Phytochemistry, Vol 24, No. 3, pp 457–459, 1985.
Printed in Great Britain.

0031–9422/85 $3.00+0.00
Pergamon Press Ltd.

NAPHTHOQUINONES PRODUCED BY *FUSARIUM OXYSPORUM* ISOLATED FROM CITRUS

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(*Revised received* 23 *July* 1984)

Key Word Index—*Fusarium oxysporum*; Fungi Imperfecti; isolates; naphthoquinones.

Abstract—Six naphthoquinone pigments are described which were produced by *Fusarium oxysporum* isolates obtained from roots of diseased citrus trees. These were 8-*O*-methylbostrycoidin, 9-*O*-methylfusarubin, 9-*O*-methylanhydrofusarubin, 5-*O*-methyljavanicin, 5-*O*-methylsolaniol and 1,4-naphthalenedione-3,8-dihydroxy-5,7-dimethoxy-2-(2-oxopropyl). These naphthoquinones had not been previously identified from *F. oxysporum* isolates.

INTRODUCTION

Fusarium oxysporum Schlect. emend Snyd. and Hans. is a soil-borne fungus causing wilt and root rot in a number of crop plants. It has been reported as a colonizer of diseased citrus fibrous roots [1], and is commonly associated with nematode lesions on these roots [2]. Fusaria, principally *Fusarium solani* (Mart.) Appel. and Wr. emend. Snyd. and Hans., and to a lesser extent *F. oxysporum*, constitute the predominant fungi isolated from diseased fibrous roots of citrus affected with blight [3]. *F. solani* produces a number of naphthoquinone pigments in culture, several of which are phytotoxic. Eleven of these compounds have been identified in cultures of *solani* from citrus [4–6]. With the exception of bostrycoidin [7], naphthoquinone compounds have not been reported as metabolites of *oxysporum*. We now report the isolation of six pigments produced by *F. oxysporum* obtained from roots of diseased citrus trees.

RESULTS AND DISCUSSION

Isolates of *F. oxysporum*, obtained from roots of field-grown citrus trees that exhibited blight symptoms, were examined for production of pigments. Under certain cultural conditions, several of these produced a number of pigments. The isolates that produced these pigments were obtained from trees at two different sites. TLC of extracts of the cultures were compared to extracts of *F. solani* and it was readily apparent that they were different. Seven pigments were isolated and identified, none of which have been previously reported as metabolites of *F. oxysporum*. They are 8-*O*-methylbostrycoidin (**1**), 9-*O*-methylfusarubin (**2**), 3-*O*-methyl,9-*O*-methylfusarubin (**3**), 9-*O*-methylanhydrofusarubin (**4**), 5-*O*-methyljavanicin (**5**), 5-*O*-methylsolaniol (**6**) and 1,4-naphthalenedione-3,8-dihydroxy-5,7-dimethoxy-2-(2-oxopropyl) (**7**). Compounds **1**, **2**, **5** and **6** were first reported from *F. moniliforme* cultures isolated from maize [8]. Compound **5** was also isolated from *F. solani* [9]. Compounds **4** and **7** have not been reported previously. A seventh compound, 3-*O*-methyl,9-*O*-methylfusarubin (**3**), was isolated and identified from our initial extracts. This compound is an artefact produced when the dried extract is dissolved in methanol. Subsequent extractions of culture filtrates from which methanol was excluded failed to yield this compound. Compound **3** was readily made by adding hydrochloric acid to **2** in cold methanol. Compound **4** was made by heating **2** in acetic acid. Anhydrofusarubin was first made this way from fusarubin [10]. The proposed structure for **7** was based on comparison of spectral data to known compounds. The ^{1}H NMR resonances for the acetonyl group in **7** are almost identical to those of **5** and of javanicin and norjavanicin [5]. The H-6 proton signal in **7** is downfield as in **5** and **6**. The methoxy signals are centred at about δ4.0 as in the rest of the compounds. The methyl at C-3 in **5** and **6** has been replaced by a hydroxyl whose signal disappears on the addition of deuterium oxide. A similar compound, 2-acetonyl-3,5-dihydroxy-7-methoxy-(1,4-naphthoquinone), had UV λ_{max} at 208, 227, 272, 313 and 380 nm (log ε = 4.4, 4.3, 4.2, 4.1, 3.7) and IR ν_{max} at 1705, 1635, 1610 and 1575, in the carbonyl region [11]. Compound **7** had UV λ_{max} at 211, 241, 270, 300 and 454 nm (log ε = 4.3, 4.2, 4.2, 4.0, 3.9) and IR ν_{max} at 1705, 1635, 1610 and 1570 cm^{-1} in the carbonyl region.

The major pigment isolated was 9-*O*-methylfusarubin (**2**), with **5**, **1**, **7**, **6** and **4** in decreasing concentration. Compounds **4** and **6** are present only as trace components. All of these compounds are red except **4**, which is purple. Compound **7** was isolated from fraction B (see Experimental).

EXPERIMENTAL

TLC solvent systems. (A) C_6H_6–nitromethane–HOAc (75:25:2); (B) $CHCl_3$–MeOH (97:3), (C) C_6H_6–Me_2CO–HOAc (35:5:1); (D) $CHCl_3$–nitromethane–HOAc (90:10:1.5); (E)

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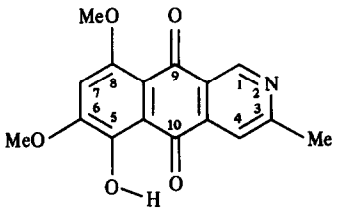
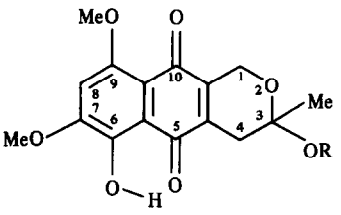
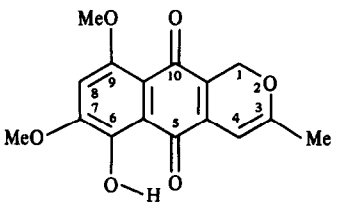


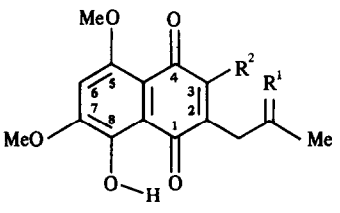
1



2 R=H
3 R=Me



4



5 R^1=O, R^2=Me
6 R^1=H,OH, R^2=Me
7 R^1=O, R^2=OH

C_6H_6–Me_2CO–HOAc (16:3:0.1); (F) $CHCl_3$–MeOH (39:1) and (G) C_6H_6–Me_2CO–HOAc (17:3:3). Systems A–D were used with 250 μm silica gel GF plates (Table 1) and E–G were used with 1 mm silica gel HF-60 plates.

Samples. F. oxysporum was isolated from fibrous roots of Florida citrus trees (*C. sinensis* Osb.) with obvious blight symptoms by plating root pieces on a modified Komada's medium [12]. For pigment production, isolates were grown as liquid still cultures on either potato–dextrose medium or a mineral salts–glucose medium. The potato–dextrose medium contained, per l., the broth from 200 *g* boiled white potatoes, 20 g glucose and 1 g KH_2PO_4. The mineral salts–glucose medium contained, in ppm: $NaNO_3$, 848; KCl, 300; $MgSO_4 \cdot 7H_2O$, 165; NaH_2PO_4, 100; $CaCl_2 \cdot 2H_2O$, 40; H_3BO_3, 5.7; $FeSO_4 \cdot 7H_2O$, 5.0; $ZnSO_4 \cdot 7H_2O$, 4.4; $MnSO_4 \cdot H_2O$, 3.1; $Na_2MoO_4 \cdot 2H_2O$, 2.5; $CuSO_4 \cdot 5H_2O$, 0.4; and glucose, 20000.

After inoculation, cultures were held at 27° in the dark for 4–6 weeks. Cultures were then filtered through cheesecloth to remove the mycelium, and the filtrate was extracted twice at pH 7 with EtOAc (fraction A). The filtrate was then adjusted to pH 2.7 with 6 M HCl and extracted twice with EtOAc (fraction B). The naphthoquinones were isolated from the EtOAc extracts by TLC.

^{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. The NMR spectra of **1**, **2**, **5** and **6** were in agreement with those reported by Steyn *et al.* [8].

Table 1. R_f values on TLC of compounds **1**–**7**

Compound	Solvent system			
	A	B	C	D
1	0.08	0.46	0.17	0.20
2	0.06	0.25	0.15	0.13
3	0.29	0.59	0.35	0.41
4	0.35	0.60	0.39	0.43
5	0.26	0.53	0.29	0.38
6	0.09	0.30	0.17	0.16
7	0.09	0.08	0 11	0.19

1. MS *m/z*: 299; red needles, mp 211–212° ($CHCl_3$; 215–216° [4]); IR ν_{max}^{KBr} cm^{-1}: 1650, 1630 sh, 1585, 1550 sh, 1460, 1425, 1400 sh, 1370, 1325, 1260, 1215, 1200 sh, 1160 w, 1130, 1090, 1025, 1010 sh, 920, 905, 825, 805; UV λ_{max}^{EtOH} nm: 249, 320, 488.

2. MS *m/z*: 320; red-brown needles, mp 137–140° (MeOH; 138–139° [8]); IR ν_{max}^{KBr} cm^{-1}: 3470, 3240, 1605, 1550 sh, 1460, 1430, 1370, 1310, 1260, 1215, 1170, 1155 sh, 1135 sh, 1100, 1075, 1045, 1005, 965, 925, 905, 870, 850, 840, 815, 800, 755; UV λ_{max}^{EtOH} nm: 227, 286, 486, 510, 545 sh.

3. MS *m/z*: 334 ($C_{17}H_{18}O_7$ requires: 334.104; found: 334.102); dark red needles, mp 184–186°; IR ν_{max}^{KBr} cm^{-1}: 1645, 1610, 1270, 1250, 1215, 1180, 1160, 1135, 1095, 1080, 1045, 1005, 970, 925, 905, 835, 820 sh, 800; UV λ_{max}^{EtOH} nm: 227, 286, 486, 510, 550 (log ε = 4.6, 4.1, 3.9, 3.9, 3.6); ^{1}H NMR: δ1.54 (3H, *s*, Me-3), 2.49 (1H, *dm*, *J* = 19 Hz, H-4 ax), 2.83 (1H, *dd*, *J* = 19, 2, H-4eq), 3.27 (3H, *s*, MeO-3), 3.97 (3H, *s*, MeO-9), 4.00 (3H, *s*, MeO-7), 4.38 (1H, *dm*, *J* = 19 Hz, H-1ax), 4.72 (1H, *dd*, *J* = 19, 1.5 Hz, H-1eq), 6.73 (1H, *s*, H-8), 13.15 (1H, *s*, OH-6).

4. MS *m/z*: 302; black needles, mp 175–177° (MeOH); IR ν_{max}^{KBr} cm^{-1}: 3450, 1620, 1570, 1460, 1425, 1375, 1300, 1265, 1235, 1215, 1185, 1135, 1115, 1065, 1010, 960, 905, 855, 835, 810, 790; UV λ_{max}^{EtOH} nm: 208, 222, 273, 503 (log ε = 4.3, 4.4, 4.2, 3.6, 3.9); ^{1}H NMR: δ2.00 (3H, *s*, Me-3), 3.97 (3H, *s*, MeO-9), 4.00 (3H, *s*, MeO-7), 5.15 (2H, *s*, CH_2-1), 5.83 (1H, *s*, H-4), 6.74 (1H, *s*, H-8), 13.14 (1H, *s*, OH-6).

5. MS *m/z*: 304; red needles, mp 193–195° (MeOH; 197–198° [8]); IR ν_{max}^{KBr} cm^{-1}: 3400 w, 1710, 1630, 1590 w, 1555 w, 1470, 1430, 1375, 1310, 1270, 1255, 1215, 1195, 1165, 1120, 1075, 1035, 1015 w, 995 w, 975 w, 945, 900 w, 840, 825, 800, 755; UV λ_{max}^{EtOH} nm: 227, 286, 488, 510 sh, 546 sh.

6. MS *m/z*: 306; red-black needles, mp 158–160° (C_6H_6; 152–154° [4]); IR ν_{max}^{KBr} cm^{-1}: 3460 s, 1610, 1480, 1435, 1380, 1325, 1300, 1265, 1220, 1150, 1125, 1100, 1065, 1050, 985, 955, 850, 815, 810, 750; UV λ_{max}^{EtOH} nm: 227, 288, 486, 510 sh, 550 sh.

7. MS *m/z*: 306 ($C_{15}H_{14}O_7$ requires: 306.074; found: 306.079); red-orange crystals, mp 226–229° (MeOH), IR ν_{max}^{KBr} cm^{-1}: 3200, 1705, 1635, 1610, 1570 sh, 1470, 1435, 1415, 1385, 1325, 1310 sh,

1275, 1215, 1165, 1090, 1050, 1030, 1015, 975, 920, 825; ^{1}H NMR: δ2.28 (3H, *s*, Me–C(=O)), 3.68 (2H, *s*, CH_2–C(=O)), 4.0 (6H, *s*, Me-O-5, Me-O-7), 6.62 (1H, *s*, H-6), 8.21 (1H, *s* (*br*), OH-3), 13.60 (1H, *s*, OH-8), addition of D_2O removes the OH signals at δ8.21 and 13.60; UV λ_{max}^{EtOH} nm: 211, 240, 270, 300, 454 (log ε 4.3, 4.2, 4.2, 4.0, 3.9).

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