1224. *Pigments from Two Fungi of the Order Sphæropsidales*

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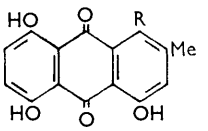
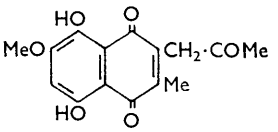
The pigments of two species of the class Deuteromycetes (Fungi Imperfecti) and Order Sphæropsidales have previously been examined. Phomazarin was first isolated from *Phoma terrestris* Hansen and examined by Kögl and his co-workers.[1] This compound was recently re-examined by Birch and his co-workers [2] and by us.[3] It was also found that some strains of the fungus lost the ability to produce phomazarin when cultured in the laboratory and produced cynodontin (Ia) instead.[4] Quilico *et al.*[5] obtained cyanodontin and helminthosporin from *Deuterophoma traceiphila*. We now report on the pigments produced by two further fungi of this Order.

Eveleigh [6] showed that one of the fungi commonly responsible for the discoloration of painted surfaces was *Phoma pigmentivora*. Pigment formation depended on light and on the medium employed. We found culture of the fungus on liquid sucrose or glucose media, and on reduced starch medium with maximum daylight illumination, to result in a slow growth of red mycelium with coloration of the medium. Non-pigmented mycelium was present below the surface. A rapid growth of reddish-purple mycelium was obtained by culture on Czapek–Dox starch medium, and extraction of the dried mycelium gave two pigments in yields of 4 and 0·02% by weight of mycelium. The major pigment was cynodontin and the minor one most probably helminthosporin (Ib) (see Experimental section).



(I) a ; R = OH
b ; R = H



(II)

Selenophoma donacis is responsible for the disease known as " eye spot " of cereals and other grasses in N. America. The fungus is stated to possess a reddish-brown to terra-cotta colour when grown on potato-dextrose–agar.[7] We found that culture on this medium

[1] F. Kögl and J. Sparenburg, *Rec. Trav. chim.*, 1940, **59**, 1180; F. Kögl and F. W. Quackenbush, *ibid.*, 1944, **63**, 251; F. Kögl, G. C. v. Wessem, and O. I. Elsbach, *ibid.*, 1945, **64**, 23.
[2] A. J. Birch, D. N. Butler, and R. W. Rickards, *Tetrahedron Letters*, 1964, 1853.
[3] K. Schofield and D. E. Wright, unpublished work.
[4] K. Schofield and D. E. Wright, *Nature*, 1960, **188**, 233.
[5] A. Quilico, C. Cardani, F. Piozzi, and P. Scrivana, *Accad. Lincei*, 1952, **12**, 650.
[6] D. Eveleigh, Ph.D. Thesis, Exeter (1959).
[7] R. Sprague, " Diseases of Cereals and Grasses in North America," Ronald Press Co., New York, 1950.

produced a very faint red pigmentation, but subsequent cultures on maltose–peptone–agar (Sabaraud's agar) produced a deep purple-red pigmentation of the mycelium and medium. Subsequent experiments showed that the presence of peptone in the medium was essential for pigment production. Extraction of the freeze-dried culture gave a single pigment in 0·4% yield. It was recalled that peptone was an essential nutritional requirement in the production of javanicin (II) by *Fusarium javanicum*,[8] and our results indicate the pigment of *S. donacis* to be javanicin, although an authentic specimen was not available for comparison.

A third fungus, *Phyllosticta sorghina*, the causative organism of " leaf spot " in sorghum and grasses,[7] was also examined. A culture on Czapek–Dox starch medium gave a rapid growth of heavily pigmented mycelium with the red pigment extending into the medium, but all attempts to isolate the pigment failed.

Experimental.—Light petroleum had b. p. 60—80° unless otherwise stated.

Phoma pigmentivora. The strain used was obtained from C.B.S. (Baarn) and labelled *Phoma pigmentivora* (= *Aposphaeria violacea* Bertel). The fungus was maintained by sub-culture on potato-glucose–agar slopes when red pigmentation of the mycelium was apparent. A culture on a Czapek–Dox starch medium at 20° with maximum daylight illumination gave a vigorous growth of pigmented mycelium on the surace of the medium. The medium itself was not pigmented. The growth rate was not accelerated and pigment production not enhanced by incubation at 25° with constant exposure to artificial light.

A medium prepared from soluble starch (750 g.) in hot water (10 l.), to which was added sodium nitrate (30 g.), magnesium sulphate heptahydrate (7·5 g.), potassium chloride (7·5 g.), and ferrous sulphate heptahydrate (1·5 g.) in water (200 ml.), and potassium dihydrogen phosphate (1·5 g.) in water (100 ml.) was diluted to a volume of 15 l. It was distributed in 100 ml. portions into 150 bottles (16 oz. medical flats), and the bottles and their contents were sterilised (15 lbs. p.s.i. for 20 min.). Whilst still fluid the contents of each bottle were brought to pH 7·0 with sterile sodium carbonate solution (5% w/v). The bottles were inoculated with blocks of agar and mycelium from a 6-week-old culture of *P. pigmentifora* on potato-dextrose–agar, and were stored horizontally in daylight for 2 months.

The contents of the bottles were then shaken with water and filtered. The red mycelium so obtained was freed from starch by washing with water and then with ethanol, then dried at 60°. The powdered mycelium (158 g.) was extracted (Soxhlet) with light petroleum (900 ml.). The extract deposited bronze needles of cyanodontin (3·2 g.), m. p. 260—263° (with sublimation) (Found: C, 62·8; H, 3·5. Calc. for $C_{15}H_{10}O_6$: C, 62·9; H, 3·5%) alone and mixed with an authentic specimen. The ultaviolet spectrum showed principal maxima at 239 and 558 mμ, and the infrared spectrum a strong band at 1580 cm.$^{-1}$ (Nujol). The acetate separated from alcohol as yellow needles, m. p. 225—226° (Found: C, 59·8; H, 4·2. Calc. for $C_{23}H_{18}O_{10}$: C, 60·7; H, 4·0%) alone and mixed with cynodontin tetra-acetate.

Concentration of the initial light petroleum extract from the mycelium gave bronze needles (35 mg.), m. p. 209—210°. Two crystallisations from ethyl acetate gave a specimen (10 mg.), m. p. 221—222°, alone and mixed with helminthosporin (m. p. 226—227°). Principal absorption occurred at 490 mμ (in MeOH), and the infrared spectrum showed a strong maximum at 1595 cm.$^{-1}$ (Nujol). Although the m. p. of the yellow acetate could not be raised above 210—215°, there was no mixed-m. p. depression with helminthosporin triacetate (m. p. 222—224°).

By treating the mycelium with 10% sulphuric acid for 24 hr., washing with water and ethanol, and drying at 60°, and then extracting with pyridine, more cynodontin (3·3 g., m. p. 260°) was isolated.

Selenophoma donacis. A strain of this fungus from C.B.S. (Baarn) was maintained on slopes of Sabaraud's agar. The freeze-dried agar and mycelium (112 g.) obtained by growing the fungus on this medium [glucose (120 g.), mycological peptone (30 g. L40, Oxoid), agar (36 g., and water (3 l.)] distributed among 60 bottles, was powdered and extracted (Soxhlet) with light petroleum. Concentration of the orange-red extract gave orange needles (470 mg.), m. p.

[8] (*a*) H. R. V. Arnstein and A. H. Cook, *J.*, 1947, 1021; (*b*) S. Gatenbeck and R. Bentley, *Biochem. J.*, 1964, **92**, 21P; (*c*) E. Hardegger, K. Steiner, E. Widmer, and A. Pfiffner, *Helv. Chim. Acta*, 1964, **47**, 2027.

199—200°. Recrystallisation from benzene raised the m. p. to 207—208° (lit.,[8] m. p. 207·5—208°) (Found: C, 62·3; H, 5·0; MeO, 10·6; C-Me, 9·8. Calc. for $C_{15}H_{14}O_6$: C, 62·1; H, 4·9; MeO, 10·7; *C*-Me, 10·3%). The 2,4-dinitrophenylhydrazone, prepared in ethanol–ethyl acetate, had m. p. 255—257° (decomp.) (lit.,[8] m. p. 256—257° (decomp.), and the derivative formed by reaction with acetic anhydride formed from ethanol yellow needles, m. p. 264—265° (Found: C, 64·5; H, 4·9; MeO, 10·5. Calc. for $C_{17}H_{14}O_6$: C, 64·9; H, 4·6; MeO, 9·9%) (lit.,[8a,9] m. p. for monoacetylanhydrojavanicin, 247—248° and 252—253°). The infrared spectrum of the pigment showed strong bands at 1602 and 1711 $cm.^{-1}$ (KBr disc), and the ultraviolet spectra of the pigment and its acetyl derivative were as reported for javanicin [8] and monoacetylanhyrojavanicin.[8]

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[9] S. Weiss and F. F. Nord, *Arch. Biochem.*, 1949, **22**, 288.