Fungal Products. Part XIII.[1] Xanthomegnin, Viomellin, Rubrosulphin, and Viopurpurin, Pigments from *Aspergillus sulphureus* and *Aspergillus melleus*

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The known fungal metabolites xanthomegnin (1) and viopurpurin (19) have been isolated from *Aspergillus sulphureus* together with the new pigments rubrosulphin (3,4,11,12-tetrahydro-9,17-dihydroxy-7-methoxy-3,12-dimethyl-2,13,16-trioxanaphth[1',2':5,6]indeno[2,1-*a*]anthracene-1,8,14,15-tetrone) (16) and viomellein {8-(3,4-dihydro-9,10-dihydroxy-7-methoxy-3-methyl-1-oxo-1*H*-naphtho[2,3-*c*]pyran-8-yl)-3,4-dihydro-6-hydroxy-9-methoxy-3-methylnaphtho[1,2-*c*]pyran-1,7,10-trione}; (10). The pigments (1), (10), and (19) were also isolated from *Aspergillus melleus*. Chemical and spectroscopic evidence for the proposed structures is presented. On this evidence structure (19) for viopurpurin is preferred to the alternative pyranobisnaphthopyran structures (22) or (23) previously suggested for this pigment.

N.m.r. evidence is presented showing that xanthomegnin (1) and viomellein (10) exist in solutions at room temperature as diastereoisomeric mixtures owing to restricted rotation about the dimeric linkage.

FOUR pigments have been isolated from the mycelium of *Aspergillus sulphureus* in yields which varied with the conditions of culture. These pigments were: xanthomegnin, previously isolated from *Trichophyton rubrum*,[2] *T. megnini*,[3] and *T. violaceum*[4] and assigned structure (1);[5,6] viomellein and rubrosulphin, two new metabolites for which structures (10) and (16) are respectively proposed; and viopurpurin, previously isolated from *T. violaceum*[4] and tentatively assigned structures (22)[4] or (23),[6] for which the revised structure (19) is now proposed. Xanthomegnin (1), viomellein (10), and viopurpurin (19) were also isolated from the mycelium of *A. melleus*.

Despite some minor differences in the n.m.r. data (Table 1) and a different m.p. for the diacetate (2), the identity of xanthomegnin (1) was established by direct comparison with an authentic sample supplied by Dr. G. Just. An interesting feature of the n.m.r. spectrum, not noted previously,[5] in which the signal for the strongly hydrogen-bonded hydroxy-group at τ −3·1 occurred as a double signal, is discussed below.

TABLE 1

Chemical shifts (τ) [a] for protons in *A. Sulphureus* pigments and derivatives

Compound	Me	CH_2	CH	6-H	1'-H	8'-H	6'-H	CH'	CH_2'	Me'	OH	OMe	OAc
(1)	8·44	6·96	5·39	2·51			2·51	5·39	6·96	8·44	−3·11 (2H) [b]	5·85 (6H)	
(2)	8·44	6·90	5·30	2·05			2·05	5·30	6·90	8·44		5·91 (6H)	7·59 (6H) [b]
(3)	8·48	6·96	5·38	2·20			2·20	5·38	6·96	8·48		5·92 (6H),[b] 6·01 (6H) [b]	
(10)	8·44	6·98	5·37	2·50	3·34	3·04		5·37	6·98	8·66	−3·88, −3·44,[b] 0·20	6·10, 6·16	
(11)	8·43	6·97	5·34	2·05	3·07	3·04		5·34	6·97	8·43	−2·90	6·14 (6H)	7·61, 7·80
(12)	8·46	6·98	5·37	2·04	2·94	2·52		5·37	6·98	8·52		6·16 (6H)	7·58, 7·62,[b] 7·81 [b]
(13)	8·45	6·98	5·40	2·17	3·03	2·70		5·40	6·98	8·45		6·00, 6·02,[b] 6·14 (6H),[b] 6·31 [b]	
(17)	8·44	6·89	5·32	2·01	3·10	2·56		5·24	6·93	8·44		5·93	7·39, 7·49
(18)	8·43	6·92	5·34	2·14	3·03	2·73		5·34	7·00	8·48		5·78, 5·88 5·91	
(20)	8·46	6·92	5·28	1·98		2·42		5·28	6·84	8·46		5·98	7·30, 7·48 (6H)
(21)	8·43	6·88	5·33	2·13		2·23		5·33	6·88	8·43		5·80, 5·87, 5·90, 6·04	

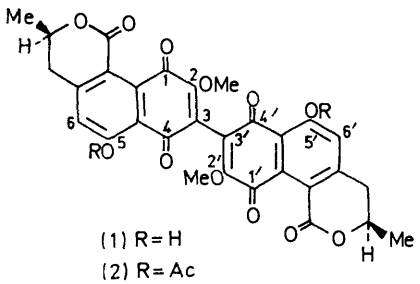
[a] In $CDCl_3$ at 100 MHz. [b] Double signals.

The n.m.r. (Table 1) and i.r. data indicated that the other three pigments had naphthoquinone–naphthalene structures in which the naphthaquinone portion was similar to that in xanthomegnin (1). This was confirmed by oxidation with alkaline hydrogen peroxide.

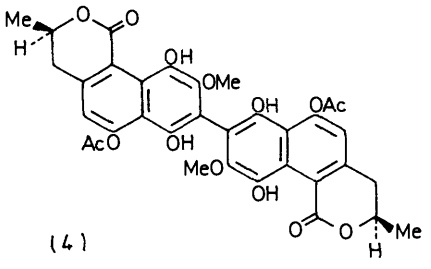
[1] Part XII, P. Hedden, J. MacMillan, and B. O. Phinney, *J.C.S. Perkin I*, 1974, 587.
[2] J. G. Wirth, P. J. O'Brien, F. L. Schmidt, and A. Sohler, *J. Invest. Dermatology*, 1957, **29**, 47; J. G. Wirth, T. E. Beesley, and S. R. Amand, *Phytochemistry*, 1965, **4**, 505.
[3] F. Blank, W. C. Day, and G. Just, *J. Invest. Dermatology*, 1963, **40**, 133.
[4] F. Blank, A. S. Ng, and G. Just, *Canad. J. Chem.*, 1966, **44**, 2873.
[5] G. Just, W. C. Day, and F. Blank, *Canad. J. Chem.*, 1963, **41**, 74.
[6] A. S. Ng, G. Just, and F. Blank, *Canad. J. Chem.*, 1969, **47** 1223.

Like xanthomegnin (1), viomellein (10) and rubrosulphin (16) were oxidised to the phthalic acid (5) which was characterised as the anhydride (8). Similarly rubrosulphin dimethyl ether (18) and viopurpurin trimethyl ether (21) were oxidised to the methyl ether (6), $M^+ + 2y$ ions after flushing the mass spectrometer source with deuterium oxide.

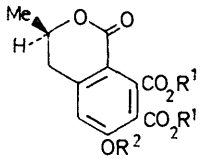
The naphthalene portion of viomellein (10) was shown to include a fused δ-lactone ring similar to that in the naphthoquinone portion by the n.m.r. and i.r. spectra



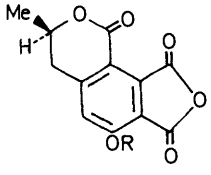
(1) R = H
(2) R = Ac
(3) R = Me



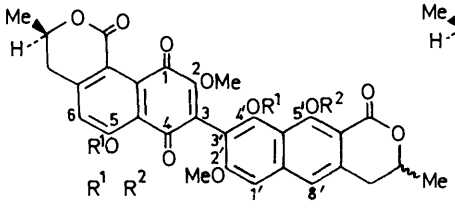
(4)



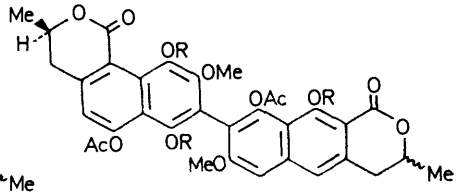
(5) $R^1 = R^2 = H$
(6) $R^1 = H$, $R^2 = Me$
(7) $R^1 = R^2 = Me$



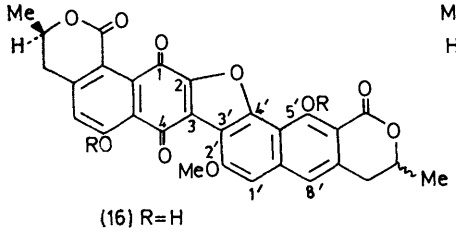
(8) R = H
(9) R = Me



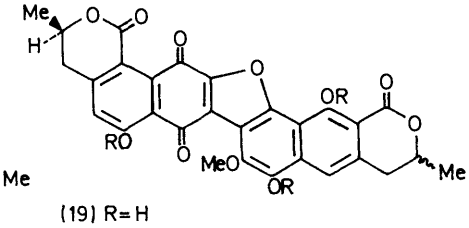
(10) H H
(11) Ac H
(12) Ac Ac
(13) Me Me



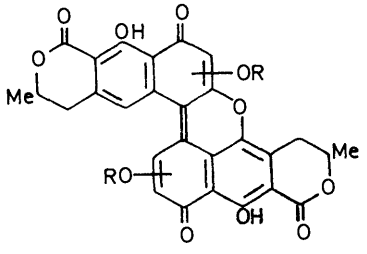
(14) R = H
(15) R = Ac



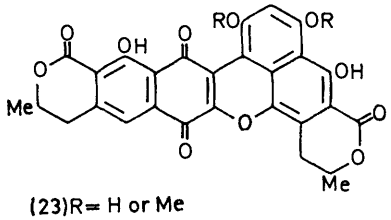
(16) R = H
(17) R = Ac
(18) R = Me



(19) R = H
(20) R = Ac
(21) R = Me



(22) R = H or Me



(23) R = H or Me

further characterised as the dimethyl ester (7) and the anhydride (9). The presence of only one quinonoid ring in viomellein (10), rubrosulphin (16), and viopurpurin (19) was also evident from the $(M^+ + y)$ ions present in their mass spectra (Table 2). The origin of these ions from the corresponding hydroquinones formed by reduction in the mass spectrometer source in the presence of water,[7] was confirmed by the presence of of the quinone and its derivatives. The n.m.r. spectra (Table 1) also showed the presence of one methoxy-group, two aromatic protons, and two hydrogen-bonded hydroxy-groups in the naphthalene nucleus. The arrangement of these substituents was deduced from the following data. The lactonic carbonyl groups in viomellein (10) and the diacetate (11) absorbed at

[7] R. T. Aplin and W. T. Pike, *Chem. and Ind.*, 1966, 2009.

ca. 1730 and 1660 cm^{-1} while the dihydrodiacetate (14) showed no absorption at 1730 cm^{-1} but increased absorption at 1660 cm^{-1}. An equivalent lowering of the lactonic carbonyl frequency occurred on reduction of xanthomegnin diacetate (2) to the tetrahydro-derivative

TABLE 2

Relative intensities of $(M + y)^+$ ion in the mass spectra of *A. sulphureus* pigments

Pigment	Relative intensity (%)				
	M^+	$M^+ + 1$	$M^+ + 2$	$M^+ + 3$	$M^+ + 4$
(1)	100	42	156	63	135
(10)	100	55	152		
(16)	100	33	24		
(19)	100	52	123		

(4). The carbonyl absorption at 1660 cm^{-1} in viomellein was therefore assigned to the δ-lactone of the naphthalene portion. This assignment was supported by the i.r. spectrum of the triacetate (12) which showed strong carbonyl absorption at 1730—1725 but none at 1660 cm^{-1}. One of the strongly bonded hydroxy-groups (τ −3·88 or −3·44) was therefore vicinal to the lactone carbonyl group in the naphthalene portion, the other being at C-5 in the naphthaquinone portion.* The less strongly hydrogen-bonded hydroxy-group (τ 0·21) was also shown to be intramolecularly bonded by its concentration-independent i.r. absorption and was placed vicinal to the strongly-bonded hydroxy-group in the naphthalene portion. This vicinal arrangement was supported by the rapid formation of the diacetate (11) and slow formation of the triacetate (12).

The two aromatic protons in the naphthalene portion of viomellein (10) gave singlets and each was shown to be *ortho* or *para* to one of the hydroxy-groups by the changes in their chemical shift upon acetylation.[8,9] The proton appearing at τ 3·34 was deshielded by 0·27 p.p.m. on formation of the diacetate (11) and assigned to C-1′ and the proton at τ 3·04 was deshielded by 0·52 p.p.m. in the triacetate (12) and assigned to C-8′. The substitution pattern deduced from the spectroscopic pattern was finally established by base-catalysed conversion of viomellein (10) into the cyclic ether, rubrosulphin (16) (see below).

Rubrosulphin (16) was too insoluble for useful n.m.r. data to be obtained but its i.r. spectrum and the n.m.r. (Table 1) and i.r. spectra of the diacetate (17) and of the dimethyl ether (18) were similar to those of the corresponding derivatives (11)—(13) of viomellein (10). These data also showed that rubrosulphin contained one methoxy- and one hydroxy-group less than viomellein (10) and one additional oxygen-containing ring. Otherwise the substitution pattern in the two pigments appeared to be analogous. Structure (16) was therefore deduced for rubrosulphin, the position of the ether bridge in the naphthalene ring being supported by the higher chemical shift of the 1′-proton in the diacetate (17) compared with that of the 1′-proton in viomellein diacetate (11). The relationship between rubrosulphin (16) and viomellein (10) was established when, from the methylation of viomellein (10) with dimethyl sulphate and base, there was obtained the rubrosulphin dimethyl ether (18) in addition to viomellein trimethyl ether (13). Treatment of viomellein (10) with base in the absence of dimethyl sulphate afforded rubrosulphin (16). This conversion presumably involves attack of the C-4′ alkoxide ion on C-2 of the quinone followed by elimination of methoxide ion (*cf.* ref. 10).

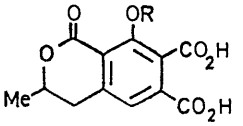
The deep purple quinone from *A. sulphureus* and *A. melleus* was identified as viopurpurin, originally isolated from *T. violaceum* by Just *et al.*,[4] from the spectroscopic data published by these authors for the quinone, its triacetate, and trimethyl ether. In particular the i.r. spectrum of the triacetate was identical with that reproduced [4] for viopurpurin triacetate. Like rubrosulphin (16), viopurpurin (19) was very insoluble but the i.r. spectra of the two pigments were very similar as were those of the acetates and methyl ethers of the two pigments. The spectroscopic data indicated that viopurpurin was a 1′- or 8′-hydroxy-derivative of rubrosulphin (16). Comparing the chemical shifts (Table 1) of the aromatic protons in the acetates and methyl ethers of rubrosulphin and viopurpurin, the lower field proton in both sets is assigned as 6-H. The proton which is absent from the spectra of the viopurpurin derivatives is that which occurs at highest field, *i.e.* 1′-H, in the rubrosulphin derivatives. The 1′-hydroxyrubrosulphin structure (19) is therefore proposed for viopurpurin. The deshielding of the 8′-proton in viopurpurin trimethyl ether (21) by 0·5 p.p.m. is precisely that expected from the presence of a *peri*(1′)-methoxy-group.[11] The observed deshielding of the 8′-proton by the *peri*(1′)-acetoxy-group is smaller; no literature data are available but the electron-withdrawing acetyl group would be expected to reduce the effect.

The two alternative structures (22) and (23), previously suggested [4,6] for viopurpurin, were based partly on the isolation of the phthalic acid (24) from the hydrogen peroxide oxidation of viopurpurin trimethyl ether. This acid (24) may come from the naphthalene portion of structure (21) although, in our hands, the isomeric phthalic acid (6) from the naphthaquinone portion was the only isolable product. Similarly we have obtained only the phthalic acids (5) and (6) from analogous oxidations of viomellein (10), rubrosulphin (16), and rubrosulphin dimethyl ether (18); no evidence for the formation of the phthalic acid (25) or its methyl ether (24) from the pigments or their methyl ethers was obtained. Further evidence which is incompatible with the structures (22) or (23) for viopurpurin, but consistent with our proposed structure (19), includes:

* The numbering system based on two naphthalene rings [see *e.g.* (1) and (10)] is used throughout the Discussion section.

[8] R. G. Cooke and I. D. Rae, *Austral. J. Chem.*, 1964, **17**, 379.
[9] A. Gaudemer and J. Polonsky, *Bull. Soc. chim. France*, 1963, 1918.
[10] G. S. Sidhu and M. Pardhasaradhi, *Tetrahedron Letters*, 1967, 4263.
[11] G. P. Dudek, *Spectrochim. Acta*, 1963, **19**, 691.

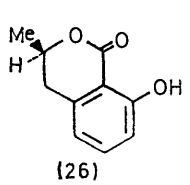
(*a*) viopurpurin shows lactonic carbonyl absorption in the i.r. spectrum at 1725 cm^{-1} whereas structures (22) and (23) should show lower (1660 cm^{-1}) absorption due



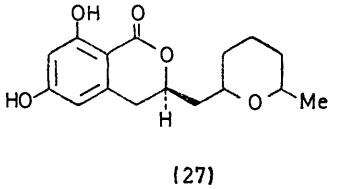
(24) R=Me
(25) R=H

to strongly hydrogen-bonded carbonyl groups; (*b*) the chemical shift (τ 2·23) of the naphthalenic proton in the trimethyl ether is too low for structure (23) in which the chemical shift should be similar to that (τ 2·70) of the 1′-proton in viomellein trimethyl ether (13); and (*c*) the vinylic or quinonoid proton of structure (22) would be expected to occur at higher field than τ 2·23 in the trimethyl ether and 2·42 in the triacetate; for example the corresponding proton in 2-methoxy-1,4-naphthoquinone absorbs [12] at τ 3·8.

The absolute stereochemistry of the asymmetric carbon in the lactone rings of xanthomegnin (1) was established by Just *et al.*[6] by degradation to (—)-(*R*)-β-hydroxybutyric acid. The asymmetric centre in the naphthaquinone portion of viomellein (10), rubrosulphin (16), and viopurpurin (19) was shown to have the same absolute configuration as in xanthomegnin (1) by c.d. The phthalic acid (5), the dimethyl ester (6), and the methyl ether dimethyl ester (7) from all four pigments showed a negative Cotton effect at *ca.* 260 nm.* The closely related (—)-(*R*)-mellein (26), another metabolite of *A. melleus*, also shows [13] a negative c.d. curve. Asperentin (27), a metabolite of *A. flavus* has the opposite lactone configuration and shows [14] a positive Cotton effect at 260 nm. The c.d. curves of xanthomegnin (1) and viomellein (10) and of their acetates (2) and (12) were complex.



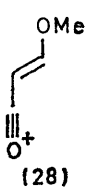
(26)



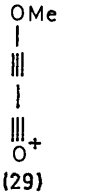
(27)

An interesting feature of the n.m.r. spectrum of xanthomegnin (1), viomellein (10), and their derivatives was the doubling of the singlets for some of the hydroxy-, acetyl, and methoxy-signals. The signals showing this doubling at 100 Hz are indicated in Table 1. At 220 MHz doubling of other signals was observed in addition to increased separation of the doublets observed at 100 MHz. Similar doubling of signals was observed in the methyl ether acetates of procyanidin dimers by Weinges *et al.*[15] who attributed the phenomenon to restricted rotation about the dimeric linkage and provided evidence for this explanation from the observed temperature dependence of the doublets. Similar evidence for restricted rotation in dimeric and trimeric derivatives of flavans has been reported by du Preez *et al.*[16] and in the natural procyanidins by Thompson *et al.*[17] In the present cases, the doublets coalesced on raising the temperature; for example, the doublets in the 100 MHz spectra of xanthomegnin (1) coalesce at 60°. Further evidence that this phenomenon is due to the mixture of diastereoisomers arising from the chirality at the 3,3′-dimeric linkage is provided by the absence of double signals of rubrosulphin (16) and viopurpurin (19) even at 220 MHz. The c.d. of xanthomegnin (1) and viopurpurin (10) did not show a large Cotton effect associated [18] with the chirality of the dimeric linkage. However, the small amplitude at 300 nm is probably due to the slight excess of one of the diastereoisomers, since the doublets observed in the n.m.r. spectrum at room temperature were not of equal intensity.

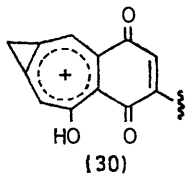
The mass spectra of the pigments show similar fragmentation patterns consistent with the proposed structures. Xanthomagnin (1) and viomellein (10) show intense ions at *m/e* 85 and 83, assigned structures (28) and (29) and derived from the quinone ring.



(28)



(29)



(30)

Rubrosulphin (16) and viopurpurin (19), which contain the ether bridge, do not show this fragmentation. Apart from these ions, the mass spectra of all four pigments show no major fragmentations after initial losses from the molecular ion. These high mass fragmentation ions which were mass-matched are rationalised for xanthomegnin (1) as follows. An initial loss of formaldehyde from the 2-methoxy-group, followed by the successive loss of carbon dioxide and a methyl radical from the lactone, gives the base peak which may be formulated as (30). The mass spectrum of viopurpurin (19) is analogous to that of xanthomegnin (1) except for the absence of the ions *m/e* 85 and 83 and of the initial loss of formaldehyde from the molecular ion. The mass spectrum of viomellein (10) is dominated

* Spectroscopic data for compounds (5)—(9) are listed in Supplementary Publication No. SUP 21159 (2 pp.). For details of Supplementary Publications see Notice to Authors No. 7 in *J.C.S. Perkin I*, 1973, Index issue.

[12] R. H. Thomson, ' Naturally Occurring Quinones,' Academic Press, London and New York, 1971.
[13] A. Arakawa, N. Torimoto, and Y. Matsui, *Annalen*, 1969, **728**, 152.
[14] J. F. Grove, *J.C.S. Perkin I*, 1972, 240.
[15] K. Weinges, J. Perner, and H. D. Marx, *Chem. Ber.*, 1970, **103**, 2344.
[16] I. C. du Preez, A. C. Rowan, D. G. Roux, and J. Feeney, *Chem. Comm.*, 1971, 315.
[17] R. S. Thompson, D. Jacques, E. Haslam, and R. J. W. Tanner, *J.C.S. Perkin I*, 1972, 1387.
[18] Y. Ohigari, N. Kobayaski, and S. Shibata, *Tetrahedron Letters*, 1968, 1881.

by the fragmentation to the ions *m/e* 85 and 83 but after the initial loss of methanol it is very similar to that of rubrosulphin (16) (*cf.* the chemical conversion of viomellein into rubrosulphin involving the elimination of methanol) and both spectra show a similar fragmentation sequence from the molecular ion, analogous to that of xanthomegnin (1) after the initial loss of formaldehyde.

EXPERIMENTAL

For general experimental details see Part V.[19]

Production of Pigments from Aspergillus sulphureus.—(*a*) *A. sulphureus* (CM1 128,939) was grown under stirred aerated conditions in Raulin–Thom medium (30 l) containing Cerulose (5%). After 7 days the mycelium was collected and extracted with acetone (3 × 6 l). The acetone was removed *in vacuo* and the aqueous residue was extracted with chloroform to give a brown oily solid (56·1 g) which was washed with light petroleum. The resultant brown powder (21·4 g) contained, by p.l.c. (see later), rubrosulphin (40%) and viopurpurin (10%).

(*b*) *A. sulphureus* was grown as surface cultures in Thompson bottles each containing 1 l of the medium specified in (*a*). After 13 days the mycelium from 45 bottles was collected, dried, and extracted with chloroform. The chloroform extract was evaporated to small volume; light petroleum was then added to give a brown solid (23 g) containing (see later) viomellein (19%), rubrosulphin (7%), xanthomegnin (26%), and viopurpurin (3%).

(*c*) *A. sulphureus* was grown as in (*b*) except that each bottle contained 350 ml medium. Extraction as before gave a brown solid (11 g) containing viomellein (19%), rubrosulphin (7%), xanthomegnin (14%), and viopurpurin (5%).

Production of Pigments by Aspergillus melleus.—*A. melleus* was grown as previously described by Mills and Turner [20] and the dried mycelium from 48 flasks was extracted as described for *A. sulphureus* cultures. The brown solid (8 g) contained viomellein (11%), xanthomegnin (50%), and viopurpurin (1%) but no rubrosulphin.

Isolation of Pigments.—The following is typical of the pigments from *A. sulphureus*. The crude mixture of pigments (800 mg) in chloroform was applied to eight silica gel plates (40 × 20 × 0·05 cm). Multiple elution with methylene dichloride–formic acid (50 : 1) gave four coloured bands, which were removed and eluted with chloroform to give the following pigments in order of increasing polarity:

(i) *Viomellein* {8-(3,4-*dihydro*-9,10-*dihydroxy*-7-*methoxy*-3-*methyl*-1-*oxo*-1H-*naphtho*[2,3-c]*pyran*-8-*yl*)-3,4-*dihydro*-6-*hydroxy*-9-*methoxy*-3-*methylnaphtho*[1,2-c]*pyran*-1,7,10-*trione*} (10) was crystallised from chloroform–light petroleum in brown beads which sintered above 260° [yield: 19% from culture conditions (*a*) and (*b*) and 0% from (*c*)] (Found: M^+, 560·137. $C_{30}H_{24}O_{11}$ requires *M*, 560·141); *m/e* 562·153 (0·41%, $M^+ + 2$, $C_{30}H_{26}O_{11}$ requires *m/e* 562·148), 561 (0·13, $M^+ + 1$), 560·137 (0·27, M^+), 530 (1), 529 (1), 528 (1), 484·119 (23, $C_{28}H_{20}O_8$ requires *m/e* 484·116), 440·121 (6, $C_{27}H_{20}O_6$ requires *m/e* 440·126), 87 (31), 85 (70), and 83 (100); $\lambda_{max.}$ 225, 264, and 395 nm (ε 16,800, 20,210, and 8200); $\nu_{max.}$ (Nujol) 3340, 2750br, 1725, 1675, 1656, 1640, 1630, 1608, 869, and 819 cm^{-1}; $\nu_{max.}$ 3390, 3000, 1730, 1680, 1648, 1603, and 1590 cm^{-1}, c.d. ($CHCl_3$) λ 411, 400, 370, 345, 304, 288, 282, 265, and 240 nm (ε −0·16, 0, +0·88, 0, −5·86, 0, +1·81, 0, and −3·6!).

(ii) *Rubrosulphin* (3,4,11,12-*tetrahydro*-9,17-*dihydroxy*-7-*methoxy*-3,12-*dimethyl*-2,13,16-*trioxanaphth*[1′,2′:5,6]*indeno*-[2,1-a]*anthracene*-1,8,14,15-*tetrone*) (16) crystallised from chloroform–light petroleum in red plates sintering above 300° (Found: M^+, 528·104. $C_{29}H_{20}O_{10}$ requires *M*, 528·106); $\nu_{max.}$ (Nujol) 3300br, 1725, 1670, 1640, 1610, 1565, 1410, 1254, 1187, 1120, 878, and 827 cm^{-1}; $\nu_{max.}$ 3350br, 1724, 1680, 1642, and 1610 cm^{-1}; *m/e* 530 (2%, $M^+ + 2$), 529 (3, $M^+ + 1$), 528 (7, M^+), 484 (100), 456 (17), 440 (17), 438 (17), and 420 (5).

(iii) Xanthomegnin (1) crystallised from chloroform–benzene in orange plates sintering above 260° (Found: C, 62·4; H, 3·8%; M^+, 574·116. Calc. for $C_{30}H_{22}O_{12}$: C, 62·7; H, 3·9%; *M*, 574·111); $\lambda_{max.}$ 222, 264, and 380 nm (ε 26,000, 19,300, and 7900); $\nu_{max.}$ (Nujol) 3300br, 1712, 1672, 1618, 1600, and 853 cm^{-1}; $\nu_{max.}$ 2280br, 1720, 1675, 1622, and 1598 cm^{-1}; *m/e* 578 (5%, $M^+ + 4$), 577 (2, $M^+ + 3$), 576 (5, $M^+ + 2$), 575 (1·5, $M^+ + 1$), 574 (3, M^+), 544·101 (52, $C_{29}H_{20}O_{11}$ requires *m/e* 544·100), 500·113 (67, $C_{28}H_{20}O_9$ requires *m/e* 500·111), 485·089 (100, $C_{27}H_{17}O_9$ requires *m/e* 485·087), 456·121 (7, $C_{27}H_{20}O_7$ requires *m/e* 465·121), 441·099 (10, $C_{26}H_{17}O_7$ requires *m/e* 441·097), 85 (57), and 83 (80); c.d. ($CHCl_3$) λ 480, 450, 430, 390, 308, 287, 278, 260, and 235 nm (ε 0, +0·16, 0, −0·87, 10·5, 0, +3·77, 0, and −6·28!).

(iv) *Viopurpurin* (3,4,11,12-*tetrahydro*-6,9,17-*trihydroxy*-7-*methoxy*-3,12-*dimethyl*-2,13,16-*trioxanaphth*[1′,2′:5,6]-*indeno*[2,1-a]*anthracene*-1,8,14,15-*tetrone*) (19) crystallised from chloroform–light petroleum in purple-black beads sintering above 310° [3, 5, and 10% yield from cultures (*a*), (*b*), and (*c*)] (Found: M^+, 544·114. $C_{29}H_{20}O_{11}$ requires *M*, 544·116); $\lambda_{max.}$ ($CHCl_3$) 274, 282, 377, and 500 nm (ε 37,200, 38,700, 8900, and 3000); $\nu_{max.}$ (Nujol) 3200; 1719, 1677, 1635, 1600, 1570, 841, and 820 cm^{-1}; $\nu_{max.}$ 3200, 1725, 1678, 1640, 1600, 1570, and 1550 cm^{-1}, *m/e* 546·122 (5%, $M^+ + 2$, $C_{29}H_{22}O_{11}$ requires *m/e* 546·116), 544 (4, M^+), 500·113 (83, $C_{28}H_{20}O_9$ requires *m/e* 500·111), 485·090 (100, $C_{27}H_{17}O_9$ requires *m/e* 485·087), 442·108 (30, $C_{26}H_{18}O_7$ requires *m/e* 442·105), and 441·100 (24, $C_{26}H_{17}O_7$ requires *m/e* 441·097).

Oxidation of Pigments with Alkaline Hydrogen Peroxide.—(a) *Xanthomegnin.* The quinone (50 mg) in 2·5N-sodium hydroxide (10 ml) was treated dropwise at 5—10° with aqueous hydrogen peroxide (30% w/v; 1 ml). After stirring for 0·25 h, more hydrogen peroxide (0·8 ml) was added and stirring was continued at 20° for 2 h. The colourless solution was acidified (pH 4) with concentrated hydrochloric acid, then aqueous sodium hydrogen carbonate was added until the solution was slightly alkaline. Chloroform extraction removed a trace of starting material. The aqueous solution was readjusted to pH 2·5 and extracted for 36 h with ether to give pale yellow rods which were recrystallised (×5) from acetone–light petroleum until homogeneous by t.l.c. The acid (5) was obtained as pale yellow rods (17 mg), m.p. 192—194° (lit.,[5] 194—195°). $[\alpha]_D^{23}$ −79·4° (*c* 0·11). Sublimation of the acid (5) at 175° and 3 mmHg gave the anhydride (8), m.p. 225—227° (lit.,[5] 227—229°) (Found: M^+, 248·031. Calc. for $C_{12}H_8O_6$: *M*, 248·032)

(b) *Viomellein.* The quinone (16 mg) in 2·5N-sodium hydroxide (5 ml) was oxidised with 30% aqueous hydrogen peroxide (0·5 ml) as in (a). Similar work-up of the bright red solution gave unchanged quinone (1·2 mg) and an oil

[19] J. MacMillan and T. J. Simpson, *J.C.S. Perkin I*, 1973, 1487.
[20] S. D. Mills and W. B. Turner, *J. Chem. Soc.* (*C*), 1967, 2242.

which was purified by p.l.c. on silica gel with ethyl acetate–light petroleum–formic acid (200 : 40 : 1). The fluorescent band at R_F 0·65 was extracted with acetone to give the acid (5), which crystallised from acetone–light petroleum in yellow rods (3 mg) identical (m.p., mixed m.p., i.r., and $[\alpha]_D^{23}$) with the acid (5) obtained in (a).

Bands from the p.l.c. at R_F 0·3, 0·56, and 0·69 gave intractable gums when extracted with chloroform.

(c) *Rubrosulphin*. The quinone (200 mg) in methanol (20 ml) and 5N-sodium hydroxide (30 ml) was oxidised with 30% hydrogen peroxide (30 ml) as in (a) to give unchanged quinone (11 mg) and the acid (5) (90 mg). The latter was purified as in (b) to give rods (28 mg), identified by m.p., mixed m.p., i.r., n.m.r., and $[\alpha]_D^{23}$.

(d) *Rubrosulphin dimethyl ether* (18). The dimethyl ether (174 mg) in methanol (20 ml) and 2·5N-sodium hydroxide (20 ml) was oxidised with 30% hydrogen peroxide (20 ml) as in (a) for 12 h. After acidification, the pale yellow solution was extracted for 48 h to give *3,4-dihydro-6-methoxy-3-methyl-1-oxo-2-benzopyran-7,8-dicarboxylic acid* (6), crystallised from methanol as needles, m.p. 206—208°, $[\alpha]_D^{23}$ +182·5° (*c* 0·4 in MeOH) (Found: C, 55·8; H, 4·5. $C_{13}H_{12}O_7$ requires C, 55·7; H, 4·3%). The *dimethyl ester* (7), prepared with diazomethane, was purified by p.l.c. on silica gel HF with ethyl acetate–light petroleum–acetic acid (9 : 10 : 1) (R_F 0·35) and crystallised from ethyl acetate–light petroleum as needles, m.p. 104—105°, $[\alpha]_D^{23}$ −176° (*c* 0·25 in MeOH) (Found: M^+, 308·090. $C_{15}H_{16}O_7$ requires *M*, 308·089).

Sublimation of the diacid (6) at 180° and 4 mmHg gave the *anhydride* (9), needles, m.p. 243—246° (Found: M^+, 262·049. $C_{13}H_{10}O_6$ requires *M*, 262·048).

(e) *Viopurpurin trimethyl ether* (21). The trimethyl ether (64 mg) was oxidised as in (d) for 22 h. Continuous extraction of the reaction mixture for 48 h with ether gave a solid (30 mg) which was methylated and purified by p.l.c. on silica gel HF with light petroleum–ethyl acetate–acetic acid (10 : 9 : 1). Recovery from the band at R_F 0·35 gave the methyl ether dimethyl ester (7) (10 mg), m.p. 103—105°, identical (mixed m.p., i.r., n.m.r., mass spectrum, and $[\alpha]_D^{22}$) with the methyl ether dimethyl ester obtained in (d).

Xanthomegnin Diacetate (2).—Prepared by treatment of the quinone (25 mg) with acetic anhydride (1·5 ml) and pyridine (8 drops) at room temperature in the dark for 24 h, the diacetate was purified by p.l.c. on silica gel with chloroform. The orange oil recovered from the band at R_F 0·35 was crystallised from chloroform–benzene as orange prisms (14 mg), m.p. 252—253° (lit.,[5] 227—228°) (Found: C, 62·1; H, 4·1%. Calc. for $C_{34}H_{26}O_{14}$: C, 62·0; H, 4·0%); $\nu_{max.}$ 1780, 1725, 1674, and 1604 cm^{-1}; c.d. ($CHCl_3$) λ 400, 340, 299, 285, 274, 265, 260, 252, and 245 nm (ε 0, −3·44, −11·8, 0, +6·24, 0, −4·3, −3·22, and −2·15).

Tetrahydroxanthomegnin Diacetate (4).—Xanthomegnin diacetate (14 mg) in ethyl acetate (15 ml) was hydrogenated at room temperature and pressure in the presence of pre-reduced palladium oxide. The usual work-up gave the tetrahydro-derivative (4), which crystallised from carbon tetrachloride as beads (7 mg), m.p. 191—192° (lit.,[5] 192—193°), $\nu_{max.}$ 3538, 3100br, 1770, 1661, 1632, and 1575 cm^{-1}.

Xanthomegnin Dimethyl Ether (3).—Xanthomegnin (80 mg), dimethyl sulphate (5 ml), anhydrous potassium carbonate (2 g), and acetone (2 ml) were refluxed for 5 h with stirring. The solid residue was removed by filtration and after distillation of the excess of acetone from the filtrate, the excess of dimethyl sulphate was hydrolysed with 5% aqueous sodium hydroxide. Extraction of the acidified solution with chloroform gave a red oil (a mixture by n.m.r.) which was fractionated by p.l.c. on silica gel HF with chloroform–methanol–formic acid (90 : 5 : 5). Recovery from the pale yellow band at R_F *ca.* 0·47 gave *xanthomegnin dimethyl ether* (3), which crystallised from acetone as a microcrystalline orange powder, m.p. 150—155° (Found: M^+, 602·144. $C_{32}H_{26}O_{12}$ requires *M*, 602·142), $\nu_{max.}$ 1724, 1673, 1618, 1595, and 1559 cm^{-1}.

Acetylation of Viomellein (10).—Viomellein (48 mg), acetic anhydride (3 ml), and pyridine (12 drops) were stirred for 18 h at room temperature in the dark. The usual work-up gave a yellow solid which was fractionated by p.l.c. on silica gel with chloroform–formic acid (400 : 1). Recovery from the band at R_F 0·73 in chloroform gave the *diacetate* (11), which crystallised from benzene–light petroleum as yellow beads (27 mg), m.p. 189—190° (Found: C, 63·8; H, 4·4. $C_{34}H_{28}O_{13}$ requires C, 63·5; H, 4·15%), $\nu_{max.}$ 3000br, 1775, 1731, 1673, 1658, 1633, and 1603 cm^{-1}.

Recovery from the band at R_F 0·40 gave the *triacetate* (12), crystallising from benzene–light petroleum in yellow beads, m.p. 195—196° (Found: C, 63·0; H, 4·5. $C_{36}H_{30}O_{14}$ requires C, 63·0; H, 4·4%), $\nu_{max.}$ 1775, 1728, 1725, 1676, 1628, and 1603 cm^{-1}; c.d. ($CHCl_3$) λ 395, 344, 322, 296, 284, 278, 274, 264, 257, 246, and 233 nm (ε 0, −1·95, −1·26, −5·4, 0, +1·72, 0, −5·44, −1·72, −1·43, and −2·3!).

Dihydroviomellein Diacetate (14).—Prepared by hydrogenation of viomellein diacetate (20 mg) in ethyl acetate (18 ml) in the presence of pre-reduced palladium oxide (8 mg) at room temperature and pressure, the *dihydro-derivative* (14) which was purified by p.l.c. (R_F 0·27) on silica gel with chloroform–formic acid (200 : 1) and crystallised from carbon tetrachloride in pale yellow beads (11 mg), m.p. 195—198° (Found: C, 60·6; H, 4·4. $C_{34}H_{30}O_{13}$ requires C, 60·3; H, 4·4%), $\nu_{max.}$ 3540, 3000br, 1775, 1660, 1632, and 1575 cm^{-1}. Acetylation in pyridine with acetic anhydride at room temperature for 39 h gave *dihydroviomellein penta-acetate* (15) which was purified by p.l.c. on silica gel with chloroform–formic acid (100 : 1) (R_F 0·22) and crystallised from benzene–light petroleum as needles, m.p. 197—199° (Found: C, 62·4; H, 4·8. $C_{40}H_{36}O_{16}$ requires C, 62·2; H, 4·7%); $\nu_{max.}$ 1776, 1720, 1630, and 1570 cm^{-1}.

Viomellein Trimethyl Ether (13).—A mixture of viomellein (10) (150 mg), anhydrous potassium carbonate (4 g), dimethyl sulphate (10 ml), and acetone (40 ml) was refluxed with stirring for 15 h. The usual work-up gave a red gum (140 mg) which was separated into two components by p.l.c. on silica gel after two elutions with chloroform–methanol–formic acid (90 : 5 : 5). Recovery from the yellow band at R_F 0·25—0·45 gave *viomellein trimethyl ether* (13) as an orange powder (69 mg), m.p. 158—160° (Found: M^+, 602·181. $C_{33}H_{30}O_{11}$ requires *M*, 602·179); $\nu_{max.}$ (Nujol) 1720, 1674, and 1591 cm^{-1}.

The lower p.l.c. band at R_F 0·15—0·25 yielded a red powder (45 mg) identical (i.r., n.m.r., and mass spectrum) with rubrosulphin dimethyl ether (18) whose preparation is described below.

Conversion of Viomellein (10) *into Rubrosulphin* (16).—Viomellein (65 mg) was added to anhydrous potassium carbonate (3 g) in acetone (30 ml) and the mixture was refluxed with stirring for 15 h. After removal of the

acetone under vacuum, water (20 ml) was added to the dark blue residue which was then acidified slowly with concentrated hydrochloric acid. Recovery of the resulting red precipitate gave a semi-solid (61 mg) which was fractionated by p.l.c. on silica gel after two elutions with chloroform–methanol–formic acid (90 : 5 : 5). Recovery from the band at R_F 0·8 gave unchanged viomellein (40 mg). Recovery from the red zone at R_F 0·7 gave rubrosulphin (16), identified by i.r., n.m.r., and mass spectra.

Rubrosulphin Diacetate (17).—Prepared by treatment of the quinone (250 mg) with acetic anhydride (4 ml) and pyridine (10 drops) at room temperature for 18 h in the dark, the *diacetate* (17) was obtained as an orange microcrystalline powder, m.p. 200—205° (Found: C, 64·3; H, 3·8. $C_{33}H_{24}O_{12}$ requires C, 64·7; H, 3·9%); $\nu_{max.}$ 1780, 1730, 1687, 1633, 1605, 1575, and 890 cm^{-1}.

Rubrosulphin Dimethyl Ether (18).—Rubrosulphin (91 mg) in acetone (40 ml) was treated with dimethyl sulphate (10 ml) and potassium carbonate (4 g) as described earlier for xanthomegnin. The crude gummy product was purified by p.l.c. on silica gel with dichloromethane–formic acid (50 : 1) to give the *dimethyl ether* (18) as a red microcrystalline powder (70 mg), m.p. 183—187° (Found: M^+, 556·138. $C_{31}H_{24}O_{10}$ requires M, 556·137); $\nu_{max.}$ (Nujol) 1720, 1675, 1621, and 1595 cm^{-1}, *m/e* 558 (52%, $M^+ + 2$), 556 (100, M^+), 541 (53), 538 (53), 523 (42), 495 (26), 465 (26), and 438 (19).

Viopurpurin Triacetate (20).—Prepared from viopurpurin (150 mg), acetic anhydride (3 ml), and pyridine (15 drops), the triacetate (120 mg) was purified by p.l.c. on silica gel with dichloromethane–formic acid (50 : 1) and obtained as a microcrystalline orange powder, m.p. 260—268° (lit.,[4] 280—285°); i.r. spectrum identical with that reproduced by Blank *et al.*[4]

Viopurpurin Trimethyl Ether (21).—Prepared from viopurpurin (200 mg), anhydrous potassium carbonate (10 mg), and dimethyl sulphate (25 ml) in acetone (100 ml) as for xanthomegnin dimethyl ether, the trimethyl ether was purified by p.l.c. on silica gel with dichloromethane–formic acid (50 : 1) and obtained as a microcrystalline red powder, m.p. 170—175° (lit.,[6] 173—174°), $\nu_{max.}$ (Nujol) 1722, 1678, 1619, and 1574 cm^{-1}.

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