# Constituents of The Higher Fungi.  Part XIV.[1]  3′,4′,4-Trihydroxypulvinone, Thelephoric Acid, and Novel Pyrandione and Furanone Pigments from *Suillus grevillei* (Klotsch) Sing. [*Boletus elegans* (Schum. per Fries)]

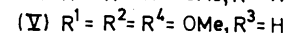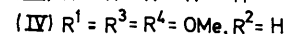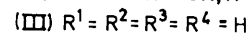By **Raymond L. Edwards** * and **Melvyn Gill**, School of Chemistry, University of Bradford, Bradford BD7 1DP
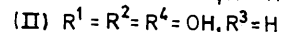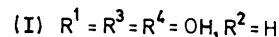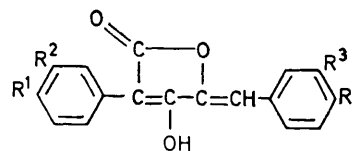
Eight crystalline pigments have been isolated from the fungus *Suillus grevillei*. 3′,4′,4-Trihydroxypulvinone, a novel pyrandione, and a novel furanone are described, and thelephoric acid is identified as the cap skin pigment of *Suillus grevillei var. badius*

THE common larch mushroom, *Suillus grevillei* (*Boletus elegans*) has yellow tubes which turn pink on bruising and a stem which shows a variable colour change but usually changes to blue or green in young specimens. During this work two distinct forms of this mushroom were encountered, one with a brown and the other with a bright yellow cap; the two forms were usually found growing together and differed only in the cap skin constituents. The former is *Suillus grevillei* (Klotsch) *var. badius* (Sing.) and the latter is the type species *Suillus grevillei* (Klotsch) Sing. The cap skins of the brown form have yielded thelephoric acid, protocatechuic acid, and protocatechualdehyde by extraction with alcohol. No thelephoric acid occurs in the skins of the bright yellow variety and the closely related, brown capped, *Boletus* (*Suillus*) *luteus* (Linn per Fr.) S. F. Gray. The latter has often been confused with *S. grevillei*.[2]

The pigments from the cap (not cap skin) and stem were isolated *via* the lead salts. The crude lead salt precipitate from this mushroom is reddish in colour; this distinguishes the species from others such as *S. bovinus*, which yields an olive green salt, and *S. variegatus* and the true boletes, which yield brown or yellow salts.

The crude extract contains at least eleven pigments; these were detected by the characteristic colours pro-

duced with sodium hydroxide solution or concentrated sulphuric acid (Table). Unlike xerocomic and variegatic acids, some of the pigments from this species yield stable red, yellow, blue, and green colours with sodium hydroxide solution. The quantity of each pigment present was small and varied with the season. However, seven pigments have been obtained crystalline and we describe here the isolation of and propose the structure for three of these ($B_3$, $B_1$, and A in the Table). Variations occurred, particularly among the pigments



(I)  $R^1 = R^3 = R^4 = OH, R^2 = H$
(II)  $R^1 = R^2 = R^4 = OH, R^3 = H$
(III)  $R^1 = R^2 = R^3 = R^4 = H$
(IV)  $R^1 = R^3 = R^4 = OMe, R^2 = H$
(V)  $R^1 = R^2 = R^4 = OMe, R^3 = H$

of group B; the pigment responsible for the intense green colour with sodium hydroxide in the 1969 crop ($B_1$) was absent in the 1971 crop and was replaced by a pale yellow compound ($B_3$) which gave only a yellow colour with sodium hydroxide solution.

Trihydroxypulvinone [$B_3$ (I)], $C_{17}H_{12}O_6$, m.p. 291° (decomp.), which separates as greenish-yellow needles

¹ Part XIII, R. L. Edwards and M. Gill, *J.C.S. Perkin I*, 1973, 1538.
² R. Watling, *Trans. Bot. Soc. Edinburgh*, 1965, **40**, 100.

and plates, forms a tetra-acetate and a tetramethyl ether. Oxidation of the tetra-acetate and hydrolysis of the oxidation products yielded a mixture of 4-hydroxy-benzoic acid and 3,4-dihydroxybenzoic acid. The u.v. spectrum was similar to those of isoxerocomic acid [3] and methyl isoxerocomate. The pigment differs from xerocomic acid, methyl xerocomate, and variegatic acid in showing no blue colour when dilute solutions are treated with ammonium hydroxide solution. The

acid analogues which possess a 3',4',4-substitution pattern.[3]

The pulvinone structure was proved by synthesis of the tetramethyl ether. Pulvinone may be readily prepared either from pulvinic acid by decarboxylation in hot quinoline in the presence of copper chromite catalyst,[5] or by thermal rearrangement of 2,5-diphenyl-cyclopentane-1,3,4-trione.[6] However, our attempts to decarboxylate trimethoxypulvinic acid by the former

| Group | Compd. | Physical state | Yield (mg) 1969 | Yield (mg) 1971 | Tube nos. | $R_F$ | | Colour reaction on silica +NaOH | +conc. $H_2SO_4$ |
|---|---|---|---|---|---|---|---|---|---|
| A | A | Red needles | 26 | 11 | 35—38 | 0·59 | Yellow | Blue ⟶ Red Violet * | Cherry red * |
| B | B₁ | Orange needles | 132 | | 50—57 | 0·52 | Yellow | Green * | Violet * |
| | B₂ | Red needles | 8 | | 50—57 | 0·47 | Grey-brown | Blue ⟶ Grey | Purple * |
| | B₃ | Lustrous yellow plates | | 194 | 58—62 | 0·49 | Pale yellow U.v.-fluorescent | Yellow | Yellow * |
| | B₄ | Yellow needles | | 20 | 45—54 | 0·53 | Pale yellow Very fluorescent | Salmon pink * | Blue |
| C | C₁ | Red gum | | | 76—86 | 0·41 | Colourless | Green * | Transient green |
| | C₂ | Dark red needles | 20 | 18 | 79—85 | 0·38 | Grey | Grey | Pale violet |
| D | D₁ | Orange needles | 39 | 18 | 101—109 | 0·23 | Orange | Red * | Red * ⟶ Orange ⟶ Yellow |
| | D₂ | Brown gum | | | 114—120 | 0·20 | Yellow-brown | Brown | Transient green |
| E | E | Brown gum | | | 132—140 | 0·10 | Yellow-green | Green * | Violet * |

* Denotes stable colour.

reagent produces a shift in absorption to 259 and 386 nm which is similar to the shift produced by alkali in the spectra of isoxerocomic acid (to 285 and 378 nm) and methyl isoxerocomate (to 263 and 386 nm) and different from the shift produced in the case of methyl xerocomate (to 246, 396, and 601 nm).

In the i.r., strong absorption at 1694 cm⁻¹ (KBr) is characteristic of a chelated ester or acid; this occurs at higher frequencies in the spectra of the tetra-acetate and the tetramethyl ether. The absence of absorption above 1800 cm⁻¹ in the spectrum of the tetra-acetate proved the absence of a dilactone structure of the type present in xerocomic or variegatic acid dilactone acetates. The low carbonyl frequency for compound (I) can be explained in terms of intermolecular hydrogen bonding; this was proved by measuring the carbonyl frequency for a solution in dioxan,[4] in this solvent the carbonyl absorption appears at 1754 cm⁻¹ and this shift is similar to that from 1697 (KBr) to 1767 cm⁻¹ (dioxan) in the case of pulvinone (III).

Structure (I) is supported by ¹H n.m.r. measurements; the methine signal appears at τ 3·5 (pulvinone, τ 3·29) and the four hydroxy-protons resonate as a single broad peak at τ 6·35. In the spectra of the tetra-acetate and the tetramethyl ether, the methine signals appear at τ 3·94 and 3·70, respectively, similar in position to the methine absorptions of O-acetylpulvinone and O-methylpulvinone (τ 3·70 and 3·62). In the aromatic region a low-field two proton doublet at τ 2·06 is coupled to a doublet at τ 3·06. This pattern and the position of one doublet near τ 2·0 compare well with the aromatic pattern observed for other pulvinic

method yielded only a complex gummy mixture. With cupric acetate in organic solvents, trimethoxy-pulvinic acid (VI) readily forms a crystalline copper chelate, $C_{42}H_{34}CuO_{16},7H_2O$, soluble in organic solvents. Heating in vacuo gave a tetrahydrate, which was readily decarboxylated in hot quinoline to yield an isomeric mixture of 2-(3,4-dimethoxyphenyl)-5-(4-methoxy-phenyl)cyclopentane-1,3,4-trione (VII) and trimethoxy-pulvinones [(IV) and (V)]. The identity of (VII) was confirmed by spectroscopic comparison with 2,5-di-phenylcyclopentane-1,3,4-trione [3] (VIII).



(VI)



(VII) R¹ = R² = R³ = OMe
(VIII) R¹ = R² = R³ = H
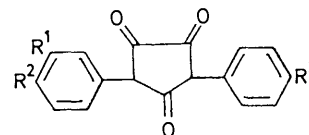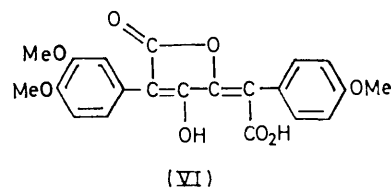
The synthetic trimethoxypulvinone mixture showed a strong carbonyl absorption at 1702 cm⁻¹ similar to that of pulvinone at 1697 cm⁻¹. In the ¹H n.m.r. spectrum

[3] R. L. Edwards and M. Gill, J.C.S. Perkin I, 1973, 1529.
[4] B. Akermark, Acta Chem. Scand., 1961, 15, 985, 1695.
[5] A. Schonberg and A. Sina, J. Chem. Soc., 1946, 601.
[6] L. Claisen and T. Ewan, Annalen, 1895, 284, 245.

two singlets at τ 3·38 and 3·40 (cf. the single absorption at τ 3·29 in pulvinone) showed the presence of two isomers, which was confirmed by the formation of two isomeric tetramethyl ethers with diazomethane; these were separated by fractional crystallisation. The isomeric ethers differ in m.p. (151 and 181°) but the large differences in u.v. and ¹H n.m.r. spectra usually encountered in normal and iso-pulvinic acid derivatives are absent. However, in the case of the lower melting isomer the four methoxy-groups give rise to four distinct singlets, whereas only two are observed in the higher melting compound. Comparison with the tetramethyl ether from (I) established the identity of the latter with the low-melting isomer.

The orientation of substituents in B₃ cannot be determined easily by chemical methods. A 3′,4′,4-orientation is suggested from the u.v. and ¹H n.m.r. spectra but conclusive proof was sought from the mass spectrum. However, before definite fragmentation assignments could be made it was necessary to examine the fragmentation of cyclopentene-1,3-diones and also
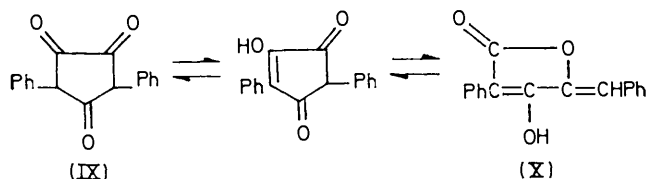


of pulvinone. Previous investigations into the behaviour of acyclic 1,3-diones,[7,8] cyclohexane-1,3-diones,[9,10] and alkylcyclopentane-1,3-diones [11] have revealed that such systems give equilibrium mixtures of diketone and enol molecular ions which exhibit their own fragmentation patterns. Fragmentation of cyclic diones involves ring fission and the expulsion of stable molecules such as CO, keten, and ethylene.[9-11] In the molten state 2,5-diphenylcyclopentane-1,3,4-trione (IX) exists in thermal equilibrium with the enolic γ-lactone, pulvinone [5] (X) and the mass spectra of both of these would therefore be expected to be identical. This is so: the spectra (Figure 1) show an abundant molecular ion (m/e 264), presumably originating from a mixture of molecular ions (a—d) which yields the primary fragments m/e 118 and 145 by either of two mechanisms (Figure 2); both of these ions then fragment further by loss of CO. An unusual fragment (m/e 191), probably the diphenylcyclopropene cation (XI), arises from the parent ion by loss of C₂HO₃.

The mass spectrum of the natural pigment is similar to that of pulvinone and an analogous analysis can be employed to explain the observed fragmentation pattern. However, since the two possible positional isomers [(I) and (II)] will be in thermal equilibrium

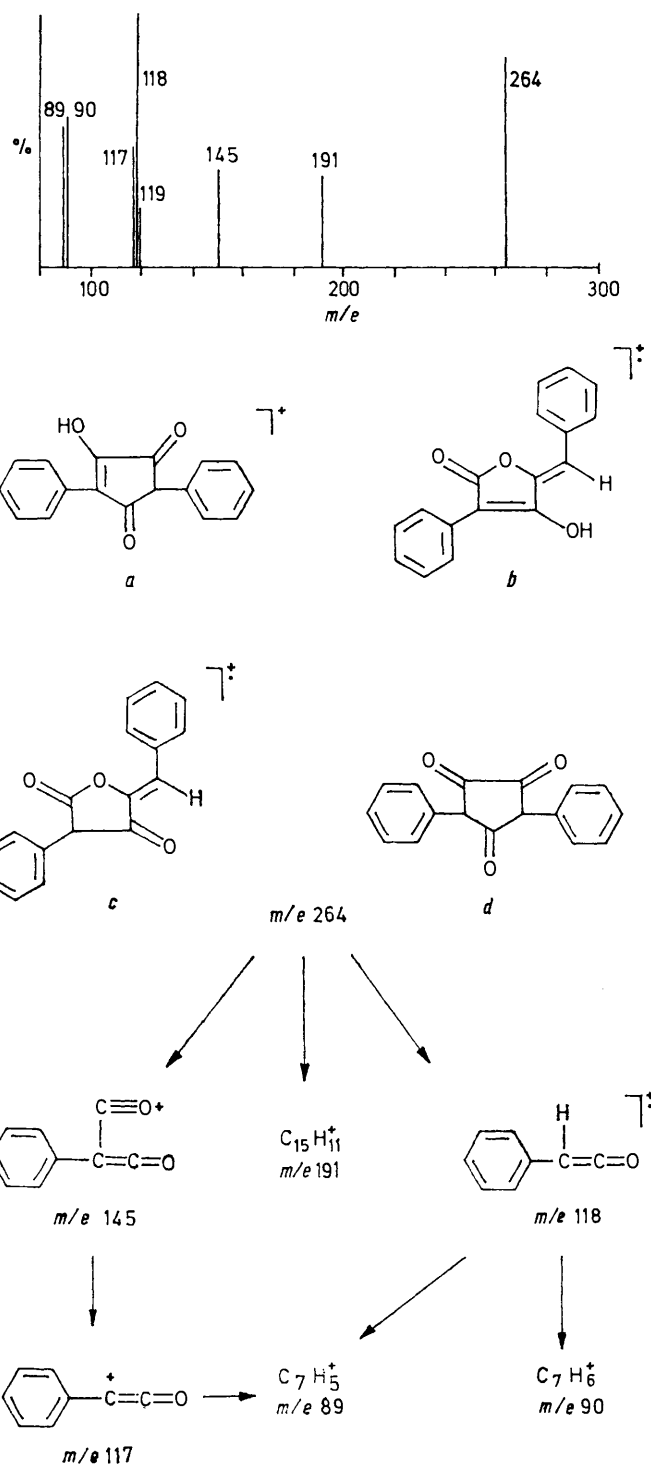with the same triketone, the mass spectrum cannot distinguish between the two structures.



FIGURE 1

Although the methyl ethers of the triketone (IX) and pulvinone (X) cannot be thermally interconverted

[7] N. Schamp and M. Vandewalle, Bull. Soc. chim. belges, 1966, 75, 539.
[8] N. Schamp and M. Francque, Bull. Soc. chim. belges, 1967, 76, 528.
[9] T. Goto, A. Tatematsu, Y. Nakajima, and H. Tsuyama, Tetrahedron Letters, 1965, 757.
[10] O. H. Mattsson, Acta Chem. Scand., 1968, 22 (8), 2479.
[11] E. Cant and M. Vandewalle, Org. Mass. Spectrometry, 1971, 5, 1197.

in the laboratory, the mass spectra of the compounds are identical and can be rationalised in terms of initial cleavage followed by successive losses of CO. As before, it is impossible to relate large fragments to a
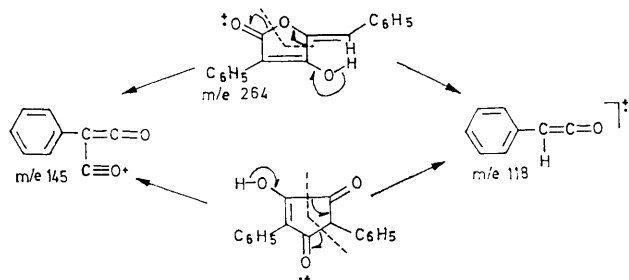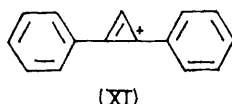


FIGURE 2



(XI)

single molecular ion. In this case the hydrocarbon ion $m/e$ 179 ($C_{14}H_{11}^+$) replaces the diphenylcyclopropene ion $m/e$ 191 ($C_{15}H_{11}^+$) found in the spectrum of the parent compounds.

By analogy it is to be expected that the two known isomeric $O$-methyl trimethoxydiphenylcyclopentenediones[3] would form a thermal equilibrium mixture with their respective $O$-methyl trimethoxypulvinone isomers. Any difference in the spectra should then permit identification of each pulvinone derivative and hence unambiguously establish the structure of the natural pigment. The diketone (XII) is isomeric with 3,4,4'-trimethoxy-$O$-methylpulvinone (XIII) and the diketone (XIV) with 3',4',4-trimethoxy-$O$-methylpulvinone (XV) and, as expected, (XII) and (XIV) show different fragmentations. Also, the spectra of the synthetic low melting tetramethyl ether and the tetramethyl ether from the natural pigment were identical with that from (XIV); this establishes structure (I) for the natural isomer. Apart from the direct comparison the same conclusion can be reached from an analysis of the fragmentation pattern.

Compound (XII) shows several fragment ions, which correspond in mass to monomethoxy- and dimethoxy-analogues of ions observed in the spectrum of the unsubstituted methyl ether. Fragmentation of (XII) involves cleavage to ions $m/e$ 148 and 205 which then lose successive molecules of CO. Ions at $m/e$ 177 and 149, and 148 and 120 correspond to dimethoxy- and monomethoxy-analogues respectively of ions at $m/e$ 117 and 89, and 118 and 90 in the spectrum of the methoxydiphenyl analogue. The ion at $m/e$ 269 appears to be a trimethoxylated analogue of the ion $m/e$ 179 in the spectrum of the unsubstituted ether and is presumably a protonated analogue of the diphenyl-acetylene fragment found in the spectra of pulvinic lactones. The spectrum of (XIV) is similar but the ion $m/e$ 148 in (XII), which appears to originate from a

molecular ion in which the methine proton is adjacent to the monomethoxylated aryl ring, is replaced by an ion $m/e$ 178, corresponding to a system with the same proton adjacent to the dimethoxylated residue.

The isolation of 3',4',4-trihydroxypulvinone from *S. grevillei* provides an example of the natural occurrence of a logical biosynthetic precursor to involutin. The orientation of the hydroxylated aryl rings in the pulvinone derivative is the same as that in involutin and in isoxerocomic acid, and supports the idea that involutin may be a product of the natural degradation of isoxerocomic acid.

The complete absence of trihydroxypulvinone ($B_3$) in one fungus crop and its apparent replacement by the orange-red pigment $B_1$ suggested a possible relation between the two pigments and prompted a chemical examination of this compound.

Pigment $B_1$ [(XX) or (XXI)], m.p. 275° (decomp.) crystallised as red or yellow solvated needles and plates. Addition of ammonia to a 10% ethanolic solution produces an intense green colouration, stable for up to 20 min; this distinguishes the compound from the 'blueing' pulvinic acid pigments, which give less stable colourations. The green colour produced on addition of sodium hydroxide solution fades over 4 min. The pigment dissolves in concentrated sulphuric acid to give a stable violet colouration.
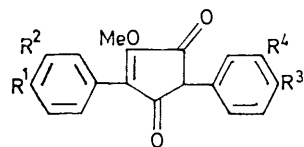


(XII) $R^1 = R^2 = R^3 =$ OMe, $R^4 =$ H
(XIV) $R^1 = R^3 = R^4 =$ OMe, $R^2 =$ H
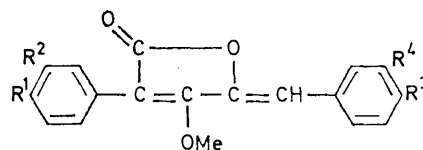


(XIII) $R^1 = R^2 = R^3 =$ OMe, $R^4 =$ H
(XV) $R^1 = R^3 = R^4 =$ OMe, $R^2 =$ H

Pigment $B_1$ forms a yellow crystalline tetra-acetate which on oxidation, followed by hydrolysis of the products, gave 4-hydroxy- and 3,4-dihydroxy-benzoic acids. Attempts to methylate the pigment did not yield crystalline products. Methylation with diazomethane gave a complicated mixture and treatment with dimethyl sulphate gave a yellow oil which decomposed during purification. Remethylation of the decomposition products gave the original yellow ether.

The mass spectrum of $B_1$ shows a close similarity to that of 3',4',4-trihydroxypulvinone (Figure 3), with common ions at $m/e$ 312, 239, 161, 150, 134, 133, 123,

122, and 105; additional ions occur however at $m/e$ 340 ($M^+$), 323, 295, and 163. The ions at $m/e$ 150, 122 and 105 and at $m/e$ 161 and 133 are characteristic of the
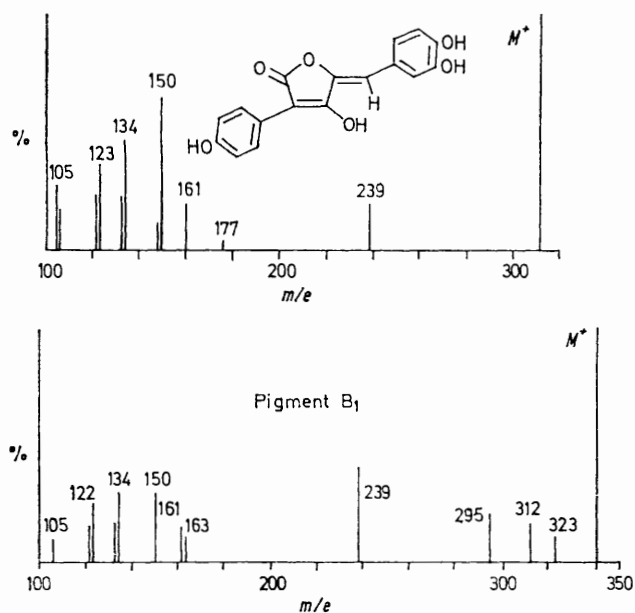


FIGURE 3

aryl residues and are indicative of the presence of a methine proton on the carbon atom adjacent to the dihydroxyaryl residue. In the $^1H$ n.m.r. spectrum hydroxy-absorption occurs at $\tau$ 1·24 (3H, broad) and 6·8 (1H, broad). The remaining eight-proton absorption between $\tau$ 2·15 and 2·97 comprises seven aromatic protons and a methine proton ($\tau$ 2·86). Similar low field methine absorption occurs in the spectrum of the acetate and this distinguishes the pigment and its

nucleus necessitates inclusion of the remaining oxygen atom as a second carbonyl group.

Of the isomeric possibilities (XVI)—(XIX), structures (XVI) and (XIX) can be dismissed since they do not possess the $(OH)_2C_6H_3$–CH–C–O sequence necessary for the ready formation of the $m/e$ 150 ion in the mass spectrum. Thus, by arranging the hydroxyaryl and hydroxy-groups in such a way that conjugation is at a maximum, and taking into consideration the possible relationship with pulvinone, two possible structures, (XX) and (XXI), can be written for $B_1$. These represent keto-lactone analogues of the 3',4',4-trihydroxy-pulvinone structure.

During the preparation of this paper, Steglich et al.[12] reported the isolation of grevillins A, B, and C from this fungus. The structure proposed for grevillin B is identical with (XXI). However, some of the physical data reported for the grevillin B differs from those reported here for $B_1$. In particular grevillin B absorbs at 1715—1705 (cf. $B_1$, 1736 cm$^{-1}$) and its methine and aromatic protons are assigned $\tau$ values 0·20—0·24 p.p.m. to higher field than those observed for $B_1$. The shift in carbonyl frequency on acetylation of $B_1$ is much smaller than in the case of grevillin B, and it is possible that $B_1$ possesses the alternative structure (XX). It is significant that methyl pyruvate shows only a single carbonyl absorption, at 1748 cm$^{-1}$.

The intense colourations produced by pigment $B_1$ with acid and with alkali are also characteristic of the red pigment A, m.p. 259—263° (decomp.), $C_{19}H_{14}O_7$. The molecular formula differs by $CH_3$ from that of $B_1$ and by COMe from that of trihydroxypulvinone ($B_3$) and the presence of an n.m.r. singlet at 5·99 (3H) suggested its identity as the monomethyl ether of $B_1$. However, u.v. absorption at 444 nm (cf. 400 nm for $B_1$)



(XVI)  (XVII)  (XVIII)  (XIX)
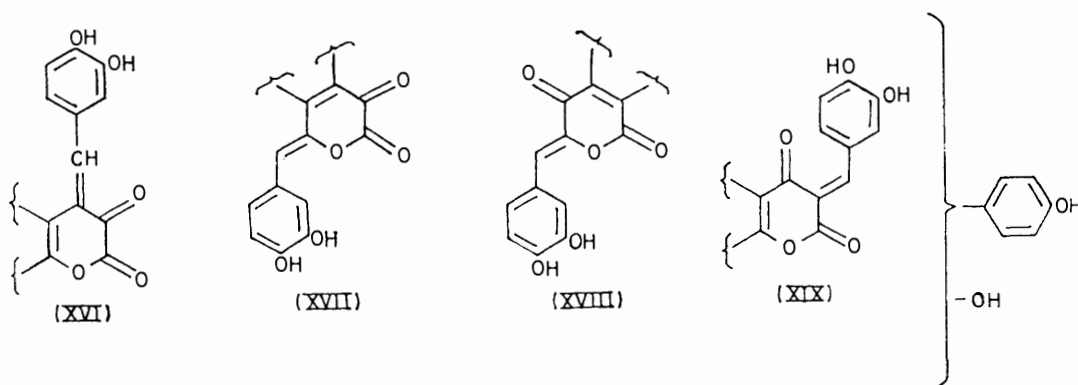
acetate from trihydroxy- and triacetoxy-pulvinone which show methine absorption at $\tau$ 3·50 and 3·94, respectively. The i.r. carbonyl absorptions of $B_1$ and its acetate [1736 and 1752 cm$^{-1}$, respectively (KBr)] indicate the presence of a lactone nucleus but the simple addition of CO to the $\gamma$-lactone nucleus of trihydroxypulvinone leads to unacceptable pulvinic aldehyde or lactol structures. The alternative $\delta$-lactone

is not consistent with simple monomethyl ether formation. In addition, the i.r. carbonyl absorption at 1724 cm$^{-1}$ is lower than that of $B_1$, and an additional carbonyl band occurs at 1670 cm$^{-1}$; the stability of the compound is also in marked contrast to the lability of the methyl ether of $B_1$.

[12] W. Steglich, H. Besl, and A. Prox, Tetrahedron Letters, 1972, 4895.

Treatment of the pigment with dilute aqueous sodium hydroxide yields an intense indigo blue colouration,



(XX)          (XXI)
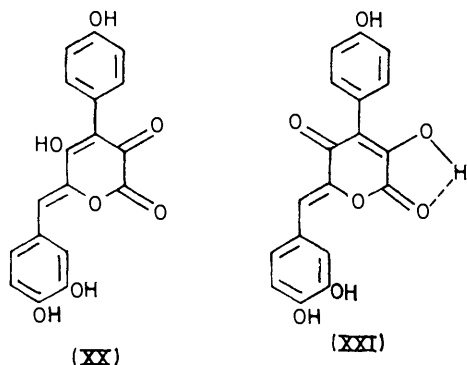
which changes to a stable red-violet over 2 min. The mass spectrum (Figure 4) resembles that of trihydroxy-pulvinone except that the ion at $m/e$ 145 replaces that at 134 in the latter; the ion at $m/e$ 239 (the trihydroxy-diphenyl cyclopropene ion) indicated the presence of both mono- and di-hydroxylated aryl rings and this was proved by oxidation of the triacetate with chromium
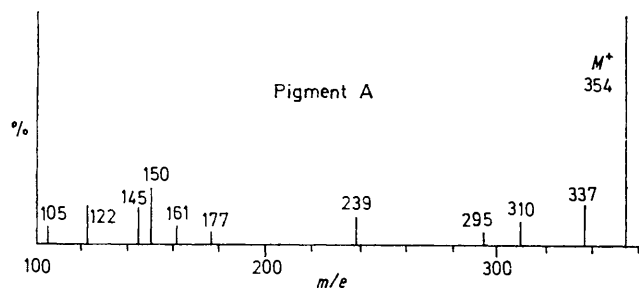


FIGURE 4   Mass spectrum of pigment A

trioxide, which yielded the acetates of 4-hydroxy- and 3,4-dihydroxy-benzoic acid. This evidence can
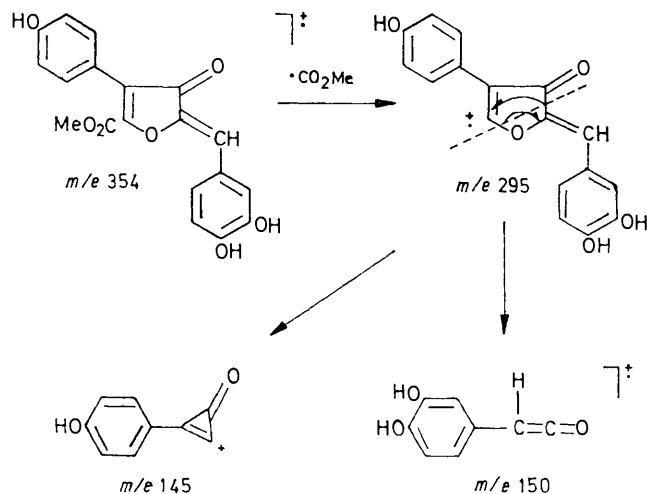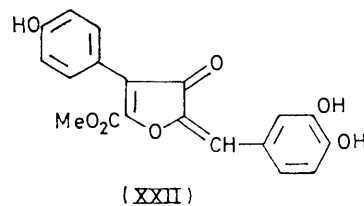


FIGURE 5

be interpreted in terms of the structure (XXII); this satisfactorily explains the absence of the ion $m/e$ 134,

characteristic of pigments $B_1$ and $B_3$, and explains its replacement by the ion $m/e$ 145 (Figure 5). The similarity of the i.r. absorptions at 1670 and 1630 cm$^{-1}$ to those of involutin (1672 and 1631 cm$^{-1}$) is also significant.



(XXII)

The nuclei of trihydroxypulvinone (I), pigment $B_1$ [(XX) or (XXI)] and pigment A (XXII) could conceivably originate from the hypothetical ketonic acid (XXIII) (see Scheme) produced by the condensation of two molecules of phenylpyruvic acid. A similar scheme
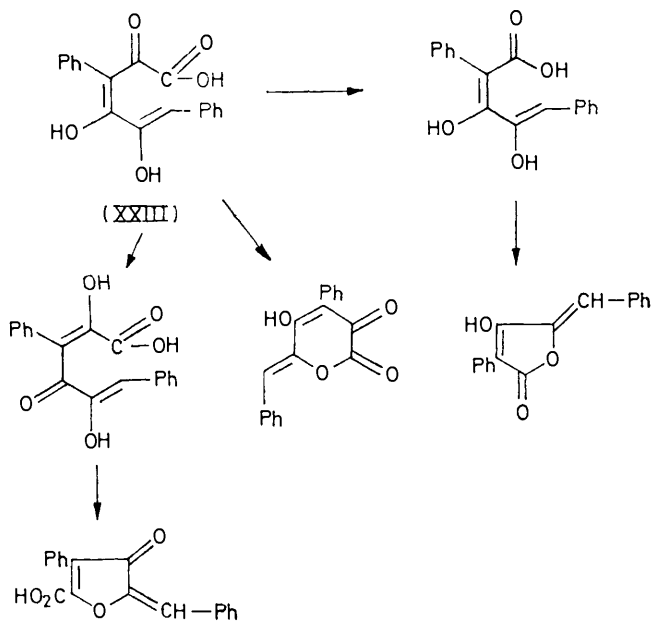


SCHEME

has been proposed as a possible route to fungal diphenylbenzoquinones.[13] Alternatively, the same compounds could originate by the route involving condensation of phenylalanine with pyridoxal phosphate.[14]

EXPERIMENTAL

M.p.s were determined on a Kofler hot-stage apparatus, i.r. spectra on a Perkin-Elmer 237 spectrophotometer, u.v. spectra on a Unicam SP 800 spectrophotometer, $^1$H n.m.r. spectra on a JEOL JNM-MH-100 spectrometer except where stated otherwise (tetramethylsilane as an internal standard), and mass spectra on an A.E.I. MS9 spectrometer. All thin-layer (t.l.c.), preparative-layer (p.l.c.), and column chromatography was done on Merck Kieselgel PF$_{256 + 366}$. Preparative layers consisted of 16 g silica gel on 20 × 20 cm glass plates. Solvent

$^{13}$ G. Read, L. C. Vining, and R. H. Haskins, Canad. J. Chem., 1962, 40, 2357.
$^{14}$ W. S. G. Maass, Phytochemistry, 1970, 9, 2477.

systems used in chromatography were (1) benzene–ethyl formate–formic acid (50 : 49 : 1) and (2) benzene–acetic acid (95 : 15).

*Isolation of 3′,4′,4-Trihydroxypulvinone* (I), *Pigment A* (XXII), *and Pigment B₁* [(XX) *or* (XXI)].—The stems from freshly collected sporophores (60 kg) were sliced and immersed in warm ethanol (60°) for 3 h. The solution was decanted and the residual stipes squeezed dry in filter cloth. The combined extracts were filtered and excess of aqueous lead diacetate was added to the filtrate. The lead salt was filtered off and washed twice with water and then twice with methanol. A vigorously stirred suspension of the salt in methanol (1 l) was decomposed by dropwise addition of methanolic hydrogen chloride (7%); periodic filtration of the mixture and preparation of a fresh slurry ensured that no excess of mineral acid could accumulate. After removal of the lead chloride, the solution was evaporated under reduced pressure. The residual gum was extracted with hot water (3 × 200 ml) and the hot extracts were decanted from the gummy residue. The solid which separated on cooling was filtered off and recrystallised from water (charcoal) to yield fumaric acid (4 g) as needles, m.p. 287°. The aqueous mother liquor was continuously extracted with benzene (16 h), then with ether (16 h), and the ethereal extract was evaporated under reduced pressure until impure fumaric acid began to separate. Filtration, and evaporation of the filtrate gave a dark red gum (18 g), which was applied in portions (1 g) in solvent system (1) to columns of silica gel (60 × 4 cm) and eluted with the same solvent. A quantity of gum (250 mg) from each portion was insoluble in the solvent and was discarded. The eluate from the column was collected in 10 ml fractions.

Fractions 35—38 deposited an orange-red solid on slow evaporation at room temperature. Recrystallisation from ethanol gave *pigment A* (26 mg) as red needles, m.p. 259—263° (decomp.) [Found: C, 64·1; H, 4·3; OMe, 9·0%; $M^+$, 354·075313. $C_{18}H_{11}O_6$(OMe) requires C, 64·4; H, 4·0; OMe, 8·8%; $M$, 354·073944]; $\nu_{max.}$ 3700—2500, 1724, and 1670 cm⁻¹; $\lambda_{max.}$ 257, 273infl, and 444 nm (log ε 4·08, 4·03, and 4·21); $\lambda_{max.}$ (10% EtOH) 255, 273infl, and 442 nm (4·07, 4·01, and 4·19); $\lambda_{max.}$ (10% EtOH + 2 drops 2N-NaOH) 504 nm (4·24); $\lambda_{max.}$ (10% EtOH + 2 drops 2N-NH₄OH) after 2 min 557 nm (4·37), and after 15 min 512 nm (4·31); τ [(CD₃)₂CO] 1·21 (3H), 2·14—2·98 (8H), and 5·99 (3H); *m/e* 354, 337, 310, 295, 239, 177, 161, 150, 145, 122, and 105.

Fractions 50—57 deposited orange-red needles of *pigment B₁* on slow evaporation at room temperature, which crystallised from formic acid or ethanol as solvated orange-red needles and from acetone as solvated yellow needles and plates, m.p. 275° (decomp.) [Found: C, 63·6; H, 4·9%; $M^+$, 340·059948. $C_{18}H_{12}O_7$,Me₂CO requires C, 63·3; H, 4·5%, $M$ ($C_{18}H_{12}O_7$), 340·063381]; $\nu_{max.}$ (KBr) 3800—2200, 3550, 1736, and 1639 cm⁻¹; $\lambda_{max.}$ 285 and 400 nm (log ε 3·92 and 3·88); $\lambda_{max.}$ (10% EtOH + 2 drops 2N-NH₄OH) 365 and 694 nm (4·01 and 3·73); $\lambda_{max.}$ (10% EtOH + 2 drops 2N-NaOH) 346 and 654 nm (3·95 and 3·85); τ [(CD₃)₂CO] 1·24 (3H), 2·15—2·97 (8H), and 6·80 (1H).

Fractions 58—62 gave lustrous yellow plates of 3′,4′,4-*trihydroxypulvinone* (B₃) (72 mg) on slow evaporation at room temperature, which crystallised from aqueous ethanol as yellow needles and rods, m.p. 291° (decomp.) (Found: C, 65·4; H, 3·9%; $M^+$, 312·063685. $C_{17}H_{12}O_6$ requires C, 65·4; H, 3·85%; $M$, 312·063381); $\nu_{max.}$ (KBr) 3350 (OH), 2350—2700 (chelated OH), 1694 (C=O), 1620, and 1610 cm⁻¹, $\nu_{max.}$ (dioxan) 1754 (C=O), 1725sh, and 1600 cm⁻¹; $\lambda_{max.}$ (EtOH) 243, 308infl, 343, and 378 nm (log ε 4·12, 3·96, 4·10, and 4·14); $\lambda_{max.}$ (10% EtOH) 253, 307infl, 335, and 356infl nm (4·11, 4·07, 4·18, and 4·08); $\lambda_{max.}$ (10% EtOH + 2 drops 2N-NH₄OH after 2 min) 259 and 386 nm (4·10 and 4·14); τ [(CD₃)₂CO] 2·02 (1H), 2·11 (1H), 2·44—3·10 (5H), 3·5 (1H), and 6·35br (4H).

Solutions from the tubes immediately preceding and succeeding those yielding pure trihydroxypulvinone, together with the mother liquors from filtration of the pure product, deposited yellow crystals contaminated with a colourless solid. The mixed solids were filtered off and washed with warm water. Evaporation of the aqueous solution and recrystallisation of the brown residue from water (charcoal) gave 3,4-dihydroxybenzoic acid (160 mg), m.p. and mixed m.p. with authentic material 199°. The washed crystalline residue was identical with trihydroxypulvinone. By combining all mother liquors from the foregoing separations and repeating the chromatography further pure trihydroxypulvinone (68 mg) was obtained.

*Isolation of Thelephoric Acid* (2,3,8,9-*Tetrahydroxybenzo-*[1,2-b:4,5-b′]*bisbenzofuran-6,12-dione*) *and Protocatechuic Aldehyde.*—(i) *Cold ethanol extraction of dark cap skins.* Freshly collected dark brown sporophores (570) were skinned and the skins were immersed in cold ethanol for 24 h. The extracts were strained through filter cloth and the solution was filtered. The turbid filtrate was evaporated at room temperature under reduced pressure until a suspension separated, and was then set aside at room temperature (48 h) and centrifuged. The black solid which separated was removed and centrifuged in turn with water (2×), acetone (4×), benzene (3×), and ethanol (4×). The aqueous extract and washings were retained. The black solid was repeatedly extracted with small volumes of hot pyridine and the extracts were combined, filtered, and set aside. Red-brown needles and plates of solvated thelephoric acid (193 mg) were deposited (m.p. >300°) which yielded solvent-free material (130 mg) on heating at 110° for 4 days, identical with authentic thelephoric acid (Found: C, 61·5; H, 2·45. Calc. for $C_{18}H_8O_8$: C, 61·4; H, 2·3%); $\nu_{max.}$ (KBr) 3500 (unchelated OH), 3320, 3210 (chelated OH), and 1645 (C=O) cm⁻¹; $\lambda_{max.}$ (pyridine) 311, 395infl, and 493 nm (log ε 4·53, 3·58, and 4·05). The aqueous extract and washings remaining after removal of the thelephoric acid were evaporated to dryness *in vacuo* and the residual brown gum was extracted repeatedly with ethyl acetate. The extracts were combined, evaporated to 100 ml, filtered, and distributed in a Craig counter-current apparatus (100 tubes) with water. The gum remaining after evaporation of the contents of tubes 72—82 was separated on silica gel [solvent system (1)] to yield 3,4-dihydroxybenzoic acid (47 mg) as needles, m.p. and mixed m.p. 198—199° (Found: $M^+$, 154·026951. Calc. for $C_7H_6O_4$: $M$, 154·026605). The gum (344 mg) remaining after the evaporation of the contents of tubes 83—91 yielded protocatechualdehyde (105 mg) after separation on silica gel [solvent system (1)]; m.p. and mixed m.p. 151—153° (Found: $M^+$, 138·032306. Calc. for $C_7H_7O_3$: 138·031690); 2,4-dinitrophenylhydrazone (Found: N, 17·9. Calc. for $C_{13}H_{10}N_4O_6$: N, 17·6%).

*Extraction of Yellow Cap Skins.*—The foregoing procedures were repeated. No thelephoric acid was isolated from

430 yellow cap skins (430). 3,4-Dihydroxybenzoic acid and 3,4-dihydroxybenzaldehyde were obtained in yields similar to those obtained from the dark cap skins.

3,4,4'-*Trimethoxypulvinic Acid* (VI).—3,4,4'-Trimethoxypulvinic lactone (1 g) was suspended in aqueous potassium hydroxide (2%; 100 ml) at room temperature. After 1 h the solution was poured into water (1 l), acidified with acetic acid, and extracted with ether for 16 h. The extract was dried and evaporated to yield a yellow solid (1 g). Crystallisation from ethanol gave 3',4',4-*trimethoxypulvinic acid* (87 mg) as glistening yellow plates, m.p. 249—253° (Found: C, 63·2; H, 4·75. $C_{21}H_{18}O_8$ requires C, 63·3; H, 4·5%); $\nu_{max.}$ (KBr) 3485, 2835, 2470, 1773, 1785sh, and 1681 cm$^{-1}$; $\lambda_{max.}$ (EtOH) 242infl, 259, and 392 nm (log ε 4·35, 4·41, and 4·15); τ [(CD$_3$)$_2$SO] 1·75 (1H), 2·86—2·96 (5H), and 6·09, 6·10, and 6·13 (each 3H).

Evaporation of the mother liquor gave a solid (760 mg) which after separation by p.l.c. [solvent system (2)] gave 3,4,4'-*trimethoxypulvinic acid* ($R_F$ 0·14; 250 mg) as orange needles, m.p. 200—205° (from ethanol) (Found: C, 63·1; H, 4·6%); $\nu_{max.}$ (KBr) 3450, 2480, 2839, 1770, and 1678 cm$^{-1}$; $\lambda_{max.}$ (EtOH) 260 and 392 nm (log ε 4·19 and 3·84); τ [(CD$_3$)$_2$SO] 2·09—2·99 (7H), 6·10 (9H), and 3',4',4-trimethoxypulvinic acid (360 mg).

*Trimethoxypulvinic Acid Copper*(II) *Chelate.*—Copper(II) acetate (2·5 g) in the minimum volume of water, was added to a stirred solution of trimethoxypulvinic acid (2·5 g) in ethanol (300 ml) at room temperature. The green solution was heated to 95°, cooled, and evaporated to dryness under reduced pressure. The yellow residue was washed free of residual copper acetate with hot water and crystallised from methanol to yield yellow hair-like needles of the *copper chelate* (600 mg). Concentration of the mother liquors gave a further crop of pure product (200 mg), m.p. 210° (darkening at 105°) (Found: C, 51·5; H, 4·7; Cu, 6·4. $C_{42}H_{48}CuO_{23}$ requires C, 51·2; H, 4·9; Cu, 6·5%). Drying for 48 h *in vacuo* gave a brown solid, m.p. 214—216° (capillary) (Found: C, 54·15; H, 4·5; Cu, 6·4. $C_{42}H_{42}CuO_{20}$ requires C, 54·3; H, 5·4; Cu, 6·8%); $\nu_{max.}$ (CHCl$_3$) 1768 and 1756 cm$^{-1}$ (C=O); $\lambda_{max.}$ 258 and 382 nm (log ε 4·65 and 4·34); $\lambda_{max.}$ (CHCl$_3$) 4·04 nm (log ε 4·36).

3,4,4'- *and* 3',4',4-*Trimethoxypulvinone* [(IV) *and* (V)].—The vacuum-dried copper chelate (810 mg) was refluxed in freshly distilled quinoline (5 ml) for 15 min. The mixture was cooled and poured with stirring into hydrochloric acid (2N; 150 ml). After 10 min the precipitate was filtered off and washed with dilute hydrochloric acid and water. Crystallisation from acetic acid gave a first crop of lemon yellow plates (140 mg), separated by p.l.c. [solvent system (2)] into two components. The slower moving component gave a mixture of 3,4,4'- and 3',4',4-trimethoxypulvinone (65 mg), crystallising from acetic acid as small, dull, yellow needles, m.p. 224—225° (capillary tube) (Found: C, 68·0; H, 5·2. Calc. for $C_{20}H_{18}O_6$: C, 67·8; H, 5·1%); $\nu_{max.}$ (EtOH) 246, 305infl, 325infl, 334, and 362 (log ε 4·31, 4·31, 4·38, 4·40 and 4·35); τ [(CD$_3$)$_2$CO] 1·92—2·98 (7H), 3·38 and 3·40 (close singlets; total 1H), 6·10 and 6·13 (9H), and 6·59 (1H). The faster moving component gave 2-(3,4-*dimethoxyphenyl*)-5-(4-*methoxyphenyl*)*cyclopentane*-1,3,4-*trione* (65 mg), crystallising from acetic acid as lemon yellow needles, m.p. 209—210° (capillary tube) (Found: C, 67·8; H, 5·3. $C_{20}H_{18}O_6$ requires C, 67·8; H, 5·1%); $\nu_{max.}$ (KBr) 3290, 3000, 2915, 2834, 1734, and 1667 cm$^{-1}$; $\lambda_{max.}$ (EtOH) 223sh, 269, and 366 nm (log ε 4·04, 3·78, and 3·81); τ (60 MHz; C$_5$-

D$_5$N) 0·95 (1H), 1·09 (1H), 2·49—3·11 (5H), 5·51 (1H), 5·54 (1H), 6·08 (3H), and 6·37 (6H).

3,4,4'- *and* 3',4',4-*Trimethoxy-O-methylpulvinone.*—The mixture of 3,4,4'- and 3',4',4-trimethoxypulvinone (150 mg) was treated with an excess of ethereal diazomethane. After 2 h, the excess of reagent was destroyed with acetic acid and the ether was evaporated off under reduced pressure. The residual brown gum was applied to a column of silica gel (35 × 2·5 cm) in solvent system (2) and the column was eluted with the same solvent. The intense brown band was eluted, the solvent was evaporated off, and the gum was crystallised from acetic acid to yield 3,4,4'-*trimethoxy-O-methylpulvinone* as tan-coloured needles (30 mg), m.p. 179—181° (Found: C, 68·3; H, 5·5. $C_{21}H_{20}O_6$ requires C, 68·5; H, 5·4%); $\nu_{max.}$ (KBr) 1751 and 1662 cm$^{-1}$; $\nu_{max.}$ (CHCl$_3$) 1752 and 1632 cm$^{-1}$; $\lambda_{max.}$ 239 and 357 nm (log ε 4·33 and 4·56), τ (CDCl$_3$) 2·14—3·09 (7H), 3·73 (1H), 6·07 (6H), and 6·15 (6H). The mother liquors from the crystallisation were evaporated to small volume and the precipitated solid was recrystallised from ethanol to yield 3',4',4-*trimethoxy-O-methylpulvinone* (40 mg) as tan-coloured leaflets, m.p. 153—155° (Found: C, 68·5; H, 5·5%); $\nu_{max.}$ (KBr) 1749 and 1655 cm$^{-1}$; $\nu_{max.}$ (CHCl$_3$) 1754 and 1630 cm$^{-1}$; $\lambda_{max.}$ 240, 259infl, and 367 nm (log ε 4·34, 4·17, and 4·60); τ (CDCl$_3$) 2·38—3·09 (7H), 3·70 (1H), 5·99 (3H), 6·10 (3H), and 6·11 (3H).

*Acetylation of Trimethoxypulvinone.*—A mixture of trimethoxypulvinone (30 mg), acetic anhydride (0·5 ml), and sulphuric acid (1 drop) was heated on a water-bath for 15 min. After cooling, the yellow needles were filtered off, washed with acetic anhydride and recrystallised from acetic acid to yield a mixture of *O*-acetyl-3,4,4'- and -3',4',4-trimethoxypulvinone (20 mg) as hair-like yellow needles, m.p. 235—237° (softening at 225°) (Found: C, 67·7; H, 5·1. Calc. for $C_{22}H_{20}O_7$: C, 67·7; H, 5·1%); $\nu_{max.}$ (KBr) 1767 cm$^{-1}$; $\nu_{max.}$ (CHCl$_3$) 1789 cm$^{-1}$.

*Methylation of Trihydroxypulvinone* (B$_3$).—Trihydroxypulvinone (100 mg) was treated with an excess of ethereal diazomethane for 2 h and then acetic acid was added to destroy the excess of reagent. Evaporation left a brown gum which was applied to a column of silica gel (40 × 3 cm) and eluted with solvent (2). The principal yellow band gave a yellow eluate; evaporation and recrystallisation of the residue from ethanol gave 3'4',4-trimethoxy-*O*-methylpulvinone (46 mg) as tan leaflets, m.p. 153—155° (Found: C, 69·5, H, 5·4, OMe, 33·4. Calc. for $C_{21}H_{20}O_6$: C, 68·5; H, 5·4; OMe, 33·7%), identical with the product already described.

*Acetylation of Trihydroxypulvinone* (B$_3$).—A mixture of trihydroxypulvinone (40 mg), acetic anhydride (0·3 ml), and sulphuric acid (1 drop) was heated on a water-bath for 15 min. The yellow crystalline solid whch separated on cooling was recrystallised from acetic acid to yield 3',4',4-*triacetoxy-O-acetylpulvinone* (18 mg) as pale yellow needles, m.p. 200—203° (Found: C, 62·7; H, 4·3. $C_{25}H_{20}O_{10}$ requires C, 62·5; H, 4·2%); $\nu_{max.}$ (KBr) 1775sh and 1756 cm$^{-1}$; $\lambda_{max.}$ (EtOH) 225infl, 252infl, and 340 nm (log ε 4·04, 3·86, and 4·18); τ (CDCl$_3$) 2·06—2·76 (7H), 3·84 (1H), 7·56 (3H), 7·66 (3H), 7·67 (3H), and 7·68 (3H).

*Pigment B$_1$ Tetra-acetate.*—A mixture of the pigment (60 mg), acetic anhydride (1 ml), and sulphuric acid (1 drop) was heated on a water-bath for 15 min, then poured into water (5 ml); the crystalline solid was filtered off, washed with water, and crystallised from ethanol to give the *tetra-acetate* (37 mg) as yellow needles, m.p. 184—186°

(Found: C, 61·6; H, 4·0. $C_{26}H_{20}O_{11}$ requires C, 61·4; H, 3·9%); $\nu_{max.}$ 1768 and 1752 $cm^{-1}$; $\lambda_{max.}$ 243 and 361 nm (log $\varepsilon$ 4·50 and 4·28); $\tau$ (CDCl$_3$) 1·94—2·71 (8H), 7·60 (6H), 7·62 (3H), and 7·67 (6H).

*Acetylation of Pigment A.*—The pigment (20 mg) was heated on a water-bath for 15 min with acetic anhydride (0·2 ml) containing a trace of concentrated sulphuric acid. The mixture was cooled and diluted with water (2 ml). The yellow amorphous *acetate*, m.p. 120—125°, could not be crystallised; $\nu_{max.}$ (CHCl$_3$) 1773sh, 1764, and 1719 $cm^{-1}$.

*Oxidation of 3′,4′,4-Triacetoxy-O-acetylpulvinone.*—A solution of chromium trioxide (40 mg) in water (0·05 ml) and acetic acid (0·5 ml) was added during 1 h to a mixture of 3′,4′,4-triacetoxy-O-acetylpulvinone (15 mg), acetic acid (0·7 ml), and acetic anhydride (0·7 ml) maintained at 60°. After a further 1·5 h, the green solution was poured into water (10 ml) and continuously extracted with ether (10 ml) overnight. Evaporation of the extract produced a colourless solid which was hydrolysed by heating on a water-bath in a mixture of ethanol (1 ml) and sulphuric

acid (2N; 0·3 ml) for 2 h. Examination of the residue on cellulose plates in the solvent system sodium formate (10 g), water (200 ml), formic acid (1 ml) (diazotised *p*-nitro-aniline spray) revealed 3,4-dihydroxybenzoic acid (yellow-brown spot), $R_F$ 0·61, and 4-hydroxybenzoic acid (red spot), $R_F$ 0·76.

Similar oxidations of $A_1$ acetate and $B_1$ tetra-acetate gave the same two aromatic acids in each case.