Viridin. Part V.[1] Structure

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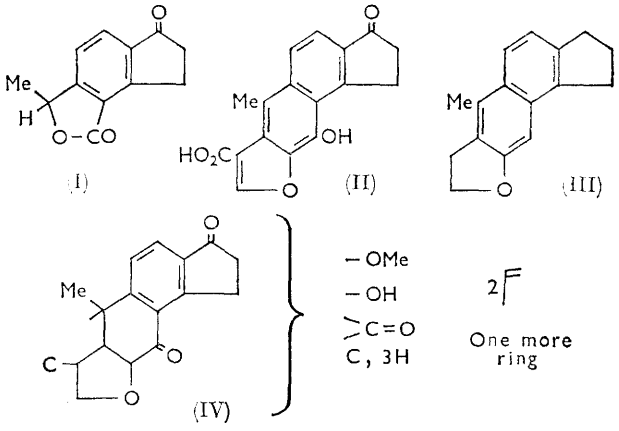
The structure of viridin, $C_{20}H_{16}O_6$, an antifungal metabolic product of *Gliocladium virens*, is elucidated.

SYNTHESIS of the oxocyclopentenophthalide [2] (I), a product of the chromic acid oxidation of viridin, established the relative positions of its ketonic group and methyl substituent. This permitted the formulation [3] of the $C_{17}H_{12}O_5$ product of oxidation of viridin with hydrogen peroxide as (II) following the synthesis [1] of its transformation product (III). If it be assumed that the skeleton of the degradation product (II) is present in viridin itself, then consideration of the nature of the functional groups [4] and spectra [1,4] of the latter and its transformation products leads to the partial structure (IV) for viridin.

Viridin has been shown [4] by stepwise reductive transformation to contain three carbonyl groups, of which two are present in the partial structure (IV). The third, which is isolated from the aromatic system and does not undergo catalytic hydrogenation to methylene, survives ($\nu_{max.}$ 1688 cm.$^{-1}$) in the α,β-unsaturated ketone [4] $C_{20}H_{22}O_3$ and in its reduction product [4] $C_{20}H_{24}O_3$ ($\nu_{max.}$ 1730 cm.$^{-1}$), and must be contained in a six-membered ring so formed from the C_2 fragment and other structural

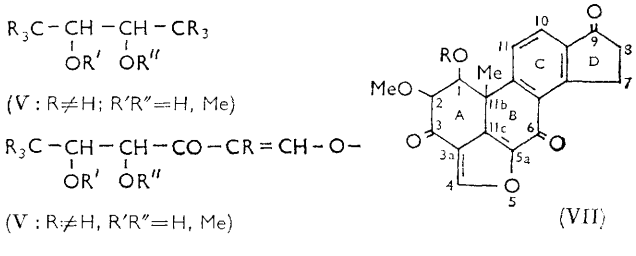


addenda of (IV) as to block the aromatisation of the ring carrying the methyl substituent. The way in

[1] Part IV, J. S. Moffatt, preceding Paper.
[2] P. McCloskey, *J. Chem. Soc.*, 1965, 3811.
[3] J. S. Moffatt, *J. Chem. Soc.* (*C*), 1966, 725.
[4] J. F. Grove, J. S. Moffatt, and E. B. Vischer, *J. Chem. Soc.*, 1965, 3803.

which this occurs was made clear by the nuclear magnetic resonance (n.m.r.) spectra of viridin and its diacetate.

The spectrum of viridin (see Table) showed three features consistent with the expression (IV). These comprised an A_2B_2 system at τ 6·2, 7·1, and an AB double doublet at τ 1·2, 1·9, J = 8 c./sec., both arising from the 4,5-disubstituted indanone system, and a three-proton singlet at τ 6·25 (OMe). In addition, there were three other features vital to the expansion of (IV): firstly, in the olefinic CH region, a one-proton singlet (τ 1·55) indicated that the two olefinic bonds are respectively tetra- and tri-substituted, with the latter present in the system $-O-CH=CR_2$, probably as $-O-CH=CR-CO-$; secondly, a three-proton singlet at τ 8·3 showed that the methyl group is tertiary; thirdly, a two-proton double doublet at τ 5·55, 6·05, J = 5 c./sec. indicated the presence of partial structure (V), and, as expected, one doublet moved downfield to τ 4·65 in acetylviridin. Diacetylviridin was considered [4] to be an enol acetate formed with the elimination of the asymmetry at an asymmetric centre adjacent to the carbonyl group involved; in confirmation of this assignment, the two-proton double doublet in the spectrum of acetylviridin at τ 4·65, 5·85 was replaced by a one-proton singlet at τ 3·90 in the spectrum of diacetylviridin.



Also, the olefinic proton at τ 1·55 moved upfield to τ 2·48, permitting expansion of (V) to (VI). Combination of partial structures (IV) and (VI) leads to (VII; R = H) for viridin in which, however, the relative positions of the hydroxyl and methoxyl substituents have still to be assigned.

The assignment shown followed from consideration of the structure of the $C_{19}H_{14}O_7$ product of chromic acid oxidation [4] of viridin. This neutral compound retained the methoxyl group and ultraviolet chromophore of viridin. The n.m.r. spectrum showed that the protons at positions 1 and 2 in (VII; R = H) had been eliminated, and that the hydroxyl group ($\nu_{max.}$ 3410 cm.$^{-1}$) is tertiary since there was no resonance, other than that attributable to the methoxyl group and indanone system, in the region τ 4·0—6·5. The C-methyl, methoxyl, and olefinic proton resonances had all moved downfield to 8·05, 5·85, and 0·70 τ, respectively, consistent with (XI),

Chemical shifts (τ values) and coupling constants (c./sec.) for protons in viridin and its transformation products.

	Position of proton [c]											Coupling constant		
Compound	1	2	4	5a	7	8	10 and 11		11b-Me	OMe	OAc	$J_{1,2}$	$J_{10,11}$	$J_{5a,11c}$
Viridin [a]	5·55d	6·05d	1·55s		7·1	6·2	1·2d	1·9d	8·27	6·25		5	8	
β-Viridin [a]	5·65s	5·65s	1·55s		7·1	6·2	1·2d	1·9d	8·20	6·10			8	
Acetylviridin	4·65d	5·85d	1·55s		7·25	6·25	1·9s	1·9s	8·12	6·32	7·60	5		
Acetyl-β-viridin	4·30d	5·70d	1·55s		7·25	6·2	2·15d	1·95d	8·20	6·30	7·65	10	8	
Diacetylviridin	3·90s		2·48s		7·3	6·3	2·2d	2·1d	8·25	6·20	7·65		8	
Deoxoketone (XV; X = Y = O)	3·85d	6·32d	6·1m	5·3d	7·0 *		2·4d	2·6d	8·50	6·60	7·80	3	8	8·5
Bisdeoxoketone (XV; X = H_2, Y = O)	3·80d	5·97d	6·3m (8)	5·4m	7·1 * (1)	(2)	2·75s (5 and 4)	2·75s	8·67 (6-Me)	6·55	7·87	2		7
Ester (XI) [b]			0·70s		7·0	6·2	1·7s	1·7s	8·05	5·85				
Ester (XII; R = Me, R′ = Ac) [a]			1·60s		7·25	6·2	2·2d	1·9d	8·05	6·05	7·90		8	

s = Singlet; d = doublet; m = multiplet. * Also protons at 9.

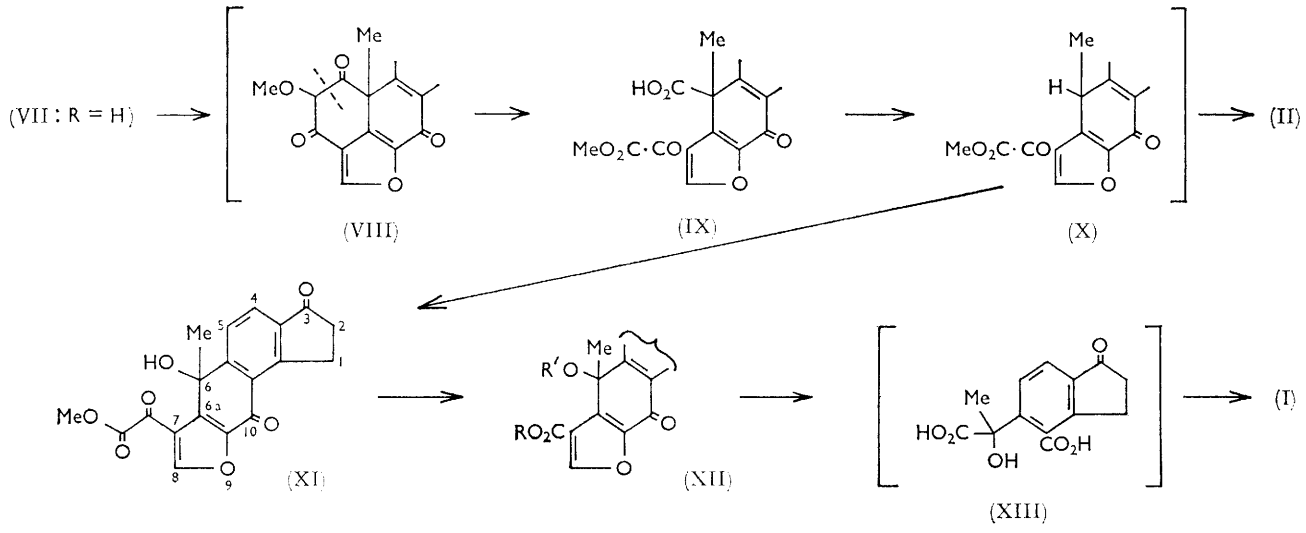
[a] In the presence of a trace of $CF_3 \cdot CO_2H$; [b] in $CDCl_3$–$CF_3 \cdot CO_2H$ (2 : 1) from which the compound was recovered; [c] corresponding protons in the esters (XI) and (XII; R = Me, R′ = Ac) are numbered as in parentheses.

in which the C-methyl residue now carries an additional oxygen substituent, the methoxyl is part of an ester group (new band at $\nu_{max.}$ 1747 cm.$^{-1}$), and the olefinic proton is heavily deshielded by the ester carbonyl of the methoxalyl side chain. Structure (XI) is derived from (VII; R = H) by a logical, hypothetical sequence (see Chart) in which the 1,3-diketone presumed to be formed by oxidation of the secondary hydroxyl group of viridin is cleaved as shown in (VIII) to give the tertiary carboxylic acid (IX), which undergoes decarboxylation to the intermediate (X) followed by oxidation of the methine hydrogen in (X) to hydroxyl, yielding (XI). The location of the methoxalyl side chain in (XI) determines the position of the methoxyl substituent in (VII; R = H).

Further oxidation of the side chain in (XI) then gives the products (XII; R = R′ = H) and, ultimately, (I). Aromatisation of the intermediate (X) is a major alternative pathway in the hydrogen peroxide oxidation of viridin and gives, after cleavage of the methoxalyl side chain, the naphtholic acid (II).[3]

Molecular models show that ring A in structure (VII; R = H) exists in a flattened chair conformation; nonetheless, the geometry of the system is such that the terms "axial" and "equatorial" may still be used to define

the orientation of the ring A substituents. The n.m.r. spectrum of β-viridin [4] was similar to that of viridin. Although the chemical shifts of the protons at positions 1 and 2 were identical, giving a two-proton singlet at τ 5·65, acetyl-β-viridin showed the familiar double doublet at τ 4·30, 5·70, $J = 10$ c./sec. This large coupling constant shows that these protons in acetyl-β-viridin are *trans*-diaxial and hence that the methoxyl group in β-viridin is equatorial. The 1,3-diaxial interaction between the 2-methoxyl and 11b-methyl groups in viridin clearly provides the driving force for the epimerisation to β-viridin. Diacetylviridin is the enol ester (XIV).

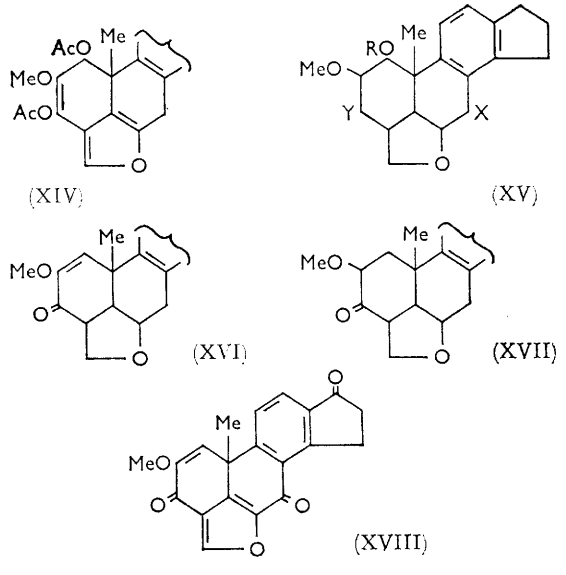


Oxidative degradation of viridin

Structure (VII; R = H) satisfactorily explains the known chemical and spectroscopic properties of viridin. In the (solution) infrared spectrum of viridin [4] the band at 1709 cm.$^{-1}$ is assigned to the 9-keto group, while absorption due to the 3- and 6-keto groups is contained in the peak at 1674 cm.$^{-1}$. In the catalytic reduction of acetylviridin [4] (VII; R = Ac) the 9-ketonic group is the first to be removed, and the $C_{22}H_{24}O_6$ and $C_{22}H_{26}O_5$ products have structures (XV; R = Ac, X = Y = O) and (XV; R = Ac, X = H_2, Y = O), respectively. In agreement with these structures a new doublet at τ 5·3, $J = 8·5$ c./sec. attributed to the proton at 5a in the n.m.r. spectrum of (XV; R = Ac, X = Y = O) became a doublet of triplets with $J = 7$ and 4·5 c./sec. in its deoxo derivative (XV; R = Ac; X = H_2; Y = O). The spectra of both compounds showed a complex multiplet at τ 6·1—6·3 arising from the magnetically non-equivalent protons at position 4.

The $C_{20}H_{24}O_3$ ketone [4] obtained by catalytic reduction of the αβ-unsaturated ketone (XVI), resulting from base-catalysed β-elimination of the elements of acetic acid from (XV; R = Ac, X = H_2, Y = O), has structure (XVII). The unusually high ($\nu_{max.}$ 1730 cm.$^{-1}$) carbonyl frequency for this ketone is attributed to the presence of the adjacent methoxyl group, which is also responsible for the high wavelength of absorption, $\lambda_{max.}$ 270 mμ [unaltered by subtraction of absorption due to the isolated benzenoid chromophore in (XVII)] of the unsaturated ketone (XVI). Ready β-elimination of the 1-acetoxyl group also occurred with acetylviridin, which was converted on neutral alumina into a mixture of acetyl-β-viridin and the unsaturated ketone (XVIII).

The curve obtained by subtraction of the ultraviolet spectrum ($\lambda_{max.}$ 216, 257, 309 mμ; log ε 4·34, 4·01, 3·53)



of the reduction product (XV; R = Ac, X = Y = O) from that of viridin ($\lambda_{max.}$ 242, 300 mμ; log ε 4·49, 4·22) had $\lambda_{max.}$ 240, 298 mμ; log ε 4·42, 4·12, but became negative below 222 mμ, indicating an interaction between

the chromophoric systems in viridin consistent with the cross-conjugated expression (VII; R = H) in which the furyl ketone system predominates.

The chromophoric system of viridin persists in the oxidation product (XI) and in the keto-acid $C_{17}H_{12}O_6$ derived from it by further oxidation [2] or by oxidation of viridin with chromic acid [2] or hydrogen peroxide; [3] this acid must therefore have the structure (XII; R = R′ = H). In support of this argument the n.m.r. spectrum of the ester (XII; R = Me; R′ = Ac) closely resembled that of the keto-ester (XI) except that the latter showed the characteristic deshielding of the olefinic proton at position 4 associated with the methoxalyl side chain. Oxidation of the furan ring in (XII; R = R′ = H) might be expected to give the hypothetical tertiary carboxylic acid (XIII) from which the keto-lactone (I) could arise by decarboxylation and lactonisation.

The production on alkaline hydrolysis of viridin of formic acid [4] (*ca.* 1 mol.) (from the α-furyl methine group) and an acidic residue, probably of diosphenol type, is readily explicable in terms of structure (VII; R = H). Unlike viridin, the keto-ester (XI) gave the iodoform reaction; [4] presumably the liberation of formic acid, in a manner analogous to that in viridin, is followed by rupture of the 6,6a-bond [for numbering see (XI)] in a retroaldol reaction which leaves a C-acetyl residue.

Viridin joins the interesting group of antibiotics, based on the steroid nucleus, which includes [5] helvolic acid, cephalosporin P, and fusidic acid. Its biogenesis may be presumed to follow, in its early stages, that of other steroids, although the possibility that viridin is derived from a diterpenoid skeleton of the cassaic acid type has not yet been excluded. When *Gliocladium virens* was grown in the presence of [2-^{14}C]-mevalonic lactone, radioactivity was incorporated to a significant extent in the viridin produced. The degradation of this labelled material should provide experimental proof of the biogenetic pathway.

In the course of this work some new derivatives of the keto-acid (XII; R = R′ = H) were prepared. Acetylation with boiling acetic anhydride furnished the mixed anhydride (XII; R = R′ = Ac) which gave, on methanolysis, the acid (XII; R = H, R′ = Ac). Methylation of this with diazomethane then gave the ester (XII; R = Me; R′ = Ac).[3]

EXPERIMENTAL

Melting points are corrected. Unless otherwise stated, ultraviolet spectra were determined in ethanol and infrared spectra in Nujol mulls. N.m.r. spectra were determined for deuteriochloroform solutions (tetramethylsilane as internal reference with τ 10·00) with a Varian Associates A.60 spectrometer (60 Mc.).

Epimerisation of Acetylviridin.—Acetylviridin (1 g.) in benzene–ether (3 : 7; 100 ml.) was caused to percolate through a column of neutral alumina [3] (30 g.). The filtrate was refiltered through the column, and this process was repeated once more. Elution of the column with the same solvent mixture (500 ml.) and with ether (400 ml.) gave an eluate which, on fractional crystallisation from acetone, afforded (i) needles (108 mg.), m. p. 295—297° (decomp.), $\lambda_{max.}$ 239, 287, 305 mμ, log ε 4·51, 4·10, 4·07, $\nu_{max.}$ 1715, 1706, 1690, 1668 cm.$^{-1}$, of 3,6,7,8,9,11b-*hexahydro*-2-*methoxy*-11b-*methyl*-3,6,9-*trioxocyclopenta*[7,8]-*phenanthro*[1,10-bc]*furan* (XVIII) (Found: C, 72·1, 71·6; H, 4·4, 4·4; OMe, 9·7. $C_{20}H_{14}O_5$ requires C, 71·9; H, 4·2; OMe, 9·3%), and (ii) prisms (266 mg.) of acetyl-β-viridin,[4] identified by comparison of the infrared spectra.

Derivatives of 2,3,6,10-*Tetrahydro*-6-*hydroxy*-6-*methyl*-3,10-*dioxo*-1H-*cyclopenta*[7,8]-*naphtho*[2,3-b]*furan*-7-*carboxylic Acid* (XII; R = R′ = H).[2]—(*a*) The keto-acid (150 mg.) was heated under reflux with acetic anhydride (3 ml.) for 9 hr. Trituration with ether of the brown semi-solid residue obtained on removal of the solvent *in vacuo* gave a crystalline product (98 mg.), m. p. 166—168°. Recrystallisation (charcoal) from benzene–light petroleum (b. p. 40—60°) gave rosettes (56 mg.), m. p. 171—173°, of the mixed *anhydride* (XII; R = R′ = Ac), (Found: C, 64·0; H, 4·1. $C_{21}H_{16}O_8$ requires C, 63·6; H, 4·1%), $\nu_{max.}$ 1817, 1745, ~1738, 1707, 1681 cm.$^{-1}$ (C=O), $\lambda_{max.}$ (in ether) 239, 296 mμ, log ε 4·49, 4·12. (*b*) The anhydride (XII; R = R′ = Ac) (26 mg.) was heated under reflux for 10 min. with methanol (0·5 ml.). The prisms (17 mg.) obtained on concentration of the solution were recrystallised from methanol (charcoal), giving the *acid* (XII; R = H, R′ = Ac) as prisms, m. p. >300° (Found: C, 64·1; H, 4·1. $C_{19}H_{14}O_7$ requires C, 64·4; H, 4·0%), $\nu_{max.}$ ~3430, ~2580 (broad OH), ~1745, ~1725, 1708, 1672 cm.$^{-1}$ (C=O), $\lambda_{max.}$ 242, 308 mμ, log ε 4·48, 4·13. Methylation of the acid (XII; R = H, R′ = Ac) with diazomethane afforded the ester (XII; R = Me, R′ = Ac) [3] identified by mixed m. p. and comparison of the infrared spectra.

[^{14}C]-*Viridin.*—*Gliocladium virens* (ACC. 941) was grown in surface culture as previously described.[6] *dl*-[2-^{14}C] Mevalonic lactone (250 μc) was distributed evenly among the culture flasks at the time of inoculation. The fermentation was harvested after 15 days. The culture filtrate (3 l.) was extracted with chloroform (3 × 1 l.) and the brown resinous product (2·9 g.) was triturated with ethanol. Repeated recrystallisation from benzene of the solid obtained gave needles (277 mg.) of [^{14}C]-viridin, identified by the infrared spectrum. Assays of radioactivity were carried out by standard methods [7] and gave relative molar activity × 10^{-4}, 317; 11 μc.

After recommendation by the Editor of the Chemical Society, the following nomenclature is adopted: viridin: 1,2,3,6,7,8,9,11bξ-octahydro-1(*eq*)-hydroxy-2(*ax*)-methoxy-11b-methyl-3,6,9-trioxocyclopenta[7,8]phenanthro[1,10-*bc*]furan; the hexahydrodeoxy derivative [4] (XV; R = Ac, X = Y = O): 1-acetoxy-1,2,3,3a,4,5a,6,7,8,9,11bξ,11c-dodecahydro-2-methoxy-11b-methyl-3,6-dioxocyclopenta-[7,8]phenanthro[1,10-*bc*]furan; the octahydrobisdeoxy derivative [4] (XV; R = Ac, X = H_2, Y = O): 1-acetoxy-1,2,3,3a,4,5a,6,7,8,9,11bξ,11c-dodecahydro-2-methoxy-11b-methyl-3-oxocyclopenta[7,8]phenanthro[1,10-*bc*]furan; the α,β-unsaturated ketone [4] (XVI): 3,3a,4,5a,6,7,8,9,11bξ,11c-decahydro-2-methoxy-11b-

[5] A. Melera, *Experientia*, 1963, **19**, 521.
[6] P. W. Brian, P. J. Curtis, H. G. Hemming, and J. C. McGowan, *Ann. Appl. Biol.*, 1946, **33**, 190.
[7] A. J. Birch, R. A. Massy-Westropp, R. W. Rickards, and H. Smith, *J. Chem. Soc.*, 1958, 360.

methyl-3-oxocyclopenta[7,8]phenanthro[1,10-*bc*]furan; the ketone [4] (XVII): 1,2,3,3a,4,5a,6,7,8,9,11bξ,11c-dodecahydro-2-methoxy-11b-methyl-3-oxocyclopenta[7,8]phenanthro[1,10-*bc*]furan: the alcohol,[4] $C_{20}H_{26}O_3$: 1,2,3,3a,4,5a,6,7,8,9,11bξ,11c-dodecahydro-3-hydroxy-2-methoxy-11b-methylcyclopenta[7,8]phenathro[1,10-*bc*]-furan; the product of oxidation with chromic acid [4] (XI): methyl 2,3,6,10-tetrahydro-6-hydroxy-6-methyl-3,10-dioxo-1*H*-cyclopenta[7,8]naphtho[2,3-*b*]furan-7-yl glyoxylate.

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