(43 2), C-14 (55.0), C-15 (22 9), C 16 (28 3), C-17 (55 9), C-18 (12.0), C-19 (12 8), C-20 (40 5), C-21 (21 1), C-22 (135.8), C-23 (131 9), C-24 (43.0), C-25 (33 2), C-26 (19 6), C-27 (20 0), C-28 (17 9), MeCO (170 5), MeCO (21 4) The carbon signals were assigned by comparison with those of related sterols in the lit [4, 10].

5-*Dihydroergosteryl* (**2**) *acetate* ^{1}H NMR. δ 0 541 (3H, *s*, C-18), 0.811 (3H, *s*, C-19), 1 016 (3H, *d*, J = 6.6 Hz, C-21), 0 836 (3H, *d*, J = 6 4 Hz, C-26), 0 820 (3H, *d*, J = 6.6 Hz, C-27), 0 913 (3H, *d*, J = 6 8 Hz, C-28), 4 69 (1H, *m*, C-3α), 5.15 (1H, *m*, C-7), 5 19 (2H, *m*, C-22, C-23) ^{13}C NMR those signals omitted from below are the same in their chemical shifts with the corresponding signals of 1-acetate, C-16 (δ_C 28 0), C-22 (135.6), C-23 (131.8), C-24 (42.7), C-25 (33 1), C-26 (19 9), C-27 (19 6), C-28 (17.6)

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PIGMENTS FROM *NECTRIA HAEMATOCOCCA*: ANHYDROFUSARUBIN LACTONE AND NECTRIAFURONE

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Key Word Index—*Nectria haematococca*, naphthoquinones, anhydrofusarubin lactone, nectriafurone

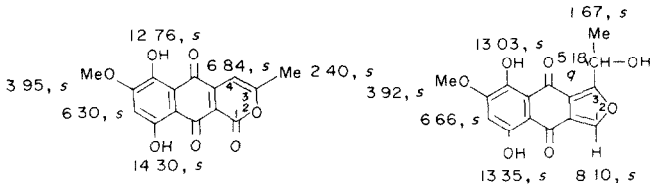
Abstract—The structures of anhydrofusarubin lactone and of nectriafurone, two new naphthoquinone pigments isolated from the fungus *Nectria haematococca*, are reported. Nectriafurone is the first natural isofuran naphthoquinone and is biogenetically related to fusarubin. In addition eight known *Fusarium* naphthoquinones were isolated. All the naphthoquinone pigments isolated from *N. haematococca* are produced in higher yields by selected mutant strains obtained from the wild strain

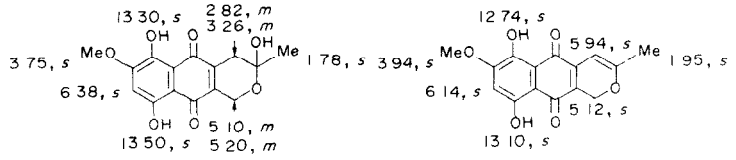
INTRODUCTION

The production of naphthoquinone pigments by fungi has been known for more than 40 years. Fusarubin (**3**) is a typical representative in this series [1–3] but more recently new antibiotics, possessing particular structural features, have also been reported, such as kalafungin [4] and the dimeric naphthoquinone lactones [5, 6] of the xanthomegnine type. Substances in this family are partly responsible for the biological activity of *Fusarium* on plants, as observed *in vitro* with tomato cuttings, as well as for cell division inhibition [7, 8].

Mutants were selected from a homothallic wild strain

This publication is dedicated to Professor Edgar Lederer on the occasion of his 75th birthday.





of *Nectria haematococca* (Berk. and Br) Wr, [9,10] which is the teleomorphic stage of *Fusarium solani* [11] They were distinguished from the original wild strain by their deep red colour and genetic analyses showed the corresponding mutations to be mapped in at least six distinct genes However, the pigments obtained from the red mutants did not reveal different chromatographic patterns, only higher concentrations This feature greatly facilitated the isolation of the pigments and, in the present publication, we report the identification of 10 naphthoquinones from *N haematococca* among which two are new anhydrofusarubin lactone (**1**) and nectriafurone (**2**)

RESULTS AND DISCUSSION

On TLC of the crude pigments obtained from wild or mutant strains of *N haematococca*, the chromatographic patterns were similar but greater quantities of the pigments were produced by the highly coloured mutant strain 58 Preparative TLC of extracts of this last strain, after preliminary fractionation on a LH-20 Sephadex column, gave a series of pigments among which eight were identified as naphthoquinones previously isolated from *Fusarium*, i e fusarubin (**3**), anhydrofusarubin (**4**), javanicin, anhydrojavanicin, norjavanicin [12–16], deoxyfusarubin and the two isomeric dihydrofusarubins Two pigments were new and for these we propose the names anhydrofusarubin lactone and nectriafurone as well as the formulae **1** and **2**, respectively

Anhydrofusarubin lactone (**1**) had a R_f intermediate between those of fusarubin and nectriafurone The IR spectrum indicated a conjugated six-membered ring lactone and the UV spectrum was consistent with a naphthoquinone chromophore of the anhydrofusarubin type, **4** In the ^{1}H NMR spectrum, the signals for the methyl and methoxy groups were readily assigned, by comparison with the resonances usually found in this series particularly for anhydrofusarubin (**4**) No signal corresponding to the two H-1 of fusarubin (**3**) was found while H-4 and the hydroxyl of one phenolic group were deshielded in contrast to their respective counterparts in the authentic anhydro derivative The NMR spectrum allowed the assignment of the 10 protons of the molecule, leading to a $C_{15}H_{10}O_7$ formula which was confirmed by the presence in the mass spectrum of $[M]^+$ at 302

Nectriafurone (**2**) $C_{15}H_{12}O_7$, was characterized on the basis of its NMR and other spectral properties It gave similar signals to fusarubin (**3**) or **1** in the NMR spectrum for the methoxyl and hydroxyl groups but a new system appeared at δ 1 64–1 67 (*d*, 3H) and 5 18 (*q*, 1H) indicating a Me–CH–OH substitution, this being further supported on the acetate of **2** The singlet observed at δ 8 10 (1H), or at 7.98 for the acetate, was characteristic of the isofuranic proton at C-1 of **2** as already established for a similar benzoquinone isofuran [17, 18] High resolution mass spectrometry performed on this acetate confirmed a triacetate, $C_{21}H_{18}O_{10}$, with the successive loss of 42 amu ($-CH_2CO$) and 60 (HOAc) amu, which analysed for 430.0875 (calc 430 0895)

The two new compounds reported in this work appear to be biogenetically related to fusarubin (**3**), **1** being an oxidation product of the methylene group in the pyran ring of anhydrofusarubin (**4**) and **2** very likely being a rearrangement product of the pyran ring of fusarubin or of a related metabolite This biogenetic relationship favours structure **2** rather than the alternative in which the Me–CH–OH group is attached to C-1

EXPERIMENTAL

Extraction and isolation of pigments The mutant strain 58, derived through selection from *Nectria haematococca* S1 wild strain, was grown at 26° in the dark for 12 days on agar synthetic medium [9, 10] The mycelial mats were cut into small pieces, the pigments extracted with Me_2CO and the aq mixture obtained by vacuum concn re-extracted (×4) with EtOAc The EtOAc residue was redissolved in $CHCl_3$ and applied to a Sephadex LH-20 column packed in $CHCl_3$–MeOH (49 1) and the pigments eluted with $CHCl_3$–MeOH (49 1) The first pigments to be eluted gave a dark violet fraction containing anhydrofusarubin as the main product Then a red fraction was obtained which was shown to be a mixture of javanicin, norjavanicin anhydrojavanicin and small amounts of as yet unidentified pigments The isofuranic substance **2** (nectriafurone), was isolated in a yellow fraction It showed a strong fluorescence in UV which distinguished this pigment from the preceding ones

Next, anhydrofusarubin lactone (**1**) was eluted as a purple fraction and last came fusarubin Minor substances remaining on the column and not further investigated were recovered by washing with increasing amounts of MeOH in $CHCl_3$

The pigments from the different fractions were submitted to

TLC on Si gel PF_{254} developed either with $CHCl_3$–MeOH (49.1) or hexane–EtOAc (1 1). Nectriafurone was finally obtained by reversed-phase HPLC by using a column (250 × 9 mm) of Lichrosorb 10 RP 18 developed with MeOH–H_2O (19 1)

In a typical expt with 300 Petri dishes, corresponding to 8 l. of medium, after 12 days of growth of the mutant strain 58, the yields (mg) were. fusarubin 350, anhydrofusarubin 90, anhydrofusarubin lactone 7, javanicin 40, norjavanicin 10, nectriafurone 8 5 All the known substances were identified by direct comparison of the physical properties (R_f, mps) and spectra (UV, IR, MS, NMR) with those of authentic standards and with data in the lit.

Anhydrofusarubine lactone (**1**) Amorphous dark purple powder; UV λ_{max}^{EtOH} nm 240, 285, 355, 500, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3250–2500, 2840, 1735, 1605, 1585, 1400, MS *m/z* (rel int) 302 $[M]^+$ (100); ^1H NMR, (250 MHz, $CDCl_3$, TMS as int standard) see formula **1**.

Nectriafurone (**2**) Obtained directly as crystals, mp 230° UV λ_{max}^{EtOH} nm 255, 320, 443, 465, IR ν_{max} cm^{-1}. 3350, 3250–2500, 2880, 2820, 1600, 1545, 1450, MS *m/z* (rel. int) 304 $[M]^+$ (100), 286 (86), ^1H NMR. see formula **2**

Triacetate of **2**. Obtained in pyridine–Ac_2O (1 1) amorphous, purified by HPLC (conditions given above) MS *m/z* (rel int) 430 $[M]^+$ (1), 388 $[M - CH_2CO]^+$ (32), 346 $[388 - CH_2CO]^+$ (12), 286 $[346 - HOAc]^+$ (100), ^1H NMR δ 1 60 (*d*, 3H, MeCHO–), 2.44 (3H, *s*, MeCOO–), 2 07 (3H, *s*, MeCOO–), 2.45 (3H, *s*, MeCOO–), 3 91 (3H, *s*, MeO–), 6.44 (*q*, 1H, MeCHO–), 6 90 (1H, *s*), 7 98 (1H, *s*)

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GLABRACHALCONE, A CHROMENOCHALCONE FROM *PONGAMIA GLABRA* SEEDS

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Key Word Index—*Pongamia glabra*, Leguminosae, seeds, chromenochalcone, glabrachalcone, synthesis

Abstract—Glabrachalcone, a new chromenochalcone has been isolated along with a known chromenochalcone from an ethanolic extract of the seed oil of *Pongamia glabra* The structure of glabrachalcone has been established as 2′-hydroxy-2,4,5-trimethoxy-6″,6″-dimethylchromeno(4′,3′:2″,3″)chalcone on the basis of spectral evidence and was confirmed by synthesis.

In an earlier paper [1] we reported the isolation of a new chromenoflavone, isopongachromene In continuation of this work, we now wish to report the isolation of two chromenochalcones **1** and **2**. Compound **1** is a new compound while **2** has been reported earlier [2] from the heartwood of *Pongamia glabra* and confirmed syntheti-