Studies in Mycological Chemistry. Part XXI.[1] The Structure of Aurofusarin, a Metabolite of Some *Fusarium* Species

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Spectroscopic, degradative, and synthetic studies indicate that aurofusarin is a dimeric quinone made up from substituted naphthopyran units.

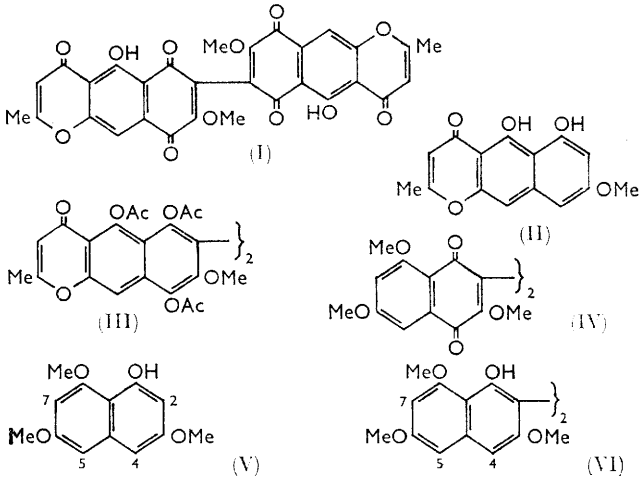
AUROFUSARIN was first isolated [2] from various strains of *Fusarium culmorum* and *F. graminearum*, moulds pathogenic to the wheat plant. The mycelium from all the strains of the moulds which were investigated yielded different proportions of aurofusarin (small orange-yellow prisms, m. p. >360°) and rubrofusarin (orange-red needles, m. p. 210—211°). The structure of rubrofusarin, $C_{15}H_{12}O_5$, has been established [3] as (II). Raistrick and his collaborators [2] concluded tentatively, from an exhaustive analytical investigation of aurofusarin (and of several of its simple derivatives), that it had the formula, $C_{30}H_{20}O_{12},H_2O$. Further, they showed that the molecule possessed two methoxyl groups, yielded a dibenzoate, and could be reduced to a tetrahydro-derivative which, in turn, gave a hexabenzoate. Structural elucidation ceased at this point and no further progress on the chemistry of aurofusarin has yet [4] been reported.

We obtained aurofusarin from a strain of *F. graminearum* Schwabe. Our analytical figures for carbon and hydrogen agreed with those quoted,[2] but they would equally well fit the formula $C_{30}H_{18}O_{12},H_2O$. It occurred to us *a priori* that aurofusarin might be a dimeric quinone produced by oxidation and oxidative dimerisation of its simpler phenolic co-metabolite, rubrofusarin (II), thus:

$$(2 \times C_{15}H_{12}O_5) + (2 \times 1\hat{O}) - (2 \times 3\hat{H}) = C_{30}H_{18}O_{12}$$

This hypothesis had the advantage of providing a rational explanation of the observation [2] (mentioned above) that, whereas aurofusarin yielded a di-benzoate, tetrahydroaurofusarin gave a hexa-benzoate. The explanation is that reduction of a dimeric quinone would produce a bis-quinol (tetrahydroaurofusarin) which would contain four extra hydroxyl groups amenable to acylation. These ideas prompted the following spectroscopic and chemical investigations.

The infrared spectrum of aurofusarin showed strong absorptions at 1678 (quinone CO) and 1664 cm.$^{-1}$ (γ-pyrone CO; cf. 1662 cm.$^{-1}$ for CO in rubrofusarin [5]). The ultraviolet spectrum (Table 1) was not very informative but a comparison of the ultraviolet spectrum of hexa-*O*-acetyltetrahydroaurofusarin (III) with that of di-*O*-acetylrubrofusarin (II; OAc for each OH) yielded important information. The $\lambda_{max.}$ values for the two compounds are distinctly similar but the ε values of the former are approximately double those of the latter. It thus seemed probable that hexa-*O*-acetyltetrahydroaurofusarin contained two isolated chromophores each of which was essentially similar to that of di-*O*-acetylrubrofusarin. The proton magnetic resonance (p.m.r.) spectrum of aurofusarin (which is interpreted in detail



below) was consistent with the idea that the metabolite was a symmetrical dimer. These results and interpretations strengthened our hypothesis concerning the structure of aurofusarin, and we turned our attention to its chemical degradation.

Alkaline hydrolysis of aurofusarin yielded methanol (identified by a colour test), acetone (identified as its 2,4-dinitrophenylhydrazone), and a residue which, after methylation, gave a red-yellow gum. By a sequence of chromatographic separations and crystallisation, a small yield of golden lenticular crystals, m. p. 259—261°, was obtained. These were identical with a synthetic sample (see below) of bis-3,3′-tri-*O*-methylflaviolin (IV). [Flaviolin [6] (2,5,7-trihydroxy-1,4-naphthaquinone) is a metabolite of *Aspergillus citricus*.] Bearing in mind the symmetrical dimeric nature of aurofusarin as indicated by the spectral evidence mentioned above, the degradation of aurofusarin by alkali may be interpreted in terms of structure (I) as shown in the annexed scheme. It should be noted that a 2(or (3)-methoxy-1,4-naphthaquinone system is hydrolysable by alkali since it possesses a vinylogous ester structure.

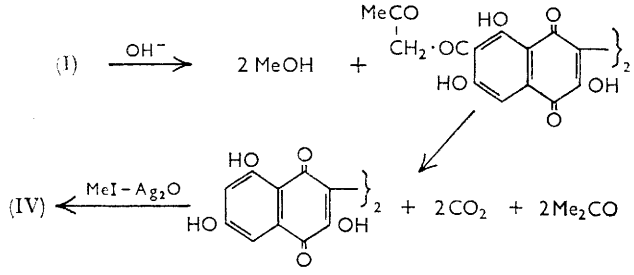
[1] Part XX, J. A. Knight, J. C. Roberts, and P. Roffey, *J. Chem. Soc.* (*C*), 1966, 1308.
[2] J. N. Ashley, B. C. Hobbs, and H. Raistrick, *Biochem. J.*, 1937, **31**, 385.
[3] G. H. Stout, D. L. Dreyer, and L. H. Jensen, *Chem. and Ind.*, 1961, 289; H. Tanaka and T. Tamura, *Tetrahedron Letters*, 1961, 151; S. Shibata, E. Morishita, and Y. Arima, *Chem. and Pharm. Bull.* (*Japan*), 1963, **11**, 821.
[4] Cf. R. P. Mull and F. F. Nord, *Arch. Biochem.*, 1944, **4**, 419.
[5] B. W. Bycroft, T. A. Dobson, and J. C. Roberts, *J. Chem. Soc.*, 1962, 40.
[6] B. W. Bycroft and J. C. Roberts, *J. Chem. Soc.*, 1962, 2063.

TABLE 1

Ultraviolet absorption spectra [$\lambda_{max.}$ (mμ) (10^{-3} ε in parentheses)]

Aurofusarin (I) (in $CHCl_3$)	248 (49·3)	269 (33·5)	381 (9·8)	422 infl. (8·42)				
Hexa-*O*-acetyltetrahydroaurofusarin (III) (in EtOH)	203 (36·1)	227 (39·4)		260 infl. (83·6)	276 (89·5)		341 (10·5)	362 (10·3)
Di-*O*-acetylrubrofusarin (in EtOH)	203 (20·3)	225 (24·5)	249 infl. (36·6)	259 (42·6)	270 (42·0)	329 infl. (5·83)	343 (7·77)	360 infl. (5·62)

Acid hydrolysis of aurofusarin gave a compound, $C_{28}H_{14}O_{12}$, to which we allocate structure (I; OH for each OMe).



The p.m.r. spectrum of aurofusarin can also be interpreted in terms of structure (I), as shown in Table 2 which also gives the values for corresponding protons in a model compound, 2-methylchromone.

TABLE 2

Proton magnetic resonance absorptions * (τ scale) (solvent, trifluoroacetic acid)

Compound	C-C*H*$_3$	O-C*H*$_3$	Ar-*H*	γ-Pyrone-*H*
Aurofusarin (I) †	7·13 (s, 6)	5·63 (s, 6)	1·87 (s, 2)	2·91 (s, 2)
2-Methylchromone	7·01 (s, 3)	—	1·3— 2·2 (m, 4)	2·54 (s, 1)

* The values refer to freshly made solutions. The nature and intensity of the signals are given in parentheses: s = singlet; m = multiplet. Internal reference, tetramethylsilane. † Signals due to 2 × O*H* are not detectable. They presumably lie beneath the " solvent peak."

molecule is not complete, otherwise the natural product would be expected to be optically active. A structure containing an angularly fused γ-pyrone ring for one or both moieties of the aurofusarin molecule is excluded because of the relationship (discussed above) between the ultraviolet spectra of hexa-*O*-acetyltetrahydroaurofusarin and di-*O*-acetylrubrofusarin. (A similar problem was considered previously.[5])

Of the thirty carbon atoms in the aurofusarin molecule, twenty-eight have been identified in the form of methanol (2), acetone (6), and bis-3,3′-tri-*O*-methylflaviolin (IV) (20). We presume that the remaining two carbon atoms are lost as carbon dioxide by the decarboxylation of γ-resorcyclic acid systems.

On the basis of the spectroscopic and degradative evidence given above, we allocate structure (I) to aurofusarin.

After several unsuccessful attempts to synthesise bis-3,3′-tri-*O*-methylflaviolin (IV) by Ullmann-type reactions, we turned our attention to a synthesis involving oxidative dimerisation in which an appropriate monomer is fused with a cupric salt.[7] The required dimeric quinone was synthesised as follows. The 2-acetyl derivative of methyl 3,5-dimethoxyphenylacetate was converted [8] into the trimethoxy-1-naphthol (V) which, on fusion [7] with cupric acetate, yielded the dimer (VI). Oxidation of the latter by means of Frémy's salt [9] then produced the desired quinone (IV).

TABLE 3

Proton magnetic resonance absorptions * (τ scale, *J* in c./sec.) (solvent, deuteriochloroform)

Compound	OC*H*$_3$	O*H*	*H*-7	*J*	*H*-2	*J*	*H*-5	*J*	*H*-4	*J*
(V)	6·01 (s, 3) 6·12 (s, 3) 6·14 (s, 3)	0·79 (s, 1)	3·64 (d, 1)	3	3·53 (d, 1)	2	3·33 (d, 1)	3	3·36 † (1)	?
	OC*H*$_3$	O*H*	*H*-7/7′	*J*			*H*-5/5′	*J*	*H*-4/4′	*J*
(VI)	6·08 (s, 6) 6·12 (s, 6) 6·21 (s, 6)	0·52 (*s*, 2)	3·64 (d, 2)	3			3·27 (d, 2)	3	3·25 ‡ (2)	—

* The nature and intensity of the signals are given in parentheses: s = singlet; d = doublet. Internal reference, tetramethylsilane. † Partly obscured by signal for *H*-5. ‡ Submerged in signal for *H*-5/5′.

Aurofusarin is optically inactive, yet the comparison (mentioned above) of the ultraviolet spectrum of hexa-*O*-acetyltetrahydroaurofusarin with that of di-*O*-acetylrubrofusarin indicates that the two halves of the molecule are not coplanar. In seems that the restriction of rotation about the bond joining the two halves of the

The structure of the dimeric naphthol (VI), with special reference to the position of coupling, followed from the considerations: (*a*) it gave a positive Gibbs test [10] ($\lambda_{max.}$ 566 mμ) indicating that there was at least one position in the molecule *para* to a hydroxyl group which was unsubstituted; (*b*) its p.m.r. spectrum (Table 3) was characteristic of a symmetrical compound

[7] W. W. Kaeding, *J. Org. Chem.*, 1963, **28**, 1063.
[8] B. W. Bycroft and J. C. Roberts, *J. Chem. Soc.*, 1963, 4868.
[9] H.-J. Teuber *et al.*, *Chem. Ber.*, 1952, **85**, 95, and later Papers.
[10] F. E. King, T. J. King, and L. C. Manning, *J. Chem. Soc.*, 1957, 563.

(2 × 13 protons); (*c*) a comparison of the p.m.r. spectrum of the monomer with that of the dimer indicated that the hydrogen atoms at position 2 of the former are removed in the oxidative coupling process to give the latter.

EXPERIMENTAL

Melting points were determined on a Kofler hot-stage apparatus. Ultraviolet spectra were measured on a Unicam spectrophotometer (S.P. 700). Infrared spectra were determined on compounds in potassium bromide discs with a Unicam spectrophotometer (S.P. 200). P.m.r. spectra were recorded on a Perkin-Elmer spectrometer (R.10, 60 Mc./sec.), tetramethylsilane being used as an internal reference; in the sequel, figures in parentheses, following the statement of the nature of the signal, indicate intensities.

The alumina used for chromatography (Spence, type H) was treated with dilute hydrochloric acid, washed thoroughly with water, dried at 170°, and mixed (immediately before use) with water (7·5 ml./100 g. of solid). Other adsorbents were silica (M.F.C., Hopkin and Williams) and magnesium trisilicate (British Drug Houses).

Aurofusarin (I).—Investigation of three strains of *F. culmorum* and of one strain of *F. graminearum* Schwabe (Commonwealth Mycological Institute, No. 89,367) showed that the last-mentioned strain gave the best yield of aurofusarin. Stock cultures of this strain were kept on potato–dextrose–agar medium, and aurofusarin (7·0 g. of crude pigment from 332 g. of dried mycelium) was obtained by the published method.[2] Repeated crystallisation from boiling glacial acetic acid (charcoal) and one crystallisation from chloroform gave pure aurofusarin, decomp. >320° [Found (on a sample dried *in vacuo* at 156°): C, 61·2; H, 3·4. Calc. for $C_{30}H_{18}O_{12},H_2O$: C, 61·2; H, 3·4%], $\nu_{max.}$ 1678, 1664, 1610, 1598 cm.$^{-1}$, etc., no significant absorption due to an OH stretching vibration being detectable.

Attempts to prepare pure derivatives of aurofusarin by methylation or by reductive acetylation were unsuccessful.

Hexa-O-*acetyltetrahydroaurofusarin* (III).—A solution of tetrahydroaurofusarin [2] (0·2 g.) in a mixture of acetic anhydride (10 ml.) and dry pyridine (5 ml.) was kept at room temperature for 2 days and then heated on a steam-bath for 4 hr. The solvents were removed *in vacuo*. The residue was dissolved in chloroform and the solvent removed *in vacuo*. This last operation was repeated twice. A solution of the residue in chloroform was chromatographed on a column (15 × 1·5 cm.) of magnesium trisilicate–diatomite (1 : 1) in subdued light. (Bright light appeared to decompose the product.) Evaporation of the solvent from the eluted yellow band produced a residue which, after three crystallisations from ethanol, yielded the *acetate* (42 mg.) as yellow micro-crystals, m. p. 270—276° (Found: C, 61·0; H, 4·45. $C_{42}H_{34}O_{18}$ requires C, 60·9; H, 4·15%), $\nu_{max.}$ 1782, 1665, 1626, 1577 cm.$^{-1}$, etc.

Acid Degradation of Aurofusarin.—Aurofusarin (0·1 g.) was boiled with glacial acetic acid (100 ml.). To the hot, filtered solution was added concentrated hydrochloric acid (1 ml.). Some (50 ml.) of the acetic acid was removed by distillation and the residual solution was kept at room temperature for 2 days. The precipitate was collected, washed with cold glacial acetic acid, and dried *in vacuo* at 156° for 12 hr., to give *didemethylaurofusarin* (18 mg.) as yellow-green needles, m. p. >350° (decomp.) [Found: C, 60·3; H, 3·0; OMe, nil. $C_{28}H_{14}O_{12},H_2O$ requires C, 60·0; H, 2·9. Found (on a portion of the previous sample which had been dried *in vacuo* at 209° for a further 12 hr.): C, 62·3; H, 2·3. $C_{28}H_{14}O_{12}$ requires C, 62·0; H, 2·6%].

Alkaline Degradation of Aurofusarin.—(*a*) A suspension of aurofusarin (0·5 g.) in 3% aqueous potassium hydroxide (50 ml.) was heated under reflux for 45 min., and the mixture was then distilled. The first runnings (5 ml.) gave a positive colour test [11] for methanol.

(*b*) To a 3% aqueous solution of potassium hydroxide (100 ml.) which was heated under reflux, was added dropwise a solution of aurofusarin (0·5 g.) in 25% aqueous tetraethylammonium hydroxide (45 ml.). The solution was heated under reflux for a further 3 hr. and then distilled. The distillate (25 ml.) was added to 60 ml. of a saturated solution of 2,4-dinitrophenylhydrazine hydrochloride (made from 0·2 g. of the base and 100 ml. of 2N-hydrochloric acid). A chloroform solution of the washed and dried precipitate was chromatographed on alumina. The fast-moving yellow band was eluted and the solvent was removed. Crystallisation of the residue from ethanol gave yellow rods (30 mg.), m. p. 116—120°, which, when sublimed at 110°/0·1 mm., yielded pure acetone 2,4-dinitrophenylhydrazone, m. p. and mixed m. p. 125—126°.

The solution which remained, after the above mentioned distillation, was acidified and extracted with ether. Evaporation of the washed and dried ethereal extracts gave a dark red-brown residue which contained no methoxyl groups (Zeisel estimation). This residue was shaken with silver oxide (2 g.) and methyl iodide (15 ml.) for 4 days at room temperature. The solids were removed by filtration and washed with chloroform. The combined filtrate and washings were evaporated, to give a red-yellow gum. A solution of this gum in chloroform was chromatographed on a column of alumina (15 × 1·5 cm.) and a yellow band was eluted. The chloroform was removed from the eluate and a solution of the residue in benzene was chromatographed on a column (15 × 1·5 cm.) of magnesium trisilicate–diatomite (2 : 1). Benzene eluted a yellow band. A second yellow band was eluted with benzene–chloroform (9 : 1). The solvents were removed from the latter eluate, to produce a residue which, after two crystallisations from benzene, gave *bis*-3,3′-(*tri*-O-*methylflaviolin*) (10 mg.) as golden lenticular prisms, m. p. (and mixed m. p. with a synthetic specimen; see below) 259—261° [Found: *M* (mass spectrum), 494·1213. $C_{26}H_{22}O_{10}$ requires *M*, 494·1213], $\lambda_{max.}$ (in $CHCl_3$) 265, 303, 381, and 402 (infl.) mμ (10^{-3} ε 31·1, 17·9, 7·38, and 6·24), $\nu_{max.}$ 1666, 1638, 1617sh, 1606, 1591, 1566 cm.$^{-1}$, etc. The p.m.r. spectrum (in $CDCl_3$) showed: (i) three closely spaced singlets (18) at τ 6·00, 6·04, and 6·09, (ii) a doublet (2) at τ 3·26 ($J \sim 3$ c./sec.), and (iii) a doublet (2) at τ 2·66 ($J \sim 3$ c./sec.).

Di-O-*acetylrubrofusarin.*—Rubrofusarin, which was concomitantly produced in the biochemical preparation of aurofusarin (see above), was separated, purified, and converted into the acetate, m. p. 260°, as previously described.[2]

3,6,8-*Trimethoxy*-1-*naphthol* (V).—This compound, m. p. 145—146°, was prepared as previously described; [8] $\lambda_{max.}$ (in $CHCl_3$) 246, 292, 302, 314, and 329 mμ (10^{-3} ε 60·0, 4·31, 4·57, 3·92, and 3·08), $\nu_{max.}$ 3337, 3145, 1643, 1633, 1621, 1595 cm.$^{-1}$, etc. It gave a positive Gibbs test [10] ($\lambda_{max.}$ 594 mμ).

Bis-2,2′-(3,6,8-*trimethoxy*-1-*naphthol*) (VI).—The

[11] F. Feigl, "Spot Tests in Organic Analysis," Elsevier, Amsterdam, 6th edn., p. 357.

going naphthol (246 mg.) was ground with cupric acetate (212 mg.). The mixture was heated (oil-bath, 200°) for 5 min. and was then cooled rapidly. The solid brown mass was exhaustively extracted with hot benzene. The filtered benzene solution was evaporated to *ca.* 8 ml. and poured on to a silica column (15 × 1·5 cm.). Development of the chromatogram with benzene removed unchanged monomer. The dimer was eluted with benzene–ethyl acetate (4 : 1). The solvent was removed from the eluate and the residue (a colourless syrup which soon solidified) was crystallised from benzene, to give the *dimeric naphthol* (103 mg., 49%) as cubes, m. p. 276—278° [Found: C, 66·6; H, 5·4%; *M* (mass spectrum), 466. $C_{26}H_{26}O_8$ requires C, 66·9; H, 5·6%; *M*, 466], $\lambda_{max.}$ (in $CHCl_3$), 253, 287, 300, 314, and 331 mμ (10^{-3} ε 98·1, 13·9, 11·5, 9·52, and 7·38), $\nu_{max.}$ 3400, 3368, 1634, 1593 $cm.^{-1}$, etc. It gave a positive Gibbs test [10] ($\lambda_{max.}$ 566 mμ).

Bis-3,3′-(*tri*-O-*methylflaviolin*) (IV).—To a solution of the foregoing naphthol (100 mg.) in acetone (100 ml.) was added slowly (at room temperature and with continuous shaking) a solution of potassium nitrosodisulphonate (1·0 g.) in aqueous potassium dihydrogen phosphate (M/6; 20 ml.). Water was then carefully added until the yellow precipitate had just dissolved. The solution was kept for 2 days at room temperature and extracted with chloroform. The chloroform solution was washed with water, dried ($MgSO_4$), and the solvent removed *in vacuo*. A solution of the residue in benzene was chromatographed on an alumina column (15 × 1·5 cm.) and the chromatogram was developed with benzene–ether (9 : 1). The slowest moving yellow band was eluted. Removal of the solvent (*in vacuo*) from this eluate and crystallisation of the residue from benzene gave the *dimeric quinone* (17 mg.) as golden lenticular prisms, m. p. 259—261° (Found: C, 62·8; H, 4·55. $C_{26}H_{22}O_{10}$ requires C, 63·2; H, 4·45%), $\lambda_{max.}$ (in $CHCl_3$) 265, 302, 379, and 402 (infl.) mμ (10^{-3} ε 31·7, 18·3, 8·0, and 6·72). This compound was unstable towards trifluoroacetic acid. Its infrared and p.m.r. spectra were virtually identical with the corresponding spectra (detailed above) of the alkaline degradtion product of aurofusarin.

When this work was virtually complete we learnt that Professor W. B. Whalley and his associates had been investigating a metabolite of *Hypomyces rosellus* which showed resemblances to aurofusarin. A comparison of the two metabolites revealed their identity and, by mutual agreement, we decided to publish our respective results simultaneously.

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