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FUSARUBINOIC ACID, A NEW NAPHTHOQUINONE FROM THE FUNGUS *NECTRIA HAEMATOCOCCA*

DENISE PARISOT, MICHEL DEVYS* and MICHEL BARBIER*

Laboratoire de Cryptogamie, Bâtiment 400, Faculté des Sciences, 91405 Orsay Cedex, France; *CNRS, Institut de Chimie des Substances Naturelles, Avenue de la Terrasse, 91198 Gif sur Yvette Cedex, France

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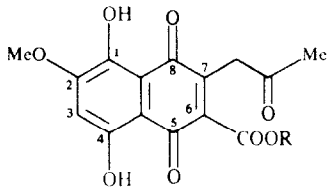
Key Word Index–*Nectria haematococca*; fungus; naphthoquinones; fusarubinoic acid.

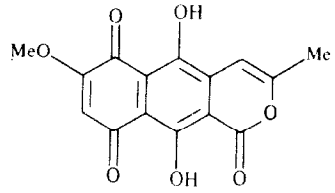
Abstract–Fusarubinoic acid, a new naphthoquinone pigment, was isolated from the culture medium of *Nectria haematococca* (Berk. and Br.) Wr. The structure **1a**, established on the basis of MS and ^{1}H NMR determinations carried out on this compound and on the methyl ester **1b**, was confirmed by a partial synthesis starting from anhydrofusarubin. Fusarubinoic acid is a candidate precursor in the biosynthesis of fusarubin from the heptaketide **6**, as of the corresponding anhydrofusarubin lactone **2**.

INTRODUCTION

Several isolates of the fungal plant pathogen *Fusarium solani* (Mart. Sacc.), were reported to produce naphthoquinone pigments of the fusarubin family [1–12]. These pigments exhibited antimicrobial [13, 14], insecticidal [15] and phytotoxic [14, 16, 17] activities. The ascomycete *Nectria haematococca* (Berk. and Br.) Wr., is the perfect stage of *F. solani* [18]. During the course of the past six years, we have isolated 13 naphthoquinone pigments released into the culture media of the wild or mutant strains [19–21] of *Nectria haematococca*. Recently, we considered the presence of naphthoquinone pigments more polar than fusarubin, and thus, 13-hydroxy-norjavanicin was found as a major compoud in the culture medium of the 169 red mutant [22]. In the present publication, we report on the isolation from this strain 169, of a more polar new pigment for which the structure **1a** is established and the name of fusarubinoic acid proposed.



1a R = H
1b R = Me



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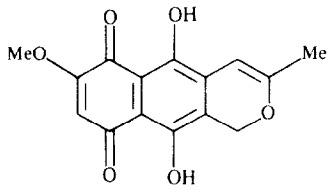

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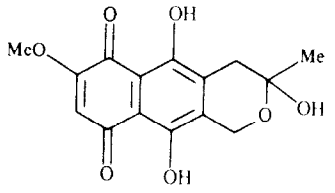

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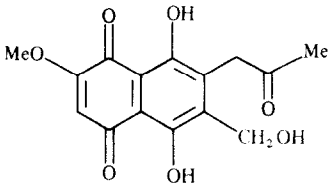

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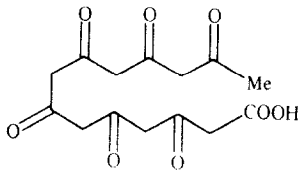

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RESULTS AND DISCUSSION

The culture medium of the fungus *Nectria haematococca* strain 169 was investigated in order to find out new polar naphthoquinone pigments related to fusarubin. Solvent extractions at different pH followed by crystallisations, gave fusarubinoic acid **1a** (12 mg/l). The EI mass spectrum gave an ion at *m/z* 302 (10%) accompanied by significant fragments at *m/z* 287 $[302-15]^+$ (20%); 259 $[302-15-28]^+$ (100%); 43 $[MeCO]^+$ (50%). That the ion at *m/z* 302 was not the molecular ion was established by the CI mass spectrum which gave an ion at *m/z* 321 $[M+1]^+$, (8%); with *m/z* 303 $[M-18]+1$, (98%); 277 $[M-44]+1$ (100%) as other significant peaks. The polarity of the substance on TLC and the $[M-44]$ ion in CIMS ($-CO_2$) suggested a carboxylic acid which could be lactonized in the apparatus to give the ion at 302. A methyl ester was prepared with an ether solution of diazomethane (new R_f on TLC's), showing a particularly characteristic mass spectrum EI. H: 334 $[M]^+$ (10%); 302 $[M-MeOH]^+$ (10%), 292 $[M-42]^+$ (10%), 260 $[M-42-MeOH]^+$ (100%; 43 [MeCO] (50%). High resolution MS, calc. for $C_{16}H_{14}O_8$ 334.0685, found 334.0687. By standing in solution, or by warming, the substance gave a small amount of a less polar compound which was identified as anhydrofusarubin lactone **2** by direct comparison with the natural or synthetic product. Hence, the structure **1a** was proposed for this acid, which is easily lactonized into **2** due to the enolizable oxo group present in the acetonyl aromatic substitution. The IR confirmed the presence of the carboxyl in **1a**, in particular by the bonded OH stretching absorption in the region 3300–2500 cm^{-1} (broad band). The ^1H NMR allowed the attribution of the remaining protons in **1a** ($C_{15}H_{12}O_8$); all protons were also respectively attributed in the corresponding methyl ester **1b** ($C_{16}H_{14}O_8$). **1a**: 2.25, *s*, 3H, (MeCO); 3.98, *s*, 2H, (CH_2); 4.04, *s*, 3H, (MeO); 6.46, *s*, 1H (aromatic proton at C-3); phenolic protons exchanged (in CD_3OD); **1b** ($CDCl_3$): 2.22, *s*, 3H, (MeCO); 3.83, *s*, 2H, (CH_2); 3.90, *s*, 6H, (ester and ether OMe); 6.15, *s*, 1H (aromatic proton at C-3); 12.40, *s*, 1H, (phenolic OH at C-1); 12.85, *s*, 1H, (phenolic OH at C-4).

The final proof of the structure **1a** for the isolated acid came from direct comparison of the properties with a compound obtained through hydrolysis of anhydrofusarubin lactone **2**. Due to the minute amounts of anhydrofusarubin lactone available from natural sources [21], we synthesized this product by the diphenyl seleninic anhydride oxidation of the acetylated anhydrofusarubin **3** (30% yield). This oxidation of the methylene group of anhydrofusarubin **3** does not occur so readily, as the yield after 2 hr was only 10% and could not be improved after 6 hr (30%) of reflux. This result is in agreement with a biological origin of the anhydrofusarubin lactone isolated from *N. haematococca* rather than an oxidation occurring during extraction.

As a hypothesis, we propose that the biosynthesis of fusarubin **4** proceeds through the sequence **6** ---> **1a** ---> **5** ---> **4** ---> **3** with the alternative **1a** ---> **2**. However, such a hypothesis will require to be confirmed by biochemical assays carried out from labelled precursors. The existence of the acid **1a** as a direct precursor to fusarubin, as of the corresponding aldehyde, was hypothetically proposed by Arsenault in 1968 [4], but these two substances had not been so far isolated as natural products.

EXPERIMENTAL

Isolation of fusarubinoic acid. Cultures of *N. haematococca* strain 169 were carried out according to the methods previously described [20–21]. The naphthoquinones were extracted from the agar medium of 7-day-old cultures as follows: after discarding the mycelium with its cellophane membrane support, the agar cakes were taken out of the Petri dishes, wrapped in plastic bags, frozen at −20° for 24 hr and then thawed at 40° on a water-bath. *Ca* 0.98 l of red liquid exuded from 60 frozen and thawed agar cakes. The exudate was filtered through a folded paper filter. The agar was further washed with H_2O, squeezed, the washings filtered and added to the exudate. The final vol. was adjusted to 1.5 l with H_2O (final pH 5.5). The aq. extract was concd to 1 l *in vacuo* and successively extracted with 3 × 1 l of hexane containing 10% EtOAc, then 4 × 1 l of EtOAc. These extractions gave a mixture of the less polar compounds (from anhydrofusarubin to 13-hydroxynorjavanicin), while more polar substances remained in the aq. residue. This aq. phase was acidified to pH 3 with HOAc and re-extracted with 3 × 1 l of EtOAc. The solvent was evapd *in vacuo* and the residue was taken up in CH_2Cl_2. After concn and standing overnight at 5°, a red pulverulent solid pptd. It was recrystallized from CH_2Cl_2, yielding 19 mg of nearly pure **1a** (still containing some traces of anhydrofusarubin lactone **2**); mp, 200–210° (with decomposition); the product is soluble in MeOH, fairly soluble in CH_2Cl_2, EtOAc, insoluble in hexane; R_f 0.15, SiO_2 TLC (Schleicher–Schüll), development by $CHCl_3$–MeOH (7:1).

By adding a soln of diazomethane in Et_2O and keeping for a few min, fusarubinoic acid **1a** gives quantitatively the corresponding methyl ester **1b**, crystallized as red needles from methanol, R_f 0.60 in the quoted conditions, mp, 187–190°, yield 100%.

Partial synthesis of anhydrofusarubin lactone **2** *and of fusarubinoic acid* **1a**. 30 mg of anhydrofusarubin diacetate (prepared by action of acetic anhydride in pyridine (4:5), 20 hr at 20°) were refluxed for 6 hr in dry C_6H_6 containing 150 mg of diphenylseleninic anhydride (a Fluka reagent). After concn of *ca* 75% of the C_6H_6 in *vacuo* the pptd reagent was filtered from the soln on a small cotton plug, washed with a few drops of C_6H_6 and the product was isolated by prep. silica gel TLC in hexane–CH_2Cl_2–MeOH (29:29:2), R_f 0.50, while the starting material had a R_f of 0.70 (obtained 9.5 mg (*ca* 30%)).MS, 386 $C_{19}H_{14}O_9$, $[M]^+$ 3%; 344 $[M-42]^+$ 10%; 302, $[M-42-42]^+$ 100%; ^1H NMR, $CDCl_3$, 2.45, *s*, 6H, (MeCOO); 2.70, *s*, 3H, (Me); 3.95, *s*, 3H, (OMe); 6.30, *s*, 1H, (aromatic proton); 6.80, *s*, 1H, (olefinic proton). This acetate was saponified in 0.5 ml EtOH containing a few drops of a satd soln of K_2CO_3 in H_2O, stirring for 1 hr at room temp. After acidification by HOAc, and adding 1 ml H_2O, the anhydrofusarubin lactone **2** was extracted by 2 × 1 ml EtOAc, washed with H_2O, dried over Na_2SO_4, yield 95%. Prep. silica gel TLC in the above solvent, gave 6.7 mg of **2** (90%), R_f 0.40, dark purple amorphous powder, MS *m/z* 302 $(M)^+$, 100%. ^1H NMR ($CDCl_3$), 2.40, *s*, 1H, (Me); 3.95, *s*, 3H, (OMe); 6.30, *s*, 1H, aromatic proton; 6.84, *s*, 1H, (olefinic proton). This product is identical in all respects to the anhydrofusarubin lactone **2** previously isolated [21] from the fungus *Nectria haematococca*. Oxidations of the acetylated **3** by diphenylseleninic anhydride, carried out for 2, 4 and 8 hr, gave respectively yields of 10, 20 and 32%. Elemental analysis, calc. for $C_{15}H_{10}O_7$, %, C:59.61, H:3.34, found C:59.84, H:3.66.

The anhydrofusarubin lactone **2** (5 mg in 2 ml MeOH at 60°) was hydrolysed into fusarubinoic acid **1a** by addition of 4 drops of KOH in MeOH (100 mg in 5 ml) and standing for 1 hr. After acidification by dilute HCl the product was extracted by $CHCl_3$, washing with H_2O, and drying over Na_2SO_4, yield 100%. This product was finally purified through silica gel TLC, development

with $CHCl_3$–MeOH (7:1), R_f 0.15, yield 70%. The synthesized fusarubinoic acid **1a** and its corresponding methyl ester **1b** were identical in all respects to the natural compounds and their derivative (cf. theoretical part for details).

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4-ETHYLGALLIC ACID FROM TWO *MIMOSA* SPECIES

B. K. MEHTA, KM. SAVITA SHARMA and AVINASH DUBEY

School of Studies in Chemistry, Vikram University, Ujjain 456 010, India

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Key Word Index—*Mimosa hamata*, *Mimosa rubicaulis* Mimosaceae, flowers, 4-ethylgallic acid.

Abstract—4-Ethylgallic acid has been identified from the flowers of *Mimosa hamata* and *M. rubicaulis*.

INTRODUCTION

The roots and leaves of *Mimosa rubicaulis* are widely used in the treatment of piles, bruises and burns [1]. The leaf extract of *Mimosa hamata* had shown significant antimicrobial and fungistatic activities [2, 3]. From the leaves of *M. hamata*, ethyl gallate and gallic acid have been reported [4]. This paper deals with the isolation and structure determination of 4-ethylgallic acid.

RESULTS AND DISCUSSION

The benzene–ether (9:1) eluate of the flowers afforded silky crystals, mp 233–234° (decomposition), (ether) $C_9H_{10}O_5$, M^+ *m/z* 198 (98.07%). λ_{max}^{MeOH} 218, 268 nm, diacetate mp 169°, (benzene), $C_{13}H_{14}O_7$. The IR spectrum showed strong absorptions at 3500, 1655, 1610 and 1270 cm^{-1} for acidic, hydroxyl, carbonyl and ether linkages, respectively, along with bands for benzene and