NEW ANTIBIOTIC PIGMENTS RELATED TO FUSARUBIN FROM *FUSARIUM SOLANI* (MART.) SACC.

II. STRUCTURE ELUCIDATIONS

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Three antibiotic pigments isolated from *Fusarium solani* and related to fusarubin (**4**) were shown to be O-ethylfusarubin (**2**), hydroxydihydrofusarubin (**3**) and O-ethylhydroxydihydrofusarubin (**1**).

Recently a cholesterol-metabolizing fungus, isolated from soil and identified as *Fusarium solani* was shown to produce four antibiotic pigments of the fusarubin type[1]. Because of interest in their possible antitumor activity[1,2] we decided to determine the structures of all four antibiotics.

Materials and Methods

The cultural conditions, production, isolation and some purification methods have been described[1].

Chromatography

All column chromatography was carried out with Mallinckrodt CC-4, 100~200 mesh, eluting with $CHCl_3$ and if necessary, increasing concentrations of ethyl acetate in $CHCl_3$. Thin-layer chromatography (TLC) utilized MN Polygram Sil G/UV_{254} and $CHCl_3$ or $CHCl_3$ - ethyl acetate (4:1).

Spectra

Ultraviolet-visible absorption spectra were recorded on a Beckman UV 5270 Spectrophotometer. Infrared spectra were obtained using the Perkin-Elmer Model 283 Spectrophotometer. NMR spectra were obtained using a Varian T-60 instrument on solutions in $CDCl_3$ with tetramethyl silane as internal standard.

Chemical interconversions. *a*, *b*, and *c* in Fig. 1

To small amounts of **1** in ethanol was added an equal volume of 10% NaOH (for *a* and *b*) or 10% HCl (for *c*). After 1 hour at room temperature the deep blue-purple alkaline solutions from *a* and *b* were acidified using acetic acid for *a* and excess HCl for *b*. All three were extracted with $CHCl_3$ and the extracts examined by TLC. The conversions of **1** to **2** and **4** were essentially complete; however from reaction *c* some unchanged **1** could be seen.

d in Fig. 1. Fusarubin ethyl ether (**2**) from fusarubin (**4**)

Pure, dry **4** obtained by preparative TLC was dissolved in anhydrous ethanol and 2 ml of anhydrous ethanolic·HCl was added. After overnight at room temperature the solution was poured into excess dilute aqueous sodium acetate and the pigments extracted with $CHCl_3$. Column chromatography of the extract removed a small amount of by-product anhydrofusarubin (**5**) which eluted before **2**.

Anhydrofusarubin (**5**)

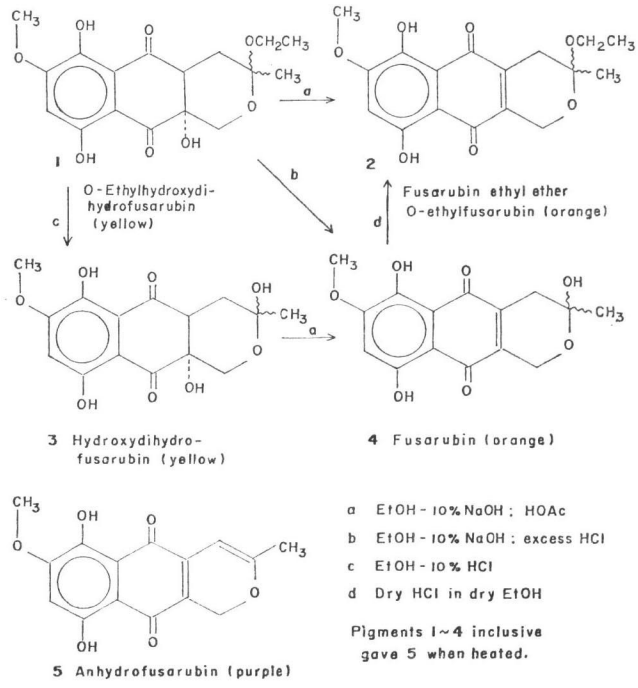
This was prepared most readily by heating dry fusarubin (**4**) for 30 minutes at 170°C. The black residue was extracted with $CHCl_3$ and pure **5** obtained by column chromatography of the $CHCl_3$ extract. The preparation of **5** from pigments **1**, **2** and **3** was similar and was demonstrated by TLC of the $CHCl_3$ extracts versus an authentic sample.

Results and Discussion

F. solani was grown first in shake flasks[1]. Pigments were readily extracted from acidified whole broth by chloroform and separated into two orange components by column chromatography or TLC (Rf=0.6 and 0.3). Both pigments were reversibly decolorized by sodium hydrosulfite and gave strong positive ferric chloride tests indicating hydroxyquinone structures. The ultraviolet-visible absorption spectra (UV-vis) λ_{max} 301, 472, 485 (sh), 498, 522 (small), 535 nm were identical and indicated a naphthoquinone chromophore since benzoquinones usually have only two maxima (*i.e.* 283, 425 nm) and anthraquinones have a closer spacing of the peaks in the visible region (*i.e.* 490, 510, 525 nm)[3]. For both pigments, the UV-vis and the conversion during the attempted melting point determination to purple crystals, λ_{max} 237, 290, 545 nm, suggested fusarubin[4]. In solution the purple substance was reversibly decolorized by sodium hydrosulfite. Fusarubin is known to dehydrate with heat and/or acid to give anhydrofusarubin, structure **5** in Fig. 1[5]. When the two orange pigments were analyzed using the TLC solvent systems developed for the classification of antitumor antibiotics[2] it was clear that the slower moving one was fusarubin and the faster moving one was different. The identity of the slower orange pigment with fusarubin was confirmed by comparison with an authentic sample. The suspected and authentic substances were identical in UV-vis, infrared spectra, TLC behavior and in conversion to anhydrofusarubin.

A structure which would allow for significantly greater TLC mobility in organic solvents but would permit other properties to be identical to those of fusarubin is an enol ether. Herbarin, O-methylherbarin and anhydroherbarin have been isolated from *Torula herbarium*[6]. The structure of herbarin is identical to that of fusarubin except that herbarin has two methoxyl groups rather than two hydroxy and one methoxy group in the left hand ring. However, the ^{1}H-NMR of our faster moving orange pigment clearly showed the methyl triplet and methylene quartet of an ethyl group as well as the expected bands for methoxyl, an aromatic proton and two strongly hydrogen bonded phenolic hydrogens (Table 1). Thus the faster moving orange pigment was the ethyl ether of fusarubin (structure **2**, Fig. 1) which had been chemically synthesized from fusarubin in 1950[5]. As a check, fusarubin ethyl ether was prepared both from authentic fusarubin and from our slower orange pigment. The products were identical with each other and identical in UV-vis, infrared spectra and TLC behavior with the faster orange pigment.

Fig. 1. Pigments related to fusarubin and their interconversions.



When the *F. solani* was grown in the fermentor[1], both the faster and slower moving components from column chromatography were seen to be mixtures of one of the above discussed orange pigments and a yellow substance, λ_{max} 272, 300 (small), 388 nm, which had a strong bluish fluorescence. Each pair of co-chromatographing yellow and orange pigments was at least partly separated by TLC. The faster moving yellow substance was obtained in greatest amount, about 150 mg from the 30-liter fermentation. TLC spots of the two yellow pigments became orange on the plate within 24 hours. Both yellow pigments when heated gave anhydrofusarubin, seen in TLC as a purple spot, Rf 0.7. It was suspected that each pair of co-chromatographing yellow and orange pigments were related to each other as are nanaomycins A and B, which had been isolated from *Streptomyces rosa*[7]. Nanaomycin B is a hydroxydihydronaphthoquinone similar to **3**. This was confirmed by the dehydration of each yellow pigment to its orange partner by dilute alkali at room temperature as had been reported for the conversion of nanaomycin B to A[7]. The chemical interconversions of the pigments are summarized in Fig. 1. In every case the identity of the product was confirmed by UV-vis and by TLC versus a previously isolated and authenticated product. Infrared spectra are summarized in Table 2.

For ^{1}H-NMR, pigments **2** and **3** were prepared in quantity from **1**. The NMR spectra are summarized and compared with that of fusarubin in Table 1. Since the NMR of **3** shows a methoxyl band like that in **1** but no peaks indicating an ethyl group, the easily hydrolyzed hemiacetal ether is ethyl not methyl. In the NMR of **2** the singlet C-methyl-band at δ 1.3 obscures somewhat the methyl triplet of the

Table 1. The ^{1}H-NMR spectra of the fusarubin group of pigments[a]

Pigment	OCH_3	Phenolic OH	Aromatic H	C-CH_3	Other[e]
1[b]	4.0	11.9, 12.2	6.63	1.4	OEt: 3.57 q, 1.2 t, J=5. $-CH_2O$: 4.2 dd. CH_2–C: see text
2[b]	4.0	12.7, 13.0	6.2	1.3	OEt: 3.7 q, 1.3 t, J=5. $-CH_2O$: 4.75 brd. CH_2–C: 2.87 brd.
3[b]	4.0	12.1, 12.2	6.75	1.5	CH_2O: 4.2 dd, 1.57 m, 2.3 m.
4[c]	[d]	[d]	6.36	[d]	methylenes: 3.1 and 5.2 brm.

a. Values given in δ units relative to tetramethyl silane (TMS)=0.
b. In $CDCl_3$.
c. In pyridine. Values taken from ref. 9.
d. Values not given in ref. 9.
e. q=quartet, t=triplet, dd=doublet of doublets, br=broad, m=multiplet, d=doublet.

Table 2. The infrared spectra of the fusarubin group of pigments.

Pigment	O-H[c]	C=O[c]	Other[c,d]
1[a]	3630s	1740w 1635s	1050, 1285, 1270, 1440, 1410, 1170, 1465~1480, 1005
3[a]	3600w	1730w 1635s	1270, 1440, 1170, 1410, 1465~1480, 1310, 1005
2[b]	—	1580sh 1600	390, 410, 445, 510, 540, 580, 650, 665, 810s, 840, 950, 970, 1055s, 1080s, 1100s, 1150s, 1170s, 1210s
4[b]	3380 3460	1575 1600	385, 410, 440, 645, 665, 705, 755s, 815s, 870s, 925, 950, 970, 1035, 1080, 1095, 1150s, 1175, 1215s

a. In $CHCl_3$. b. KBr disk.
c. w=weak, s=strong, sh=shoulder, Values given in cm^{-1}.
d. For **1** and **3** values are listed in order of decreasing intensity.

ethyl group but the methylene quartet of the ethyl group is clearly apparent at δ 3.7. The NMR of **1** clearly proves the position of the angular hydroxy group as shown since the $\underline{CH_2}$-O-methylene at δ 4.2 is definitely an AB type. It has four lines and is symmetrical[8]. Thus there is no coupling with any adjacent hydrogen; there is no adjacent hydrogen. On the other hand the–$\underline{CH_2}$–C-methylene is an AMX type[8] which can be seen as three doublets of doublets: the axial H of the $-CH_2$ at δ 1.57, the equatorial H of the $-CH_2$ at δ 2.33 and the H next to carbonyl at δ 2.97. The coupling constants which can be derived from the observed splittings, $J_{AM}=14$, $J_{AX}=4.5$; $J_{MX}=10$-11 imply that the H next to carbonyl is axial. The trans diaxial stereochemistry explains the facile dehydration.

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