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STRUCTURE AND BIOSYNTHESIS OF 5-DEOXYFUSARUBIN AND ANHYDRO-5-DEOXYFUSARUBIN, NAPHTHAQUINONE PIGMENTS FROM *NECTRIA HAEMATOCOCCA*

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Key Word Index—*Nectria haematococca*; fungus; biosynthesis; heptaketide; naphthaquinones; deoxyfusarubin; anhydrodeoxyfusarubin.

Abstract—Two naphthaquinone pigments, 5-deoxyfusarubin and anhydro-5-deoxyfusarubin, were isolated from a yellow strain of the fungus *Nectria haematococca*. The ^{13}C NMR spectra of **2** biosynthetically labelled from [1-^{13}C]acetate confirmed the structures of the pigments and established the heptaketide origin of the molecules.

INTRODUCTION

Nectria haematococca (Berk and Br.) Wr., the teleomorph of the fungus *Fusarium solani*, produces in culture a series of naphthaquinone pigments [1]. These pigments are all members of the fusarubin family [2, 3]. They are produced in higher yields by mutant strains obtained from the wild type through selection [4, 5]. We previously reported the presence in *N. haematococca* of 10 pigments belonging to this series [1], among which were two new compounds, namely, anhydrofusarubin lactone and nectriafurone. More recently new mutants were isolated, particularly yellow mutants, and in order to obtain high yields of yellow pigments, the overproducer double-mutant *ann A** 58. *yel J1* was constructed through appropriate crosses [5]. In this publication, we show that the major pigment produced by the double-mutant is 5-deoxyfusarubin (**1**). This mutant also produces the corresponding anhydro derivative of **1**.

RESULTS AND DISCUSSION

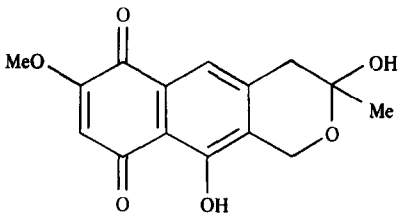
The naphthaquinone pigments, 5-deoxyfusarubin (**1**) and anhydro **1** (**2**), were isolated from the *ann A** 58. *yel J1* strain of *N. haematococca*. As the anhydrodeoxyfusarubin was easily prepared by acetic acid dehydration of deoxyfusarubin due to the instability of the tertiary hydroxy group in **1**, further studies made use of the anhydro compound.

The IR and UV spectra were consistent with the naphthaquinone chromophore. In particular, anhydro deoxyfusarubin (**2**) could be directly related to anhydrofusarubin which has a similar structural feature. In the ^1H NMR spectrum, the signals for the methyl and methoxyl groups were readily assigned by comparison with the resonance found in fusarubin and its anhydro derivative. Mass spectrometry of **1** and **2** indicated that they were deoxy derivatives of fusarubin and its anhydro derivative respectively. The formulae $C_{15}H_{14}O_6$ and $C_{15}H_{12}O_5$ were assigned to **1** and **2** on the basis of MS and ^1H and ^{13}C NMR studies carried out on these substances and their acetates.

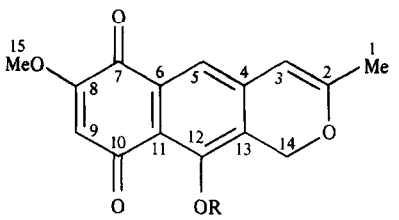
As neither vicinal nor 1,3-protons (as in the altersolanols [6], dactylariol or dactylarin [7]) were present, we had to consider structures in which the two aromatic H atoms were located in different rings of the molecules. This restricted the possibilities to the phenolic hydroxyl group being either next to the olefinic proton or next to the oxymethylene group as represented in **2**. This last possibility was favoured by the fact that the CH_2O protons were shifted in the acetate **3** whereas the signals for the olefinic proton and the methyl group were unchanged, cf. the acetylation of anhydrofusarubin which produced a shift of both the olefinic and the CH_2O protons [8, 10]. The ^1H NMR data of **1**–**3** are presented in Table 1. Nevertheless, this evidence did not seem conclusive enough and final proof of the structures was obtained from biosynthetic experiments.

The ^{13}C NMR spectrum of ^{13}C-labelled anhydrodeoxyfusarubin prepared by growing the fungus in the presence of [1-^{13}C]acetate established that the anhydrodeoxyfusarubin had structure **2**, as the C-1 label of the acetate units enhanced all signals but one at any oxygen bearing position. The relative ^{13}C-labelling intensities of **2** were established by comparison with the natural abundance determined for the methoxyl carbon atom which is known to originate from methionine (Table 2). These results thus revealed the heptaketide origin of the molecule as represented in **4**. Similar conclusions had been drawn from similar biosynthetic studies on marticin [9] and dihydrofusarubin [10]. The fact that the properties of the isolated **2** were identical to those of the anhydro derivative prepared through dehydration of deoxyfusarubin, led us to propose structure **1** for this closely related metabolite. On this basis, the ^{13}C NMR of this product was then unambiguously interpreted (Table 2).

We have numbered the C atoms in **1** and **2** in a logical sequence based on the heptaketide (**4**) origin of these molecules. In this system, the hydroxyl group is missing from C-5, which as it turns out corresponds to C-5 of the fusarubin numbering system according to IUPAC recom-



1



2 R=H
3 R=OAc

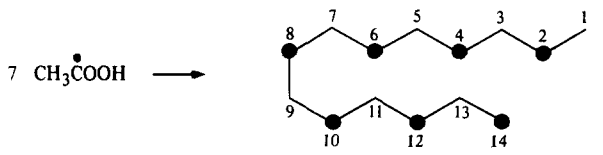


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Table 1. ^{1}H NMR spectra of 5-deoxyfusarubin (1), anhydro-5-deoxyfusarubin (2) and anhydro-5-deoxyfusarubin acetate (3) ($CDCl_3$)

H	1	2	3
1	1.65 *s*, 3H	1.96 *s*, 3H	1.97 *s*, 3H
3	2.96 *s*, 2H	5.62 *s*, 1H	5.60 *s*, 1H
5	7.43 *s*, 1H	7.16 *s*, 1H	7.54 *s*, 1H
9	6.04 *s*, 1H	6.02 *s*, 1H	5.98 *s*, 1H
OH-12	12.25 *s*, 1H	12.36 *s*, 1H	
14	4.92 *s*, 2H	5.24 *s*, 2H	5.14 *s*, 2H
15	3.94 *s*, 3H	3.91 *s*, 3H	3.85 *s*, 3H
AcO			2.45 *s*, 3H

Table 2. ^{13}C NMR spectra of anhydro-5-deoxyfusarubin acetate (3) and 5-deoxyfusarubin (1) (δ_c, ppm).

C	3 ($CDCl_3$)	% Enrichment from [1-^{13}C]acetate	1 (DMSO)
1	19.80		28.19
2	156.14	2.07	93.17
3	100.55		28.83
4	144.70	4.42	141.02
5	111.36		109.24
6	132.72	4.28	130.05
7	179.77		178.55
8	159.97	2.86	161.07
9	118.28		119.25
10	183.27	4.88	190.67
11	120.05		110.86
12	138.71	3.00	155.64
13	125.75		128.40
14	63.46	3.40	57.72
15	56.33		56.74
$\underline{C}H_3COO$	21.05		
$CH_3\underline{C}OO$	168.99		

mendations [11, 12]. Hence, we propose the names 5-deoxyfusarubin and of anhydro-5-deoxyfusarubin for substances 1 and 2.

EXPERIMENTAL

Mps (Kofler block, microscope) corrected; MS: AEI MS 50; ^{1}H and ^{13}C NMR: Cameca 240, Bruker 90 and 400 MHz, TMS as int. standard. The ^{13}C-acetate was obtained from the C.E.A. Saclay, France.

Extraction and isolation of the pigments. The mutant strain *ann A* 58. yel J1* derived from *Nectria haematococca* S1 wild strain was grown at 26° in the dark for 12 days on an agar-containing synthetic medium [1]. The agar, after removal of the fungus, was extracted with Me_2CO. The Me_2CO extract was concd *in vacuo* and extracted with EtOAc. The EtOAc extract was subjected to flash chromatography on silica gel (Merck 7734) developed with mixtures of hexane–EtOAc. Compound 1 was eluted with hexane–EtOAc (3:1) whilst 2 needed EtOAc and 1% EtOAc–AcOH for elution. The two fractions were then submitted to TLC (on silica gel) developed with $CHCl_3$–MeOH (19:1) followed (on the same plate) by $CHCl_3$–MeOH (49:1) (compounds recovered with EtOAc). The yellow product 1 was obtained as crystals, but the vermilion coloured 2 needed further purification on HPLC (reverse phase) (Partisil M 10-9/50 C8 developed with MeOH–H_2O, 3:1). From a typical experiment with 40 Petri dishes (1L agar) 17 mg 1 and 4 mg 2 were finally isolated.

5-Deoxyfusarubin (1). Mp 208–212° (yellow needles); UV λ_{max}^{EtOH} nm (ε, $\times 10^{-3}$): 218 (9), 250 (6.95), 295 (5.3), 410 shoulder, (2.15), 423 (2.20), 445 shoulder (1.65); IR ν_{max}^{KBr} cm^{-1}: 1680 (weak), 1620 (intense; 1,4-quinone chelated with an OH group in the peri position); $[\alpha]_D^{20} = 0°$ (pyridine); MS *m/z*: 290 $[M]^+$, $C_{15}H_{14}O_6$; ^{1}H NMR: Table 1; ^{13}C NMR: Table 2. On boiling for 20 min in AcOH, 1 gave anhydro-5-deoxyfusarubin (2) which was purified by preparative chromatography.

Anhydro-5-deoxyfusarubin (2). Mp 205° (vermilion needles), formation of prisms and second mp at 210–213°; UV λ_{max}^{EtOH} nm (ε, 10^{-3}): 235 (3.39), 295 (5.2), 340, shoulder, (0.95), 450 (1.02); IR ν_{max}^{KBr} cm^{-1}: 1680 (weak), 1630 (intense; 1,4-quinone chelated with an OH group in the peri position); MS *m/z* 272 $[M]^+$,

$C_{15}H_{12}O_5$. Mono-acetate 3 (Ac_2O–pyridine), 20 hr, 20°, prisms which sublime starting from 200° and melt at 250° (new prismatic form); MS *m/z*: 314 $[M]^+$, $C_{17}H_{14}O_6$, 272 $[M-CH_2CO]^+$; ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Biosynthesis of 2 *from* [1-^{13}C]*acetate.* Pilot experiments carried out with ^{14}C-labelled acetate, with or without the addition of larger amounts of unlabelled acetate, showed that the incorporation of acetate into the pigments was best after 68 hr of culture and that it was repressed when acetate was added in increasing concentration. Consequently, 250 mg [1-^{13}C]acetate (90% enrichment) was introduced into cultures of *N. haematococca* (700 ml) after 68 hr followed by a further 250 mg after 100 hr. The experiment was stopped on the eighth day after inoculation. After the usual extraction–isolation procedure, the ^{13}C-deoxyfusarubin was transformed into its anhydro derivative (boiled with AcOH for 20 min) and mixed with ^{13}C-2. The crude product was purified by TLC (silica gel), acetylated and subjected to HPLC on a column of Lichrosorg Si 60-7 developed with hexane–EtOAc (7:3).

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