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THREE FURTHER NAPHTHOQUINONES PRODUCED BY *FUSARIUM SOLANI*

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Key Word Index—*Fusarium solani*; Hyphomycetes; citrus root blight; naphthoquinones; pigments; interconversion.

Abstract—Three naphthoquinone pigments are described which were produced by *Fusarium solani*. They are 2,3-dihydro-5,8-dihydroxy-6-methoxy-2-hydroxymethyl-3-(2-hydroxypropyl)-1,4-naphthalenedione, 2,3-dihydro-5-hydroxy-4-hydroxymethyl-8-methoxy-naphtho[1,2-b]furan-6,9-dione and 5,8-dihydroxy-2-methoxy-6-hydroxymethyl-7-(2-hydroxypropyl)-1,4-naphthalenedione. One of these pigments was shown to be the precursor of the other two.

INTRODUCTION

Fusaria, principally *Fusarium solani* (Mart.) Appel. and Wr. emend. Snyd. and Hans., and to a lesser extent *F. oxysporum* (Schlect. emend. Snyd. and Hans) constitute the predominant fungi isolated from diseased fibrous roots of citrus affected with Blight [1]. Blight is a disease of unknown etiology that threatens most of the citrus plantings of Florida. These Fusaria produce a number of naphthoquinone pigments in culture, some of which are phytotoxic. Seventeen of the compounds have been identified in cultures of Fusaria from citrus [2–5]. We now report the isolation of three new pigments produced on synthetic media of *F. solani* originally obtained from roots of diseased citrus trees.

RESULTS AND DISCUSSION

Large quantities of naphthoquinones were required for field testing. The isolated compounds are to be first tested on radish seeds for toxicity, then on potted citrus plants. When this is completed they will be tested on grown trees in the field. In shake culture *F. solani* had produced more compounds that were of interest than in still cultures. In an attempt to scale-up production we tried using a large holding tank for growing the culture. Various parameters were tested until growth products were identical to those obtained from shake cultures [6]. In one procedure we observed a large quantity of a new metabolite that had only been observed in trace quantities in shake cultures. From this metabolite fraction we isolated three new compounds. They are 2,3-dihydro-5,8-dihydroxy-6-methoxy-2-hydroxymethyl-3-(2-hydroxypropyl)-1,4-naphthalenedione (**1**), 2,3-dihydro-5-hydroxy-4-hydroxymethyl-8-methoxy-3-methyl-naphthol[1,2-b]furan-6,9-dione (**2**) and 5,-8-dihydroxy-2-methoxy-6-hydroxymethyl-7-(2-hydroxypropyl)-1,4-naphthalenedione (**3**). The production was monitored daily by TLC and it was apparent that 3,4,4a,10a-tetrahydro-3,6,9-trihydroxy-7-methoxy-3-methyl-1H-naphtho[2,3-c]pyran-5,10-dione (**4**, dihydrofusarubin) was the precursor to **1** [3, 7]. We have previously shown that **4** and its isomer were the precursor to five naphthoquinones produced by *F. solani* [3]. When an ethyl acetate extract containing all of the naphthoquinones was stored at 5° for a month, most of **1** was converted to **2** and a small amount of **3**. When a portion of the extract was separated on a preparative silica gel HF plate and the plate left overnight in the hood most of **1** was converted to **2** and **3**.

We propose structures for compounds **1**, **2** and **3** based mainly on NMR and mass spectral data and comparison with the NMRs of twelve other naphthoquinones produced by *F. solani* [3]. Compound **1** has a UV spectrum identical to that of **4**. The NMR spectra were determined in deuterochloroform at 270 MHz and the observed data are listed in the Experimental. When **1** was treated with D_2O the hydroxyls at δ12.58, 12.22 and 2.42 disappeared. This treatment also caused the H-1 distorted *dd* at δ4.24 to become a sharp *dd*, J = 11.2, 2.5 and the 2H multiplet at δ3.86 became rounded with a projecting *dd* out the top (J = 11.2, 4.5 Hz) indicating a CH_2 coupled to a hydroxyl. Decoupling of **1** at δ4.24 changed the 2H multiplet at δ3.86 to a multiplet and the 1H multiplet at δ2.90 to a multiplet showing only that they are coupled. Decoupling at δ3.86 changed the *dd* at δ4.24 to a broad singlet, the 1H multiplet at δ2.90 became a *dd* (J = 6.2, 2.5 Hz), the 1H multiplet at δ1.96 became a *dd* (J = 15, 7.5 Hz), the 1H multiplet at δ1.79 became a broad *d* (J = 15 Hz) and the 3H doublet at δ1.26 became a singlet. The C-9 carbon is CH_2–OH at δ4.24, 3.86 and is split by a single hydrogen at δ2.90 on C-2 and distorted by the hydroxyl. The other H at δ3.86 is coupled to CH_2 at δ1.96 and 1.79 and a methyl at δ1.26. Decoupling of the 1H multiplet at δ3.28 changed the multiplet at δ2.90 to a *dd* (J = 4.5, 2.5 Hz), the multiplet at δ1.96 became a broad doublet (J = 15 Hz) and the multiplet at δ1.79 became a *dd* (J = 15, 10 Hz). Decoupling of the 1H multiplet at δ2.90 caused the *dd* at δ4.22 to become a doublet (J = 11.2 Hz) and the multiplet at δ3.86 to show a distorted doublet (J = 11.2 Hz). The C-10 carbon is CH_2 at δ1.96, 1.79 which is attached to the C-3 position and is split by the C-3 hydrogen at δ3.28 and the

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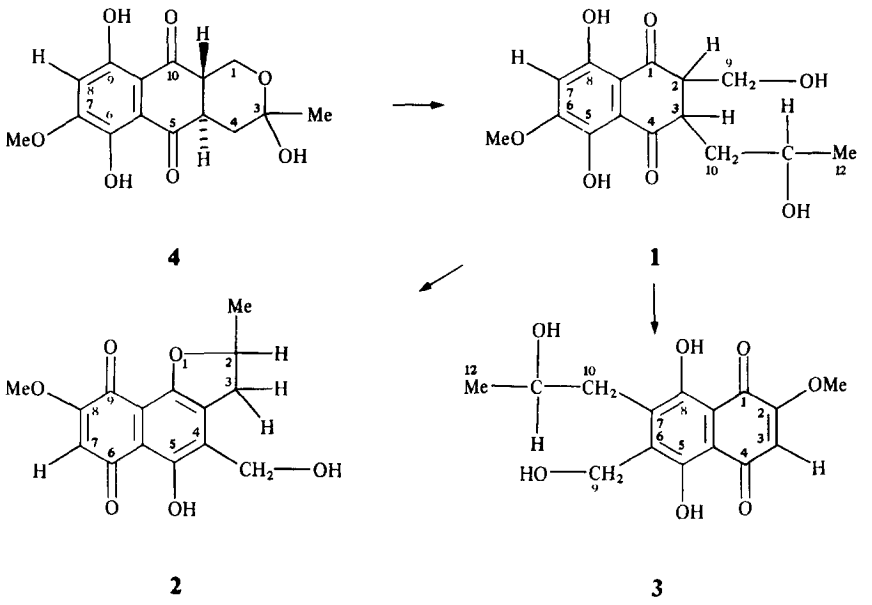

Fig. 1. Pathway of formation for *Fusarium* metabolites.

C-11 hydrogen at δ3.86. The C-12 is a methyl that is split by the C-11 hydrogen. The H-2 and H-3 hydrogens were shown to be coupled and establishes their relative positions. The small coupling constant of 6.2 indicates they are in axial–equatorial position. The methoxy resonance at δ3.95 is assigned to the 6 position. The lone unsplit hydrogen at δ6.70 is at the C-7 position on the aromatic portion of the ring [8]. The mass spectrum showed an [M − 18] base peak with a small signal at *m/z* 310. A chemical ionization spectrum with isobutane gave an [M + 1] peak at 311 and [M + 1 − 18] at 293.

The NMR spectrum of **2** has an eight line multiplet with 1H at δ5.21. When the spectrum is decoupled at δ5.21 the 1H *dd* at δ3.46 (J = 17.9 Hz) becomes a *d* (J = 17 Hz), the 1H *dd* at 2.92 (J = 17, 6.5 Hz) becomes a doublet (J = 17 Hz) and the 3H doublet at δ1.59 becomes a singlet. Thus, a single hydrogen is split by a CH_2 and a methyl. Decoupling the 2H doublet at δ4.8 (J = 6 Hz) causes the triplet at δ2.67 (J = 6 Hz) to become a singlet. A CH_2 is split by a single H. Decoupling the 3H doublet at δ1.6 causes the multiplet at δ5.21 to become a *dd* (J = 9, 6.5 Hz). When D_2O was added the singlet at δ13.60 and the triplet at 2.67 disappear and the 2H doublet at δ4.74 became a singlet. This indicates a Me–C–H attached to CH_2 and an isolated CH_2 attached to a hydroxyl. A single unsplit hydrogen at δ6.07 is on a quinone ring [8] at C-7 while a singlet 3H at δ3.90 indicates a methoxy at the C-8.

The NMR spectrum of **3** is listed in the Experimental and is unambiguous. A single unsplit hydrogen at δ6.20 indicates that it is on the quinone ring [8]. The C-9 and C-11 hydroxyls were not observed.

When compounds **1** through **4** were viewed on a thin-layer plate with ultraviolet light, **1** and **4** were yellow fluorescent and **2** and **3** were red fluorescent. Their colours in visible light were the same as with ultraviolet. Thus, the structures of compounds **1–3** were established based on their NMR and mass spectral data, the ease of formation of **2** and **3** from **1**, and the formation of **1** from a compound of known structure (**4**) [7].

EXPERIMENTAL

TLC solvent systems. (A) $CHCl_3$–nitromethane–MeOH–HOAc (170:20:6:1); (B) $CHCl_3$–CH_2Cl_2–HOAc–MeOH (140:50:4:4). TLC plates were 250 μm silica gel GF and 1 mm silica gel HF 60. Column chromatography: Kieselgel 60 reinst (70–230 mesh ASTM), 5 × 20 cm. The silica was deactivated with HOAc and H_2O, washed with Me_2CO and then $CHCl_3$. The sample was loaded in $CHCl_3$, then eluted with $CHCl_3$, $CHCl_3$–Me_2CO, 2, 5, 10 and 15%. UV light 254 and 360 nm.

Samples. Isolates of *F. solani* were obtained from fibrous roots of Florida citrus trees. These isolates were grown in a 1200 l. tank containing 660 l. of mineral salts/sucrose liquid medium [6].

^{1}H NMR 270 MHz, TMS as internal standard in $CDCl_3$ and MS were obtained through the Chemistry Department of Florida State University. Mps are uncorr. 2,3-Dihydro-5,8-dihydroxy-6-methoxy-2-hydroxymethyl-3(2-hydroxypropyl)-1,4-naphthalenedione (**1**). MS *m/z* 292 $[M-18]^+$; MS (CI isobutane) *m/z* 311 $[M+1]^+$. $C_{15}H_{18}O_7$ requires 310.1051, found 310.1046; light red crystals from EtOAc, 135° on preheated block; IR ν_{max}^{KBr} cm^{-1}: 3300, 1610, 1570 sh, 1470, 1430, 1400, 1380, 1315, 1290, 1270, 1220, 1205, 1175, 1125, 1095, 1065, 1040, 1020, 1000 sh, 990, 925, 900, 870 w, 825, 805 sh; ^{1}H NMR: δ1.22 (3H, *d*, J = 6 Hz, Me-12), 1.79 (1H, *m*, H-10), 1.96 (1H, *m*, H-10), 2.42 (1H, *s*, OH), 2.90 (1H, *m*, H-2), 3.28 (1H, *m*, H-3), 3.86 (2H, *m*), 3.95 (3H, *s*, MeO-6), 4.24 (1H, *dd*, J = 11.2, 2.5 Hz), 6.70 (1H, *s*, H-7), 12.22 (1H, *s*, OH-5), 12.58 (1H, *s*, OH-8), addition of D_2O removes the OH signals at δ2.42, 12.22 and 12.58; UV λ_{max}^{EtOH} nm: 213, 244, 277, 303 sh, 391, 400 sh (log ε3.98, 4.21, 3.83, 3.64, 3.88, 3.83). 2,3-Dihydro-5-hydroxy-4-hydroxy-methyl-8-methoxy-naphtho[1,2-b]furan-6,9-dione (**2**). MS *m/z* 290, $C_{15}H_{14}O_6$ requires 290.0789, found 290.0795; red crystals 202–203° (CH_2Cl_2–hexane); IR ν_{max}^{KBr} cm^{-1}: 3490, 1660, 1625, 1585, 1420, 1400, 1390 sh, 1355, 1315, 1220, 1160, 1075, 1040, 1015, 955, 915,

885, 855, 830, 815, 780; ^{1}H NMR: δ1.59 (3H, *d*, J = 6 Hz, Me), 2.67 (1H, *t*, J = 6 Hz, CH_2–OH), 2.92 (1H, *dd*, J = 17, 6.5 Hz, H-3), 3.46 (1H, *dd*, J = 17, 9 Hz, H-3), 3.90 (3H, *s*, MeO-8), 4.74 (2H, *d*, J = 6 Hz, H-4), 5.21 (1H, *m*, H-2), 6.07 (1H, *s*, H-7), 13.60 (1H, *s*, C-5); addition of D_2O removes the OH signals at δ2.67 and 13.60. UV λ_{max}^{EtOH} nm: 223, 297, 485, 502, 538 (log ε4.06, 3.62, 3.50, 3.47, 3.17). 5,8-Dihydroxy-2-methoxy-6-hydroxymethyl-7-(2-hydroxypropyl)-1,4-naphthalenedione (**3**). MS m/z 308. $C_{15}H_{16}O_7$ requires 308.0895. Found 308.0862; red crystals 206–212° (dec. EtOAc); IR ν_{max}^{KBr} cm^{-1}: 3330, 1615, 1550, 1450, 1420, 1390, 1295, 1265, 1245, 1220, 1205, 1180 w, 1150, 1115, 1105, 1075, 1025, 1010, 995 w, 940 w, 855, 825, 815 sh; ^{1}H NMR: δ1.39 (3H, *d*, J = 6 Hz, Me-12), 2.86 (1H, *dd*, J = 13.5, 10 Hz, H-10), 3.13 (1H, *dd*, J = 13.5, 3 Hz, H-10), 3.95 (3H, *s*, MeO-2), 4.16 (1H, *m*, H-11), 4.63 (1H, *d*, J = 12 Hz, H-9), 4.97 (1H, *d*, J = 12 Hz, H-9), 6.20 (1H, *s*, H-3), 12.86 (1H, *s*, OH-5), 13.35 (1H, *s*, OH-8); addition of D_2O removes the OH signals at δ12.86 and 13.35, the other OHs were not observed; UV λ_{max}^{EtOH} nm: 227, 306, 452 sh, 482, 509, 546, (log ε4.38, 3.87, 3.56, 3.76, 3.80, 3.59).

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