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THE STRUCTURE OF AUROFUSARIN

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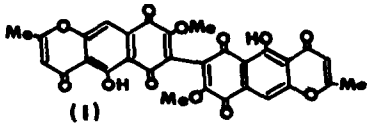
Recently, Baker and Roberts (1) have put forward a structure (I) for aurofusarin, a colouring matter of Fusarium spp., which was first isolated and studied by Ashley, Hobbs and Raistrick (2).



(I)

This prompts us to report our study on the structure of aurofusarin which has led to the same conclusion as being represented by the formula (I). Aurofusarin was obtained along with rubrofusarin (II) (2,3,4,5) and unidentified minor pigment from the mycelia of Fusarium culmorum (W.G. Smith)Sacc. No.F-16, which was cultivated on Raulin-Thom medium at 25° for 30 days at initial pH 3.4 and final pH 6.0. The pigments were separated by the column chromatography over silica gel impregnated with oxalic acid with varying solvent systems, chloroform, benzene-acetone (9:1), and benzene-acetone (4:1) to eluate rubrofusarin, the minor pigment and aurofusarin, respectively.

Aurofusarin, $C_{30}H_{18}O_{12}$, golden yellow plates (from $CHCl_3$ and

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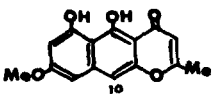
MeOH), m.p. >330°, $[\alpha]_D^{26}$: 0°, $[\alpha]_{700-450}^{26}$: 0° (in $CHCl_3$)(Anal. calcd. for $C_{30}H_{18}O_{12}\cdot CH_3OH$: C, 61.85; H, 3.68. Found: C, 61.89, 61.71, 62.01; H, 3.34, 3.47, 3.36), is very sparingly soluble in usual organic solvents and shows intensive colour change into violet in alkalis, though it does not dissolve in them.

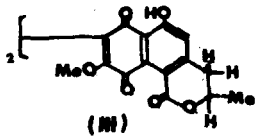
Similar colour change was observed in xanthomegnin (III) of Trichophyton megnini Blanchard (6), which gave an almost parallel U.V. curve ($\lambda_{max}^{Dioxane}$ mμ (log ε): 229 (4.71), 285 (4.25) inflex., 390 (4.05)) with that of aurofusarin ($\lambda_{max}^{Dioxane}$ mμ(log ε) : 243 (4.69), 267 (4.52), 372 (4.05)) suggesting a close similarity in the structures of both compounds. The positive magnesium acetate reaction in ethanol (red) which is characteristic for α-hydroxy quinonic structure also supported the 8-hydroxynaphthoquinone system in aurofusarin. The I.R. spectrum of aurofusarin ($\nu_{max}^{CHCl_3}$ cm^{-1}: 1680 (non-chelated C=O), 1665 (chelated C=O), 1615, 1600 (aromatic), no OH band) showed that the hydroxyl is strongly hydrogen bonded with carbonyl.

The N.M.R. spectrum of aurofusarin indicated the presence of methoxyl (τ 5.89). Alkaline degradation of aurofusarin yielded acetone and acetic acid to suggest the presence of an α-methyl-γ-pyrone ring system which would be rationalized biogenetically by the co-occurrence of rubrofusarin (II), and was supported by the N.M.R. spectrum (τ 7.57 (s) $C-CH_3$ in γ-pyrone ring).

Aurofusarin diacetate, $C_{34}H_{22}O_{14}$ (Anal. calcd.: C, 62.44; H, 3.39. Found: C, 62.81; H, 3.46), yellow plates, m.p. >330° (from $CHCl_3$-benzene), $[\alpha]_D$: 0° (in $CHCl_3$), was prepared by the action of acetyl chloride in dried chloroform and pyridine



(II)



(III)

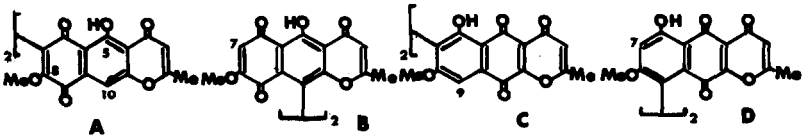
under ice cooling. The U.V. spectrum of the diacetate (λ_{max}^{EtOH} mμ (log ε): 223 (4.74), 270 (4.71), 350 (4.12)) resembled closely with that of triacetate of flaviolin (= 3,6,8-trihydroxynaphthoquinone) (IV) (7) (λ_{max}^{EtOH} mμ (log ε): 255 (4.25), 350 (3.65)). The N.M.R. spectrum (in $CDCl_3$) showed the signals of τ 1.90 (s. 2H), 3.86 (s, 2H (ββ'-H of γ-pyrone ring)), 5.90 (s, 6H (aromatic OCH_3)), 7.55 (s, 6H (αα'-CH_3 in γ-pyrone ring) broad, long range coupling with ββ'-H), 7.59 (s, 6H (acetyl)).

On methylation with MeI and Ag_2O in dried chloroform, aurofusarin afforded dimethyl ether, $C_{32}H_{22}O_{12}$ (Anal. calcd.: C, 64.21; H, 3.68. Found: C, 64.17; H, 4.03), yellow needles, m.p. 250-251° (decomp.)(from MeOH), U.V. λ_{max}^{EtOH} mμ (log ε): 227 (4.72), 269 (4.66), 360 (4.08); I.R. $\nu_{max}^{CHCl_3}$ cm^{-1}: 1682 (sh.), 1665, 1657 (sh.), 1595; N.M.R. (in $CDCl_3$): τ 2.08 (s, 2H), 3.88 (s, 2H (ββ'-H of γ-pyrone ring)), 5.92 (s, 6H), 6.04 (s, 6H) (aromatic OCH_3), 7.61 (s, 6H (αα'-CH_3 in γ-pyrone ring)); the molecular weight determination by osmometer (Calcd.: 598. Found: 588-888**) indicated a dimeric structure whose monomeric moieties were proved to be equivalent, since all the signals in the N.M.R. spectrum were exactly doubled.

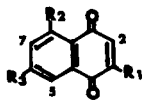
A possibility of o-quinone structure of aurofusarin was excluded by the negative reaction with o-phenylenediamine.

** Owing to the instability of the compound in solution, the results fluctuated.

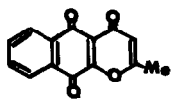
Consequently, the following possible structures of aurofusarin (A∼D) have been deduced:



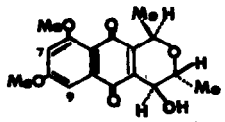
The N.M.R. signal of aromatic ring proton of aurofusarin diacetate and dimethyl ether appears at τ 1.90 and 2.08, respectively, which is in very lower field than expected for the proton at $C_{(7)}$ of the formula B in comparison with the signal of the corresponding proton at $C_{(2)}$ of naphthoquinone (VI)(τ 3.03) and flaviolin trimethyl ether (V)(τ 4.00)(7). It is also too low for assigning it to the proton at $C_{(7)}$ of the formula D in comparison with the corresponding proton at $C_{(7)}$ of V (τ 3.28) and naphthoquinone dimethyl ether A (VIII) (τ 3.27) derived from protoaphin-fb (9). Thus the formulae B and D are improbable for aurofusarin.



(IV) $R_1=R_2=R_3=$ $OCOCH_3$
(V) $R_1=R_2=R_3=$ OCH_3
(VI) $R_1=R_2=R_3=H$
(XI) $R_1=OH$, $R_2=R_3=$ OCH_3



(VII)

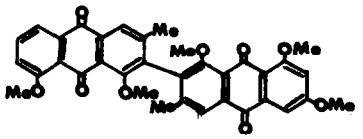


(VIII)

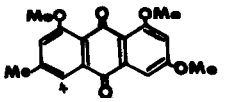
The formula C was excluded by the comparison of U.V. spectra of the diacetate and the dimethyl ether of aurofusarin with that of 2-methyl-4H-naphtho(1,2-b)pyran-4,5,6-trione (VII) (8)which exhibited a quite different type of absorption curve (λ_{max}^{EtOH} mμ

(log ε): 233 (4.32),241 (4.37), 253 (4.31 inflex.), 270 (3.92 inflex.), 305 (3.78). The expected chemical shift of the N.M.R. signal of $C_{(9)}$-proton in the formula C would appear around 2.78 or 2.74 from the data given by V and VIII.

β-Dimerization would not give so remarkable effect to the chemical shift of ring proton in the m-position to the ββ'-C-C linkage of the two moieties, since such a proton signal in the spectrum of a ββ'-bimolecular compound, cassiamin pentamethyl ether (IX) (τ 2.10) (10) showed only a slight lower shift in comparison with the proton at $C_{(4)}$ of a monomer, emodin trimethyl ether (X) (τ 2.34).



(IX)



(X)

The unusual lower shift of the N.M.R. signal of the aromatic ring proton of aurofusarin derivatives would reasonably be explained by the formula A. It has already been known that the proton at $C_{(10)}$ of rubrofusarin derivatives shows the signal in down field, at τ 2.46~3.10 by the deshielding effect of ring current. The quinonic carbonyl at $C_{(9)}$ of aurofusarin derivatives would give an anisotropic effect to intensify much lower shift of the signal (τ 1.90~2.08). The presence of aromatic proton at $C_{(5)}$ and hydroxyl at $C_{(10)}$ in aurofusarin would not be ruled out regarding only from the down field shift of the aromatic proton signal. However, it would be improbable from biogenetical point of view of co-occurrence of rubrofusarin, as well as by the resistance in acetylation and methylation

of aurofusarin by usual methods. It can only be explained by the presence of hydroxyl at $C_{(5)}$, which is strongly hindered by the neighbouring carbonyls. The strong hydrogen bonding of the hydroxyl of aurofusarin which gave no hydroxyl band in the I.R. spectrum in $CHCl_3$ would also be rationalized when the hydroxyl exists at $C_{(5)}$, rather than at $C_{(8)}$. This has also been supported by the strong positive (red) magnesium acetate reaction of aurofusarin in comparison with the almost negative colouration of flaviolin dimethyl ether (3-OH free)(XI) with this reagent. All the evidences mentioned above have led to the structure (I) for aurofusarin.

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