Nectriachrysone, a New Metabolite Related to Fusarubin produced by the Fungus *Nectria haematococca*

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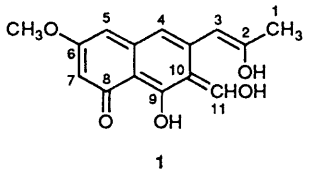
An extensively conjugated enol system, **1**, is proposed as the structure for nectriachrysone, a yellow pigment isolated from a mutant of the fungus *Nectria haematococca*.

Nectria haematococca [Berk. and Br.)Wr. produces, in cultures, a wide range of naphthoquinone pigments related to the antibiotic fusarubin.[1] Previous work has shown that the pigments have as their key intermediate a heptaketide,[2,3] fusarubinoic acid. For our investigation, the selection of mutants in order to find producers of putative biosynthetic intermediates derived from the heptaketide[4,5] has been invaluable. Of particular interest has been the yellow yelJl mutant. In order to secure sufficient amounts of the pigments, the latter mutant was crossed with the pigment overproducing redD169 strain[6] and the deep yellow-redD169.yelJl double-mutant was selected for isolation and chemical analysis. This yielded three previously identified compounds, 5-deoxyfusarubin, anhydro-5-deoxyfusarubin, and 5-deoxyjavanicin,[2] together with a new compound for which we propose a structure, **1**, and a name, nectriachrysone. Up to now,



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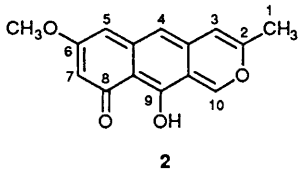

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nectriachrysone seems to be the earliest metabolite produced from the heptaketide in the biosynthetic pathway of fusarubin.

The physicochemical data for nectriachrysone (UV, IR, MS, ^{1}H NMR) suggest that it is an extensively conjugated enol system. This structure is typically polyenic, with a hyperconjugated carbonyl function, three hydroxy groups (one is vinylic), a methoxy and a methyl group. The ^{1}H NMR signals are all singlets and can be attributed unambiguously by comparison with reported values for similar systems (for example mitorubrin,[7] mitorubrinol, mitorubrinic acid,[8] rubropunctatin and monascorubin).[9,10] Nectriachrysone is unstable and rearranges readily during manipulation into a series of other unidentified products. This can be related to an equilibrium between the oxo and enol forms in the side chain, as previously established by cyclizations to give anhydrofusarubin lactone or lactol.[3,11] Nectriachrysone dehydrates very easily [the base peak is at m/z M − 18$^+$ in the mass spectrum (MS)] to give the corresponding anhydronectriachrysone **2**. Under reflux in MeOH–HCl, the dehydration of **1** is quantitative and gives the γ-pyrone **2** as the sole product (this also favours the existence of tautomeric forms of **1**). The high resolution MS of **2** supports the formula $C_{15}H_{12}O_4$, and gives rise to two main fragments, M − CHO$^+$ and M − CH_3CO^+ (that is, both ions contain the same oxygen atom), a fragmentation typical of a pyrone ring. The ^{1}H NMR spectrum shows 4 singlets corresponding to 3-, 4-, 5-, 7-H together with a fifth singlet as reported for the 10-H in the γ-pyran substructure.[7–10] Anhydronectriachrysone is thus a quinonoid, hyperconjugated γ-pyrone in which all protons on a double bond are those of a polyenic system. Structure **2** is corroborated by the ^{13}C NMR spectrum which shows the expected 7 H together with eight quaternary C-atoms (spin echo technique). Structures **1** and **2** are the only ones which are supported by all the reported physicochemical data and properties. It is clear that structure **1** is possible only because of the stabilizing effect exerted by the *exo* vinyl double bond. It is probable that, as with mitorubrin,[7] the biogenetic origin of nectriachrysone **1** involves reduction of the terminal carboxylic acid in the original heptaketide to an aldehyde, a

system known to be stabilized as the corresponding lactol.[10] Recently,[12] a scheme was advanced in which the biosynthesis of hyperconjugated γ-pyrone pigments isolated from fungi have, as a fundamental step, internal addition of an aldehyde function to an enolate to give lactols.

Experimental

Nectriachrysone **1**. This was isolated from 4 day cultures of the redD169.yelJl double mutant of *Nectria haematococca* (in liquid GAMS medium) according to a previously reported method.[5] The culture filtrates were collected and extracted successively with pentane–ethyl acetate (1:1) and ethyl acetate. The pentane–ethyl acetate fraction was concentrated under reduced pressure and chromatographed on preparative SiO_2 plates developed in pentane–ethyl acetate (1:1). A slow moving broad yellow fluorescent band (R_f 0.33) was eluted with ethyl acetate and submitted to a second preparative TLC as above, giving **1**, precipitated by concentration of an ethyl acetate solution (yellow amorphous powder, *ca.* 1 mg l^{-1} of culture medium); λ(MeOH)/nm 210, 278, 289, 337, 359 and 498; ν(KBr)/cm^{-1} 3300, 1708, 1640 and 1591; *m/z* (%) 274 M^+ (15), 256, M − 18$^+$, (100) and 232 M − CH_2CO^+, (20); δ_H([$^2H_6$]-DMSO) 2.10 (s, 3 H, 1-CH_3), 3.75 (s, 3 H, 6-OCH_3), 5.90 (s, 1 H, 4-H), 6.05 (s, 1 H, 5-H), 6.20 (s, 1 H, 7-H), 6.50 (s, 1 H, 3-H), 6.52 (s, 1 H, 10-H), 9.2 (s, 1 H, 9-OH) and 10.10 (s, 1 H, 10-OH).

Anhydronectriachrysone **2**. This product was obtained by heating under reflux for 30 min a solution of **1** (10 mg) dissolved in MeOH (2 ml) containing 1 drop of concentrated HCl. The reaction mixture was diluted with water (6 ml) and extracted with ethyl acetate (6 ml × 2). The organic phase was dried (Na_2SO_4) and concentrated and the residue was chromatographed [TLC: SiO_2, $CHCl_3$–MeOH (49:1)] to give **2** as red needles (8 mg), m.p. 210–215 °C; λ_{max}(MeOH)/nm 210, 277, 290, 327 and 359; ν_{max}(KBr)/cm^{-1} 3460, 1682, 1635, 1619, 1577, 1398, 1290, 1183 and 1160; *m/z* 256 (M^+, 100); *m/z* (%) 256.0742 (100; Calc. for $C_{15}H_{12}O_4$: 256.0748), 227.0701 [16; Calc. for $C_{14}H_{11}O_3$: (M − CHO^+) 227.0738] and 213.0534 [8; Calc. for $C_{13}H_9O_3$: 213.0551 (M − CH_3CO^+)]; δ_H($CDCl_3$) 2.25 (s, 3 H, 1 − Me), 3.90 (s, 3 H, 6-OMe), 6.20 (s, 1 H, 4-H), 6.20 (s, 1 H, 5-H), 6.25 (s, 1 H, 7-H), 6.35 (s, 1 H, 3-H), 8.40 (s, 1 H, 10-H) and 13.90 (s, 1 H, 9-OH); δ_C($CHCl_3$, 250 MHz) 19.06 (C-1), 142.88 (C-2), 98.13 (C-3), 110.77 (C-3a), 103.07 (C-4), 116.59 (C-4a), 106.77 (C-5), 166.45 (C-6), 55.61 (OCH_3), 110.01 (C-7), 184.79 (CO at C-8), 125.17 (C-8a), 152.86 (C-9), 128.11 (C-9a) and 155.43 (C-10).

Acknowledgements

Thanks are due to Drs. B. C. Das, C. Girard and J. P. Dupuis for the EIMS determinations, to the Centre Régional de Mesures Physiques de l'Ouest, Rennes University, for the high resolution MS, and to Mrs. C. Pasquier and C. Fontaine for the ^1H NMR spectroscopy carried out at Gif sur Yvette.

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Paper 1/02702F
Received 7*th May* 1991
Accepted 12*th June* 1991