Constituents of the Higher Fungi. Part XVI.[1] Bulgarhodin and Bulgarein, Novel Benzofluoranthenequinones from the Fungus *Bulgaria inquinans* (Fries)

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Three crystalline quinones have been isolated from the fungus *Bulgaria inquinans*. Two of these have been identified as derivatives of benzo[*j*]fluoranthene and the third characterised as 4,9-dihydroxyperylene-3,10-quinone. This is the first recorded isolation of a natural product with structure based on the benzofluoranthene nucleus.

The bark fungus *Bulgaria inquinans* forms black, disc-shaped fruiting bodies, 1—3 cm in diameter, on the surface of freshly felled oak. The fungus was originally examined by Zopf,[2] who reported the presence of a number of colouring matters; these included a water-insoluble red pigment which crystallised from chloroform, a water-insoluble blue amorphous colouring matter, a red-brown resin, a yellow fat, and water-soluble red and yellow compounds. The red crystalline colouring matter was named bulgariin.

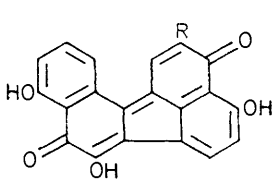
We have re-examined the colouring matters from this fungus and now report the isolation of three crystalline compounds. Two of these, bulgarhodin (I) and bulgarein (II), belong to a new class of naturally occurring quinones with structures based on the benzo[*j*]fluoranthene nucleus, and the third has been identified as 4,9-dihydroxyperylene-3,10-quinone (III). Compound (III) occurs in the fruiting body of the fungus *Daldinia concentrica*.[3]

[1] Part XV, R. L. Edwards and M. Gill, *J.C.S. Perkin I*, 1975, 351.

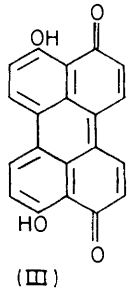
[2] W. Zopf, *Beitrage zur Physiologie und Morphologie Die Niedere Organismen*, 1892, **2**, 17.

[3] D. C. Allport and J. D. Bu'Lock, *J. Chem. Soc.*, 1958, 4090.

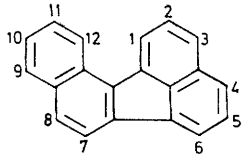
Bulgarhodin does not appear to be the same as the crystalline pigment (bulgariin) isolated by Zopf. We have been unable to crystallise bulgarhodin from chloroform, and the colour reactions with strong acid are not the same as those described for the Zopf pigment. Considerable quantities of a black insoluble polymeric material separated with the pigments during the



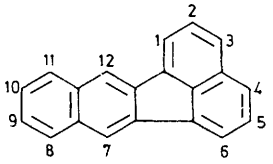
(I) R = OH
(II) R = H



(III)



(IV)



(V)

extraction and this rendered their purification difficult. Also, the yield varied considerably with different batches of fungi. The best yields of crystallisable pigment were obtained from rapidly air-dried material; freeze drying or prior defatting of the air-dried material with light petroleum reduced the quantity of solvent-extractable material.

Extraction of the air-dried fungus with chloroform gave an intensely yellowish-red solution from which a black amorphous solid separated. Re-extraction of the latter with chloroform over a long period removed a sparingly soluble red colouring matter which could only be crystallised from dimethyl sulphoxide. The crystalline material consists of a mixture of three pigments (I)—(III) which could not be separated by crystallisation. Purification was achieved by chromatography of the mixed leucoacetates; the pure acetates were then hydrolysed and the resulting quinol solutions oxidised in air. Bulgarhodin (I) is the major component of the mixture.

Bulgarhodin (I), $C_{20}H_{10}O_6$, M^+ 346, crystallised from dimethyl sulphoxide as purple hair-like needles. The pigment is sparingly soluble in common organic solvents, yielding pale red solutions and dissolves in sulphuric acid to yield a solution with an intense green colouration. Absorption in the visible and u.v. is broad and not very characteristic. Bulgarhodin dissolves in aqueous sodium hydroxide to yield a green solution which quickly deposits a green sodium salt. This property is characteristic of extended quinones.[4] On exposing the alkaline solution to the air the sodium salt slowly redissolves and a solution with a purple colouration is produced.

Bulgarein (II), $C_{20}H_{10}O_5$, M^+ 330, is more soluble in organic solvents than bulgarhodin. Saturated ethanolic solutions are purple and more dilute solutions are intensely blue. In the u.v.–visible region, the relatively sharp band at 375 nm is accompanied by broad absorption at 570 and 630 nm; on dilution the 375 nm band becomes more broad and the 570 nm absorption disappears. Like those of bulgarhodin, solutions of the pigment in sulphuric acid are green, but unlike those of the former pigment, alkaline solutions possess a stable blue colour.

4,9-Dihydroxyperylene-3,10-quinone (III), the third and least abundant constituent of the mixture can be readily distinguished from bulgarhodin and bulgarein by the red colouration produced in sulphuric acid.

Bulgarhodin (I) and bulgarein (II) readily produce leucoacetates on reductive acetylation. Bulgarhodin-leucohexa-acetate, $C_{32}H_{24}O_{12}$, and bulgarein leucopenta-acetate, $C_{30}H_{22}O_{10}$, both yield yellow solutions which exhibit a green fluorescence; the fluorescence is less intense than that shown by solutions of tetra-acetoxyperylene. The u.v.–visible absorptions of both these compounds are significantly different from that of the perylene derivative (Figure 1); high intensity absorption in the 325 nm region is absent in the spectrum of tetra-acetoxyperylene. The u.v. absorption of a quinone leucoacetate is generally accepted to be similar to that of the parent hydrocarbon, and this rules out the possibility that (I) and (II) are perylene derivatives.

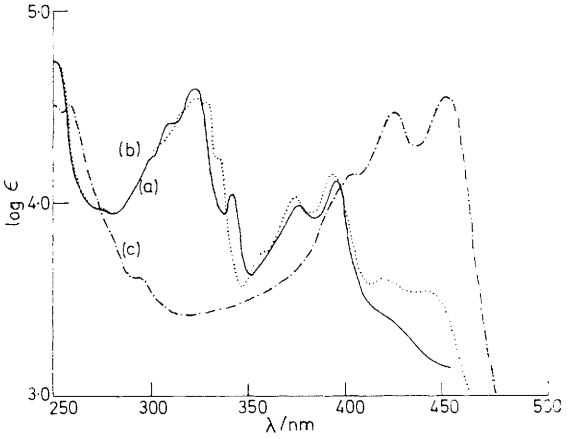


FIGURE 1 U.v. spectra of (a) bulgarhodin leucohexa-acetate, (b) bulgarein leucopenta-acetate, and (c) tetra-acetoxyperylene

The analytical figures and molecular weight determinations show that all the available oxygen atoms are accounted for in the formation of the hexa- and penta-acetates, and this eliminates systems containing bridging oxygen atoms, *e.g.* perixanthenoxanthenes, which show similar absorption in the 325 nm region.[5] The molecular formulae limit the number of possible hydrocarbon skeletons that can be assigned to (I) and (II), and a

[4] A. Calderbank, A. W. Johnson, and A. R. Todd, *J. Chem. Soc.*, 1954, 1285.
[5] R. L. Edwards and N. Kale, *Tetrahedron*, 1965, **21**, 2095.

comparison of the spectra of the leucoacetates with the spectra of the isomeric benzofluoranthenes (Figures 2 and 3) indicated the presence of a benzo[*j*]fluoranthene

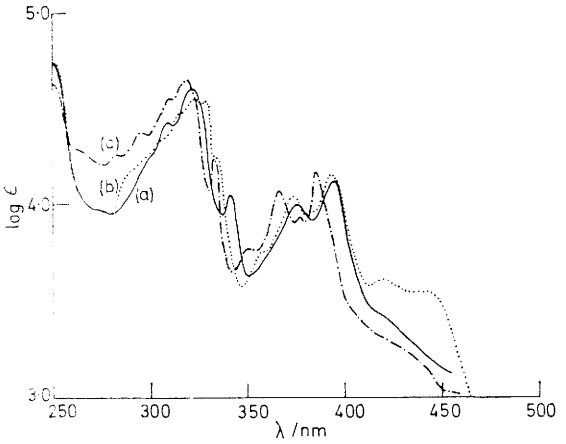


FIGURE 2 U.v. spectra of (a) bulgarhodin leucohexa-acetate, (b) bulgarein leucopenta-acetate, and (c) benzo[*j*]fluoroanthene

nucleus (IV). Bands at 334, 319, and 310 nm in the spectrum of benzo[*j*]fluoranthene correspond closely to similar bands at 341, 322, and 309 nm in that of bulgarhodin leucoacetate and at 336, 328, and 303 nm in that of bulgarein leucoacetate. Also, solutions of the hydrocarbon show green fluorescence similar to those of the leucoacetates. The corresponding absorptions of the benzo[*k*]isomer (V) are at shorter wavelength (325, 309, and 298 nm) and solutions of this hydrocarbon show blue fluorescence. The absence of low intensity absorption above 404 nm in the benzo[*k*]isomer is also significant (Figure 3).

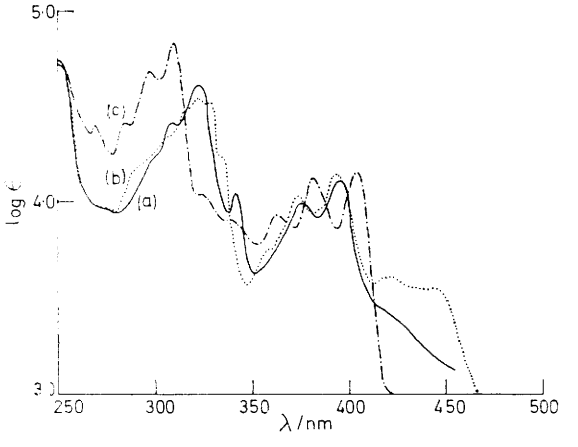


FIGURE 3 U.v. spectra of (a) bulgarhodin leucohexa-acetate, (b) bulgarein leucopenta-acetate, and (c) benzo[*k*]fluoranthene

Bulgarhodin forms a red crystalline tetra-acetate (VI) with acetic anhydride and pyridine and with diazomethane in chloroform solution produces a dimethyl ether (VII). A mixture of mono- and di-bromo-derivatives is produced with bromine in chloroform. Both the pigment and the dimethyl ether yield a green colouration with boric acetic anhydride. The crystalline acetylborates have not been isolated but their evident formation locates at least one and more probably two hydroxy-groups *peri* to a carbonyl group. The pigment does not form a phenazine derivative with *o*-phenylenediamine in boiling acetic acid during 6 h.

Bulgarhodin absorbs in the hydroxy and carbonyl regions of the i.r. at 3 350, 1 660sh, 1 640, and 1 610 cm^{-1}; these bands are absent in the spectrum of the leucoacetate. In the spectrum of the dimethyl ether the reduced hydroxy-absorption at 3 350 cm^{-1} and the positions of the carbonyl absorptions at 1 645 and 1 610 cm^{-1} are as expected for a compound containing *peri*-chelated carbonyl groups. The tetra-acetate absorbs weakly at 1 690 and strongly at 1 660 cm^{-1}; the position of the latter unchelated carbonyl absorption is characteristic of extended quinones. The significance of the weak 1 690 cm^{-1} absorption is, however, unclear; the position is consistent with the presence of *ortho*-quinonoid carbonyl groups but the absence of any similar absorption in the spectrum of the acetylated dimethyl ether (VIII) could indicate the presence of potential tautomerism within the parent pigment. Methylation of *o*-hydroxy-groups prior to acetylation would effectively block such tautomerism and a band at 1 690 cm^{-1} would not be expected in (VIII) (Scheme 1). Tautomerism could also explain our failure to obtain other pure derivatives of (I). Methylations with methyl iodide and dimethyl sulphate under various conditions gave only complex mixtures which have not been separated. Also, a bright blue, unstable monoacetate is produced with acetic anhydride–triethylamine. The high solubility and intense colour of this derivative in comparison with the parent pigment and its other crystalline derivatives suggests that this compound is derived from an alternative tautomeric structure.

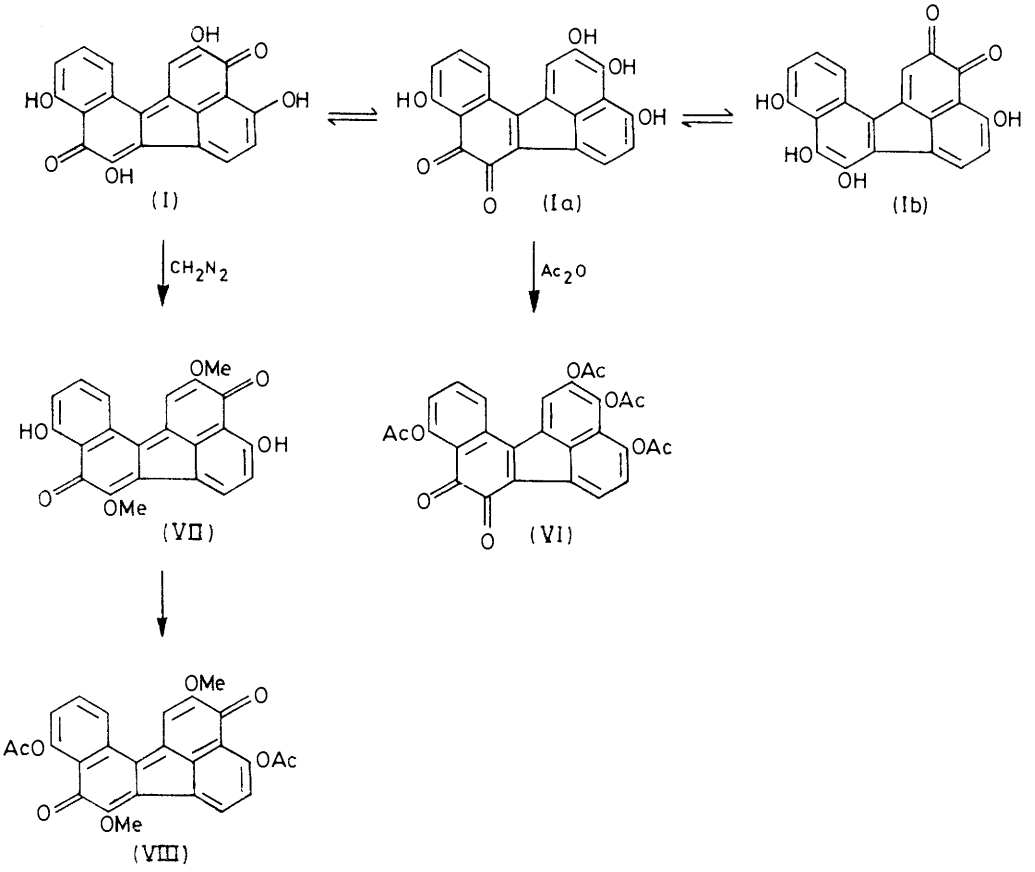
The i.r. spectrum of bulgarein is similar to that of

U.v. absorption maxima $\lambda_{max.}$/nm (log ε)

Benzo[*k*]fluoranthene (V)	249 (4.74)	269 (4.39)	285 (4.41)	298 (4.68)		309 (4.83)		325 (4.03)	338 (391)	363 (3.93)	382 (4.13)		404 (4.15)			
Benzo[*j*]fluoranthene (IV)	245 (4.64)	283 (4.26)	295 (4.37)	310 (4.56)		310 (4.56)		334 (4.25)		351 (3.78)	367 (4.04)	377 (4.11)	386 (4.16)	414 (3.25)	438 (3.00)	
Bulgarhodin leucohexa-acetate	248 (4.76)	274 (3.96)	295 (4.16)	309 (4.41)		322 (4.60)		341 (4.04)			376 (3.99)		395 (4.11)	420 (3.45)	450 (3.15)	
Bulgarein leucopenta-acetate	248 (4.80)	274 (3.96)	284 (4.15)	303 (4.32)	315 (4.47)	323 (4.54)	328 (4.52)	336 (4.24)		358 (3.75)	374 (4.02)	385 (3.89)	393 (4.14)	420 (3.66)	444 (3.53)	
3,4,9,10-Tetra-acetoxyperylene	251 (4.48)	295 (3.47)								403 (4.15)	425 (4.46)		452 (4.54)			
	259 (4.48)															

bulgarhodin but the individual carbonyl absorption bands are less well resolved at 1 640sh and 1 615 cm^{-1}.

Because of solubility difficulties it has only been possible to determine the ^1H n.m.r. spectra of the leucoacetates. Both spectra are relatively simple, and both only show signals (except for acetate absorptions) in the aromatic region between δ 7.24 and 8.61. The acetate protons in the hexa-acetate resonate at δ 2.54 (9 H), 2.58 (6 H), and 2.64 (3 H). Two of the six protons in the aromatic region resonate at lower field than the other four (see Figure 4). One of these signals occurs as a well defined doublet at δ 8.57 (J 8.5 Hz) and the other as a singlet at δ 8.38. A proton giving rise to a triplet at δ 7.68 is coupled to the low-field proton and also to a proton (doublet) resonating at highest field at δ 7.28. A doublet at δ 7.93 (J 8.5 Hz, 1 H) shows coupling to a proton resonating at δ 7.4 (J 8.5 Hz).



SCHEME 1

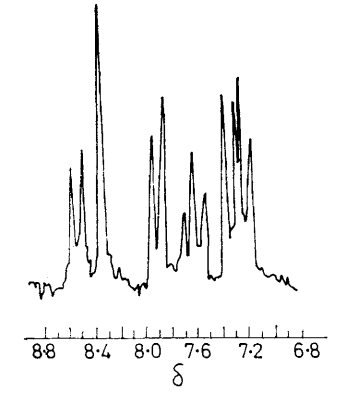


FIGURE 4 Aromatic proton resonances of bulgarhodin leucohexa-acetate in trifluoroacetic acid

The spectrum of bulgarein leucopenta-acetate is similar but the low field singlet is replaced by a doublet at δ 8.34 (J 7.5 Hz) which is coupled to a one-proton doublet at δ 7.31 (J 7.5 Hz). Acetate absorption occurs at δ 2.52 (3 H) and 2.40 (12 H).

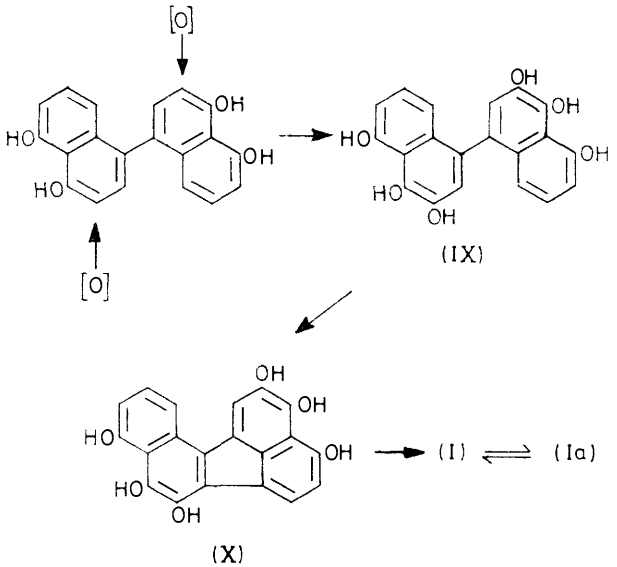
The n.m.r. spectra of benzo[*k*]- and benzo[*ghi*]fluoranthene have been analysed [6,7] but there does not appear to have been any attempt to analyse the complex spectrum of the benzo[*j*]isomer. Also, there is a lack of knowledge of the chemistry of this hydrocarbon; the simple substitution reactions do not appear to have been examined and no quinones have been described.

As an aid to interpreting the spectra and the chemical properties of the two pigments, the biosynthetic origin of the benzo[*j*]fluoranthene nucleus might be considered. It has been established that perylene derivatives can be produced by oxidative coupling of naphtha-

[6] M. L. Heffernan, A. J. Jones, and P. J. Black, *Austral. J. Chem.*, 1967, **20**, 589.

[7] K. D. Bartle, D. W. Jones, and J. E. Pearson, *J. Mol. Spectroscopy*, 1967, **24**, 330.

lene derivatives,[8] and 4,5,4′,5′-tetrahydroxy-1,1′-binaphthyl is considered to be the precursor of 4,9-dihydroxyperylene-3,10-quinone in *Daldinia concentrica*.[3] Because this latter quinone occurs with bulgarhodin and bulgarein in *Bulgaria inquinans* it is reasonable to assume that similar precursors are involved in their formation. In order to produce the benzo[*j*]fluoranthene nucleus, a *para,meta*-coupling of a binaphthyl derivative would be required (Scheme 2); this is unlikely in 4,5,4′,5′-tetrahydroxy-1,1′-binaphthyl itself but should be possible if further hydroxy-groups are introduced into



SCHEME 2

the 3- and 3′-positions (IX). The phenol (X) produced from (IX) by coupling would yield the quinones (I) and/or (Ia) on oxidation.

Structure (I) [and/or (Ia)] for bulgarhodin does explain the ^{1}H n.m.r. spectrum of the leucoacetate remarkably well. In common with the angular protons in other angular hydrocarbons, the proton at position 12 would be expected to resonate at lowest field,[9] and the doublet at δ 8.57 is assigned to this proton. The expected additional splitting of this doublet due to H-10 must be small and is not observed. The triplet centred at δ 7.68 is assigned to H-11 and the doublet (also unsplit) at δ 7.28 to H-10. The singlet, also at low field would then be due to H-1 and the remaining two protons at positions 5 and 6 would contribute the AB double doublet. The spectrum of bulgarein leucoacetate is also explained: replacement of the 2-hydroxy-group in bulgarhodin leucoacetate by hydrogen will result in the replacement of the low-field singlet by a doublet and the appearance of a new doublet at higher field. The structure (I) for bulgarhodin also explains the formation of the tetra-acetate and the dimethyl ether, and explains the i.r. spectra of these compounds.

The mass spectrum of bulgarhodin shows a prominant molecular ion at 346 (100%); the only other significant peaks are at 347 (M + 1, 25%) and 318 (M − 28, 15%). Bulgarein similarly shows peaks at 330 (M, 100%), 331 (M + 1, 25%), and 302 (M − 28, 18%). The molecular ion of bulgarhodin leucohexa-acetate (*m/e* 600) loses successively six acetate groups as keten to yield the parent quinol ion at 348 (50%); loss of a proton from this ion produces the base peak at 347 and further loss of a proton produces the ion at 346 (47%) corresponding to the quinone. A weak peak corresponding to the loss of 28 mass units from the quinone occurs at 318 (18%). The fragmentation of bulgarein leucopenta-acetate is similar: successive loss of five keten units from the molecular ion at *m/e* 542 produces the quinol ion at 332 (83%), this then loses a proton to give 331 (100%) and then a further proton to give the weak 330 ion (15%) corresponding to the quinone. In the spectrum of the dimethyl ether (VII) the molecular ion at 374 is the base peak and the only other significant peaks occur at 359 (M − 15, 24%) and 345 (M − 15 − 14, 30%). The fragmentation of the acetylated dimethyl ether (VIII) follows a similar pattern; the molecular ion at 458 loses two keten units to yield the base peak at 374 which then loses successively 15 and 14 mass units to yield ions at 359 (24%) and 345 (30%).

The fragmentation of bulgarhodin tetra-acetate (VI) is different: the molecular ion appears at 516 (M + 2) and this successively loses two keten units to yield 474 and 432. The latter ion is accompanied by an ion at 430 of equal intensity and it is this 430 ion which successively loses two more keten units to yield ions at 388 and 346. The 346 peak is the base peak and is the molecular ion of bulgarhodin.

Bulgarein triacetate behaves similarly, producing a molecular ion at 458 (M + 2) which loses successively two keten units to yield ions at 416 and 374. There is also an ion at 372 which is of the same intensity as the 374 ion. The 372 ion loses 42 mass units to give the base peak at 330 corresponding to bulgarein.

This fragmentation pattern supports our suggestion that bulgarhodin tetra-acetate and bulgarein triacetate are derivatives of an *ortho*-quinonoid structure; it is well established that *ortho*-quinones yield a prominent M + 2 ion owing to reduction of the quinone by water in the mass spectrometer[10] and in the case of these particular acetates the M + 2 ion is the only molecular ion observed. If the sequence of loss involves first the acetate from the position *peri* to the carbonyl group and then the acetate from position 3 (Scheme 3) then it is only after the loss of the latter acetate group that the molecule can revert to the more stable *para*-quinonoid structure; this results in the appearance of the normal quinone diacetate ion. Loss of two keten units from this ion then produces the quinone ion at 346. The spectrum of bulgarein triacetate can be explained in a similar way and this fragmentation pattern is supported

[8] D. W. Cameron and H. W. S. Chan, *J. Chem. Soc.*, 1966, 1825.

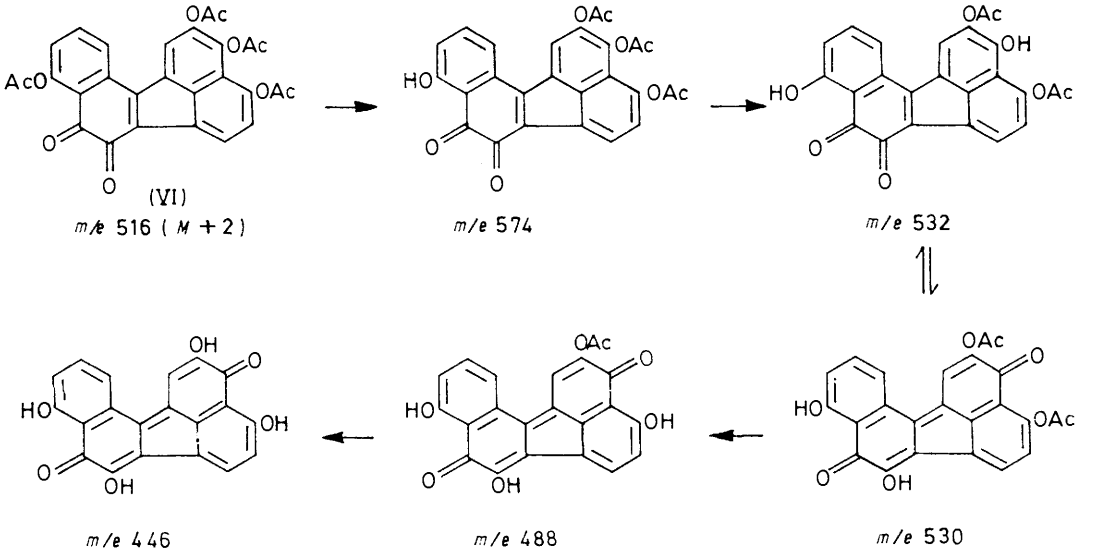
[9] R. H. Martin, *Tetrahedron*, 1964, **20**, 897.

[10] K. P. Zeller, ' The Chemistry of the Quinonoid Compounds, Part I,' ed. S. Patai, Wiley, London, 1974, p. 248.

by a change in the intensity of the peaks and the appearance of $M + 4$ peaks when D_2O is introduced with the sample into the mass spectrometer.[11]

To our knowledge there has been no previous report of the occurrence of benzo[*j*]fluoranthene derivatives as natural products. The hydrocarbon is a known carcinogen and most work has been directed towards detecting the hydrocarbon either as an atmospheric contaminant or as a product of atmospheric contamination. The parent hydrocarbon has been detected in phytoplankton [12] and in tree leaves [13] and in the latter case the largest quantities were found to occur in yellowing autumn leaves; the actual origin of the hydrocarbon from these sources is however unknown. An examination of certain forest soil samples taken from 5 cm below ground level has shown the presence of the hydrocarbon and in this case the origin is considered to be phytochemical.[14,15] Most of these examinations have used methods which are only applicable to the separation and detection of the hydrocarbon, and such methods are unlikely to detect quinonoid compounds of the type described in this paper. It is reasonable to assume that other compounds derived from this nucleus do occur naturally. We have examined extracts from the fungus *Daldinia concentrica* for compounds based on this nucleus without success.

Work on the other pigments from this fungus and on the chemistry of benzo[*j*]fluoranthene will be published later.



SCHEME 3

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus, i.r. spectra with a Perkin-Elmer 237 spectrophotometer, u.v. spectra with a Unicam SP 800 spectrophotometer, ^1H n.m.r. spectra with a JEOL JNM-MH-100 spectrometer for solution in CF_3CO_2H, and mass spectra with an A.E.I. MS9 spectrometer. All thin-layer (t.l.c.), preparative layer (p.l.c.), and column chromatography was performed on Merck Kieselgel $PF_{255+366}$. Preparative layers consisted of silica gel (16 g) on 20 × 20 cm glass plates.

Isolation of the Pigment Mixture.—Freshly collected apothecia of *Bulgaria inquinans* were quickly air-dried, and the dried material (1.5 kg) was extracted (24 h) with chloroform (3 l) (Soxhlet). The black amorphous solid (0.5 g) which separated during the extraction was filtered off and re-extracted (3 days) (Soxhlet) with chloroform until the extracting solvent was almost colourless. The crude pigment deposited (150 mg) was crystallised from dimethyl sulphoxide (10 ml) to yield small purple hair-like needles which were only sparingly soluble in cold dimethyl sulphoxide and were almost insoluble in other commonly used solvents.

Bulgarhodin Leucohexa-acetate, Bulgarein Leucopentaacetate and 3,4,9,20-Tetra-acetoxyperylene.—A suspension of the crude pigment (500 mg) (from the initial extraction) in acetic anhydride (30 ml) was heated under reflux. Fused sodium acetate (10 mg) was added; the purple solution quickly turned blue. Zinc dust (50 mg) was added in portions over 15 min; during the addition the colour changed through red to a fluorescent yellow-green. Heating was continued for 30 min, then the solution was filtered hot and the residual zinc washed with acetic acid (20 ml). Hydrolysis of the combined filtrates in water (250 ml) yielded the yellow leucoacetate mixture (345 mg). The crude solid was extracted with benzene–ethyl formate–formic acid (75 : 25 : 1, 3 × 50 ml); bulgarhodin leucohexa-acetate remained undissolved (88 mg). The combined extracts were applied to a column of silica gel (80 × 3.5 cm) and the column was eluted with the same solvent mixture. Three yellow bands were eluted. Band 1 gave a yellow crystalline solid which after recrystallisation from

[11] S. Ukai, K. Hirose, A. Talematsu, and T. Goto, *Tetrahedron, Letters*, 1967, 4999.
[12] J. Borneff and R. Fischer, *Arch. Hyg. Bacteriol.*, 1962, **146**, 334.
[13] W. Graef and H. Diehl, *Arch. Hyg. Bacteriol.*, 1966, **150**, 49.
[14] J. Borneff and R. Fischer, *Arch. Hyg. Bacteriol.*, 1962, **146**, 430.
[15] J. Borneff and H. Kunte, *Arch. Hyg. Bacteriol.*, 1963, **147**, 401.

acetic anhydride gave yellow needles of tetra-acetoxyperylene (57 mg), m.p. >300° (Found: C, 69.7; H, 4.35. Calc. for $C_{28}H_{20}O_8$: C, 69.4; H, 4.1%). The yellow crystalline solid from band 2 gave yellow needles of *bulgarein leucopenta-acetate* (70 mg), m.p. 234—237° after recrystallisation twice from acetic acid (Found: C, 66.7; H, 3.8%; M^+, 542.124 257. $C_{30}H_{22}O_{10}$ requires C, 66.4; H, 4.1%; M, 542.121 284); $\nu_{max.}$ 1 770 cm^{-1}. The yellow crystalline solid from band 3 gave yellow needles of *bulgarhodin leucohexa-acetate* (64 mg), m.p. 274—276° after recrystallisation twice from acetic acid (Found: C, 64.3; H, 3.9%; M^+, 600.122 862. $C_{32}H_{24}O_{12}$ requires C, 64.0; H, 4.0%; M, 600.126 761); $\nu_{max.}$ 1 775 cm^{-1}.

Bulgarhodin (2,4,7,9-*Tetrahydroxybenzo*[j]*fluoranthene*-3,8-*quinone*) (I).—A mixture of bulgarhodin leucohexa-acetate (125 mg), ethanol (50%; 20 ml), and sodium hydroxide solution (2N; 2 ml) was refluxed for 4 h under nitrogen. After cooling, sulphuric acid (2N; 2.5 ml) was added, and air was passed through the solution for 36 h. The black solid slowly deposited was filtered off and washed with water (10 ml) and then with ethanol (10 ml). Crystallisation and recrystallisation from dimethyl sulphoxide gave long purple hair-like needles (32 mg) of *bulgarhodin*, m.p. >300° (Found: C, 69.05; H, 3.2%; M^+, 346.047 621. $C_{20}H_{10}O_6$ requires C, 69.4; H, 2.9%; M, 346.047 731); $\lambda_{max.}$ (H_2SO_4) 258, 324, 348, 410, 450sh, and 670 nm (log ε 4.45, 3.97, 3.91, 4.03, 3.80, and 4.00), $\lambda_{min.}$ 305, 340, 372, and 514 nm (log ε 3.91, 3.91, 3.84, and 3.50); $\lambda_{max.}$ ($CHCl_3$, sat. soln.) 254, 294, 312, 338sh, 359, 402, 514sh, 542, and 670 nm, $\lambda_{min.}$ 282, 303, 323, 377, 452, 600, and 750 nm.

Bulgarein (4,7,9-*Trihydroxybenzo*[j]*fluoranthene*-3,8-*quinone*) (II).—A mixture of the leucopenta-acetate (22 mg), ethanol (3 ml), and sulphuric acid (2N; 2 ml) was refluxed for 6 h. The acetate slowly dissolved to produce an orange solution exhibiting a green fluorescence. Air was passed through the cooled solution for 18 h, and the black precipitate (11.5 mg) was filtered off, washed, and dried. Crystallisation from nitrobenzene over several days gave purple needles (8 mg) of *bulgarein*, m.p. >300° (Found: M^+, 330.052 179. $C_{20}H_{10}O_5$ requires M, 330.052 817); $\lambda_{max.}$ (EtOH) (purple soln.) 253, 300sh, 372, 565sh, and 660 nm, $\lambda_{min.}$ 326 and 460 nm; $\lambda_{max.}$ (EtOH) (blue soln.) 253, 300sh, 372, 400, and 636, $\lambda_{min.}$ 330 and 470 nm.

Bulgarhodin 2,7-*Dimethyl Ether* (VII).—A suspension of bulgarhodin (21 mg) in chloroform (60 ml), was heated under reflux for 30 min. Excess of ethereal diazomethane was added to the cooled suspension and the mixture set aside for 3 h. The solution was filtered and the dark red solid washed with ethanol (10 ml). The residue (8 mg) was crystallised from dimethyl sulphoxide to yield red needles of the *ether* m.p. >300° (Found: M^+, 374.080 800. $C_{22}H_{14}O_6$ requires M, 374.079 030); $\nu_{max.}$ 3 405br, 1 645, 1 610, 1 590, and 1 530 cm^{-1}. The ether dissolves in 2N-hydroxide to yield a blue-green solution which rapidly deposits an insoluble green sodium salt.

Bulgarhodin Tetra-acetate (VI).—Acetic anhydride (6 ml) was added to a suspension of bulgarhodin (74 mg) in pyridine (3 ml) and the mixture set aside for 90 min at room temperature. The bulgarhodin quickly dissolved over 5 min and a red crystalline solid was slowly deposited. The solution was filtered and the crystalline residue washed with acetic anhydride to yield red needles of the *tetra-acetate* (35 mg), m.p. >300° [Found: C, 65.45; H, 3.4%; (M^+ + 2), 516.109 638. $C_{28}H_{18}O_{10}$ requires C, 65.35; H, 3.5%); (M + 2), 516.105 635]; $\nu_{max.}$ 1 770, 1 690, 1 660, and 1 590 cm^{-1}.

Acetylation of Bulgarhodin Dimethyl Ether.—Acetic anhydride (1 ml) was added to a solution of the ether (3 mg) in pyridine (0.25 ml) at room temperature. After 1 h the dark red crystalline solid was filtered off and dried to yield the *diacetate* (2.7 mg), m.p. 238° (Found: M^+, 458.100 474. $C_{26}H_{18}O_8$ requires M, 458.100 157); $\nu_{max.}$ 1 765, 1 630, and 1 595 cm^{-1}.

Bulgarhodin Dibromide.—Excess of bromine in chloroform was added to a suspension of bulgarhodin (16.2 mg) in chloroform (10 ml) maintained at 60 °C. The mixture was set aside for 12 h. The pigment dissolved over 20 min and after 30 min a black solid began to separate. The solution was filtered and the solid (15 mg) recrystallised from nitrobenzene to yield purple hair-like needles (8 mg) of the dibromide. Analysis and mass spectroscopy showed the presence of some monobromo-derivative, which could not be removed by recrystallisation (Found: M^+, 501.872 07. Calc. for $C_{20}H_8Br_2O_6$: M, 501.868 86).

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