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

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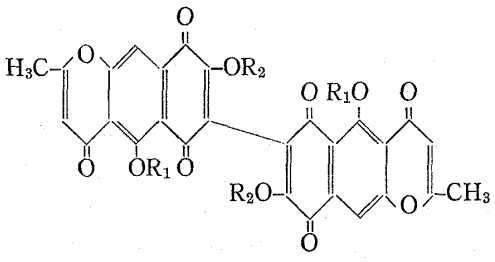
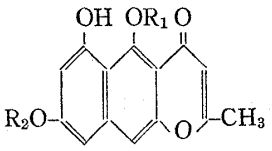
The structure of aurofusarin (Ia) was established by the biogenetical type synthesis. Rubrofusarin (IIa) was partially methylated to yield monomethyl ether A (IIb) which was converted into IIIa by oxidation with Fremy's salt. Partial demethylation of IIIa to IIIb followed by oxidative coupling using potassiumpersulphate as the reagent afforded 7,7′-bis(8-hydroxy-5-methoxy-2-methyl-4*H*-naphtho[2,3-*b*]pyrane-4,6,9-(6*H*, 9*H*)trione] (Ic), whose methyl ether was proved to be identical with aurofusarin dimethyl ether (Ib).

In the previous paper,[4] we proposed a structural formula (Ia) for aurofusarin, a golden yellow pigment of *Fusarium culmorum* (W.G. Smith) Sacc.[5]

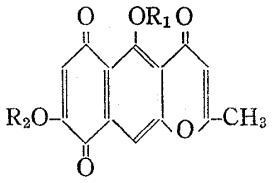


Ia : R_1=H, R_2=CH_3
Ib : R_1=R_2=CH_3
Ic : R_1=CH_3, R_2=H

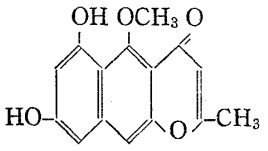
The structure (Ia) of aurofusarin has been established by the biogenetical type synthesis, starting with rubrofusarin (IIa)[5] which occurs in the same fungus.



IIa : R_1=H, R_2=CH_3
IIb : R_1=R_2=CH_3



IIIa : R_1=R_2=CH_3
IIIb : R_1=CH_3, R_2=H



IV

Rubrofusarin (IIa) was methylated with diazomethane to yield rubrofusarin monomethyl ether A (IIb) whose structure was synthetically proved by us[6]. On oxidation of IIb with Fremy's salt[7] at room temperature, 5,8-dimethoxy-2-methyl-4*H*-naphtho[2,3-*b*]pyrane-4,6,9(6*H*,9*H*)-trione (IIIa), mp 254—256° (decomp.) was obtained in a yield of 50.5%. The UV, IR and NMR spectra of IIIa and aurofusarin dimethyl ether (Ib)[4] were compared (Table I).

1) Part XXVIII, S. Shibata, E. Morishita, T. Takeda, and K. Sakata, *Chem. Pharm. Bull.*(Tokyo), **16**,405 (1968).
2) (1) see 1)
3) Location: *Hongo, Tokyo.*
4) S. Shibata, E. Morishita, T. Takeda, and K. Sakata, *Chem. Pharm. Bull.* (Tokyo), **16**, 405 (1968).
5) J.N. Ashley, B.C. Hobbs, and H. Raistrick, *Biochem. J.*, **31**, 385 (1937).
6) S. Shibata, E. Morishita, and Y. Arima, *Chem. Pharm. Bull.* (Tokyo), **11**, 821, (1963); *ibid.*,**15**, 1757 (1967).
7) H.A. Anderson, J. Smith, and R.H. Thomson, *J. Chem. Soc.*, **1965**, 2141.

Table I

Compound	IIIa	Ib
UV λ_{max}^{EtOH} mμ (log ε)	225(4.39), 265(4.45), 347(3.68)	227.5(4.72), 269(4.66), 360(4.08)
IR $\nu_{max}^{CHCl_3}$ cm^{-1}	1665(broad), 1655(sh), 1624, 1595	1665(broad), 1657(sh), 1595
NMR in $CDCl_3$ (τ)		
Arom. H-$C_{(10)}$	2.03(H)(s)	2.08(2H)
$C_{(7)}$	3.81(H)(s)	
$C_{(3)}$	3.84(H)(s)	3.88(2H)
Arom. $OC\underline{H}_3$	5.92(3H)(s)	5.92(6H)
	6.08(3H)(s)	6.04(6H)
Arom. $C\underline{H}_3$	7.59(3H)(s)	7.61(6H)

i) The UV spectral curves of both compounds are almost superimposable; IIIa showed slightly hypsochromic shifts of the absorption maxima in comparison with Ib.

ii) Ib is a C_7–$C_{7'}$ dimer of IIIa as indicated in the previous report[4]; the comparison of NMR signals of aromatic protons of IIIa and Ib agreed with that conclusion.

iii) The $C_{(10)}$–H signal is shifted to the lower field (τ 2.03—2.08) by the anisotropic effect of the neighbouring carbonyl at $C_{(9)}$. On heating IIIa with 10% H_2SO_4 at 90° for 1 hr, 8–methoxyl was demethylated to give 8–hydroxy–5–methoxy–2–methyl–4*H*–naphtho[2,3–*b*]pyrane–4,6,9(6*H*,9*H*)–trione (IIIb), which was also derived from nor–rubrofusarin–5–methyl ether (IV)[6] by the air oxidation in caustic alkali.

By the oxidative coupling using $K_2S_2O_8$ by Thomson's method,[8] IIIb afforded 7,7′–bis[8–hydroxy–5–methoxy–2–methyl–4*H*–naphtho[2,3–*b*]pyrane–4,6,9(6*H*,9*H*)–trione](Ic), mp> 300°, in a yield of 12.5%. On methylation with CH_3I and Ag_2O, Ic gave the 8,8′–dimethyl ether (Ib), mp 250—251° (decomp.), which was proved to be identical with aurofusarin dimethyl ether.[4] Consequently, the structure of aurofusarin has been established as being 7,7′–bis[5–hydroxy–8–methoxy–2–methyl–4*H*–naphtho[2,3–*b*]pyrane–4,6,9(6*H*,9*H*)–trione] (Ia).

Experimental

6–Hydroxy–5,8–dimethoxy–2–methyl–4*H*–naphtho[2,3–*b*]pyrane–4–one (IIb) (Rubrofusarin Monomethyl Ether A) ——IIa (2.7 g) was methylated with ethereal CH_2N_2 (prepared from N–nitrosomethylurea (27 g)] by the method as described previously by Raistrick, *et. al.*[5] Purification by chromatography on silica gel using benzene: acetone (4:1) as a solvent gave pale yellow needles (from EtOH), mp 203—204° (1.96 g, 69%).

5,8–Dimethoxy–2–methyl–4*H*–naphtho[2,3–*b*]pyrane–4,6,9(6*H*, 9*H*)–trione (IIIa)——IIb (1 g) dissolved in dioxane (200 ml) was added dropwise into 2% aqueous solution of Fremy's salt (3.2 g). After stirring for 24 hr at room temperature, the reaction mixture was poured into H_2O and extracted with $CHCl_3$ repeatedly. The extract was washed with H_2O, dried over anhyd. Na_2SO_4 and concentrated, which was chromatographed, in benzene: acetone (4:1) as a solvent, on a column of silica gel. Elution of the first yellow band gave a quinone which was crystallized from $CHCl_3$–EtOH to give pale yellow needles, mp 254—256° (decomp.) (530 mg, 50.5%). *Anal.* Calcd. for $C_{16}H_{12}O_6$: C, 64.05; H, 4.03. Found: C, 64.17; H, 4.34.

8–Hydroxy–5–methoxy–2–methyl–4*H*–naphtho[2,3–*b*]pyrane–4,6,9(6*H*, 9*H*)–trione (IIIb)——i) Acid hydrolysis of IIIa: To a solution of IIIa (400 mg) in dioxane (40 ml) and EtOH (130 ml), 10% H_2SO_4 (60 ml) was added. The mixture was refluxed for 1 hr at 90° on a water bath. After cooling, it was poured into H_2O and extracted with $CHCl_3$. The concentrated extract was purified by chromatography on silica gel using $CHCl_3$:acetone (9:1) as the developing solvent. The second yellow band was eluted and recrystallized from EtOH to give yellow needles, mp 215—217° (decomp.) (150 mg, 39%). From the first yellow band, the starting material (IIIa) (160 mg, 41%) was recovered. The product is soluble in 5% NaOH giving an orange red colour and no colour change with $Mg(OAc)_2$ in EtOH. *Anal.* Calcd. for $C_{15}H_{10}O_6$: C, 62.99; H,

8) R.H. Thomson and A.G. Wylie, Private Communication (Nov. 1966).

3.53. Found: C, 62.86; H, 3.63. UV λ_{max}^{EtOH} mμ (log ε): 221 (4.38), 267 (4.37), 325 (3.97), 350 (3.81). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440 (OH), 1657 (sh), 1665, 1655 (sh) (C=O).

ii) Aerial Oxidation of IV: Oxygen-stream was passed through a solution of IV (60 mg) dissolved in 1% NaOH (10 ml) for 4 hr under ice-cooling. Finally the reaction mixture showed a dark red colour. Acidification with 5% HCl separated orange yellow solids which were collected and dissolved into $CHCl_3$; the filtrate was also extracted with $CHCl_3$. The combined $CHCl_3$ solution was washed with H_2O and dried over anhyd. Na_2SO_4. After removal of the solvent, it was purified by chromatography, in benzene:acetone (4:1) on a column of silicic acid. From the second yellow band, yellow needles (from EtOH), mp 215—217° (decomp.) (20 mg) (32%) were obtained. *Anal.* Calcd. for $C_{15}H_{10}O_6$: C, 62.99; H, 3.53. Found: C, 63.15; H, 3.58. It was identified with the substance obtained by acid hydrolysis of IIIa by the comparison of UV and IR spectra (KBr-Tab.) and thin-layer chromatograms.

7,7′-Bis[8-hydroxy-5-methoxy-2-methyl-4*H*-naphtho[2,3-*b*]pyrane-4,6,9(6*H*,9*H*)-trione] (**Ic**)——A warm solution of IIIa (200 mg) in H_2O (20 ml) containing 2N-NaOH (0.35 ml) was gradually dropped into $K_2S_2O_8$ (0.19 g) dissolved in H_2O (6 ml) for 10 min at 95° under vigorous stirring. After cooling, H_2O was added into the reaction mixture to separate brown solids, which were collected, washed with H_2O and dried *in vacuo*. It was chromatographed on a column of silicic acid using $CHCl_3$:acetone (2:1) as solvent. From the first yellow band, a small amount of the starting material (12 mg, 6%) was recovered, and from the second orange yellow band, yellow plates, mp>300° (recrystallized from MeOH), were obtained (Yield: 50 mg, 12.5%). It dissolves in 5% NaOH giving an orange brown colour, shows the same colour with 1% $FeCl_3$ and exhibits a negative $Mg(OAc)_2$ test. *Anal.* Calcd. for $C_{30}H_{18}O_{12}\cdot CH_3OH$: C, 61.85; H, 3.68. Found: C, 62.30; H, 3.69. UV λ_{max}^{EtOH} mμ (log ε) 223 (4.65), 268 (4.59), 331 (4.27), 366 (4.09). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3330 (OH), 1675 (sh), 1665, 1645 (sh) (C=O).

7,7′-Bis[5,8-dimethoxy-2-methyl-4*H*-naphtho[2,3-*b*]pyrane-4,6,9(6*H*,9*H*)-trione] (**Ib**)——Ic (50 mg) was methylated with CH_3I (8 ml) and Ag_2O (200 mg) in dried $CHCl_3$ by refluxing for 1 hr. Removal of Ag_2O by filtration, and then evaporation of the solvent *in vacuo* left a brown oily substance which was purified by chromatography, in benzene:acetone (4:1), on silica gel to afford pale yellow needles, mp 250—251° (decomp.) (from MeOH containing a small amount of $CHCl_3$) (20 mg, 36%). It was proved to be identical with the dimethyl ether derived from natural aurofusarin by the comparison of IR spectra (KBr-Tab) and thin-layer chromatograms. *Anal.* Calcd. for $C_{32}H_{22}O_{12}$: C, 64.21; H, 3.68. Found: C, 64.02; H, 3.80. UV λ_{max}^{EtOH} mμ (log ε): 227.5 (4.77), 270 (4.73), 360 (4.21). IR ν_{max}^{KBr} cm^{-1}: 1668 (broad), 1650 (sh), 1635 (sh) (C=O).

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