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Metabolic Products of Fungi. XXX[1] The Structure of Fuscofusarin

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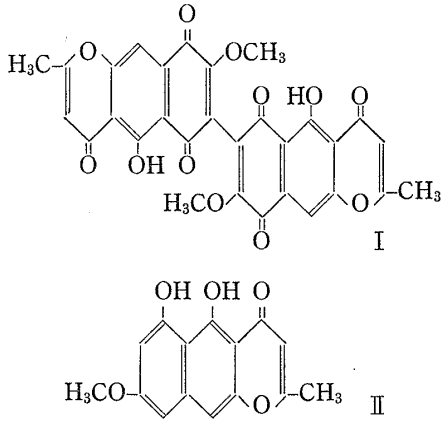
The structure of fuscofusarin, $C_{30}H_{20}O_{11}$, mp >300°, which was isolated from *Fusarium culmorum* (W.G. Smith) Sacc. along with aurofusarin (I) and rubrofusarin (II) has been established to be formulated as III.

Previously we reported[3] the structure of aurofusarin (I) which was isolated from the mycelia of *Fusarium culmorum* (W.G. Smith) Sacc.,[4] along with rubrofusarin (II).[5,6,7] The present paper concerns with a minor pigment named fuscofusarin of the same mould. Fuscofusarin, $C_{30}H_{20}O_{11}$, mp >300°, optically inactive brown red crystals (from methanol), showed a positive quinonic reaction (brown with magnesium acetate) and a positive Gibbs' reaction. The ultraviolet (UV) (Fig. 1) and infrared (IR) spectra (Table I) of fuscofusarin suggested its close structural correlation with aurofusarin and rubrofusarin.



I

II

Table I. IR–Absorptions $\nu(cm^{-1})$ in $CHCl_3$

	Fusco-fusarin	Auro-fusarin	Rubro-fusarin
Non–chelated C=O	1665	1680	—
γ–Pyrone C=O	1650	1665	1660
Chelated C=O	1620	1615	—
Aromatic Double bond	1603	1600	1600

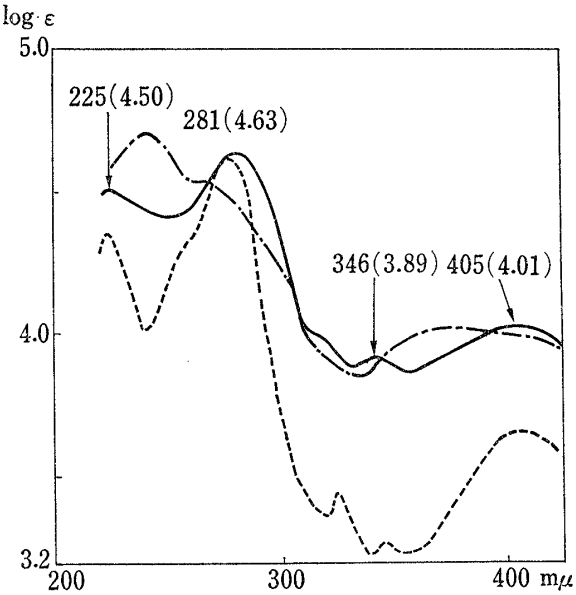


Fig. 1

aurofusarin
fuscofusarin
rubrofusarin

1) Part XXIX: E. Morishita, T. Takeda and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **16**, 411 (1968).
2) Location: *Hongo, Tokyo.*
3) S. Shibata, E. Morishita, T. Takeda and K. Sakata, *Chem. Pharm. Bull.* (Tokyo), **16**, 405 (1968); E. Morishita, T. Takeda and S. Shibata, *ibid.*, **16**, 411 (1968).
4) Aurofusarin has been isolated also from *Fusarium rigidiuseura.*
5) G.H. Stout and D.L. Dreyer, *Acta Cryst.*, **15**, 451 (1961); *Chem. Ind.* (London), **1961**, 289.
6) H. Tanaka, Y. Ohno and T. Tamura, *Tetrahedron Letters*, **1961**, 151; *Agr. Biol. Chem.* (Tokyo), **27**, 48 (1963).
7) S. Shibata and E. Morishita, *Chem. Pharm. Bull.* (Tokyo), **11**, 821 (1963).

On acetylation with acetic anhydride and pyridine, fuscofusarin yielded diacetate, orange needles, mp 270° (decomp.) and triacetate, yellow needles, mp 269° (decomp.), whose IR-spectra are shown below.

Table II. IR Spectra (in $CHCl_3$, cm^{-1}) of Fuscofusarin Diacetate and Fuscofusarin Triacetate

	Fuscofusarin diacetate	Fuscofusarin triacetate
Phenolic acetate C=O	1770	1780
Non-chelated C=O	1668	1670
γ-Pyrone C=O	1650	1660
Chelated C=O	1634	—
Aromatic double bond	1607	1615, 1610

From these data it has been suggested that fuscofusarin is a dimeric compound consisting of rubrofusarin and a monomeric half of aurofusarin.

This has also been supported by the NMR spectral analysis of fuscofusarin acetates.

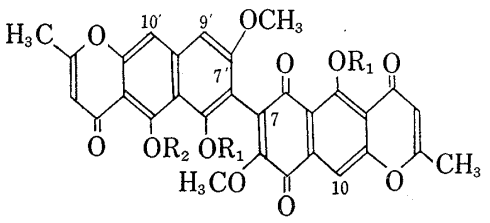
Table III. NMR Spectra (in $CDCl_3$, τ Value)

Fuscofusarin diacetate			Fuscofusarin triacetate		
τ			τ		
7.76	(3H s)	CH_3COO (6′)	7.81	(3H s)	CH_3 COO (6′)
7.60	(9H s)	CH_3COO (5) CH_3 (2) (2′)	7.55	(12H s)	CH_3 COO (5) (5′) CH_3 (2) (2′)
6.10	(6H s)	CH_3O (8) (8′)	6.12	(6H s)	CH_3O (8) (8′)
3.97	(1H s)	H (3′)	4.04	(1H s)	H (3′)
3.86	(1H s)	H (3)	3.88	(1H s)	H (3)
3.05	(1H s)	H (9′)	2.95	(1H s)	H (9′)
2.93	(1H s)	H (10′)	2.38	(1H s)	H (10′) (down-field shifted by
1.90	(1H s)	H (10)	1.94	(1H s)	H (10) acetylation of *p*-hydroxyl)
−4.74	(1H s)	OH (5′)			

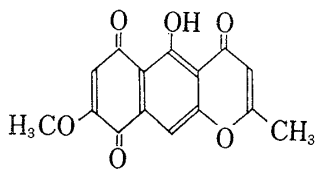
The proton signals at τ 1.90 and τ 1.94 of fuscofusarin di- and triacetate, respectively, correspond to $H_{(10)}$ of aurofusarin.

Since acetylation of *p*-hydroxyl caused down-field shift of aromatic proton, the proton signal at τ 2.93 in the diacetate which corresponds to the signal at τ 2.38 in the triacetate would be assigned to $H_{(10')}$. The proton signals at τ 3.97 and 3.86 in the diacetate and τ 4.04 and τ 3.88 in the triacetate would correspond to the pyrone-ring protons.

Since the proton signal corresponding the $C_{(7)}$ of the monomer of aurofusarin, mp 260°, (VI) was not observed in the NMR spectrum of fuscofusarin acetates, the $C_{(7)}$ of the monomeric aurofusarin moiety is linking with $C_{(7)}$ or $C_{(9)}$ of the rubrofusarin moiety.



III : $R_1=R_2=H$
IV : $R_1=Ac, R_2=H$
V : $R_1=R_2=Ac$



VI

Finally, fuscofusarin has been oxidized with Fremy's salt, and the product has been identified with aurofusarin (I). Thus fuscofusarin, the diacetate and the triacetate are formulated as III, IV and V, respectively.

Experimental

Isolation and Purification of Fuscofusarin (III)——The crude pigment (360 mg) was isolated by the extraction with chloroform from the mycelia of *Fusarium culmorum* (W.G. Smith) Sacc. F-16 strain (200 g) cultivated on Raulin-Thom medium at 25° for 30 days at initial pH 3.4 and final pH 6.0. On chromatography over silica gel impregnated with 0.5 N oxalic acid using varying solvent systems, chloroform and benzene–acetone (9:1) fuscofusarin was separated, which was recrystallized from methanol. Brown powder mp >300° (Yield: 260 mg (0.13%)). It is insoluble in alkali and gives a brown colouration with magnesium acetate in ethanol. *Anal.* Calcd. for $C_{30}H_{20}O_{11}$: C, 64.75; H, 3.60. Found: C, 64.47; H, 3.77.

Diacetate (IV)——Fuscofusarin (100 mg) was acetylated with acetic anhydride (20 ml) and pyridine (4 ml) at room temperature allowing to stand overnight. The reaction mixture poured into water was extracted with chloroform.

On chromatography over silica gel using benzene–acetone (4:1) as the solvent, fuscofusarin diacetate was eluted from the first yellow band which was recrystallized from ethanol to form orange needles, mp 268—270° (decomp.) (Yield: 15 mg). *Anal.* Calcd. for $C_{34}H_{24}O_{13}\cdot 2H_2O$: C, 60.76; H, 4.20. Found: C, 61.28; H, 4.29. UV λ_{max}^{EtOH} mμ (log ε): 222 (4.49), 257 (sh) (4.48), 278 (4.71), 323 (3.75), 350 (3.75).

Triacetate (V)——From the second band of chromatogram fuscofusarin triacetate was isolated, which was recrystallized from methanol to form yellow needles, mp 267—269° (decomp.) (Yield: 30 mg). *Anal.* Calcd. for $C_{36}H_{26}O_{14}\cdot H_2O$: C, 61.71; H, 4.00. Found: C, 61.80; 61.90, H, 4.22, 4.25. UV λ_{max}^{EtOH} mμ (log ε): 226 (4.62), 272 (4.86), 346 (4.12). $[\alpha]_{700-450}^{24°}$—450: 0° (in $CHCl_3$).

Oxidation of Fuscofusarin (III) to Aurofusarin (I) (7,7′-Bis[5-hydroxy-8-methoxy-2-methyl-4*H*-naphtho-[2,3-*b*]pyrane-4,6,9(6*H*, 9*H*)-trione])——Fuscofusarin (III) (50 mg) dissolved in dioxane (10 ml) was added dropwise into 2% aqueous solution of Fremy's salt (140 mg).

After stirring for 24 hr at room temperature, the reaction mixture was poured into water and extracted with chloroform repeatedly.

The extract was washed with water, dried over anhydr. Na_2SO_4 and concentrated, which was chromatographed in benzene–acetone 4:1 on a column of silica gel impregnated with 0.5 N oxalic acid.

Elution of the first yellow band gave a quinone which was crystallized from chloroform–methanol to give golden yellow prisms (15 mg) (29.3%).

It was proved to be identical with aurofusarin by the comparison of IR spectra (KBr–Tab.) and thin-layer chromatograms. *Anal.* Calcd. for $C_{30}H_{18}O_{12}\cdot MeOH$: C, 61.85; H, 3.68. Found: C, 61.80; H, 3.42.

5-Hydroxyl-8-methoxy-2-methyl-4*H*-naphtho[2,3*b*]pyrane-4,6,9(6*H*, 9*H*)-trione (VI)——Rubrofusarin (II) (100 mg) dissolved in dioxane (20 ml) was added dropwise into 2% aqueous solution of Fremy's salt (270 mg).

The chloroform extract was chromatographed in benzene–acetone 4:1 as a solvent on a column of silicic acid.

Elution of the third yellow band gave a quinone which was crystallized from methanol to give yellow prisms, mp 258—260° (decomp.) (60 mg) (57.1%). *Anal.* Calcd. for $C_{15}H_{10}O_6$: C, 62.94; H, 3.52. Found: C, 62.88; H, 3.61. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1685, 1665, 1625, 1603. UV $\lambda_{max}^{dioxane}$ mμ: 240, 264, 340. NMR τ (in $CDCl_3$) (100 Mc): 2.36 (H,s), 3.76 (H,s), 3.80 (H,s), 6.09 (3H,s), 7.56 (3H,s), OH nondetectable.

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