Viridin. Part 8.[1] Structures of the Analogues Virone and Wortmannolone

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The secondary metabolic products virone (from *Gliocladium virens*) and wortmannolone (from *Penicillium wortmannii*) are shown to be pregna-5,8-dieno[6,5,4-*bc*]furan-3,7,20-trione and 1α,2α-epoxy-3β-hydroxyandrosta-5,8-dieno[6,5,4-*bc*]furan-7,17-dione, respectively.

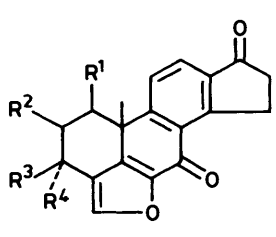
Viridin (1; R^1 = OH, R^2 = OMe, R^3R^4 = O),[2] a steroidal secondary metabolite of the fungus *Gliocladium virens* Miller,[3] CMI 24039 (a fungus erroneously described in the early work[4,5] as *Trichoderma viride*), has conventionally been produced[5] in surface culture on Raulin Thom medium at 25 °C. When this fermentation is carried out at 32 °C, viridin is obtained in much reduced yield (5—10 mg/l as opposed to 25 mg/l) but is accompanied (2 mg/l) by a less polar metabolite of composition $C_{22}H_{24}O_4$ to which we have assigned the trivial name virone. Virone is not produced at all at 25 °C.

The related steroidal metabolite wortmannin (2)[6,7] is produced by some strains of *Penicillium wortmannii* Klocker,[8] but not by all. With one strain (CMI 44277), wortmannin is accompanied by a metabolite (trivial name, wortmannolone) of composition $C_{20}H_{20}O_5$. This paper is concerned with the chemistry and structural elucidation of virone (3) and wortmannolone (4; R^1R^2 = O, R^3 = OH, R^4 = H).

In common with viridin[3] and its analogues,[9] wortmannolone and its derivatives tenaciously retained solvent of crystallisation and solvent-free material could not always be obtained for analysis. Wortmannolone (4; R^1R^2 = O, R^3 = OH, R^4 = H) contained two carbonyl groups ($\nu_{max.}$ 1 715 and 1 665 cm^{-1}) and a secondary hydroxy group ($\nu_{max.}$ 3 360 cm^{-1}) which was readily acetylated to give acetylwortmannolone (4; R^1R^2 = O, R^3 = OAc, R^4 = H) ($\nu_{max.}$ OH absent; δ_H 6.16), and readily oxidised by the chromic oxide–sulphuric acid reagent to a dehydro derivative (4; R^1R^2 = R^3R^4 = O). Dehydrowortmannolone contained three carbonyl groups ($\nu_{max.}$ 1 735, 1 703, and 1 667 cm^{-1}; δ_C 218.0, 186.8, and 173.6). The u.v. absorption of wortmannolone, $\lambda_{max.}$ 264 and 309 nm, was similar to that of wortmannin, suggesting the presence of the same furanocyclohexadienone chromophore: the dehydro derivative (4; R^1R^2 = R^3R^4 = O) showed an additional peak at 230 nm, consistent with the new carbonyl group being in the 3-position. The deshielding effect of a 3-one on 30-H,* previously noted in the viridin series, is apparent when the 30-H chemical shifts for dehydrowortmannolone (δ_H 8.12), acetylwortmannolone (δ_H 7.75), and the acetal (4; R^1R^2 = O, R^3 = R^4 = OMe) (δ_H 7.60) are compared. The u.v. data show that one of the carbonyl groups ($\nu_{max.}$ 1 665) of wortmannolone is located at position 7: the second group ($\nu_{max.}$ 1 735; δ_C 218.0 in the dehydro derivative) is placed, in a five-membered ring, at position 17. These assignments account for four of the five oxygen atoms of wortmannolone. The fifth oxygen atom is present as an epoxide (in the acetyl derivative δ_H 3.60 and 3.52; δ_C 51.5 and 54.8) which must be located at the 1,2 position since decoupling experiments indicated the presence of the partial structure (7).

The ring A configuration of this partial structure was deduced as follows. Treatment of wortmannolone with sodium boro-

* Because of the biosynthetic implications of this work, triterpenoid rather than viridin numbering[2] is used.



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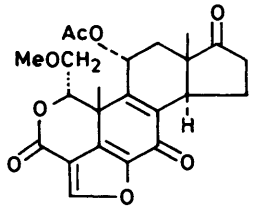

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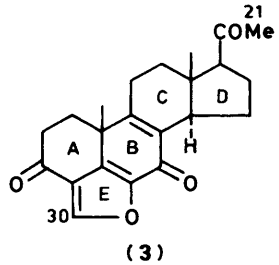

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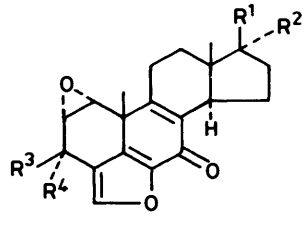

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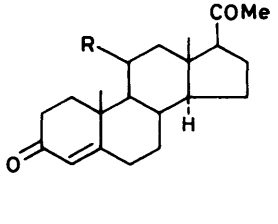

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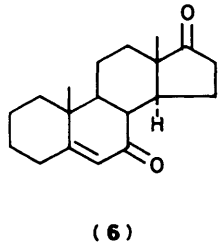

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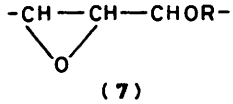


(7)

Table 1. ^{1}H N.m.r. resonances (δ, *J* in parentheses) for virone (**3**) and acetylwortmannolone (**4**; R^1R^2 = O, R^3 = OAc, R^4 = H) and their relatives

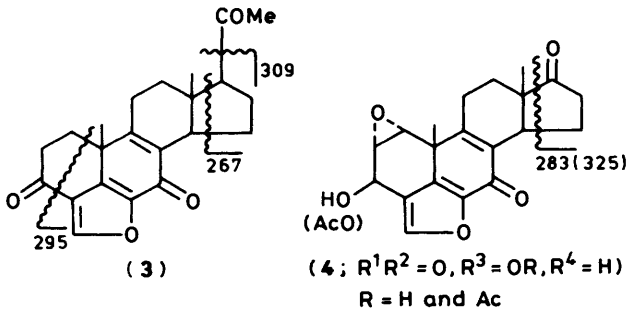
	Position												
Compd.	1	2	3	11	12	14	15	16	17	18	19	30	Other
(**3**)	α, 1.96, td (13.0, 5.0) β, 2.28, ddd (13.2, 5.3, 2.1)	α, 2.68, ddd (18.8, 5.0, 2.0) β, 2.87, ddd (18.8, 13.2, 5.3)		2.59, m	1.6—1.7, m 1.9—2.0, m	2.43 ddt (12.8, 7.3, 2.8)	α, 2.87, m β, 1.69, m	2.25, m	2.57, t (9.6)	0.71, s	1.59, s	8.09, s	2.18, s, 21-H
(**1**; $R^1 = R^2 = H$, $R^3R^4 = O$)[21]	α, 2.24, td (13.5, 5) β, 2.74, m	α, 2.81, ddd (18, 5, 2) β, 3.00, ddd (18, 13.5, 5.3)		7.62, d (8)	7.9, d (8)		3.69 3.80	2.7			1.65, s	8.20, s	
(**2**)	4.77, dd (7.0, 1.9)	3.02, dd (11.2, 7.0) 3.46, dd (11.2, 1.9)		6.16, ddd (8.7, 7.6, 2.7)	1.60, dd (12.9, 8.7) 2.60, dd (12.9, 7.6)	2.90, ddd (12.6, 6.0, 2.7)	α, 3.16, ddd (13, 9, 6) β, 2.06, tt (13, 9)	α, 2.26, dt (19, 9) β, 2.60, dd (19, 8)		0.97, s	1.75, s	8.27, s	2.15, s, OAc 3.19, s, OMe
(**4**; $R^1R^2 = R^3R^4 = O$)	3.95, d (3.6)	3.58, d (3.6)		2.80, dddd (20, 10, 8, 3) 2.92, dddd (20, 8, 3, 1.5)	1.70, dt (13, 9) 2.06, ddd (13, 8, 1.5)	2.70, ddt (12.7, 6.0, 2.8)	α, 3.13, dddd (13, 9, 6, 1) β, 2.08, tt (13, 9)	α, 2.30, dt (19, 9) β, 2.63, ddd (19, 9, 1)		0.92, s	1.64, s	8.12, s	
(**4**; R^1R^2 = O, $R^3 = R^4$ = OMe)[a]	3.50, s	3.50, s								0.80, s	1.43, s	7.60, s	3.20, s, OMe 3.40, s, OMe
(**4**; R^1R^2 = O, R^3 = OAc, R^4 = H)[a]	3.60, d (3.5)	3.52, dd (3.5, 2.5)	6.16, d (2.5)							0.91, s	1.64, s	7.75, s	2.14, s, OAc
(**4**; $R^1 = R^3$ = OAc, $R^2 = R^4$ = H)	3.60, d (3.6)	3.51, dd (3.6, 2.6)	6.15, d (2.6)	2.65, dddd (20, 10, 7.5, 3) 2.80, dddd (20, 7, 3, 1)	1.56, m 1.95, ddd (13, 7.5, 1)	2.40, ddt (12.5, 6.7, 2.8)	α, 2.79, m β, 1.79, qd (12.5, 6)	α, 2.33, dtd (13, 10, 6) β, 1.67, ddd (13, 7, 2.5)	4.76, dd (9.4, 7.3)	0.84, s	1.62, s	7.71, s	2.08, s, OAc 2.14, s, OAc
(**4**; $R^1 = R^4$ = OAc, $R^2 = R^3$ = H)	3.67, d (4.1)	3.63, dd (4.1, 1.6)	6.21, t (1.6)						4.76, dd (9.3, 7.2)	0.82, s	1.50, s	7.48, d (1.6)	2.08, s, OAc 2.24, s, OAc

[a] At 100 MHz.

Table 2. ^{13}C N.m.r. resonances (δ, number of bonded H in parentheses) for virone (3) and acetylwortmannolone (4; R^1R^2 = O, R^3 = OAc, R^4 = H) and their relatives

Position	(3)	(5; R = H)[d,22]	(1; R^1 = R^2 = H, R^3R^4 = O)[15]	(4; R^1R^2 = O, R^3 = OAc, R^4 = H)	(4; R^1R^2 = R^3R^4 = O)	(1; R^1 = R^2 = R^4 = H, R^3 = OAc)[15]	(6)[16]
1	34.1 (2)[a]	35.1	32.6	51.5 (1)[a]	55.6 (1)[a]	26.1	39.0
2	36.6 (2)[a]	33.5	36.2	54.8 (1)[a]	58.6 (1)[a]	28.3	24.0
3	191.5 (0)	197.7	191.5	62.5 (1)	186.8 (0)	64.1	26.8
4	122.0 (0)	123.2	121.8	115.9 (0)	118.3 (0)	121.0	32.7
5	146.9 (0)[b]	170.6	144.3	145.6 (0)	146.7 (0)	144.7	169.2
6	145.8 (0)[b]	31.9	146.0	139.6 (0)[b]	141.3 (0)	144.3	124.3
7	174.3 (0)	31.6	172.5	173.9 (0)	173.6 (0)	173.5	200.8
8	135.0 (0)	34.9	129.4	134.7 (0)[b]	135.1 (0)	130.5	44.3
9	160.1 (0)	53.1	156.7	157.9 (0)	157.0 (0)	157.7	45.7
10	37.7 (0)	38.1	36.4	40.7 (0)	41.1 (0)	37.0	39.2
11	27.3 (2)[c]	20.5	125.8	24.4 (2)[c]	25.2 (2)[b]	124.7	20.1
12	33.0 (2)[a]	37.9	126.5	27.7 (2)[c]	27.8 (2)[b]	126.9	30.7
13	44.0 (0)	43.2	136.4	47.8 (0)	47.8 (0)	137.1	47.8
14	48.7 (1)	55.3	157.3	44.2 (1)	44.3 (1)	158.3	50.4
15	24.7 (2)[c]	22.3	27.9	22.9 (2)[c]	22.9 (2)[b]	32.9	21.8
16	25.0 (2)[c]	23.8	35.7	36.8 (2)	36.7 (2)	36.5	35.5
17	60.7 (1)	62.5	205.1	218.9 (0)	218.0 (0)	206.4	220.0
18	12.9 (3)	12.9		13.9 (3)	13.9 (3)		13.7
19	25.0 (3)	16.8	30.0	28.5 (3)	29.9 (3)	32.4	17.4
30	146.9 (1)		149.7	147.4 (1)	146.9 (1)	148.2	
20	209.1 (0)	208.2		Ac 170.0 (0)		Ac 171.1	
21	31.3 (3)	31.0		20.1 (3)		21.1	

[a,b,c] Assignments may be interchanged. [d] In $(CD_3)_2SO$.



Scheme. Mass spectral fragmentation of virone (3) and wortmannolone (4; R^1R^2 = O, R^3 = OH, R^4 = H)

hydride in methanol at 0 °C furnished the diol (4; R^1 = R^3 = OH, R^2 = R^4 = H), more conveniently isolated as the diacetate (4; R^1 = R^3 = OAc, R^2 = R^4 = H), in which reduction of the 17-one had taken place from the less-hindered α-face of the molecule.[10] Similar treatment of dehydrowortmannolone afforded a non-identical but isomeric diacetate, the ^1H n.m.r. spectrum of which showed allylic coupling (1.6 Hz) between 30-H and 3-H. Molecular models show that this diacetate must have the 3α-OAc structure (4; R^1 = R^4 = OAc, R^2 = R^3 = H) in which the allylic angle Θ is *ca.* 90°. Irradiation of 19-H showed a significant (9%) n.O.e. with 3-H, an effect not shown by the diastereoisomer (4; R^1 = R^3 = OAc, R^3 = R^4 = H). It follows that the configuration of the 3-ol in wortmannolone is β. This results in some deshielding of 19-H, as can be seen when the spectra of the diastereoisomeric diacetates are compared (Table 1).

The borohydride reduction of unsubstituted steroidal 3-ketones occurs mainly from the less hindered α-face to give the 3β-ol and this is the only product in the presence of a 4-ene[10] or a 1β,2β-epoxide.[11] Steric hindrance by a 1α,2α-epoxide of the approach of the reagent to the α-face leads to the formation of some 3α-ol[12] and with a 1α,2α-epoxy-4-ene,[13] the 3α-ol is the only product. It follows from this summary of an earlier review[11] that the formation of a 3α-ol on borohydride reduction of dehydrowortmannolone is consistent only with the presence of a 1α,2α-epoxide. This conclusion is supported by a significant (5%) n.O.e. between 19-H and 1-H in the diacetate (4; R^1 = R^4 = OAc, R^2 = R^3 = H).

This work determines the structure of rings A, B, and E of wortmannolone: that rings C/D have the same *trans* fusion as that in wortmannin (2) follows from a careful comparison of the relevant chemical shifts and coupling constants revealed by the ^1H n.m.r. spectra. At 400 MHz a complete analysis of the steroidal CH/CH_2 envelope is possible though difficult.[14] However, with viridin analogues the 8-en-7-one system effectively removes the allylic hydrogens at positions 11 and 14 from this envelope and, additionally, 15α-H is deshielded by the 7-one and is separated from 15β-H by 1 p.p.m. Moreover, there is homoallylic coupling (2.5—3 Hz) between 14-H and both hydrogens at position 11. These factors greatly simplify the analytical problem, and first-order interpretation can be used to assign (Table 1) all the signals in the range δ 1.5—3.5 in wortmannin: only the signal from 15α-H is partially masked (by the OMe singlet) and the presence of the 11α-OAc group provides further simplification. These assignments and coupling constants differ in some respects from those reported previously.[6] The hydrogens at position 16 are readily recognised by the geminal coupling constant of 19 Hz, and an associated zero vicinal coupling constant is consistent with φ15α–16β being close to 90°. The same signals from 12-H, 14-H, 15-H, and 16-H, with essentially the same coupling constants, are present in the spectrum of dehydrowortmannolone (4; R^1R^2 = R^3R^4 = O). It is concluded that wortmannolone has the 1α,2α-epoxy-3β-hydroxyandrosta-5,8-dieno[6,5,4-*bc*]furan-7,17-dione structure (4; R^1R^2 = O, R^3 = OH, R^4 = H).* This conclusion is reinforced by a comparison (Table 2) of the ^{13}C n.m.r. spectra of acetylwortmannolone and dehydrowortmannolone with the spectra of the demethoxyviridin derivatives (1; R^1 = R^2 = R^4 = H, R^3 = OAc) and (1; R^1 = R^2 = H, R^3R^4 = O),[15] for

* *Note added in proof*: this was confirmed by *X*-ray crystallography of wortmannolone (P. B. Hitchcock, personal communication).

C-3—C-10 and C-30, and with the spectrum of the androstenedione (**6**)[16] for C-12—C-18.

The mass spectra of wortmannolone and its derivatives showed, in addition to ions resulting from the loss of $CH_3^{\bullet}$ and CO, the expected fragmentation (see Scheme) with loss of 57 mass units (ring D + H).

Molecular models show that ring A of wortmannolone is forced into a shallow boat conformation and that the 3β-substituent is attached by a flagpole bond instead of the equatorial bond normally associated with 5α-steroidal 3β-substituents. With the 19-methyl group making its normal contribution the approach to the β-face of ring A is sterically hindered and the epoxide is resistent to rearside nucleophilic attack. Both wortmannolone and its acetate were recovered after 7 days in methanol at room temperature in the presence of boron trifluoride;[17] and wortmannolone was recovered after 2 days in methanol at room temperature in the presence of perchloric acid[18] and after 2 h under reflux in methanol–acetic acid (9:1).[19]

These steric restrictions do not apply to dehydrowortmannolone. Nevertheless, the only product obtained from this compound by the action of boron trifluoride in methanol was the acetal (**4**; R^1R^2 = O, R^3 = R^4 = OMe). Other reaction conditions were complicated by extensive side reactions and no simple epoxide ring-opened product was obtained.

Three of the four oxygen atoms of virone were present as C=O groups [$\nu_{max.}$ 1 700, 1 660, and 1 660 cm^{-1}; δ_C 209.1, 191.5, and 174.3]. The fourth is assigned to a furan ring since the u.v. absorption [$\lambda_{max.}$ 230, 264, and 300 nm (log ε 4.00, 3.92, and 3.83)] was almost identical to that of the oxo substituted furanocyclohexadienone chromophore present in dehydrowortmannolone (**4**; R^1R^2 = R^3R^4 = O). This chromophore accounts for two of the three C=O groups present in virone: the third ($\nu_{max.}$ 1 700 cm^{-1}; δ_C 209.1) is located in a COMe group (δ_H 2.18), suggesting a pregnan-20-one skeleton.

The ^1H (Table 1) and ^{13}C (Table 2) n.m.r. spectra of virone were wholly consistent with structure (**3**). In addition to the characteristic singlet at δ 8.09, ascribed to 30-H (*cf.* dehydrowortmannolone, δ 8.12), the ^1H n.m.r. spectrum showed two 3H-singlets at δ 0.71 (calc.[20] for 17β-acetyl-14α-androsta-5,8-diene-3,7-dione, δ 0.61) and δ 1.59, ascribed to 18-H and 19-H, respectively. The chemical shifts and coupling constants for the hydrogens at positions 1 and 2 (Table 1) agreed closely with those reported[21] for the demethoxyviridin derivative (**1**; R^1 = R^2 = H, R^3R^4 = O). The signals at δ 1.6 and 1.9 from 12-H, and at δ 1.7 and 2.87 from 15-H, were masked by other resonances and the multiplicities could not be determined with certainty, but the chemical shifts and coupling constants for 17-H (δ 2.57, t, *J* 9.6 Hz) and 14-H (δ 2.43, ddt, *J* 12.8, 7.3, and 2.8 Hz) agreed well with those found in 11β-hydroxyprogesterone (**5**; R = OH)[14] (δ 2.43, t, *J* 9.4 Hz) and the wortmannolone derivative (**4**; R^1 = R^3 = OAc, R^2 = R^4 = H) (δ 2.40, ddt, *J* 12.5, 6.7, and 2.8 Hz), respectively. Second-order spectra were obtained from 11-H and 16-H, but the chemical shifts were as expected.

The ^{13}C n.m.r. spectrum showed signals at δ 48.7(1) and 60.7(1), assigned to C-14 and C-17 respectively, by comparison with progesterone (**5**; R = H)[22] which provided an analogy for the carbon atoms 12—18, 20, and 21, associated with rings C/D. The demethoxyviridin derivative (**1**; R^1 = R^2 = H, R^3R^4 = O) likewise provided[15] an analogy for the carbon atoms 1—10, 19, and 30, associated with rings A/B.

The mass spectrum of virone had a fragmentation pattern (see Scheme) which was also consistent with structure (**3**). In addition to an ion (*m/z* 337) resulting from the loss of $CH_3^{\bullet}$, it showed a loss of 43 mass units (17-side chain) followed by 42 mass units (ring D + H) to give ions $C_{20}H_{21}O_3^+$ and $C_{17}H_{15}O_3^+$ at *m/z* 309 and 267, respectively; and from ring A, loss of 57 mass units to give an ion $C_{19}H_{19}O_3^+$ at *m/z* 295.

Virone is therefore considered to have the pregna-5,8-dieno-[6,5,4-*bc*]furan-3,7-20-trione structure (**3**).

Virone and wortmannolone are of interest since they are pregnane and androstane analogues of viridin (**1**; R^1 = OH, R^2 = OMe, R^3R^4 = O). The compounds are possible intermediates on the biosynthetic pathways from lanosterol to viridin and wortmannin respectively, and they provide circumstantial evidence that the formation of the furan ring precedes at least the final steps in the degradation of the sterol side chain.

Experimental

M.p.s were taken on a Kofler hot-stage apparatus and are corrected. I.r. spectra were determined for Nujol mulls and u.v. spectra for solutions in methanol. N.m.r. spectra were obtained in $CDCl_3$ at 360 MHz (^1H) and 90.55 MHz (^{13}C). Chemical shifts are recorded as δ (p.p.m.) from $SiMe_4$ (internal standard). *J* Values are taken from line separations (first-order interpretation). CH_3/CH and CH_2/Cq ^{13}C sub-spectra were generated by spin-echo pulse sequences. Molecular weights were taken from the mass spectra. Optical rotations were measured in a 1 dm cell. Merck silica gel HF_{254} was used in analytical t.l.c. with chloroform–methanol (98:2). Acetylations were carried out in pyridine with acetic anhydride at room temperature during 24 h.

Fermentations.—(*A*) Gliocladium virens. (*a*) Conical flasks (1 l) containing Raulin Thom medium (200 ml) were inoculated with a spore suspension of *G. virens* CMI 24039, as previously described.[5] After 20 days at 32 °C the fermentation was harvested and the culture filtrate (10 l) was extracted with chloroform to give a brown oil (2.6 g). The oil, in benzene, was chromatographed on a column of acid alumina pH 2.5[3] (100 g) in intermittent u.v. light. After (i) a yellow fluoroscent band giving an oil (138 mg) had been eluted with benzene (500 ml), benzene–methanol (200:1; 500 ml) eluted (ii) a green fluorescent band giving a solid (281 mg) from which viridin[3] (104 mg) was obtained by recrystallisation from benzene.

Crystallisation of fraction (i) from benzene gave *virone* (*pregna-5,8-dieno*[6,5,4-bc]*furan-3,7,20-trione*) (**3**) as plates (15 mg), m.p. 276—280 °C (decomp.), $[\alpha]_D^{20}$ −80° (*c* 0.05 in ethanol), R_F 0.58 (Found: C, 75.1; H, 6.8%; *M*, 352.1669. $C_{22}H_{24}O_4$ requires C, 75.0; H, 6.9%; *M*, 352.1674); $\nu_{max.}$ 3 150w and 3 120w (=CH), 1 700, 1 660 (C=O), 1 630w, and 1 530 cm^{-1}; $\lambda_{max.}$ 230, 264, and 300 nm (ε 9 920, 8 350, 6 740); *m/z* (% base peak) 352 (22), 337 (42), 309.1498 (14), 295.1342 (100), 279 (8), 267.1046 (16), 253 (10), and 43 (80). $C_{20}H_{21}O_3^+$, $C_{19}H_{19}O_3^+$, and $C_{17}H_{15}O_3^+$ require 309.1491, 295.1334, and 267.1021, respectively. It sublimed at 200 °C/10^{-2} mmHg, gave a yellow solution in 2M-hydroxide, and reduced ammoniacal silver nitrate on warming.

(*b*) In carefully controlled parallel experiments, separate batches of 25 flasks were incubated for 16 days (i) at 32 °C and (ii) at 25 °C. The oils obtained by extraction (0.6 g from both fermentations) were dissolved in benzene (20 ml) and chromatographed on a column of alumina (pH 2.5, 15 g, 18 × 1.2 cm) made up in benzene. Elution with benzene (80 ml) followed by benzene–methanol (200:1; 50 ml) gave oils, (i) 35 mg and (ii) 67 mg which were discarded. Elution with benzene–methanol (100:1, 5 × 10 ml) then gave a series of solid fractions, (i) 54 mg and (ii) 156 mg. Recrystallisation of these fractions from benzene afforded (i) virone (11 mg) followed by viridin (16 mg) and (ii) viridin (121 mg).

(*B*) Penicillium wortmannii (CMI 44277). This was grown, in surface culture, on Raulin Thom medium with added glucose (5%).[8] After 13 days at 25 °C, the fermentation was harvested and the culture filtrate was extracted with chloroform, giving a

brown oil, which solidified to give a brown powder. Initially, wortmannin (R_F 0.64) and wortmannolone (R_F 0.44) were obtained by preparative t.l.c. of this powder (silica, 1 mm layer, with benzene–methanol, 83:17) but subsequently, a column chromatographic method was developed. In a typical separation the crude extract (3 g powder), adsorbed onto silica, was added to the column (silica, 120 g, packed with dichloromethane), which was developed with increasing concentrations of acetone in dichloromethane. Wortmannin (255 mg) and wortmannolone (624 mg) were eluted with dichloromethane–acetone (95:5) and dichloromethane–acetone (85:15) respectively.

Two other strains of *P. wortmannii* (CMI 60036; CMI 91023) were grown in surface culture in the same manner as strain 44277. Analytical t.l.c. of the crude extracts failed to furnish any evidence for the production of wortmannin or wortmannolone.

Wortmannolone (1α,2α-*epoxy*-3β-*hydroxyandrosta*-5,8-*dieno*-[6,5,4-bc]*furan*-7,17-*dione*) (**4**; R^1R^2 = O, R^3 = OH, R^4 = H) tenaciously retained water and organic solvents within the crystal lattice and analyses were unsatisfactory. It was obtained from acetone as a microcrystalline powder (decomp.) (subl. >235 °C), $[\alpha]_D^{22}$ −63° (*c* 0.100 in ethanol) [Found (dried 1.5 h at 100 °C): C, 66.3; H, 5.9%; *M*, 340.1305. $C_{20}H_{20}O_5 \cdot H_2O$ requires C, 67.0; H, 6.2%; *M*, 340.1311; (dried 6 h at 100 °C) C, 68.5; H, 6.0. $C_{20}H_{20}O_5 \cdot 0.5H_2O$ requires C, 68.75; H, 6.05%; $C_{20}H_{20}O_5$ requires C, 70.6; H, 5.9%]; R_F 0.20; *m/z* 340 (60), 325 (78), 312 (35), 297 (58), and 283.0997 (100). $C_{17}H_{15}O_4^+$ requires *m/z*, 283.0970; $\nu_{max.}$ 3 360, 3 120w, 1 715, 1 665, 1 635, and 1 550 cm^{-1}; $\lambda_{max.}$ 264 and 308 nm (ε 8 400 and 6 950). It could be sublimed at 190 °C/10^{-1} mmHg. It was insoluble in aqueous sodium hydrogen carbonate and sodium hydroxide at room temperature but dissolved in the latter reagent on heating to give a brown solution. It gave a silver mirror immediately on warming with ammoniacal silver nitrate. With concentrated sulphuric acid it gave a yellow colour which became orange on warming. It gave no colour with iron(III) chloride.

The *acetate* (**4**; R^1R^2 = O, R^3 = OAc, R^4 = H) crystallised from ethyl acetate in plates, m.p. *ca.* 190 °C (transition) and 248—253 °C (decomp.) [Found (dried at 20 °C): C, 67.7; H, 5.9%; *M*, 382.1413. $C_{22}H_{22}O_6 \cdot 0.5C_4H_8O_2$ requires C, 67.6; H, 6.1%; *M*, 382.1416]. R_F 0.64; $\nu_{max.}$ OH absent, 3 120w, 1 730, 1 660, 1 630, and 1 550 cm^{-1}; $\lambda_{max.}$ 263 and 305 nm (ε 9 550 and 7 650); *m/z* 382 (43), 367 (60), 354 (30), 339 (32), 325 (72), and 43 (100).

Dehydrowortmannolone (**4**; $R^1R^2 = R^3R^4$ = O).—Wortmannolone (50 mg) in acetone (10 ml) at 0 °C was treated with the 4M-chromic oxide–sulphuric acid reagent (0.10 ml) and the solution was allowed to warm to room temperature during 30 min. It was then concentrated at room temperature under reduced pressure after which water was added. The precipitate of felted needles (32 mg) was filtered off, dried, and recrystallised from ethyl acetate to give *dehydrowortmannolone* (**4**; $R^1R^2 = R^3R^4$ = O) as prisms which sublimed without melting at 280 °C; $[\alpha]_D^{25}$ +27° (*c* 0.130 in chloroform); R_F 0.60 (Found: C, 71.1; H, 5.2%; *M*, 338. $C_{20}H_{18}O_5$ requires C, 71.0; H, 5.4%; *M*, 338); $\nu_{max.}$ OH absent, 3 120w, 1 735, 1 703, 1 667, 1 640, and 1 530 cm^{-1}; $\lambda_{max.}$ 231, 261, and 300 nm (ε 10 300, 9 200, and 6 510).

Extraction of the aqueous filtrate with ethyl acetate gave a gum (16 mg), separated into neutral and acidic fractions by extraction with sodium hydrogen carbonate and recovery. The neutral fraction (3 mg) gave dehydrowortmannolone (2 mg) on crystallisation from ethyl acetate. The acid fraction was intractable.

Dehydrowortmannolone (8 mg) in methanol (3 ml) was treated at room temperature with boron trifluoride–diethyl ether (0.1 ml) and the solution was set aside for 18 h. After concentration under reduced pressure followed by the addition of water, extraction with chloroform afforded an amorphous solid which crystallised from ethyl acetate, to give the *acetal* (**4**; R^1R^2 = O, R^3 = R^4 = OMe) as prisms (6 mg), m.p. 250 °C (Found: C, 69.0; H, 6.5%; *M*, 384. $C_{22}H_{24}O_6$ requires C, 68.7; H, 6.3%; *M*, 384); $\nu_{max.}$ 1 730, 1 660, 1 630, and 1 548 cm^{-1}; $\lambda_{max.}$ 261 and 304 nm (ε 7 850 and 6 000).

Reduction of Wortmannolone and Dehydrowortmannolone with Sodium Borohydride.—(*A*) Wortmannolone (10 mg) in methanol (2 ml) at 0 °C was treated with sodium borohydride (5 mg) and the solution was allowed to warm to room temperature during 15 min. After concentration under reduced pressure, water (2 ml) was added and the solution was neutralised with 2M-hydrochloric acid. Extraction with ethyl acetate afforded a resinous solid which crystallised (charcoal) from ethyl acetate to give the *diol* (**4**; R^1 = R^3 = OH, R^2 = R^4 = H) as large prisms (4 mg), m.p. 280—283 °C (decomp.) (Found: C, 69.8; H, 6.4%; *M*, 342. $C_{20}H_{22}O_5$ requires C, 70.2; H, 6.5%; *M*, 342); $\nu_{max.}$ 3 390, 3 240, 1 655, 1 622, and 1 540 cm^{-1}; $\lambda_{max.}$ 263 and 307 nm (ε 9 050 and 7 100). The m.p. was depressed on admixture with the diol hydrate (**4**; R^1 = R^4 = OH, R^2 = R^3 = H).

The yield of the diol (**4**; R^1 = R^3 = OH, R^2 = R^4 = H) from this procedure was variable and in another experiment the resinous product (16 mg) from wortmannolone (20 mg) was acetylated and solid product (13 mg) was twice recrystallised from ethyl acetate to give the *diacetate* (**4**; R^1 = R^3 = OAc, R^2 = R^4 = H) as prisms, m.p. 260—263 °C (decomp.) R_F 0.64 (Found: C, 67.0; H, 6.0%; *M*, 426. $C_{24}H_{26}O_7$ requires C, 67.6; H, 6.15%; *M*, 426); $\nu_{max.}$ 1 740, 1 722, 1 667, 1 635, and 1 550 cm^{-1}.

(*B*) Dehydrowortmannolone (6 mg) in methanol (3 ml) was treated with sodium borohydride (6 mg) as described above. The product crystallised from ethyl acetate to give the *diol hydrate* (**4**; R^1 = R^4 = OH, R^2 = R^3 = H) as small prisms, m.p. 148—150 °C (Found: C, 66.2; H, 6.6%; *M*, 342. $C_{20}H_{22}O_5 \cdot H_2O$ requires C, 66.6; H, 6.7%; *M*, 342); $\nu_{max.}$ 3 490, 3 260, 1 655, 1 620, and 1 550 cm^{-1}; $\lambda_{max.}$ 265 and 309 nm (ε 8 200 and 6 550). The i.r. spectra of the isomeric diols were significantly different in the region 7—14 μm, but the mass spectra were virtually identical. The R_F of the two diols were identical (0.17) in benzene–methanol (85:15).

In another experiment the crude product (32 mg) from the reduction of dehydrowortmannolone (30 mg) was acetylated and the solid product (22 mg) was crystallised from ethyl acetate to give the *diacetate* (**4**; R^1 = R^4 = OAc, R^2 = R^3 = H) as needles, double m.p. 235—236 °C and 251—252 °C (decomp.), R_F 0.65 (Found: C, 66.6; H, 6.1%; *M*, 426. $C_{24}H_{26}O_7 \cdot 0.5C_4H_8O_2$ requires C, 66.4; H, 6.4%; *M*, 426. $C_{24}H_{26}O_7$ requires C, 67.6; H, 6.15%); $\nu_{max.}$ 1 725, 1 672, 1 640, and 1 555 cm^{-1}.

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