## NOTES

# METABOLITES OF *FUSARIUM SOLANI* RELATED TO DIHYDROFUSARUBIN*

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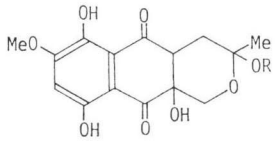
Fusarubin, a red antibiotic obtained from *Fusarium solani*[1] and shown[2] to be identical to oxyjavanicin isolated earlier from *Fusarium javanicum*[3], was characterized as **I** by RUELIUS and GAUHE[1]. We have recently shown that this quinonoid product accumulates in cultures of *F. solani* as the result of a non-enzymatic reaction[4]. The normal biosynthetic end-products are the diastereoisomeric dihydroquinones (**IIa**) which are formed by head-to-tail condensation of seven two-carbon units[5].

Two pigments (designated **3** and **1** in reference 6), which were isolated in low yield along with fusarubin and fusarubin 3-O-ethyl ether from the cholesterol-metabolizing strain PP 96 of *F. solani* and tentatively assigned structures **IIIa** and **IIIb**[6], can now be recognized as the diastereoisomeric




**IIIa**, R=H
**IIIb**, R=Et

dihydrofusarubins and their 3-O-ethyl ethers, respectively. Insufficient material was available for direct comparison but the close correspondence between the spectral characteristics of the dihydrofusarubins[4] and pigment **3**[6] is persuasive. A chemical ionization mass spectrum of compound **1** with methane as carrier gas gave five peaks: at *m/z* 377 (1.5%, M+41), 365 (23%, M+29), 337 (100%, M+1), 319 (19%, M+1−$H_2O$) and 291 (93%, M+1−EtOH). This corresponds to the molecular composition $C_{17}H_{20}O_7$ rather than $C_{17}H_{20}O_8$ and is consistent with structure **IIb** rather than the originally proposed **IIIb**. Fusarubin 3-O-ethyl ether can be prepared by treating fusarubin with anhydrous acidic ethanol[1] and 3-O-ethyl derivatives of dihydrofusarubins form during crystallization of the parent compounds from ethanol. Ethanol was not used *per se* during isolation of the pigments from strain PP 96 but may not have been completely removed from the chloroform used for their extraction and chromatographic purification. Thus the possibility that the 3-O-ethyl ethers are laboratory artifacts cannot be excluded. Alternatively, they might have been formed non-enzymatically in the culture from metabolic ethanol, although the substantial yield of ketal derivatives[6] suggests that a biological ethylation is more likely to have been responsible.

Under alkaline conditions the dihydrofusarubins oxidize spontaneously in air to fusarubin[4]. Whether fusarubin or the dihydrofusarubins accumulate in cultures of *F. solani* is, therefore, determined by the pH of the extracellular fluid into which the metabolites are excreted. In cultures grown in a maltose-ammonium tartrate-salts solution where the carbon: nitrogen ratio was varied by changing the initial maltose concentration, dihydrofusarubins were found in all cultures harvested 2 days after inoculation. However, at 6 days, cultures with a low initial carbon: nitrogen ratio contained mainly fusarubin. Conversion of dihydrofusarubins to fusarubin correlated with the sharp rise in culture pH observed after the third day (Fig. 1A). Cultures containing a high initial maltose concentration failed to show this pH change (Fig. 1B), which is attributed to exhaustion of the carbohydrate

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Fig. 1. Fusarubin and dihydrofusarubin production and pH changes in cultures of *F. solani* grown in ammonium tartrate (4.6 g/liter)-salts media containing maltose at (A) 20 g/liter and (B) 50 g/liter.

The composition of the salt solution and culture conditions are given in Table 1.

Cultures were analysed for metabolites by extracting the filtrate with ethyl acetate and measuring the absorbance at 390 nm (dihydrofusarubins) and 495 nm (fusarubin).

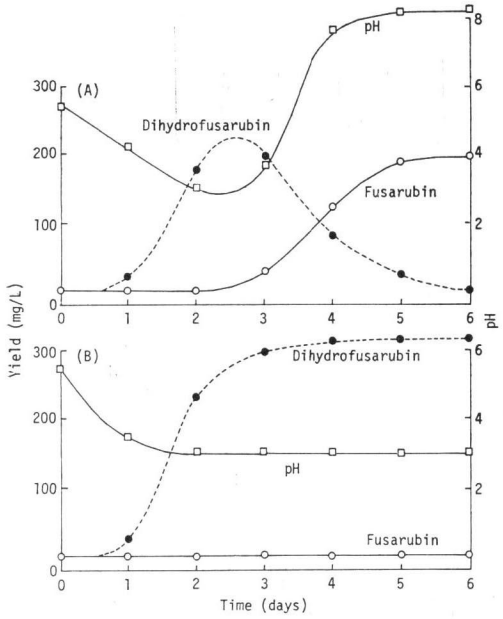



Table 2. Yields (mg/liter) of metabolites from cultures of *F. solani* grown in media with a low carbon: nitrogen ratio.*

| Metabolite | Ammonium tartrate | | |
|---|---|---|---|
| | 4.6 g/liter | | 6.9 g/liter |
| | 2 days | 6 days | 6 days |
| Dihydrofusarubin A** | 151 | 0 | 0 |
| Dihydrofusarubin B** | 74 | 0 | 0 |
| Javanicin | 7 | 7 | 0 |
| Fusarubin | 0 | 189 | 30 |
| Anhydrofusarubin | 0 | 9 | 2 |
| Norjavanicin | 0 | 4 | 0 |
| Bostrycoidin | 0 | 15 | 21 |

* Media contained maltose (20 g/liter) and ammonium tartrate as indicated. The remaining medium constituents, growth conditions and extraction procedure were as given in Table 1.

** Dihydrofusarubins A and B have structures **2** and **3** respectively, in reference 4.

Table 1. Yields (mg/liter) of metabolites from *F. solani* cultures grown in media with different carbon: nitrogen ratios.*

| Metabolite | Maltose (g/liter) | | | |
|---|---|---|---|---|
| | 30 | 40 | 50 | 60 |
| Dihydrofusarubin A** | 21 | 115 | 186 | 216 |
| Dihydrofusarubin B** | 9 | 59 | 94 | 117 |
| Javanicin | 5 | 12 | 41 | 45 |
| Fusarubin | 74 | 0 | 0 | 0 |
| Anhydrofusarubin | 11 | 0 | 0 | 0 |
| Norjavanicin | 4 | 0 | 0 | 0 |
| Bostrycoidin | 13 | 0 | 0 | 0 |

* Media contained (g/liter); ammonium tartrate (9.2), $KH_2PO_4$ (1.0), $MgSO_4 \cdot 7H_2O$ (0.5), $FeSO_4 \cdot 7H_2O$ (0.01), NaCl (0.01), $CaCl_2$ (0.01) and a trace mineral solution providing (mg/liter) $ZnSO_4 \cdot 7H_2O$ (8.8), $CuSO_4 \cdot 5H_2O$ (0.40), $MnSO_4 \cdot H_2O$ (0.06), $H_3BO_3$ (0.06) and $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.04). Cultures (50 ml per 250-ml Erlenmeyer flask) were incubated at 26°C on a rotary shaker and harvested at 6 days. Filtrates (1 liter) were extracted with ethyl acetate at pH 3.0 and the products separated by chromatography on silicic acid using benzene-ethyl acetate mixtures for elution. The elution sequence was: anhydrofusarubin (12 : 1), javanicin (9 : 1), norjavanicin and bostrycoidin (7 : 1), dihydrofusarubin A (5 : 1) and dihydrofusarubin B (3 : 1). Norjavanicin and bostrycoidin were separated by thin-layer chromatography on silica gel.

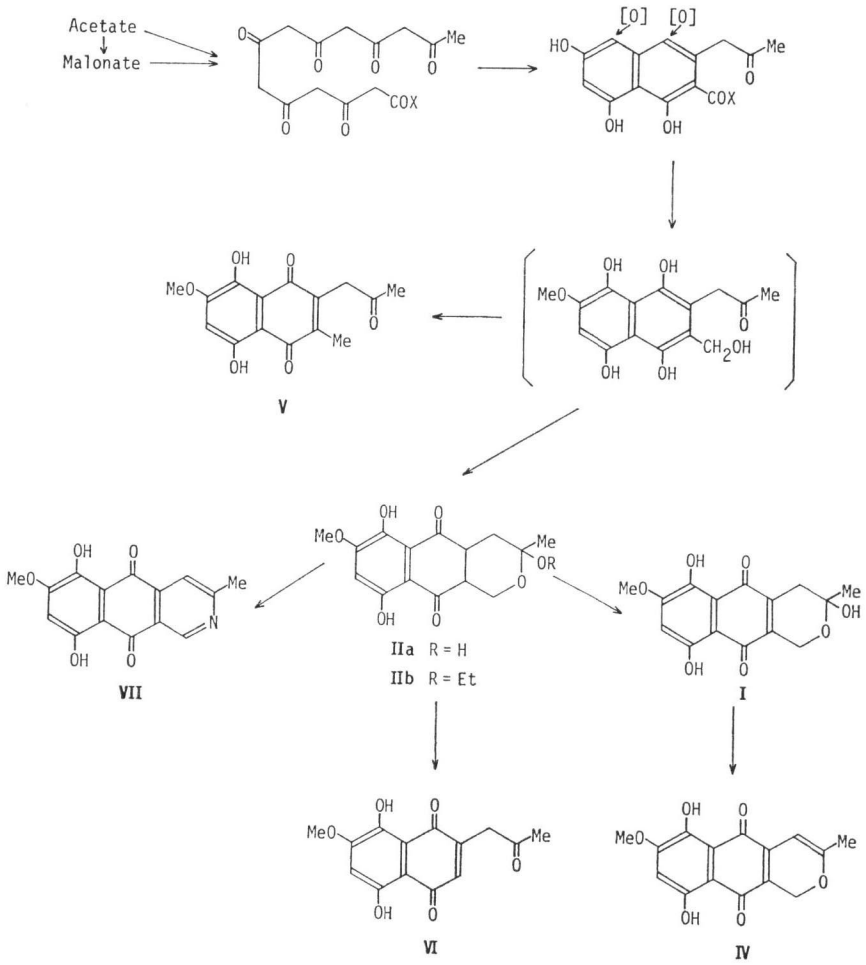
** Dihydrofusarubins A and B have structures **2** and **3**, respectively, in reference 4.

carbon source. Such cultures also failed to produce fusarubin.

Accompanying fusarubin and the dihydrofusarubins as secondary metabolites of *F. solani* were several substances of related structure. These were identified as anhydrofusarubin (**IV**), javanicin (**V**), norjavanicin (**VI**) and bostrycoidin (**VII**). All have been described previously as naphthoquinone products of *Fusarium* species[1,3,7,8]. The relative yields of these compounds were influenced by the initial carbon: nitrogen ratio of the medium (Table 1).

Javanicin was formed in highest yield in the high-carbon: nitrogen-ratio media that favoured dihydrofusarubin synthesis. Since it also appeared in the early stages of the fermentation while the culture was acidic (Table 2), it is a co-

Fig. 2. Proposed route for the formation of naphthoquinonoid metabolites in *F. solani* cultures.




metabolite, rather than a conversion product, of these compounds. In contrast, anhydrofusarubin, norjavanicin and bostrycoidin were formed only under conditions where fusarubin accumulated. The presence of anhydrofusarubin is readily accounted for by the known facility with which fusarubin dehydrates[1,2,7]. However, norjavanicin does not form from fusarubin. *In vitro* tests have indicated that it accompanies fusarubin among the products of aerobic oxidation of the dihydrofusarubins under alkaline conditions[9]. Bostrycoidin has a similar origin and is formed when the oxidation takes place in solutions containing ammonia. Significantly, the highest yields of fusarubin were obtained in media with 20 g/liter of maltose and 4.6 g/liter of ammonium tartrate whereas bostrycoidin was formed in largest amount when the initial ammonium tartrate concentration was 6.9 g/liter (Table 2).

A plausible sequence of reactions accounting for the formation of these compounds, based on the results of this and earlier studies[5,10], is presented in Fig. 2.

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