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Metabolic Products of Fungi. XXVIII.[1] The Structure of Aurofusarin. (1)[2]

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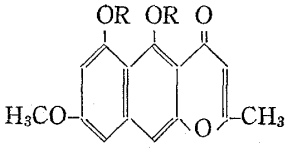
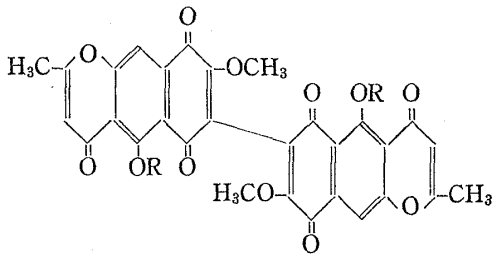
The structure of aurofusarin, a golden yellow pigment of *Fusarium culmorum* (W.G. Smith) Sacc., has been studied. A dimeric naphthoquinonepyrone structure (IIa) has been put forward for aurofusarin by the comparative spectrometric studies of diacetate (IIb) and dimethyl ether (IIc) with some reference compounds.

In 1937, Raistrick and his coworkers[4] studied the metabolites of *Fusarium culmorum* (W.G. Smith) Sacc., a pathogenic fungus which grows parasitically on wheat causing foot rot, and a related fungus, *Fusarium graminearum* Schwabe. An orange red pigment, named rubrofusarin and a golden yellow pigment, aurofusarin were isolated along with a colourless compound, culmorin.

The structure of rubrofusarin (Ia) was established X-ray crystallographically by Stout, *et al.*[5] and chemically by Tamura *et al.*[6] Culmorin, $C_{15}H_{26}O_2$, has recently been proved to be a sesquiterpene whose structure has been forwarded by Barton, *et al.*[7]



Ia : R=H
Ib : R=CH_3
Ic : R=$COCH_3$



IIa : R=H IIb : R=CH_3 IIc : R=$COCH_3$

Raistrick, *et al.*[4] obtained aurofusarin as golden yellow prisms, mp >360°, and gave a molecular formula $C_{30}H_{20}O_{12}\cdot H_2O$, involving two methoxyls and two active hydrogen atoms. They prepared dibenzoate, and the hydrogenated derivative and its benzoate.

We found that aurofusarin which was isolated from *Fusarium culmorum* and purified by chromatography on silica gel impregnated with oxalic acid gave a molecular formula $C_{30}H_{18}O_{12}$.

By the present study we have reached to the structure of aurofusarin as being formulated as IIa. Almost the same time Roberts, *et al.*[8] also proposed the same structure to aurofusarin isolated from *Fusarium graminearum*, while Whalley, Weiss and their collaborators[9] reached

1) Part XXVII, *Chem. Pharm. Bull.* (Tokyo), **15**, 1772 (1967).
2) Preliminary Report, *Tetrahedron Letters*, No. 40, 4855 (1966).
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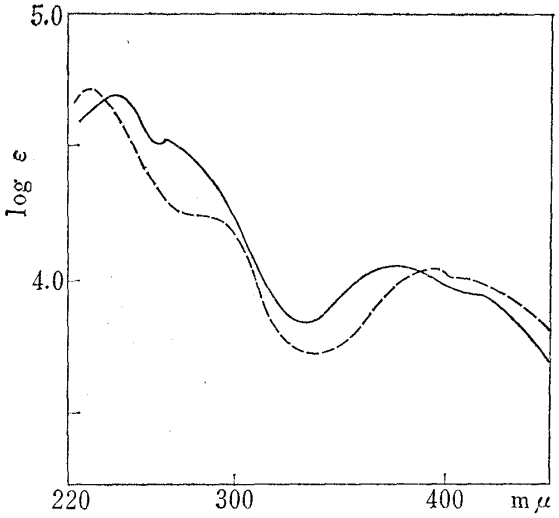
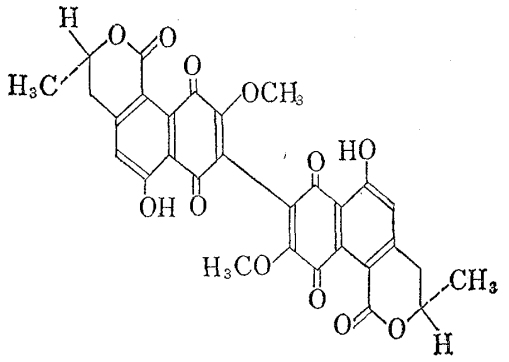
4) J.N. Ashley, B.C. Hobbs, and H. Raistrick, *Biochem. J.*, **31**, 385 (1937).
5) G.H. Stout, D.L. Dreyer, and L.H. Jensen, *Acta Cryst.*, **15**, 451 (1961).
6) H. Tanaka, T. Ohno, N. Ogawa, and T. Tamura, *Tetrahedron Letters*, No. 4, 151 (1961); *Agr. Biol. Chem.* (Tokyo), **27**, 48 (1963).
7) D.H.R. Barton and N.H. Werstink, *Chem. Comm.*, **1967**, 30.
8) P.M. Baker and J.C. Roberts, *J. Chem. Soc.*, **1966**, 2234.
9) G.R. Birchall, K. Bowden, U. Weiss, and W.B. Whalley, *J. Chem. Soc.*, **1966**, 2237.

to almost the same conclusion about the metabolite of *Hypomyces rosellus* (Alb. et Schio) Tul. and *Dactylium dendroides*)(Bull. Fr.), which was identified with aurofusarin.

Aurofusarin is sparingly soluble in organic solvents and shows yellow colour in acid and red to violet colour in alkalis forming sparingly soluble salts. A positive magnesium acetate reaction (red) revealed an α-hydroxy-quinonic structure; similar properties are shown by xanthomegnin (III), an orange pigment of *Trychophyton megnini* Blanchard.[10] The almost superimposable UV spectral curves of both compounds suggested a close similarity of the structures (Chart 1).





III

Chart 1. The UV-absorption curves (in dioxane) of:
– – – – – Xanthomegnin ——— Aurofusarin

The IR-spectrum of aurofusarin showed the presence of nonchelated carbonyl, chelated carbonyl, carbonyl of γ-pyrone, strongly hydrogen bonded hydroxyl and aromatic ring system.

Table I. Comparison of UV and IR Spectra of Aurofusarin and Xanthomegnin

	Aurofusarin (IIa)	Xanthomegnin (III)
UV $\lambda_{max}^{Dioxane}$ mμ (log ε)	249 (4.69), 267 (4.52), 372 (4.05)	229 (4.71), 285 (4.25), 390 (4.05)
IR $\nu_{max}^{CHCl_3}$ cm^{-1}		1716 (6 membered ring lactonic C=O)
	1680 (non-chelated C=O)	1680 (non-chelated C=O)
	1665 (γ-pyrone C=O[a])	
	1615 (chelated C=O)	1622 (chelated C=O)
	1600 (aromatic double bond)	1600 (aromatic double bond)

a) Rubrofusarin (Ia) showed γ-pyrone ring C=O band at 1660 cm^{-1}.

On alkaline degradation, aurofusarin gave acetone and acetic acid. This suggests the presence of α-methyl-γ-pyrone structure in aurofusarin, which might be rationalized biogenetically by the co-occurrence of rubrofusarin (Ia) in the same fungus.

The acetate and methyl ether of aurofusarin were hardly prepared by the usual methods, whereas by the action of acetyl chloride and pyridine in chloroform under ice-cooling diacetate, $C_{30}H_{16}O_{10}(OCOCH_3)_2$, mp>330°, was afforded and by the action of methyl iodide and silver oxide in chloroform, dimethyl ether, $C_{30}H_{16}O_{10}(OCH_3)_2$, mp 250—251° (decomp.), was yielded.

10) G. Just, W.C. Day, and F. Blank, *Can. J. Chem.*, **41**, 74 (1963); J.C. Wirth, J.E. Beesley, and S.R. Anand, *Phytochem.*, **4**, 505 (1965).

The molecular weight of dimethyl ether was determined osmometrically to prove the dimeric structure. Aurofusarin diacetate gave an UV absorption curve which resembles closely to that of flaviolin triacetate (Va)[11] suggesting the presence of naphthoquinone system in the molecule. The NMR spectra of aurofusarin diacetate and dimethyl ether were compared with that of rubrofusarin dimethyl ether (Ib).

Table II. The NMR Spectra of Aurofusarin Diacetate, Aurofusarin Dimethyl Ether and Rubrofusarin Dimethyl Ether (τ in $CDCl_3$)

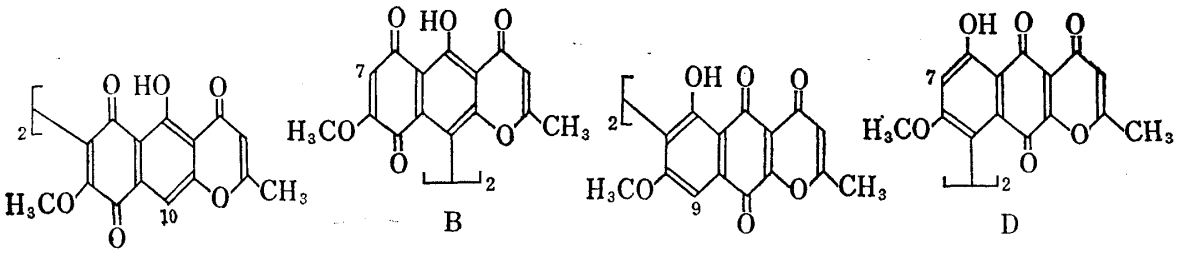
	Aurofusarin		Rubrofusarin Dimethyl Ether (Ib)
	Diacetate (IIc)	Dimethyl Ether (IIb)	
Arom. H	1.90(2H)(s)	2.08(2H)(s)	2.66(H)(s) 3.38(H)(d, J=3 cps) 3.58(H)(d, J=3 cps)
β,β'-H of 2-Methyl-γ-pyrone ring	3.86(2H)(s)	3.88(2H)(s)	4.18(H)(s)
Arom. $OC\underline{H}_3$	5.90(6H)(s)	5.92(6H)(s) 6.04(6H)(s)	6.05(3H)(s) 6.11(6H)(s)
α,α'-$C\underline{H}_3$ of 2-Methyl-γ-pyrone ring	7.55(6H)(broad s)	7.61(6H)(s)	7.71(3H)(s)
Arom. $OCOC\underline{H}_3$	7.59(6H)(s)		

The above results showed the presence of the following functional groupings or ring systems in the molecule of aurofusarin:

i) Two aromatic methoxyls and two hydrogen bonded hydroxyls.

ii) 2-Methyl-γ-pyrone ring system.

All the NMR signals of aromatic protons, methoxyls, and methyls of aurofusarin derivatives gave double magnitude; aurofusarin is a dimer of symmetric structure. Relating to the structure of rubrofusarin and their biogenetical relation, aurofusarin would possibly be a dimer of quinonic derivative of rubrofusarin, for which the following 4 possible formulas (A–D) would be considered:



A B C D

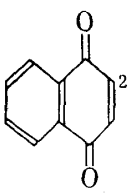
The appearance of aromatic proton signals in aurofusarin diacetate (τ 1.90) and dimethyl ether (τ 2.08) in the very low field is noted for determination of the structure.

The aromatic proton at $C_{(7)}$ of formula B would correspond to the $C_{(2)}$–H of naphthoquinone (IV) and $C_{(2)}$–H of flaviolin trimethyl ether (Vb), which appears at τ 3.03 and 4.00, respectively.

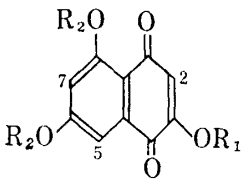
The aromatic proton at $C_{(7)}$ of formula D (OCH_3 instead of OH) should correspond to that at $C_{(7)}$ in flaviolin trimethyl ether (Vb) and at $C_{(6)}$ in naphthoquinone dimethyl ether A (VII)[12] derived from protoaphin-f_b, which shows the signal at τ 3.28 and 3.27, respectively.

11) B.D. Astill and J.C. Roberts, *J. Chem. Soc.*, **1953**, 3302; J.E. Davis, F.E. King, and J.C. Roberts, *J. Chem. Soc.*, **1955**, 2782; B.W. Bycroft, and J.C. Roberts, *J. Chem. Soc.*, **1962**, 2063.

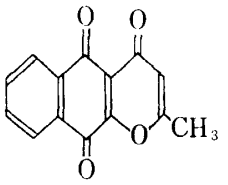
12) D.W. Cameon, D.G.I. Kingston, N. Sheppard, and Lord Todd, *J. Chem. Soc.*, **1964**, 98.



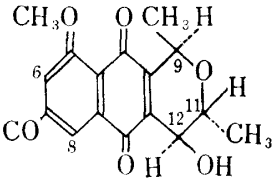
IV



Va : $R_1=R_2=COCH_3$
Vb : $R_1=R_2=CH_3$
Vc : $R_1=H, R_2=CH_3$

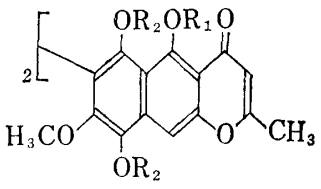


VI



VII

The $C_{(9)}$–proton of formula C (OCH_3 instead of OH) should correspond to the proton at $C_{(5)}$ of flaviolin trimethyl ether (Vb) and at $C_{(8)}$ of naphthoquinone dimethyl ether A (VII) which shows the signal at τ 2.78 and 2.74, respectively. All these τ–values of aromatic proton signal of reference compounds are higher than those of aurofusarin diacetate and dimethyl ether. Hence the formulas B,C, and D are ruled out. The UV–spectrum of 2–methyl–4*H*–naphtho[2,3–*b*]pyrane–4,5,10(5*H*,10*H*)–trione (VI)[13] [λ_{max}^{EtOH} mμ (log ε) 233 (4.32) (inflex), 241 (4.37), 253 (4.31) (inflex), 270 (3.92) (inflex), 3.05 (3.78)] which shows a remarkable difference from those of aurofusarin diacetate and dimethyl ether, also revealed that formulas C and D are incompatible with aurofusarin. The formula A (OCH_3 instead of OH) is the most probable for aurofusarin dimethyl ether whose $C_{(10)}$–H would be corresponding to $C_{(10)}$–H of rubrofusarin dimethyl ether (Ib) (τ 2.66). The anisotropic effect of neighbouring quinonic C=O at $C_{(9)}$ in the formula A would cause a lower shift of the signal of $C_{(10)}$–H to τ 2.08.



VIIIa : $R_1=H, R_2=COCH_3$
VIIIb : $R_1=R_2=COCH_3$

Reduction of aurofusarin diacetate with zinc dust in acetic anhydride and pyridine afforded tetrahydroaurofusarin tetraacetate, which gave a positive ferric chloride (green) and diazo reaction (orange red). On acetylation, it yielded hexaacetate, pale yellow plates, mp 260—261° (decomp.), whose UV spectral curve is almost parallel with that of rubrofusarin diacetate (Ic)[4] indicating that the fundamental structure of aurofusarin is a linear 2–methyl–γ–pyrone–naphthoquinone (Table III).

Table III. Comparison of UV and NMR Spectra of Tetrahydroaurofusarin Hexaacetate and Rubrofusarin Diacetate

	VIIIb	Ic
UV λ_{max}^{EtOH} mμ (log ε)	230(4.54), 268(4.92), 340(4.01) (inflex), 360(4.00)	224(4.27), 258(4.54), 269(4.51) (inflex), 342(3.80)
NMR in $CDCl_3$ (τ)		
Arom. C_{10}–H	2.27(2H)(s)	2.42(H)(S)
C_9–H		3.02(H)(d, J=3 cps)
C_7–H		3.18(H)(d, J=3 cps)
C_3–H	3.97(2H)(S)	4.05(H)(S)
Arom. $O\underline{CH_3}$	6.28(6H)(S)	6.04(3H)(S)
Arom. $C\underline{H_3}$ and Arom. O–$COCH_3$	7.42(6H)(S)	7.44(3H)(S)
	7.46(6H)(S)	7.58(3H)(S)
	7.62(6H)(S)	7.62(3H)(S)
	7.94(6H)(S)	

13) S. Fukushima, Y. Akahori, and A. Ueno, *Chem. Pharm. Bull.* (Tokyo), 12, 316 (1964).

The comparison of NMR spectra of tetrahydroaurofusarin hexaacetate (VIIIb) and rubrofusarin diacetate (Ic)[4] showed that the signals corresponding to $C_{(7)}$–H and $C_{(9)}$–H of the latter disappeared in the former (Table III).

The C–C linkage connecting two monomeric moieties of aurofusarin would therefore be located at $C_{(9-9')}$ or $C_{(7-7')}$. The possibility of *o*–quinone structure of aurofusarin was rejected since it did not react with *o*–phenylenediamine. Thus it has been concluded that aurofusarin is a *p*–quinone dimer connecting at $C_{(7-7')}$. The position of hydroxyl at $C_{(5)}$ of aurofusarin has been assigned in comparison with flaviolin dimethyl ether (Vc)[11] by the magnesium acetate colour reaction and IR–spectra. (Table IV).

Table IV

	Aurofusarin (IIa)	Flaviolin dimethyl ether (Vc)
$Mg(OAc)_2$ in EtOH	red	negative (pale pink)
IR $\nu_{max}^{CHCl_3}$ cm^{-1}	—	3440

All the evidences mentioned have supported the structural formula A(=IIa) to represent aurofusarin.

Experimental

Isolation and Purification of Aurofusarin (IIa)——According to the method reported previously by Raistrick, *et al.*, a crude pigment (2 g) was obtained by repeated extraction with $CHCl_3$ from the mycelia of *Fusarium culmorum* (W.G. Smith) Sacc. No. F–16 (200 g), which was cultivated on Raulin–Thom medium at 25° for 30 days at initial pH 3.4 and final pH 6.0.

Purification by chromatography over silica gel impregnated with 0.5 N oxalic acid using varying solvent systems, $CHCl_3$ and benzene–acetone [(9:1) and (4:1)], gave rubrofusarin (Ia) (500 mg, 0.25%), minor pigment and almost pure aurofusarin (IIa), which was recrystallized from $CHCl_3$–MeOH to form golden yellow plates, mp>330° (700 mg, 0.35%). It is insoluble in various alkalis, but shows intensive colour change from red to violet and exhibits a red colour with $Mg(OAc)_2$ in EtOH. $[\alpha]_D^{26}$: 0°, $[\alpha]_{700-450}^{26}$: 0° (saturated solution in $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{18}O_{12}\cdot CH_3OH$: C, 61.85; H, 3.68. Found: C, 61.89, 61.71, 62.01; H, 3.34, 3.47, 3.36. NMR in $CDCl_3$ (τ): 5.89 (S) (arom. $OC\underline{H}_3$), 7.57 (S) (α,α'–$C\underline{H}_3$ in γ–pyrone ring).

Alkaline Degradation of Aurofusarin——A suspension of IIa (500 mg) in 5% KOH (100 ml) was heated for 2 hr on a water bath under N_2–stream. The liquor distilled off was proved to be acetone, which was identified as 2,4–dinitrophenylhydrazone, orange prisms (from EtOH), mp 126° (50 mg). After cooling, the reaction mixture was acidified with 2 N HCl and dark brown resinous solids (400 mg) were separated off which were unstable and unsuccessful to crystallize. The filtrate was saturated with NaCl and then extracted with ether. Evaporation of the solvent left a brown oily substnace, from which AcOH was proved as ethylamine salt by paper chromatography (*Rf* value=0.26) using a saturated BtOH of H_2O as thedeve loping solvent.

An Attempt to prepare Quinoxaline Derivative (Reaction of IIa with *o*-Phenylenediamine)——A mixture of IIa (50 mg) and *o*–phenylenediamine (12 mg) in AcOH (15 ml) was warmed for 30 min on a water bath. After standing for 1 hr at room temperature, the reaction mixture was poured into ice–water and extracted with $CHCl_3$.

Removal of solvent recovered the starting material (IIa).

Aurofusarin Diacetate (IIc)——To a solution of IIa (100 mg) in dried $CHCl_3$ (80 ml) and pyridine (2 ml), AcCl (2 ml) was added slowly dropwise for 1 hr under stirring in an ice–bath. After further addition of pyridine (1 ml) and AcCl (1 ml) for 1 hr by the same way as described above, the reaction mixture was allowed to stand for 1 hr at room temperature and poured into ice–water. The $CHCl_3$ layer separated was washed with a small amount of 1% $NaHCO_3$ and water, and dried with anhydrous Na_2SO_4.

The concentrated solution was chromatographed on silica gel using mixture of $CHCl_3$:acetone (9:1) as the solvent. Elution of the second yellow band from the bottom gave diacetate, which solidified with an addition of a small amount of MeOH and recrystallized from $CHCl_3$–benzene forming orange yellow plates, mp>330° (darken from 290°), (60 mg, 52%). $[\alpha]_D^{16}$: 0 (c=1, in $CHCl_3$). *Anal.* Calcd. for $C_{34}H_{22}O_{14}$: C, 62.39; H, 3.36. Found: C, 62.32; H, 3.75. UV λ_{max}^{EtOH} mμ (log ε); 223 (4.74), 270 (4.71), 350 (4.12). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1772 (acetyl C=O), 1680 (sh), 1665, 1655 (sh) (C=O).

Aurofusarin Dimethyl Ether (IIb)——IIa (200 mg) was methylated with CH_3I (16 ml) and Ag_2O (800 mg) by refluxing in dried $CHCl_3$ (160 ml) for 3 hr. After removal of Ag_2O by filtration, the reaction mixture

was concentrated and chromatographed on silica gel using benzene–acetone (9:1) as the solvent. The first yellow band was eluted and recrystallized from MeOH to give pale yellow needles, mp 250—251° (decomp.) (80 mg; 38%). *Anal.* Calcd. for $C_{32}H_{22}O_{12}$: C, 64.21; H, 3.68. Found: C, 64.02; H, 3.80. MW Calcd.: 598. Found: 588—888[14] (by osmometry). UV λ_{max}^{EtOH} mμ (log ε): 227 (4.72). 269 (4.66), 360 (4.08). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1682 (sh). 1665, 1657 (sh) (C=O).

Tetrahydroaurofusarin Hexa-acetate (VIIIb)——To a suspension of IIc (200 mg) and zinc dust (400 mg) in AcO_2 (15 ml), 15 drops of pyridine were added dropwise for 30 min under stirring at room temperature. After further stirring for 2 hr, the reaction mixture was kept to stand for 4 hr and then poured into ice water. The yellow precipitates separated were collected and chromatographed, in benzene:acetone (9:1), on silica gel. Elution of the first yellow fluorescent band gave orange yellow solids, (110 mg, 44%), which were unsuccessful to crystallize from usual organic solvents. It is insoluble in 5% NaOH and exhibits a green colour with 1% $FeCl_3$ and the positive diazo reaction. UV λ_{max}^{EtOH} mμ: 224, 260 (sh), 282, 388. This substance would be tetrahydroaurofusarin tetraacetate formulated as (VIIIa).

VIIIa (100 mg) obtained above was reacetylated with a mixture of Ac_2O (5 ml) and pyridine (2 ml) by refluxing at 110—120° for 4 hr. After treatment by usual way, the reaction mixture was extracted with $CHCl_3$ and washed with water and dried. Removal of solvent afforded a brown residue which was purified by chromatography on silica gel using a mixture of benzene:acetone (9:1) and (4:1).

From the second pale blue fluorescent band from the bottom, pale yellow plates, mp 260—261° (decomp.) (50 mg, 45%) were obtained which were recrystallized from MeOH containing a small amount of $CHCl_3$. It gives a negative $FeCl_3$ and diazo reactions. *Anal.* Calcd. for $C_{42}H_{34}O_{18}\cdot CH_3OH$: C, 60.14; H, 4.43. Found: C, 60.29; 60.54; H, 4.40, 4.18. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1780 (acetyl C=O), 1658 (pyrone C=O) 1620 (aromatic).

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14) Owing to the instability of the compound, the results fluctuated.