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NAPHTHOFURANS PRODUCED BY *FUSARIUM OXYSPORUM* ISOLATED FROM CITRUS

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Key Word Index—*Fusarium oxysporum*; Fungi imperfecti; isolates; naphthofurans.

Abstract—The naphthofuran, nectriafurone [5,8-dihydroxy-4,9-dione-3-(2-hydroxyethyl)-7-methoxy-naphtho-[2,3-c]-furan], and its 8-methyl ether, have been found in *Fusarium oxysporum* isolates obtained from roots of diseased citrus trees. The 5-methyl ether was prepared by methylation of nectriafurone.

INTRODUCTION

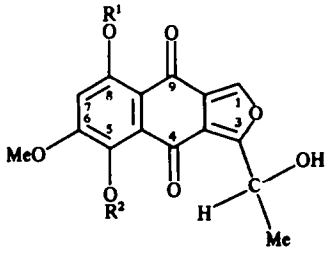
We have continued the study of the metabolites produced by *Fusarium oxysporum* Schlect. emend Snyd. and Hans. *F. oxysporum* was isolated from diseased fibrous roots of citrus affected with blight [1]. *F. solani* and *F. oxysporum* have been shown to cause wilt and root rot in a number of crop plants [2, 3]. These *Fusaria* produced a number of naphthoquinone pigments in culture, some of which are phytotoxic. Twenty-one of these compounds have been identified in cultures of *Fusaria* from citrus [1, 4–8]. We now report the isolation of two new pigments produced by *F. oxysporum* and one related derivative.

RESULTS AND DISCUSSION

We recently reported the isolation of six naphthoquinone pigments produced by *F. oxysporum* [1]. Five of these were the normal *F. solani* metabolites with an extra methoxyl group attached to the molecule. Four of these compounds had previously been identified as metabolites of *F. moniliforme* [9]. We have now identified two more metabolites, **1** and **2**, from *F. oxysporum* which are naphthofurans. Of the eight compounds identified from *F. oxysporum*, only compound **1** does not have the extra methoxy group. Compound **1** was first identified from *Nectria haematococca* [10]. Our data are in agreement with those previously described [10] (Experimental) except that part of the NMR spectrum associated with the Me–CH–OH group. We observed a five-line multiplet for the H because it was split by the hydroxy group which gives rise to a doublet at δ4.83 ($J = 7$) as well as being split by the methyl group. Decoupling of the methyl at δ1.64 gave a doublet at δ5.19 ($J = 7$). Addition of D_2O to the sample removed the three hydroxyl signals from the spectrum and gave a quartet for the hydrogen at δ5.19. This same pattern was seen for all three compounds shown in Fig. 1. The UV spectrum of **1** showed absorption at 242, 258 and 324 nm, compared to the previously reported [10] values of 255 and 320 nm. The second metabolite identified in this study, compound **2**, is the 8-*O*-methyl ether of **1**.

Proof of this is based upon the fact that treatment of **1** with diazomethane gave only one product (**3**). From *F. oxysporum* we have isolated eight naphtho-quinones, including compounds **1** and **2** [1]. Seven of these compounds were *O*-methylated β to the 6-methoxy group. When we methylated 5,8-dihydroxy-2-methoxy-6-methyl-7-(2-oxypropyl)-1,4-naphthoquinone (javanicin) we obtained two products, neither of which was 8-*O*-methyl-javanicin. Methylation of 2,5,8-trimethoxy-6-methoxy-3-(2-oxypropyl)-1,4-naphthoquinone, gave three products without the methyl group added at the eight position or β to the 6-methoxy group [8]. On the three compounds shown in Fig. 1, the proton resonance at C-7 was found above δ6.70 and this proves that the protons are on a benzenoid ring [11]. The centre ring in all three compounds has to contain two keto groups so we have both isomers of the outer ring.

Compound **1** was tested for antibiotic activity in a broth microdilution assay against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhi*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia coli*, and *Klebsiella pneumoniae*. None of the organisms were inhibited by compound **1** at the upper limit of 128 μg/ml.



	R^1	R^2
1	H	H
2	Me	H
3	H	Me

Fig. 1. Fusarium metabolites.

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EXPERIMENTAL

^{1}H NMR (270 MHz, $CDCl_3$, TMS as internal standard) and MS were obtained through the Chemistry Department, Florida State University. Mps are uncorr. For growth of cultures and isolation, see [1].

Compound 1. MS *m/z*: 304 ($C_{15}H_{12}O_7$ requires 304.058; found 304.063); yellow-brown crystals, mp 222–225° MeOH (230° [10]); IR ν_{max}^{KBr} cm^{-1}: 3420, 3100, 1605, 1560, 1450, 1425, 1410, 1360w, 1300, 1250, 1215, 1175, 1160, 1110, 1007 (sh), 1005, 975, 950, 900w, 865, 850, 820w, 810; UV λ_{max}^{EtOH} nm: 242, 258, 324, 444, 468 (log ε 4.26, 4.23, 3.84, 4.07, 3.97); ^{1}H NMR: δ1.64 (3H, *d*, *J* = 7, Me), 3.99 (3H, *s*, MeO-6), 4.83 (1H, *d*, *J* = 7, OH), 5.19 (1H, *m*, 51, H), 6.70 (1H, *s*, H-7), 8.08 (1H, *s*, H-1), 13.07 (1H, *s*, OH-5), 13.39 (1H, *s*, OH-8).

Compound 2. MS *m/z*: 318 ($C_{16}H_{14}O_7$ requires 318.0738; found 318.0743); yellow-brown needles mp 214–222° dec MeOH; IR ν_{max}^{KBr} cm^{-1}: 3430, 3120, 1645, 1625, 1600, 1540, 1470, 1435, 1375, 1345, 1295 (sh), 1265 (sh), 1245, 1215, 1165, 1120 (sh), 1110, 1070, 1035, 1005w, 955, 895, 855, 820, 810, 765; UV λ_{max}^{EtOH} nm: 236, 255 (sh), 322, 443 (log ε 4.37, 4.21, 3.90, 4.02); ^{1}H NMR: δ1.64 (3H, *d*, *J* = 7, Me), 4.00 (3H, *s*, MeO-6), 4.03 (3H, *s*, MeO-8), 4.78 (1H, *d*, *J* = 7, OH), 5.19 (1H, *m*, 51, H), 6.83 (1H, *s*, H-7), 7.99 (1H, *s*, H-1), 13.30 (1H, *s*, OH-5).

Compound 3. MS *m/z*: 318 ($C_{16}H_{14}O_7$ requires 318.0738: found 318.0740); yellow needles mp 202–203° MeOH; IR ν_{max}^{KBr} cm^{-1} 3360, 3110, 1655, 1620, 1555, 1475, 1450, 1430, 1405, 1345 (w), 1295, 1245 (sh), 1225 (sh), 1210, 1175 (w), 1160, 1115, 1105, 1090 (w), 1020 (w), 1010, 995, 955, 940, 895, 855, 845, 795, 770; UV λ_{max}^{EtOH} nm: 227, 258, 303, 406 (log ε 4.28, 4.23, 3.83, 4.00); ^{1}H NMR: δ1.62 (3H, *d*, *J* = 7, Me), 3.88 (3H, *s*, MeO-5), 3.96 (3H, *s*, MeO-6), 5.15 (1H, *m*, 51, H), 5.33 (1H, *d*, *J* = 7, OH), 6.72 (1H, *s*, H-7), 8.02 (1H, *s*, H-1), 13.66 (1H, *s*, OH-8).

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