallographic determination of its molecular weight.

For the purification of viridin by chromatography on alumina the adsorbent must be strongly acidic.[2] On somewhat less strongly acidic (pH 3) alumina viridin is partly converted into an isomer, m. p. 240—245° (decomp.), $[\alpha]_D^{19}$ −23°, which we regard as being identical with β-viridin, previously reported [2] to occur as an impurity in crude viridin. The isomeric viridins showed characteristic differences in the OH and C=O stretching regions and in the 8·8—10·5 μ region of the infrared spectra; in solution the OH and C=O stretching frequencies were identical but the differences persisted in the 8·8—10·5 μ region where strong bands at 1090 and 1032 cm.$^{-1}$ in the spectrum of viridin were absent from that of β-viridin.

Viridin contains one C–Me and one OMe group.[3] It contains one hydroxyl group ($\nu_{max.}$ 3390 cm.$^{-1}$) because on acetylation in acidic media (acetic acid–perchloric acid [4] or trifluoroacetic anhydride [5]) it gave a monoacetyl derivative, $C_{22}H_{18}O_7$ ($\nu_{max.}$ OH absent). As with viridin itself, analyses of this compound were not consistently reliable owing to solvation; however, the aggregated results on this, the similarly prepared propionyl- and isobutyryl-viridin and also on their highly crystalline carbonyl derivatives, supported the formulation of the acetyl derivative. That only one acetyl group is introduced into viridin under these conditions was further shown by acetylation with [^{14}C]acetic acid in the presence of trifluoroacetic anhydride and analysis of the resulting labelled acetylviridin.

Acetylation of viridin with acetic anhydride and pyridine gave a diacetate [2] which we now formulate as $C_{24}H_{20}O_8$; this was also produced from β-viridin and acetylviridin under the same conditions. That the diacetate contains an enol acetate grouping is supported by its ultraviolet and infrared spectra; the former ($\lambda_{max.}$ 295, 310, 317 mμ, log ε 3·97, 4·02, 4·02) showed a small but significant bathochromic shift compared with viridin and acetylviridin ($\lambda_{max.}$ 300 mμ, broad, log ε 4·1—4·2) and the latter showed a band at 1648 cm.$^{-1}$, attributed to an ethylenic bond, which is absent from the spectrum of viridin. Zerewitinoff determinations on viridin supported the presence in it of an enolisable carbonyl group; one active hydrogen was obtained in anisole and two in pyridine.

The infrared spectrum of the enol acetate, which showed C=O bands at 1702 and 1673 cm.$^{-1}$ in addition to the broad ester absorption at 1748 cm.$^{-1}$, suggests that viridin contains three carbonyl groups and experimental proof of this assignment was obtained by the stepwise reductive elimination, from acetylviridin, of all three groups (see below). Although it formed a water-soluble Girard complex,[2] viridin gave indefinite products with the common carbonyl reagents; [2,3] however, the presence in it of two reactive ketonic groups is inferred from the formation of a bisphenylhydrazone by acetylviridin.

* Incorrectly [1] described as *Trichoderma viride* Pers. ex Fries (P. W. Brian, personal communication).

[1] P. W. Brian and J. C. McGowan, *Nature*, 1945, **156**, 144.
[2] E. B. Vischer, S. R. Howland, and H. Raudnitz, *Nature*, 1950, **165**, 528.
[3] P. W. Brian, P. J. Curtis, H. G. Hemming, and J. C. McGowan, *Ann. Appl. Biol.*, 1946, **33**, 190.
[4] H. Burton and P. F. G. Praill, *J.*, 1950, 1203.
[5] E. J. Bourne, M. Stacey, J. C. Tatlow, and J. M. Tedder, *J.*, 1949, 2976.

Catalytic reduction of viridin under various conditions gave complex mixtures of products but neither the previously reported [3] violet compound $C_{20}H_{22}O_6$ nor the methoxyl-free $C_{19}H_{24}O_4$ hydrogenolysis product [3] were encountered in the present work. With Adams catalyst in acetic acid (hydrogen uptake 7 mol.) the only crystalline product isolated (6%) was a decahydrobisdeoxy-derivative, $C_{20}H_{26}O_4$, m. p. 263—266° ($\nu_{max.}$ 3500, 3260 cm.$^{-1}$; no C=O absorption). The ultraviolet spectrum ($\lambda_{max.}$ 269, 278 mμ, log ε 2·90, 2·92) of this derivative was typically that of a benzenoid compound, and, indeed, oxidation of viridin with boiling dilute nitric acid or with hot aqueous potassium permanganate, gave benzene-1,2,3,4-tetracarboxylic acid. The ultraviolet and infrared absorption of viridin is consistent with the presence of a benzene ring, and a strong band at 1587 cm.$^{-1}$ in addition to that at 1622 cm.$^{-1}$ suggests [6] that the ring is conjugated with a C=O group.

Hydrogenation of acetylviridin proceeded more uniformly than with viridin to give, in the presence of palladium and ethanol, first, a hexahydrodeoxy-derivative (40%), $C_{22}H_{24}O_6$ ($\nu_{max.}$ no OH absorption; C=O at 1736 and 1690 cm.$^{-1}$) whose ultraviolet spectrum ($\lambda_{max.}$ 257, 309 mμ, log ε 4·01, 3·53) was characteristic of the presence of conjugated benzenoid and C=O chromophores. Further hydrogenation of this derivative in the presence of palladium and acetic acid–perchloric acid yielded, secondly, an octahydrobisdeoxy-product (80%), $C_{22}H_{26}O_5$ ($\nu_{max.}$ 1738 cm.$^{-1}$, $\lambda_{max.}$ 269, 277 mμ, log ε 2·81, 2·77).

Reduction of this product with lithium aluminium hydride gave a mixture, readily separated by chromatography, of two stereoisomeric diols $C_{20}H_{26}O_4$, m. p. 149—150° (55%) ($\nu_{max.}$ 3330 cm.$^{-1}$, no C=O) and m. p. 262—265° (12%). The latter was identical with the above-mentioned minor product of direct hydrogenation of viridin.

These results indicate that viridin contains a benzene ring, two easily reducible double bonds, and two carbonyl groups which successively undergo reduction to methylene in the formation of the $C_{22}H_{24}O_6$ and $C_{22}H_{26}O_5$ hydrogenation products of acetylviridin. The formation of the diols from the $C_{22}H_{26}O_5$ product clearly involves, in addition to reductive removal of the acetyl group, reduction of a third carbonyl group, additional evidence for the presence of which is adduced from the transformation outlined below. The diols retain the OMe group present in viridin and the remaining, inert, O atom in these transformation products and in viridin is presumed to be contained in an ether linkage. On this basis viridin is pentacyclic.

Examination of the diols was confined to the predominant isomer, m. p. 149—150°, which was stable to periodate and to cold methanolic alkali. Similar alkaline treatment of its precursor, the $C_{22}H_{26}O_5$ hydrogenation product, resulted in the elimination of its acetoxyl group and the formation of an oily, αβ-unsaturated ketone, $C_{20}H_{22}O_3$ ($\lambda_{max.}$ 270 mμ, log ε 4·00; $\nu_{max.}$ 1688, 1630 cm.$^{-1}$). This transformation is interpreted as indicating the presence of a β-acetoxyketonic grouping in the hydrogenation products of acetylviridin and hence that in viridin the hydroxyl group is situated β to a carbonyl group.

Prolonged catalytic hydrogenation of the αβ-unsaturated ketone gave a ketone $C_{20}H_{24}O_3$ ($\nu_{max.}$ 1726 cm.$^{-1}$; $\lambda_{max.}$ 268, 277 mμ, log ε 2·95, 2·97) which on reduction with lithium aluminium hydride gave a mixture from which an alcohol, $C_{20}H_{26}O_3$ ($\nu_{max.}$ 3510 cm.$^{-1}$, no C=O) was readily isolated.

Viridin was recovered from solution in acetic acid or pyridine. It was stable to boiling 5% methanolic sulphuric acid, and to cold concentrated hydrochloric acid but was partially degraded by boiling 2N-hydrochloric acid. A solution of viridin in aqueous ethanol was neutral but potentiometric titration with sodium hydroxide solution showed the presence of one very weakly acidic function (inflection in the curve at pH 10·2), presumably the enolisable carbonyl group. Potentiometric back-titration of the resulting solution with dilute sulphuric acid showed that two additional acidic functions were produced as a result of the alkaline treatment and a brown amorphous solid was precipitated. Steam distillation

[6] R. S. Rasmussen, D. D. Tunnicliff, and R. R. Brattain, *J. Amer. Chem. Soc.*, 1949, **71**, 1068.

of the acid suspension yielded formic acid (1 mol.) followed, slowly, by a second, unidentified, volatile acid. The " apparent acetyl " content [3] of viridin and its acetyl derivatives therefore depends on whether acidic or basic conditions are used and on the manner in which the hydrolysis is carried out. Reduction of Fehling's solution and ammoniacal silver nitrate by viridin [2] may be attributed to the liberation of formate by alkaline media; lability to alkali and reducing properties were also shown by the acetyl derivatives of viridin.

Contrary to earlier statements [2,3] viridin readily reacted (*a*) with diazomethane to give a homologue, $C_{21}H_{18}O_6$ ($\nu_{max.}$ 3405 cm.$^{-1}$) which contained only one OMe group, and (*b*) with bromine in chloroform at room temperature to give an intractable product. Viridin formed a complex with osmium tetroxide–pyridine but was recovered after treatment with selenium dioxide, periodic acid, or silver oxide in chloroform. Oxidation with chromic oxide in acetic acid gave an insoluble neutral product, $C_{19}H_{14}O_7$ (30%), together with a number of water-soluble acidic products whose investigation is described later.[7] The compound $C_{19}H_{14}O_7$ was similar to viridin in its lability to alkali, reducing properties, and ultraviolet absorption ($\lambda_{max.}$ 306 mμ, log ε 4·10) but its infrared spectrum showed an additional C=O band at 1747 cm.$^{-1}$. One carbonyl group was removed on reductive acetylation in the presence of zinc which gave a monoacetate $C_{21}H_{18}O_8$.

Since the same $C_{19}H_{14}O_7$ oxidation product was also formed by β-viridin it seems probable that in viridin the enolisable carbonyl is attached to an asymmetric centre which epimerises to give the β-isomer but whose asymmetry is destroyed both in the oxidation product and in diacetylviridin.

Experimental

Melting points are corrected. Unless otherwise stated, optical rotations were determined in chloroform (*c* 1·0), ultraviolet spectra in ethanol, and infrared spectra in Nujol mulls. For the preparation of neutral alumina the material (Spence type H) was washed with 2N-nitric acid, repeatedly with distilled water, and then with methanol. For acidic alumina, this product was suspended in 0·1N-nitric acid and then collected. Both types were dried at 40° *in vacuo* and then activated at 180° *in vacuo* for 30 min. The pH of the adsorbent refers to the value found for a slurry of the product (1 g.) and water (10 ml.) after being shaken for several hours.

For analysis, specimens were dried *in vacuo* over phosphoric oxide.

Purification of Viridin.—A solution of crude viridin [3] (1 g.) in hot benzene (200 ml.) was cooled to room temperature and then chromatographed on acid alumina (freshly prepared, pH 2·3; 30 g.). The column was successively washed with benzene (400 ml.), benzene–ether (9 : 1, 500 ml.) (7 : 3, 500 ml.), ether (200 ml.), and ether–chloroform (7 : 3, 300 ml.). The filtrate was collected in 100 ml. portions which were separately evaporated. Solid material appeared first in fraction 8 and last in fraction 17 after which only traces of gums were eluted. Material from fractions 8 and 9, 12 and 16, and 17 showed $[\alpha]_D^{20}$, respectively, −212, −224, and −201°. These fractions were combined; the solid (0·84 g.) was thrice crystallised from benzene giving *viridin*, prisms, m. p. 245° (decomp.; varies with rate of heating), $[\alpha]_D^{19}$ −224° (Found: C, 68·3, 68·3; H, 4·7, 4·7; OMe, 8·8; active H (in anisole), 0·2, 0·3; (in pyridine), 0·5. $C_{20}H_{16}O_6$ requires C, 68·2; H, 4·6; OMe, 8·8%; 1 active H, 0·3; *M*, 352. $C_{19}H_{16}O_6$ requires C, 67·0; H, 4·7%) $\nu_{max.}$ 3390 (OH), 3145w (C=CH); 1692, 1675 (C=O), 1622, 1587 (aryl); 1532 cm.$^{-1}$, (C=C–O) and in the 8·8—10·5 μ region bands at 1126, 1090, 1070, 1032, 1019, 1005, 983, and 970 cm.$^{-1}$; $\nu_{max.}$ (ethylene chloride), 1709, 1674, 1616, 1585, 1529 cm.$^{-1}$; $\lambda_{max.}$ 242, 300 mμ, log ε 4·49, 4·22. The unit cell was orthorhombic, space group $P2_12_12_1$ or $P22_12_1$, a = 6·203, b = 16·10, c = 16·17 Å, d 1·450, M (4 molecules per unit cell), 352·6.

Viridin crystallised from methanol in plates, m. p. 140 and 242° (decomp.), of the *hemimethanolate*, $\nu_{max.}$ 3450 OH, 3105w (C=CH), 1709, 1675 (C=O), 1622, 1587, 1532 cm.$^{-1}$, and in the 8·8—10·5 μ region bands at 1120, 1085, 1064, 1015, 1001, 982, and 970 cm.$^{-1}$ [Found (dried at 20 or 63°): C, 67·3; 66·9; H, 5·1, 4·9. $C_{20}H_{16}O_6$,0·5MeOH requires C, 66·9; H, 4·9; (dried at 100°): C, 68·4; 68·3; H, 4·7, 4·9%]. The infrared spectrum of the powder obtained by drying the hemimethanolate at 100° was identical with that of solvent-free viridin. The

[7] Part II, P. McCloskey, following Paper.

hemimethanolate slowly lost solvent on storage at room temperature. Viridin formed needles, m. p. 222—224° (decomp.) from acetone [Found (dried at 20°): C, 67·2; H, 5·2. $C_{20}H_{16}O_6,C_3H_6O$ requires C, 67·3; H, 5·4; (dried at 78°): C, 68·1; H, 4·5%) and from aqueous acetic acid Found (dried at 20°): C, 67·0; H, 4·9. $C_{20}H_{16}O_6,0·5H_2O$ requires C, 66·5; H, 4·7; (dried at 100°); C, 68·2; H, 4·7%]. Viridin crystallised from glacial acetic acid in prisms, m. p. 200—205° (decomp.) $\nu_{max.}$ 3484 (OH); 3105 (C=CH); 1711, 1679 (C=O); 1623, 1584, 1531 cm.$^{-1}$ [Found (dried at 20°): C, 64·4; H, 4·95. $C_{20}H_{16}O_6{\cdot}MeCO_2H$ requires C, 64·1; H, 4·9%]. These on storage for some days showed an infrared spectrum identical with that of solvent-free viridin.

A solution of viridin in ethanol was neutral to the Universal indicator. Viridin was insoluble in dilute sodium hydrogen carbonate but dissolved in dilute sodium hydoxide to give an orange-red solution from which viridin was not recovered on acidification. With concentrated sulphuric acid viridin gave an orange-red colour which changed to deep red on warming. It reduced Fehling's solution and ammoniacal silver nitrate at room temperature. Tests with Schiff's reagent, 1,4-dihydroxynaphthalene, ferric chloride, titanous chloride in methanol, tetranitromethane, and alkaline hypochlorite–potassium iodide were negative.

Crude viridin was conveniently purified on a larger (10 g.) scale by dissolution in hot benzene (1·5 l.) and filtration of the cooled solution through a column of alumina (pH 2·3; 100 g.). Elution with benzene (1·5 l.) followed by benzene–ether (1 : 1, 1·5 l.) and crystallisation of the eluate from acetone gave needles, m. p. 220—225° (decomp.), $[\alpha]_D^{20}$ −210°, of viridin which was sufficiently pure for further transformation. The yield (50—85%) varied according to the quality of the starting material.

Chromatography of Viridin on Less Acidic Alumina.—Crude viridin (1·00 g., $[\alpha]_D^{16}$ −183°) in benzene (2 l.) was chromatographed on alumina (pH 3·0; 1·5 × 15 cm.) and the column was viewed intermittently in ultraviolet light. The following solid fractions were collected (eluant in parentheses): (i) pale yellow band fluorescing dull green (benzene, 1 l.) 0·80 g., m. p. 242° (decomp.), $[\alpha]_D^{19}$ −216°; (ii) interband [benzene–methanol (200 : 1), 100 ml.]; 0·03 g. (iii) pale yellow band fluorescing pale green [benzene–methanol (100 : 1); 200 ml.], 0·03 g., m. p. 190—195° (decomp.), $[\alpha]_D^{19}$ −41°. Further elution of the column with benzene–methanol yielded a series of intractable yellow or orange oils (0·04 g.).

Fraction (i) was recrystallised three times from benzene giving viridin, prisms, m. p. 245° (decomp.), $[\alpha]_D^{19}$ −224°. Fraction (iii) was recrystallised three times from benzene giving prisms or felted needles of β-*viridin*, m. p. 240—245° (decomp.), $[\alpha]_D^{16}$ −23° (Found: C, 68·5, 68·5; H, 4·8, 4·5; OMe, 8·8; active H, 0·3. $C_{20}H_{16}O_6$ requires C, 68·2; H, 4·6; OMe, 8·8; 1 active H, 0·3), $\nu_{max.}$ 3480 (OH); 3105w (C=CH); 1705, 1665 (C=O); 1622, 1587 (aryl); 1532 cm.$^{-1}$ (C=C–O); and in the 8·8—10·5 μ region bands at 1126, 1075, ~1068, 1046w, 1019, 995, 980, 961 cm.$^{-1}$; $\nu_{max.}$ (ethylene chloride), 1711, 1678, 1610, 1589, 1530 cm.$^{-1}$; $\lambda_{max.}$ 243, 300 mμ, log ε 4·45, 4·25. The decomposition point of viridin was undepressed on admixture with β-viridin. β-Viridin crystallised from methanol in long needles of the *hemimethanolate*, m. p. 140° and 240—245° (decomp.) [Found (dried at 20°): C, 66·6; H, 4·9. $C_{20}H_{16}O_6,0·5MeOH$ requires C, 66·9; H, 4·9%], $\nu_{max.}$ 3607, 3480 broad, 3343 broad (OH); 3115 (C=CH); 1710, 1685 (C=O); 1625, 1589 (aryl); 1536 cm.$^{-1}$ (C=C–O); and in the 8·8—10·5 μ region bands at 1118, 1060, 1006, 971 cm.$^{-1}$ Solvent-free β-viridin was obtained by drying the hemimethanolate at 100° (Found: C, 68·0; H, 4·8%). Vischer *et al.*[2] gave m. p. 140°, $[\alpha]_D^{20}$ −50° for β-viridin.

β-Viridin showed chemical properties similar to those described above for viridin.

Chromatography of purified viridin on alumina (pH 3·0) gave similar results; viridin (74%); an intermediate band, $[\alpha]_D^{21}$ −153° (7%) and β-viridin (3%) were isolated. Chromatography on alumina, pH 3·6, yielded viridin (32%), an intermediate fraction, $[\alpha]_D^{19}$ −120° (34%), and β-viridin (26%). With chromatography on alumina, pH 4·1, the uniform, orange colour of the adsorbent precluded the progress of individual bands being followed by inspection in ultraviolet light. Fractional elution with benzene followed by benzene–methanol (100 : 1) gave viridin (17%), an intermediate fraction $[\alpha]_D^{21}$ −170° (16%), and β-viridin (26%).

β-Viridin was recovered in yields of 77 and 63% after chromatography on alumina pH 3·2 and 4·1, respectively.

Derivatives of Viridin and β-Viridin.—Acetylviridin. (*a*) A mechanically stirred suspension of viridin (3 g.) in acetic acid (30 ml.) and acetic anhydride (9 ml.) was cooled at 5° and then treated dropwise with perchloric acid solution (10·2N; 0·15 ml.). The mixture was stirred at 5—8° for 30 min. The resulting yellow solution was treated with iced water (10 ml.), stored

at room temperature for 15 min., and then poured into water (300 ml.). The precipitate (m. p. 125—145°; 2·9 g.) was collected and dried *in vacuo*. Chromatography of a sample of the crude product, in benzene, on alumina (pH 2·3), followed by elution with benzene–ether (1 : 1) and crystallisation of the eluate from ether–light petroleum (b. p. 40—60°) gave the *acetyl derivative* as a micro-crystalline solid, m. p. 121—126°, $[\alpha]_D^{17}$ −176° (Found: C, 67·2; H, 4·7. $C_{22}H_{18}O_7$ requires C, 67·0; H, 4·6), $\nu_{max.}$ OH absent; 3120 (C=CH), 1745, 1710, 1675 (C=O), 1623, 1587, 1532 cm.$^{-1}$; $\lambda_{max.}$ 240, 300 mμ, log ε 4·54, 4·14. Crystallisation of the remainder (2·8 g.) of the crude product from methanol gave faintly yellow needles (2·1 g.), m. p. 151—152°, of the *hemihydrate* [Found (dried at 55°): C, 65·3, 65·6, 65·6; H, 4·7, 4·6, 4·65; OMe, 9·3; "apparent acetyl," 21·9. $C_{22}H_{18}O_7,0{\cdot}5H_2O$ requires C, 65·5; H, 4·75; OMe, 9·5; 1MeCO, 10·7%].

(*b*) A suspension of viridin (100 mg.) in acetic acid (2 ml.) was treated with trifluoroacetic anhydride (1 ml.). After 15 min., the resulting solution was poured into water (10 ml.). Crystallisation of the resulting precipitate (83 mg.) from methanol gave needles, m. p. 151—152°, of the acetyl derivative hemihydrate [Found (dried at 78°): C, 65·4, 65·8; H, 5·0, 4·7%] whose m. p. was not depressed on admixture with the material obtained as described in (*a*). Acetylation of viridin (196 mg.) with an excess of $Me^{14}CO_2H$ (activity 0·121 ± 0·0028 μc/mmole) in the presence of trifluoroacetic anhydride gave needles (from methanol), m. p. 151—152°, of [^{14}C]acetylviridin hemihydrate. The CO_2 produced by combustion in a current of oxygen (4 determinations) was isolated, measured volumetrically, and its radioactivity assayed. The results (Found: C, 65·4%; activity 0·119 ± 0·0015 μc/mmole) showed that only one acetyl group had been introduced into the viridin molecule.

Acetylviridin reduced ammoniacal silver nitrate at room temperature. It gave a red colour with concentrated sulphuric acid and it readily dissolved in dilute sodium hydroxide to give a red solution.

Acetylviridin with 4-nitrophenylhydrazine in ethanol and acetic acid gave yellow needles (from ethanol), m. p. 226—227° (decomp.), of a *4-nitrophenylhydrazone hydrate* (Found: C, 61·7; H, 4·6; N, 7·6. $C_{28}H_{23}N_3O_8,H_2O$ requires C, 61·4; H, 4·6; N, 7·7%). With Brady's reagent acetylviridin gave a *2,4-dinitrophenylhydrazone*, orange needles (from ethyl acetate), m. p. 250—253° (decomp.) (Found: C, 58·4; H, 3·8; N, 9·4. $C_{28}H_{22}N_4O_{10}$ requires C, 58·5; H, 3·9; N, 9·75%). *Acetylviridin bis-phenylhydrazone* formed yellow needles (from methanol), m. p. 241—244° (decomp.) (Found: C, 70·7; H, 5·2; N, 9·5. $C_{34}H_{30}N_4O_5$ requires C, 71·1; H, 5·3; N, 9·75%).

Acetyl-β-viridin was obtained on acetylation of β-viridin as described above in (*a*) for viridin. It formed prisms (from methanol), m. p. 130—134°, resetting and remelting at 185—190° (decomp.), $[\alpha]_D^{24}$ +49° (*c* 0·2) (Found: C, 66·7; H, 4·7. $C_{22}H_{18}O_7$ requires C, 67·0; H, 4·6%), $\nu_{max.}$ OH absent, 3120 (C=CH), 1755, 1704, 1687, 1676 (C=O), 1625, 1586, 1525 cm.$^{-1}$.

Propionylviridin was prepared by the action of propionic acid on viridin in the presence of trifluoroacetic anhydride. Purified by chromatography in benzene solution on a column of activated silica it formed needles (from methanol), m. p. 114—115°, of the *hemihydrate* [Found (dried at 65°): C, 65·9; 65·9; H, 4·85, 5·0. $C_{23}H_{20}O_7,0{\cdot}5H_2O$ requires C, 66·2; H, 5·1%], $\nu_{max.}$ 1742, 1715, 1678 cm.$^{-1}$. *Propyionylviridin 2,4-dinitrophenylhydrazone*, yellow plates (from ethyl acetate), m. p. 245—246° (decomp.) (Found: C, 59·2; H, 4·1; N, 9·5. $C_{29}H_{24}N_4O_{10}$ requires C, 59·2; H, 4·1; N, 9·5%), and *propionylviridin bis-phenylhydrazone*, yellow prisms (from methanol), m. p. 203—207° (decomp.) (Found: C, 71·3; H, 5·4; N, 9·5. $C_{35}H_{32}N_4O_5$ requires C, 71·4; H, 5·5; N, 9·5%) were prepared similarly to acetylviridin derivatives. With isobutyric acid and trifluoroacetic anhydride viridin yielded *isobutylviridin*, needles (from methanol), m. p. 109—112° (Found: C, 68·1; H, 5·3. $C_{24}H_{22}O_7$ requires C, 68·2; H, 5·3%) which gave *isobutyrylviridin 2,4-dinitrophenylhydrazone*, yellow prisms (from ethyl acetate), m. p. 250—252° (decomp.) (Found: C, 59·7; H, 4·3; N, 9·3. $C_{30}H_{26}N_4O_{10}$ requires C, 59·8; H, 4·4; N, 9·3%) and *isobutyrylviridin bisphenylhydrazone*, prismatic needles (from methanol), m. p. 174—176° (decomp.) (Found: C, 71·3; H, 5·6; N, 9·3. $C_{36}H_{34}N_4O_5$ requires C, 71·7; H, 5·7; N, 9·3%).

Diacetylviridin.—Viridin (400 mg.) in pyridine (5 ml.) and acetic anhydride (5 ml.) was set aside for 18 hr. at room temperature. The precipitate (445 mg.) obtained by pouring the solution into 2N-hydrochloric acid (20 ml.) at 0° was collected and dried at 20° *in vacuo* over phosphoric oxide. It was then dissolved in benzene and fractionally precipitated by the addition of light petroleum (b. p. 40—60°). The pale yellow powder, m. p. 131—134° (decomp.) (330 mg.),

obtained after rejection of earlier highly coloured fractions, was thrice crystallised from ethanol giving the *diacetyl* derivative as yellow prisms (236 mg.), m. p. 135—136° (decomp.) (Kofler block, m. p. 141—144° decomp.), $[\alpha]_D^{18}$ +18° (Found: C, 66·0, 66·0; H, 4·7, 4·8; OMe, 7·2; active H, nil. $C_{24}H_{20}O_8$ requires C, 66·05; H, 4·6; OMe, 7·1%), $\nu_{max.}$ OH absent; 3080w (C=CH); 1748 (broad, ester C=O); 1702, 1673 (C=O); 1648 (C=C); 1627, 1578 (aryl); 1525w cm.$^{-1}$ $\lambda_{max.}$ (in $CHCl_3$) 242, ~295, 310, 317 mμ, log ε 4·50, 3·97, 4·02, 4·02, respectively. It reduced Fehling's solution at room temperature but more slowly than did viridin. It gave no colour with ferric chloride.

Acetylation of acetylviridin with acetic anhydride in pyridine gave diacetylviridin, m. p. 128—133° (decomp.), identified by its infrared spectrum.

Acetylation of β-viridin under the same conditions also gave diacetylviridin, m. p. and mixed m. p. 132—135° (decomp.) (Kofler hot-stage apparatus m. p. 140—142° decomp.), the identification being confirmed by comparison of the infrared spectra and preparation of the dinitrophenylhydrazone.

The 2,4-*dinitrophenylhydrazone* formed orange needles, m. p. 279—280° (decomp.), from ethyl acetate (Found: C, 58·0; 58·1; H, 4·1, 3·6; N, 9·1; OMe, 5·1. $C_{30}H_{24}N_4O_{11}$ requires C, 58·4; H, 3·9; N, 9·1; OMe, 5·0%). Vischer *et al.*[2] give m. p. 145—150° (decomp.) and 271—273° for diacetylviridin and its 2,4-dinitrophenylhydrazone, respectively.

Action of Mineral Acid on Viridin.—(*a*) Viridin (400 mg.) was refluxed under nitrogen with 5% sulphuric acid in 80% methanol (30 ml.) for 30 hr. No volatile acids or carbonyl compounds were detected (barium hydroxide and dinitrophenylhydrazine traps). On cooling, viridin (275 mg.) crystallised in plates, m. p. 205—220° (decomp.), which on recrystallisation from acetone formed needles, m. p. 210—218° (decomp.), $[\alpha]_D^{22}$ −204°. The mother-liquor, after being diluted with water (10·0 ml.), was concentrated *in vacuo* and then extracted with chloroform. Evaporation of the extract and treatment of the oily residue (103 mg.) with chloroform–light petroleum (b. p. 40—60°) gave prisms, m. p. 190—220° (decomp.) which on further purification by means of chromatography on acidic alumina yielded viridin (70 mg.), m. p. 213—217° (decomp.), $[\alpha]_D^{20}$ −210°.

(*b*) Heating a solution of viridin (100 mg.) in concentrated hydrochloric acid (*d* 1·18; 2·5 ml.) to 100° in a sealed tube for 1 hr. yielded a black, insoluble resin.

Action of Sodium Hydroxide Solution on Viridin.—Viridin (174·8 mg.) was heated under reflux for 1 hr. with sodium hydroxide (0·1N, 20·0 ml.) in nitrogen. The cooled orange-red solution was back-titrated potentiometrically with 0·1016N-sulphuric acid. Below pH 6 a brown amorphous solid began to separate and the supernatant solution became pale yellow. The titration curve showed three inflexions at pH *ca.* 10, 6·8, and 4·8 corresponding to the formation of 2·85, 1·70, and 1·12 equiv. of acid from the viridin molecule. The aqueous suspension (pH 3·5) was steam-distilled in 50 ml. fractions and a 10 ml. aliquot portion of each was tested for formic acid (reduction to formaldehyde and reaction with chromotropic acid).[8] Formic acid was detected in the first five fractions. Titration of the remaining 40 ml. portions with 0·1N-sodium hydroxide showed that a total of 1·1 equiv. of volatile acid had been liberated. The next 300 ml. distillate contained a further 0·72 equiv. volatile acid.

Similar potentiometric back-titration curves were obtained after viridin had been set aside in 0·1N-sodium hydroxide at room temperature for 0·5 and for 6 hr.

Action of Diazomethane on Viridin.—An excess of diazomethane in ether was added in portions to viridin (200 mg.) in ethanol (15 ml.) at room temperature. Nitrogen was evolved and the orange-yellow solution turned red after each addition and then slowly faded. After 24 hr. the solvent was removed *in vacuo* and the recovered orange oil was chromatographed in benzene (20 ml.) on alumina (8 × 1 cm.). Elution with benzene–ether (1 : 1) in ultraviolet light gave the following fractions: (i) pale yellow-green fluorescent band (80 ml.), 2 mg.; (ii) yellow fluorescent band (10 ml.), 47 mg.; (iii) orange-yellow band (50 ml.), 93 mg., m. p. 220—225° (decomp.), identified as viridin. Continued elution of the column with benzene–methanol (200 : 1) furnished intractable brown gums.

Fractions (i) and (ii) solidified on trituration with ether. Two recrystallisations from methanol gave lemon-yellow prisms (15 mg.), m. p. 205—210° (decomp.) of a *ketone* (Found: C, 68·75; H, 5·0; OMe, 8·3. $C_{21}H_{18}O_6$ requires C, 68·8; H, 4·95; OMe, 8·5%), $\nu_{max.}$ 3405 (OH); 1709, 1668 (C=O); 1618, 1585 (aryl); 1558 cm.$^{-1}$; $\lambda_{max.}$ 243, 312·5, ~318 mμ, log ε 4·53, 4·16, 4·16.

[8] F. Feigl, " Spot Tests," 3rd edn., Elsevier, New York, 1947, p. 397.

Action of Bromine on Viridin.—Addition of bromine in chloroform (1·8% w/v; 2·6 ml.; 1 mol.) to viridin (100 mg.) in chloroform (5 ml.) at room temperature gave a colourless solution within 10 min. The solution was stored for 20 min. and then evaporated under reduced pressure. Treatment of the residue with acetone gave crystalline material (58 mg.; m. p. 138—145°). Repeated recrystallisations from acetone raised the m. p. to 190—202° (decomp.) but the resulting needles (Found: Br, 13%) were not homogeneous.

Addition of 3 mol. bromine to viridin in chloroform resulted in slow decolourisation of the solution and the formation of an intractable gum.

Reaction of Viridin with Osmium Tetroxide and Pyridine.—A solution of viridin (680 mg.) in benzene (100 ml.) was treated with osmium tetroxide (520 mg.) followed by pyridine (680 mg.) Separation of the complex began within 30 min. After 5 days the brown microcrystalline powder (1·34 g., 89%), m. p. 135—140° (decomp.), was collected. Attempted decomposition of the complex with (*a*) aqueous mannitol and dilute sodium hydroxide or sodium hydrogen carbonate (*b*) sodium sulphite in aqueous ethanol or (*c*) ascorbic acid and dilute sulphuric acid gave intractable products.

Oxidation of Viridin.—(a) *With potassium permanganate.* A suspension of viridin (1 g.) in water (30 ml.) through which carbon dioxide was continually bubbled was heated under reflux on a water-bath and treated portionwise, during 7 hr., with potassium permanganate solution (3%; 220 ml.). The mixture was cooled and then decolourised by the addition of methanol. It was filtered and the residue extracted with boiling water (2 × 30 ml.). The combined filtrate and washings were made more alkaline by the addition of potassium carbonate and then extracted with ether. The aqueous layer was acidified with phosphoric acid and concentrated at 40° under reduced pressure to small bulk (40 ml.). Continuous extraction with ethyl acetate for 24 hr. afforded a yellow oil (724 mg.) which, in methanol, was treated with an excess of ethereal diazomethane. Fractional distillation of the product gave an oil (352 mg.), b. p. 160—170° (bath temp.)/0·25 mm., which, on chromatography on alumina, afforded crystalline material (220 mg.; m. p. 109—125°). Four recrystallisations from methanol gave needles, m. p. 128—131°, of methyl benzene-1,2,3,4-tetracarboxylate (Found: C, 54·3; H, 4·5; OMe, 37·3. Calc. for $C_{14}H_{14}O_8$: C, 54·2; H, 4·6; 4 OMe, 40·0%) which were identical (mixed m. p. and infrared spectrum) with an authentic specimen prepared [9] from 1,2,3,4-tetramethylbenzene and which showed the previously noted [10] property of turning purple on storage.

Similar results were obtained on oxidation of viridin with boiling dilute nitric acid.

(b) *With chromic oxide in acetic acid.* Viridin (350 mg.) in acetic acid (15 ml.) was treated dropwise during 5 min. with chromic oxide (0·5 g.) in water (2 ml.) and the solution was set aside at room temperature for 36 hr. The crystalline precipitate (100 mg., m. p. 295° decomp.) was collected and recrystallised from acetic acid giving a *ketone*, m. p. 298—300° (decomp.) (Found: C, 64·1, 64·2; H, 4·1, 4·3; OMe, 8·3. $C_{19}H_{14}O_7$ requires C, 64·4; H, 4·0; OMe, 8·75%), $\nu_{max.}$ 3410 (OH); 3040 (C=CH); 1747, 1710, 1679 (C=O); 1607, 1595 (aryl), 1521 cm.$^{-1}$; $\lambda_{max.}$ 240, ~287, 306, log ε 4·50, 4·10, 4·14. A series of oxidation products which will be described later [7] were isolated from the filtrate.

The ketone was insoluble in all common solvents except acetic acid and 2-methoxyethanol. It was insoluble in sodium hydrogen carbonate but slowly dissolved in sodium hydroxide forming a brown solution. It gave a yellow colour with concentrated sulphuric acid which became orange on warming. It gave no colour with ferric chloride or titanous chloride. It rapidly reduced both Fehling's solution and ammoniacal silver nitrate at room temperature, and decolourised alkaline potassium permanganate. With potassium iodide and sodium hypochlorite it gave a precipitate of iodoform. Attempted acetylation gave an intractable product.

Oxidation of β-viridin exactly as described above gave prisms, m. p. 298—300° (decomp.), of the same ketone identified by comparison of the infrared spectra.

Reductive Acetylation of the Ketone $C_{19}H_{14}O_7$.—Zinc dust (600 mg.) and fused sodium acetate (600 mg.) were added to the ketone (300 mg.) in acetic anhydride (3 ml.) and the mixture was boiled under reflux for 5 min. (complete decolourisation of the red solution). The hot, filtered solution was poured into ice–water and the resulting precipitate was extracted with chloroform. The extract was washed successively with sodium hydrogen carbonate, 2N-hydrochloric acid, and water. The recovered yellow oil (345 mg.) afforded a solid (164 mg.) on trituration with

[9] L. Ruzicka, H. Schellenberg, and M. W. Goldberg, *Helv. Chim. Acta*, 1937, **20**, 791.
[10] L. I. Smith and E. J. Carlson, *J. Amer. Chem. Soc.*, 1939, **61**, 288.

benzene. Repeated recrystallisation from benzene gave needles, m. p. 219—220° of an *acetate* (Found: C, 63·1; H, 4·9; OMe, 7·7. $C_{21}H_{18}O_8$ requires C, 63·3; H, 4·55; OMe, 7·8%), $\nu_{max.}$ 3330 (OH); 1757, 1716, 1672 (C=O); 1612, 1593 (aryl); 1538 cm.$^{-1}$.

It slowly dissolved in 2N-sodium hydroxide to give a pink solution.

The 2,4-*dinitrophenylhydrazone* formed orange needles, m. p. 270—273° (decomp.), from acetic acid (Found: C, 56·2; H, 4·2; N, 9·5. $C_{27}H_{22}N_4O_{11}$ requires C, 56·05; H, 3·8; N, 9·7%).

Catalytic Reduction of Viridin.—Viridin (200 mg.) in acetic acid (40 ml.) was added to a reduced Adams catalyst (100 mg.) in acetic acid (10 ml.). After an uptake of 4 mol. hydrogen (2 hr.), hydrogenation proceeded more slowly and ceased after 2 days (total uptake, 6·5 mol.). After filtration and removal of the solvent *in vacuo*, the residual oil, in chloroform, was washed with dilute sodium hydrogen carbonate. Recovery yielded an oil (195 mg.) which was chromatographed in benzene (10 ml.) on alumina (6 × 0·5 cm.) (fractional elution technique), giving the following oily fractions (eluant in parentheses): (i) 11 mg. (benzene, 140 ml.); (ii) 23 mg. [benzene–ether (9 : 1), 60 ml.]; (iii) 18 mg. [benzene–ether (1 : 1), 60 ml.]; (iv) 38 mg. (chloroform, 80 ml.); (v) 22 mg. (methanol, 80 ml.).

Fractions (i)—(iii) and (v) were intractable. Fraction (iv) crystallised from acetone in needles (11 mg.), m. p. 263—266°, of the *decahydrobisdeoxy-derivative* (Found: C, 72·6; H, 7·9; OMe, 9·5. $C_{20}H_{26}O_4$ requires C, 72·7; H, 7·9; OMe, 9·4%), $\nu_{max.}$ 3500, 3260 (OH); C=O absent; 827 cm.$^{-1}$, $\lambda_{max.}$ 269, 278 mμ, log ε 2·90, 2·92.

Catalytic Reduction of Acetylviridin.—A suspension of acetylviridin (2 g.), in ethanol (400 ml.), was hydrogenated in the presence of 20% palladium–charcoal (1 g.) for 24 hr. (uptake about 5 mol.). The mixture was heated to boiling, and then filtered. The filtrate was evaporated under reduced pressure. Treatment of the residual gum with ethanol gave a solid (1·02 g., m. p. 194—209°) which on recrystallisation from ethanol yielded plates, m. p. 213—214°, $[\alpha]_D^{20}$ −25° (*c* 1·36), of the *hexahydrodeoxy-derivative* (Found: C, 68·6; 68·7; H, 6·5, 6·3; OMe, 8·1; " apparent " MeCO, 21·9. $C_{22}H_{24}O_6$ requires C, 68·75; H, 6·3; OMe, 8·1; 1MeCO, 11·2%), $\nu_{max.}$ 1736, 1726 (sh), 1690, 1595, 1580 (in $C_2H_4Cl_2$) 1752, 1734, 1698 cm.$^{-1}$; $\lambda_{max.}$ 216, 257, 309 mμ, log ε 4·34, 4·0 1,3·53, respectively. The compound gave a violet solution in 0·5N-methanolic potassium hydroxide.

Catalytic Reduction of the Compound $C_{22}H_{24}O_6$.—A solution of the compound (2·1 g.), in glacial acetic acid (210 ml.) which contained perchloric acid (42 mg.), was hydrogenated in the presence of 20% palladium–charcoal (2·1 g.) for 24 hr. Removal of the catalyst by filtration and evaporation of the filtrate under reduced pressure gave a semi-solid residue which was dissolved in benzene (12 ml.). The solution was percolated through a column of activated silica (11 g.) which was then washed with benzene–ether (9 : 1, 250 ml.). Evaporation of the combined filtrates yielded a crystalline residue (1·81 g.) which, on recrystallisation from methanol, gave needles (1·74 g.), m. p. 154—156°, $[\alpha]_D^{20}$ ±1°, of an *octahydrobisdeoxy-derivative* (Found: C, 71·4, 71·5; H, 7·2, 7·2; OMe, 8·6. $C_{22}H_{26}O_5$ requires C, 71·3; H, 7·1; 1OMe, 8·4%), $\nu_{max.}$ 1738 cm.$^{-1}$; $\lambda_{max.}$ 269, 277 mμ, log ε 2·81, 2·77.

Lithium Aluminium Hydride Reduction of the Compound $C_{22}H_{26}O_5$.—A solution of the compound (250 mg.) in anhydrous benzene (15 ml.) was added dropwise, with shaking, to a solution, cooled at 0°, of lithium aluminium hydride (100 mg.) in anhydrous ether (20 ml.). The mixture was stored at room temperature for 2 hr., cooled to 0°, and then decomposed with 2N-hydrochloric acid (10 ml.). The organic layer was separated and the aqueous layer was further extracted with chloroform (2 × 25 ml.). The extracts were combined and evaporated. The residue was dissolved in warm benzene (25 ml.). On cooling, crystals (i) (24 mg.; m. p. 245—255°) separated and were filtered off. The filtrate was chromatographed on a column of neutral alumina (7 g.). Elution with benzene–ether (7 : 3, 75 ml.), ether (50 ml.), and ether–chloroform (7 : 3, 75 ml.) gave (ii), needles (145 mg.; m. p. 148—150°). Elution with chloroform–methanol (9 : 1) gave (iii), rhombs (10 mg.; m. p. 250—252°). Fractions (i) and (iii) were combined and recrystallised from acetone giving needles (30 mg.), m. p. 262—265°, of a diol (Found: C, 73·1; H, 7·8%) which was identical (mixed m. p., ultraviolet and infrared spectrum) with the above-mentioned decahydrobisdeoxy-derivative obtained by hydrogenation of viridin. Recrystallisation of fraction (ii) from acetone–light petroleum (b. p. 40—60°) gave needles (132 mg.), m. p. 149—150°, of an isomeric *diol* (Found: C, 72·5; 72·4; H, 7·5, 7·9. $C_{20}H_{26}O_4$ requires C, 72·7; H, 7·9%), $\nu_{max.}$ 3350, $\lambda_{max.}$ 269, 277 mμ, log ε 2·91, 2·93. The latter diol gave a *bisphenylurethane*, prisms (from aqueous methanol), m. p. 239—240° (Found: C, 72·1; H, 6·4; N, 5·0. $C_{34}H_{36}N_2O_6$ requires C, 71·8; H, 6·4; N, 4·9%). The diol, in dioxan, did not reduce periodic acid solution.

Storage of a solution of the diol (28 mg.), in 0·1N-methanolic potassium hydroxide (2·0 ml.) for 20 hr. and recovery yielded starting material (20 mg.).

Hydrolysis of the Compound $C_{22}H_{26}O_5$.—The compound (1 g.) in methanol (50 ml.) was treated with potassium hydrogen carbonate (1 g.) in water (5 ml.) and then refluxed for 3 hr. The mixture was concentrated to small bulk under reduced pressure. It was diluted with water and extracted with benzene. Recovery and distillation gave an *αβ-unsaturated ketone* (830 mg.) as a pale yellow syrup, b. p. 230—235° (bath temp.)/0·01 mm., $[\alpha]_D^{20}$ +202° (Found: C, 76·7; H, 7·2. $C_{20}H_{22}O_3$ requires C, 77·4; H, 7·1%), $\nu_{max.}$ 1688, 1630, $\lambda_{max.}$ 270 mμ, log ε 4·00. It gave a 2,4-*dinitrophenylhydrazone*, red prisms (from ethyl acetate), m. p. 241—242° (decomp.) (Found: C, 63·8; H, 5·6; N, 11·6. $C_{26}H_{26}N_4O_6$ requires C, 63·7; H, 5·3; N, 11·4%).

Catalytic Reduction of the αβ-Unsaturated Ketone $C_{20}H_{22}O_3$.—The compound (250 mg.) in glacial acetic acid (15 ml.) was hydrogenated in the presence of 20% palladium–charcoal (130 mg.) for 25 hr. The mixture was filtered and the solvent was removed under reduced pressure. Crystallisation of the residue from methanol afforded rhombs (132 mg.), m. p. 207°, $[\alpha]_D^{16}$ +46°, of a *ketone* (Found: C, 76·7; H, 7·9; OMe, 10·3. $C_{20}H_{24}O_3$ requires C, 76·9; H, 7·7; 1OMe, 9·9%), $\nu_{max.}$ 1726 cm.$^{-1}$; $\lambda_{max.}$ 268, 277, log ε 2·95, 2·97. It gave a 2,4-*dinitrophenylhydrazone*, orange prisms (from methanol), m. p. 172—173° (Found: C, 63·9; H, 5·6; N, 11·4. $C_{26}H_{28}N_4O_6$ requires C, 63·4; H, 5·7; N, 11·4%).

Lithium Aluminium Hydride Reduction of the Foregoing Saturated Ketone.—The compound (100 mg.) in anhydrous benzene (5 ml.) was gradually added, with shaking, to lithium aluminium hydride (50 mg.) in anhydrous ether (10 ml.). After being set aside for 1 hr. the mixture was poured into ice-cold N-hydrochloric acid (25 ml.) and then extracted with benzene. The extract was washed with water. Recovery gave a gum which formed crystals (73 mg.; m. p. 124—128°) from methanol. Repeated recrystallisation from aqueous methanol gave plates, m. p. 131—133°, of an *alcohol* (Found: C, 76·3; H, 8·2; OMe, 9·6. $C_{20}H_{26}O_3$ requires C, 76·4; H, 8·3; OMe, 9·9%), $\nu_{max.}$ 3510 cm.$^{-1}$, $\lambda_{max.}$ 268, 277 mμ, log ε 2·91, 2·92.

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