A NEW DEOXYFUSARUBIN PRODUCED
BY THE FUNGUS Nectria haematococca

SYNTHESIS OF THE TWO ISOMERIC
DEOXYANHYDROPHANTHOOPYRANONES
FROM TORALACTONE

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The fungus Nectria haematococca (Berk. and Br.) Wr., the sexual stage of the phytopathogenous fungus Fusarium solani, produces in culture a wide series of naphthoquinone pigments related to the antibiotic fusarubin1). The reaction occurring between anhydrofusarubin lactol and ammonia was shown on one other side to be responsible for the formation of the antibiotic bostrycoidin2). This 2-aza-anthraquinone was accompanied by a 6-O-demethyl-5-deoxy derivative in a selected mutant of N. haematococca). The structural variations observed in the various naphthopyranones result from subsequent methylations or hydroxylations of the original heptaketide40. In order to study such biogenetic correlations, mutants of Nectria haematococca blocked at different points of their pigment production were selected5). These mutants are all mapped in a single chromosomal region consisting of at least three genes6) responsible for the transformations of the heptaketide. It has been possible to establish that two of these genes (YEL Y and YEL J) were controlling respectively O-methylations and hydroxylations of the cyclized heptaketide-derived precursor1 ~ 3> 5 ~ 8). In this context, the 4-deoxyfusarubin (1) and the corresponding anhydro compound 2 were previously isolated8) from Nectria haematococca. (The classical numbering system for the naphthopyranone structure has been adopted here, as shown on the scheme; previous publications used a different system, more adapted to biosynthetic studies and starting from the methyl group of the last acetate unit). As the selected mutants of this fungus were producing only small amounts of pigments in cultures, they were crossed with an overproducing strain obtained from the wild strain. In the course of analyzing the double mutants recovered from the progeny of crosses between naphthoquinone overproducing strains and the yen JI blocked mutants, a family of compounds differing from the wild-type pigments by the lack of hydroxyl groups has been characterized confirming that the YEL J gene controls a hydroxylation step in the biosynthesis1-6).

In the present article, we report on the isolation and identification of the new 5-deoxyfusarubin (3) and 5-deoxyanhydrofusarubin (4), isomers of the previously known4-7) 4-deoxyfusarubin (1) and 4-deoxyanhydrofusarubin (2) from the redD169.yel JI double mutant of N. haematococca.

The redD169.yel J1 strain was grown for 12 days at 26°C in still liquid cultures and the medium filtrate was extracted with ethyl acetate in the previously described condition4). The concentrated organic phase was left overnight at 4°C in order to allow the 4-deoxyfusarubin (1) to precipitate. The supernatant was submitted to a SiO2 thin layer chromatography (TLC) in the mixture CH2Cl2-MeOH 99:1 giving a number of yellow, purple and red bands, which were extracted from the scraped layer by ethyl acetate. The yellow 5-deoxyfusarubin (3) was purified by repeating this process in the same conditions, and finally crystallized from heptane-CH2C12 3:1 yielding needles mp 178~180°C (10 liters of culture medium gave 5mg of 3 and 118mg of the compound 1).

3: Electron impact MS, m/z, (%): 290 (M)+ (30), 272 (M-18)+ (100), 43 (60); high resolution MS: calcd for C15H14O6 290.0790, found 290.0782; 1H NMR (deuterated DMSO): δ ppm = 1.47, s, 3H (CH3), 2.50, m, 2H (CH2), 3.93, s, 3H (OCH3), 4.53, d, 2H (CH2), 6.13, s, 1H and 6.83, s, 1H, aromatic protons, 7, s, 1H, OH at C-2, 12.1, s, 1H (chelated OH); IR (KBr) ν cm-1: 3472-3395 (OH), 2959, 1729, 1658 (CO), 1623 (CO), 1314, 1159; UV (MeOH) λ nm: 218, 266, 285 (sh), 425. The properties of this pigment are quite similar to those of the monomethyl derivative of 6-O-demethyl-5-deoxyfusarubin4).

The corresponding 5-deoxyanhydrofusarubin (4) was obtained on preparative TLC from a purple band slightly less polar than 2. The purification was carried out by further TLC in pentane·CH2Cl2·MeOH 1:4:0.1, giving dark violet crystals, recrystallized from ethyl acetate·heptane; mp
124~128°C (the amount of the two isomeric anhydro compounds 2 and 4 were 85 and 7 mg from 10 liters of culture medium).

4: EI-MS, m/z, (%): 272 (M)+ (100), 257 (M-15)+ (15), 43 (CH₃CO)+ (60); high resolution MS: calcd for C₁₅H₁₂O₅ 272.0767, found 272.0778; ¹H NMR, CDCl₃: δ = 2.04, s, 3H (CH₃), 3.90, s, 3H (OCH₃), 5.13, s, 2H (CH₂), 5.93, s, 1H, olefinic proton, 6.64, d, 1H and 7.17, d, 1H, aromatic protons C-7 and C-5, 12.4, s, 1H, chelated phenolic OH. This product was identical to the monomethyl derivative 6-O-demethyl-5-deoxyanhydrofusarubin⁹ previously described. The structure of the new 5-deoxyfusarubin (3) and of its anhydro derivative (4) were confirmed by total synthesis, comparing the physico-chemical data. The properties of the previously described compounds 1 and 2 are not reported in this paper. However, as the synthesis of the anhydro derivative 4 also produces the isomer 2, the proposed structure is being now confirmed.

The total synthesis of the two isomeric deoxyanhydrofusarubins 2 and 4 was carried out from methoxytriacetic lactone and ethyl dimethylorsellinate as starting material. As reported¹⁰, the LDA-promoted condensation of these two reagents gave the 8-methyl toralactone 5b with a 42% yield (product directly obtained crystallized from AcOEt, yellow needles, fluorescent in UV, mp 212~214°C, MS m/z 286 (100); ¹H NMR). The toralactone 5a¹¹ is prepared from 5b by partial demethylation, using an equimolecular amount of BBr₃ in CH₂Cl₂ (30 minutes at -10°C, 1 hour at 20°C, hydrolysis by cold H₂O, extraction with CH₂Cl₂). A mixture of demethylated products is obtained, in which toralactone 5a is the main representative, isolated by crystallization in AcOEt-pentane (Rf 0.35, TLC in pentane-CH₂Cl₂-AcOEt 10:10:3), yield 54%, mp 254~256°C, yellow needles; MS (%): 272 (M)+ (20), 257 (M-15)+ (25), 43 (CH₃CO)+ (100); high resolution MS: found 272.0677, calcd for C₁₅H₁₂O₅ 272.06847; ¹H NMR¹¹. The toralactone 5a (1 mm) is dissolved in anhydrous THF (100 ml) and reduced by an excess of LiAlH₄ (3 mm, 2 hours stirring at 20°C, hydrolysis by 2 n HCl; addition of an equal volume of AcOEt, washing with H₂O, drying over Na₂SO₄). Attempts to isolate the products from this reduction failed due to decompositions occurring during preparative SiO₂ TLC and extraction from the scraped layer. Consequently, the resulting solution was stirred for 5 days over Na₂SO₄, leading to the slow cyclization of the diol resulting from the reduced lactone and to air oxidation to a naphthoquinone. Control TLC indicated the progressive formation of the two deoxy naphthopyranones 2 and 4. These products have been isolated by SiO₂ TLC (CH₂Cl₂), 2: Rf 0.60, red brown needles, orange solutions, mp 212~214°C (reported² 210~213°C), yield 11%; 4: 0.80, dark violet crystals, purple solutions, mp 124~128°C, yield 8%.
The MS and $^1$H NMR spectra of these substances 2 and 4 are identical to those of the natural products isolated from the fungus Nectria haematococca. SiO$_2$ thin layer co-chromatography confirmed the identity. HRMS of 2: found 272.0668, calc'd for C$_{15}$H$_{12}$O$_5$ 272.0647; 4: found 272.0668, calc'd for C$_{15}$H$_{12}$O$_5$ 272.0647. The formation of the two naphthopyranones 2 and 4 is of course the result of oxidations bearing on the C-8 or C-9 hydroxy groups, the corresponding naphthoquinones being stabilized by chelation with the neighbor remaining OH function. It has not so far been possible to produce the two deoxynaphthopyranones 2 and 4 by oxidizing the reduced toralactone by other reagents.

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References