A NEW DEOXYFUSARUBIN PRODUCED BY THE FUNGUS *Nectria haematococca*

SYNTHESIS OF THE TWO ISOMERIC DEOXYANHYDRONAPHTHOPYRANONES FROM TORALACTONE

DENISE PARISOT

Laboratoire de Cryptogamie
Bâtiment 400, Faculté des Sciences,
91405 Orsay Cedex, France

MICHEL DEVYS and MICHEL BARBIER*

Institut de Chimie des Substances Naturelles,
CNRS, Avenue de la Terrasse,
91198 Gif sur Yvette Cedex, France

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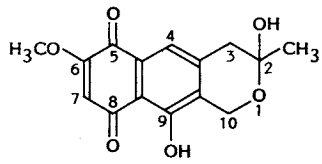
The fungus *Nectria haematococca* (Berk. and Br.) Wr., the sexual stage of the phytopathogenous fungus *Fusarium solani*, produces in culture a wide series of naphthoquinone pigments related to the antibiotic fusarubin[1]. The reaction occurring between anhydrofusarubin lactol and ammonia was shown on an other side to be responsible for the formation of the antibiotic bostrycoidin[2]. This 2-aza-anthraquinone was accompanied by a 6-*O*-demethyl-5-deoxy derivative in a selected mutant of *N. haematococca*[3]. The structural variations observed in the various naphthopyranones result from subsequent methylations or hydroxylations of the original heptaketide[4]. In order to study such biogenetic correlations, mutants of *Nectria haematococca* blocked at different points of their pigment production were selected[5]. These mutants are all mapped in a single chromosomal region consisting of at least three genes[6] 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 precursor[1~3,5~8]. In this context, the 4-deoxyfusarubin (**1**) and the corresponding anhydro compound **2** were previously isolated[4] 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 *yel J1* 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 biosynthesis[1,6].

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 known[4,7] 4-deoxyfusarubin (**1**) and 4-deoxyanhydrofusarubin (**2**) from the *redD169.yel J1* double mutant of *N. haematococca*.

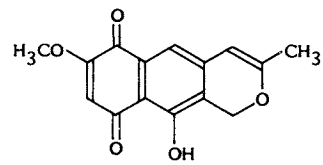
The *redD169.yel J1* strain was grown for 12 days at 26°C in still liquid cultures and the medium filtrate was extracted with ethyl acetate in the previously described condition[4]. The concentrated organic phase was left overnight at 4°C in order to allow the 4-deoxyfusarubin (**1**) to precipitate. The supernatant was submitted to a SiO_2 thin layer chromatography (TLC) in the mixture CH_2Cl_2-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-CH_2Cl_2 3:1 yielding needles mp 178~180°C (10 liters of culture medium gave 5 mg of **3** and 118 mg of the compound **1**).

3: Electron impact MS, *m/z*, (%): 290 $(M)^+$ (30), 272 $(M-18)^+$ (100), 43 (60); high resolution MS: calcd for $C_{15}H_{14}O_6$ 290.0790, found 290.0782; ^1H NMR (deuterated DMSO): δ ppm = 1.47, s, 3H (CH_3), 2.50, m, 2H (CH_2), 3.93, s, 3H (OCH_3), 4.53, d, 2H (CH_2), 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-deoxyfusarubin[4].

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-CH_2Cl_2-MeOH 1:4:0.1, giving dark violet crystals, recrystallized from ethyl acetate-heptane; mp



1



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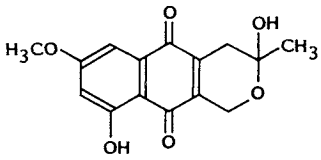

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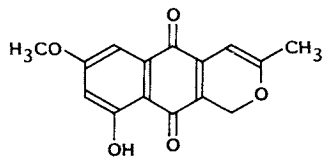
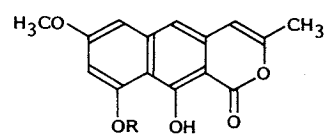


4

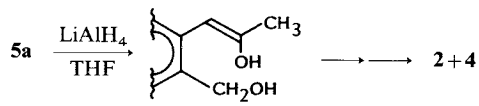
124~128°C (the amount of the two isomeric anhydro compounds **2** and **4** were 85 and 7 mg from 10 liters of culture medium).

4: EI-MS, *m/z*, (%): 272 $(M)^+$ (100), 257 $(M-15)^+$ (15), 43 $(CH_3CO)^+$ (60); high resolution MS: calcd for $C_{15}H_{12}O_5$ 272.0767, found 272.0778; ^1H NMR, $CDCl_3$: $\delta=2.04$, s, 3H (CH_3), 3.90, s, 3H (OCH_3), 5.13, s, 2H (CH_2), 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-deoxyanhydrofusarubin[9] 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 reported[10], 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 **5a**[11] is prepared from **5b** by partial demethylation, using an equimolecular amount of BBr_3 in CH_2Cl_2 (30 minutes at −10°C, 1 hour at 20°C, hydrolysis by cold H_2O, extraction with CH_2Cl_2). A mixture of demethylated products is obtained, in which toralactone **5a** is the main representative, isolated



5a R=H
5b R=CH_3



by crystallization in AcOEt - pentane (Rf 0.35, TLC in pentane - CH_2Cl_2 - AcOEt 10:10:3), yield 54%, mp 254~256°C, yellow needles; MS (%): 272 $(M)^+$ (20), 257 $(M-15)^+$ (25), 43 $(CH_3CO)^+$ (100); high resolution MS: found 272.0677, calcd for $C_{15}H_{12}O_5$ 272.06847; ^1H NMR[11]. The toralactone **5a** (1 mM) is dissolved in anhydrous THF (100 ml) and reduced by an excess of $LiAlH_4$ (3 mM, 2 hours stirring at 20°C, hydrolysis by 2 N HCl; addition of an equal volume of AcOEt, washing with H_2O, drying over Na_2SO_4). Attempts to isolate the products from this reduction failed due to decompositions occurring during preparative SiO_2 TLC and extraction from the scraped layer. Consequently, the resulting solution was stirred for 5 days over Na_2SO_4, 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 SiO_2 TLC (CH_2Cl_2), **2**: Rf 0.60, red brown needles, orange solutions, mp 212~214°C (reported[4] 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, calcd for $C_{15}H_{12}O_5$ 272.06847; **4**: found 272.0668, calcd for $C_{15}H_{12}O_5$ 272.06847. 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.

Acknowledgments

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