Phytochemistry, Vol. 26, No. 3, pp. 795–798, 1987.
Printed in Great Britain.

0031-9422/87 $3.00 + 0.00
Pergamon Journals Ltd.

NAPHTHOQUINONES AND DERIVATIVES FROM *FUSARIUM*

J. H. TATUM,* R. A. BAKER and R. E. BERRY

U.S. Citrus and Subtropical Products Laboratory,† P.O. Box 1909, Winter Haven, FL 33883, U.S.A.

(*Revised received* 15 *June* 1986)

Key Word Index—*Fusarium solani*; *F. oxysporum*; isolates and derivatives; 1,4-naphthoquinones.

Abstract Ten 1,4-naphthoquinones are described; two were produced by *Fusarium solani*, two by *F. oxysporum* and six are derivatives. The substitution patterns of the 1,4-naphthoquinones are as follows: 2,5,8-trihydroxy-6-methoxy-3-(2-oxopropyl); 8-hydroxy-2,5,6-trimethoxy-3-(2-oxopropyl); 8-hydroxy-2,5,6-trimethoxy-7-(2-oxopropyl); 2,5-dihydroxy-6,8-dimethoxy-3-(2-oxopropyl); 5-hydroxy-2,6,8-trimethoxy-3-(2-oxopropyl); 5,8-dihydroxy-2-methoxy-6-methyl-7-(2-oxopropyl); 8-hydroxy-2,5-dimethoxy-6-methyl-7-(2-oxopropyl); 8-hydroxy-5,6-dimethoxy-2-methyl-3-(2-oxopropyl); 5,8-dimethoxy-5-hydroxy-2-methyl-3-(2-oxopropyl); and 5,8-dihydroxy-2,6-dimethoxy-7-(2-oxopropyl). The NMR, IR and UV spectra are presented.

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]. *F. solani* and *F. oxysporum* have been shown to cause wilt and root rot in a number of crop plants [2, 3]. These Fusaria produce a number of naphthoquinone pigments in culture, some of which are phytotoxic. Twenty of these compounds have been previously identified in cultures of Fusaria from citrus [4–8]. We now report the isolation of one new pigment (**1**) produced by *F. solani* and the preparation of six new related derivatives (**2**, **3**, **5**, **7**, **8**, **12**).

RESULTS AND DISCUSSION

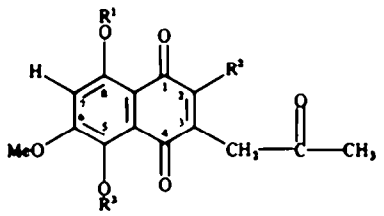
During the large-scale production of naphthoquinones [8], we isolated a new metabolite (**1**) from *F. solani*. The culture used produced large amounts of the marticins, two isomeric naphthoquinone acids that are strongly pathogenic to tomatoes and peas [2]. The structures, NMR data and UV data of the compounds identified are shown in Fig. 1, Table 1 and Table 2, respectively. Compound **1** contains three acidic hydroxyl groups and was difficult to clean and purify by TLC. When it was treated with excess diazomethane, two of the four possible trimethoxy products (**2**, **3**, **5** and **10**) were made. Compound **3** was the major product and **2** the minor product. We recently identified **4**, the 8-*O*-methyl derivative of **1**, from a *F. oxysporum* culture [7]. Treatment of the known **4** with diazomethane gave **5**. This gave us three of the four possible trimethoxy derivatives of **1**. We were unable to make the fourth (**10**). Acid hydrolysis of **4** gave **1**; for further proof of structure see ref. [9].

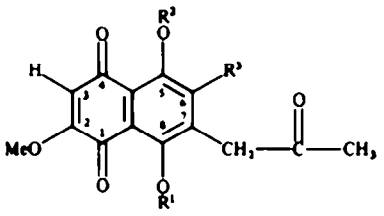
Compounds **1**, **6** and **12** can exist as tautomers in solution and the aromatic proton at the C-3 or C-7 position can be correlated with the quinoid or benzenoid nature of the rings [10]. If the proton resonance is between 6.00 and 6.20 ppm (Table 1) in the NMR spectrum, then the hydrogen can be assigned to the quinone ring, and if it is between 6.50 and 6.80 ppm, it is on the benzenoid portion of the ring. Compound **3** is the major product in the methylation of **1** with excess diazomethane because the C-2 hydroxyl is the most acidic [9] and is methylated first. After methylation the ring is disubstituted with methoxyl and oxopropyl groups and becomes a benzenoid ring which is then methylated a second time if excess diazomethane is present [9, 10]. The acidity of the 2-hydroxyl was confirmed by adding an ethereal diazomethane solution to **1**, 1 ml at a time and checking the reaction by TLC between additions [9]. Only compound **12** was produced in enough quantity to be isolated. Traces of **2** and **3** were seen when **12** was separated by TLC from the starting material. Javanicin (**6**), a well-known metabolite of *F. solani* [2, 5], was treated with diazomethane and two of the four possible monomethyl ethers were obtained. Compound **7** was the major product as expected, since javanicin's major tautomer has the hydroxyls on the disubstituted ring which would be methylated first. Compound **8** was the minor product. The third isomer, 8-*O*-methyl javanicin (**9**), was previously isolated from *F. oxysporum* and *F. moniliforme* [7, 11]. The fourth isomer (**11**) was not isolated. Acid hydrolysis [9] of **9** gave **6** and 5-hydroxy-8-methoxy-2,4-dimethylnaphtho[1,2-b]furan-6,9-dione (anhydrojavanicin).

Synthesis from known compounds, NMR and mass spectra gave proof of the structures for **1**, **5**, **8** and **9** (see Fig. 1 and Table 1). More data were needed to distinguish between the isomeric pairs **3**/**10** and **7**/**11**. Molecular models show that the oxopropyl carbonyl on **3** and **7** as well as javanicin (**6**) would be hydrogen-bonded to the C-8 hydroxyl group. All the structures shown in Fig. 1 except **3** and **7** have either a C-4 carbonyl and a C-5 hydroxyl or a C-1 carbonyl and a C-8 hydroxyl that would chelate

*To whom correspondence should be addressed.

†South Atlantic Area, U.S. Department of Agriculture, Agricultural Research Service.

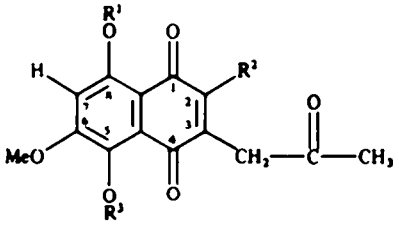




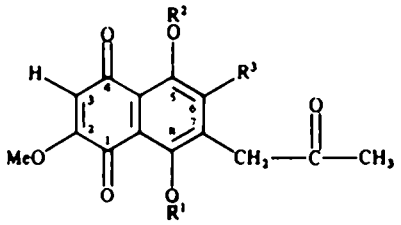
	R^1	R^2	R^3
1	H	OH	H
2	H	OMe	Me
4	Me	OH	H
5	Me	OMe	H
8	H	Me	Me
9	Me	Me	H

	R^1	R^2	R^3
3	H	Me	OMe
6	H	H	Me
7	H	Me	Me
10*	Me	H	OMe
11*	Me	H	Me
12	H	H	OMe

* Isomers not synthesized or isolated



A



B

Fig. 1.

Table 1. ^{1}H NMR spectral data of naphthoquinones

	1	2	3	4	5	6	7	8	9	12
H-7	6.52	6.62		6.62	6.74			6.64	6.75	
H-3			6.03			6.18	6.08			6.27
CH_2–C(=O)–O	3.72	3.69	3.86	3.68	3.66	3.88	3.76	3.75	3.75	3.82
Me–C	2.30	2.30	2.29	2.28	2.28	2.28	2.30	2.31	2.28	2.28
Me-2								2.10	2.12	
Me-6						2.22	2.23			
MeO-2		4.09	4.03		4.18	3.91	3.92			3.94
MeO-5		3.82	3.86				3.94	3.83		
MeO-6	4.00	3.94	3.91	4.04	3.98			3.92	3.98	4.11
MeO-8				4.04	3.98				4.00	
OH-5	13.20			13.60	13.30	13.21			13.04	13.18
OH-8	12.05	12.83	13.33			12.82	13.35	13.14		12.94
OH-2	n.o.				n.o.					

n.o., Not observed.

readily with aluminium chloride. The UV and visible spectra are given in Table 2. All compounds except 3 and 7 showed a major shift in the spectra measured 5 min after the addition of aluminium chloride. Compounds 3 and 7 showed only a slight change after 5 min. After 1 hr there was almost an equivalent change. These shift results correlate with the proposed structures of 3 and 7.

The IR spectrum of 2 contained four bands in the carbonyl region and was identical in relative intensity and position of bands to the spectrum of 8. The IR spectra of 3 and 7 indicated four bands in the carbonyl region and they were identical in relative intensity and position. The IR spectra in the carbonyl region of 5 and 9 were not identical; 5 had three bands in the carbonyl region and 9 had only two bands.

The UV spectra obtained on compounds 1 and 4

Table 2. UV spectral data of naphthoquinones

Compound	λ_{max}^{MeOH} nm	$\lambda_{max}^{MeOH + AlCl_3}$ nm
1	212, 238, 261, 320, 470, 490, 520	246, 342, 354, 368, 476, 503, 540
2	221, 277, 306, 454	230, 278, 322, 450 inf, 474, 501, 530
3	226, 245 sh, 302, 418, 435, 454	No change
4	211, 241, 270, 300, 454	259, 344, 355, 367, 486, 513, 552
5	228, 274, 305, 480, 506, 542	236, 329, 486 inf, 518, 550, 590
6	227, 305, 478, 504, 541, 572	239, 343, 489, 522, 562
7	223, 244 sh, 297, 418, 438, 456, 486	No change
8	223, 275, 296 sh, 450	229, 284, 306, 452 inf, 482, 505, 534
9	227, 286, 488, 544 inf	230, 305, 496 inf, 530, 561, 604
12	231, 314, 474, 500, 534	242, 348, 359, 376, 454 inf, 483, 515, 554

Six drops of aluminium chloride solution (1 g/20 ml) were added and after 5 min the spectra were recorded again.

depend upon the concentration of the sample. With compound **4**, if the absorbance (A) = 0.9 for band 2, it is at 235 nm and band 4 is at 306 nm. If A = 0.25, band 2 is at 244 nm and band 4 is at 300 nm. Compound **1** shows a shift for band 2 from 237 to 246 nm and band 4 from 318 to 324 nm. The hydroxyl in the 2-position is apparently responsible for these shifts.

In addition to being phytotoxic, a number of naphthoquinone derivatives are known to have significant antibacterial activity. We are currently testing several of the above compounds for *in vitro* activity against several Gram-positive and Gram-negative pathogens.

EXPERIMENTAL

TLC solvent systems. (A) C_6H_6–Me_2CO–HOAc (160:40:1); (B) $CHCl_3$; (C) C_6H_6–nitromethane (3:1). Silica gel GF 250 μm plates were used for separation of reaction products.

Methylation. The sample to be methylated was dissolved in EtOH and Et_2O and added to a CH_2N_2 soln which had been prepared from diazald by a standard procedure [12]. The reaction was stopped by adding HOAc after 3–20 min.

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

2,5,8-Trihydroxy-6-methoxy-3-(2-oxopropyl)-1,4-naphthoquinone (**1**). Red crystals ($CHCl_3$), mp 231–234°. MS *m/z* 292; $C_{14}H_{12}O_7$ requires: 292.0581; found: 292.0609; IR ν_{max}^{KBr} cm^{-1}: 3300, 1710, 1630, 1600, 1570, 1480, 1430 w, 1405, 1360, 1320, 1280, 1210, 1190 w, 1085, 1045, 1025, 975, 885, 840 w, 820 sh, 810, 760; UV λ_{max}^{EtOH} nm: 212, 238, 261, 320, 470, 490, 520 (log ε 4.22, 4.14, 4.07, 3.88, 3.84, 3.82, 3.66). Treatment of 15 mg **1** with CH_2N_2 gave 2.7 mg **2** and 5.4 mg **3**. They were isolated by TLC using solvent system A.

8-Hydroxy-2,5,6-trimethoxy-3-(2-oxopropyl)-1,4-naphthoquinone (**2**). Yellow-orange needles (MeOH), mp 149–150°. MS *m/z* 320; $C_{16}H_{16}O_7$ requires: 320.0894; found: 320.0898; IR ν_{max}^{KBr} cm^{-1}: 1705, 1655, 1640, 1605, 1480, 1420, 1395, 1360 w, 1325 w, 1285, 1265, 1250, 1215, 1170, 1155, 1090, 1055 w, 1025 w, 1005, 990, 945 w, 925, 870 w, 815; UV λ_{max}^{MeOH} nm: 221, 277, 306, 454 (log ε 4.40, 4.05, 4.05, 3.67).

8-Hydroxy-2,5,6-trimethoxy-7-(2-oxopropyl)1,4-naphthoquinone (**3**). Yellow-orange needles (MeOH), mp 164–166°. MS *m/z* 320; $C_{16}H_{16}O_7$ requires: 320.0894; found: 320.0864; IR ν_{max}^{KBr} cm^{-1}: 1705, 1680, 1625, 1595, 1475, 1460, 1420, 1400 w, 1370, 1355, 1305 w, 1275, 1225, 1150, 1080, 1065, 1005, 935 w, 865, 815; UV λ_{max}^{MeOH} nm: 226, 245 sh, 302, 418, 435, 454 (log ε 4.33, 3.96, 3.93, 3.58, 3.67, 3.64).

2,5-Dihydroxy-6,8-dimethoxy-3-(2-oxopropyl)-1,4-naphthoquinone (**4**) [7]. Fifteen mg was treated with CH_2N_2 to yield 11 mg **5**, which was cleaned up by TLC using solvent B.

5-Hydroxy-2,6,8-trimethoxy-3-(2-oxopropyl)-1,4-naphthoquinone (**5**). Red needles (EtOAc–hexane), mp 163–164°. MS *m/z* 320; $C_{16}H_{16}O_7$ requires: 320.0894; found: 320.0864; IR ν_{max}^{KBr} cm^{-1}: 1710, 1650, 1620, 1570, 1480, 1430, 1380, 1360 sh, 1310, 1270, 1220, 1165 sh, 1150, 1095, 1065, 1035, 1020, 995, 980, 915, 865, 835, 825 sh; UV λ_{max}^{MeOH} nm: 228, 274, 305, 484, 504, 542 (log ε 4.34, 3.95, 3.96, 3.81, 3.74, 3.41).

8-Hydroxy-2,5-dimethoxy-6-methyl-7-(2-oxopropyl)-1,4-naphthoquinone (**7**). Yellow-orange needles (MeOH), mp 189–194°. MS *m/z* 304; $C_{16}H_{16}O_6$ requires: 304.0945; found: 304.0972; IR ν_{max}^{KBr} cm^{-1}: 1705, 1680, 1625, 1595, 1440, 1410, 1370, 1355, 1315, 1260, 1220, 1170, 1145, 1090, 1065, 1015, 900, 865, 840; UV λ_{max}^{MeOH} nm: 222, 244 sh, 297, 418, 438, 456, 486 (log ε 4.46, 3.99, 4.00, 3.66, 3.76, 3.75, 3.54).

8-Hydroxy-5,6-dimethoxy-2-methyl-3-(2-oxopropyl)-1,4-naphthoquinone (**8**). Light red needles (MeOH), mp 196–199°. MS *m/z* 304; $C_{16}H_{16}O_6$ requires: 304.0945; found: 304.0963; IR ν_{max}^{KBr} cm^{-1}: 1705, 1655, 1645, 1605, 1480, 1435, 1410, 1390, 1365, 1315, 1275, 1255, 1225, 1165, 1145, 1015, 995, 945, 905 w, 865, 840; UV λ_{max}^{MeOH} nm: 222, 275, 296 sh, 450 (log ε 4.53, 4.05, 3.87, 3.74).

5,8-Dihydroxy-2,6-dimethoxy-7-(2-oxopropyl)-1,4-naphthoquinone (**12**). Red-brown crystals (MeOH), mp 180–182°. MS *m/z* 306; $C_{15}H_{14}O_7$ requires: 306.0738; found: 306.0710; IR ν_{max}^{KBr} cm^{-1}: 1720, 1590, 1480, 1435, 1410, 1310, 1280, 1210, 1165, 1090, 1065 w, 1025, 985, 970, 915, 870, 815, 795; UV λ_{max}^{MeOH} nm: 231, 314, 474, 500, 534 (log ε 4.44, 3.93, 3.65, 3.88, 3.70).

REFERENCES

1. Nemec, S. (1975) *Plant Dis. Rep.* **59**, 210.
2. Kern, H. (1978) *Ann. Phytopathol.* **10**, 327.
3. Sherbakoff, C. D. (1953) *Phytopathology* **43**, 395.
4. Baker, R. A., Tatum, J. H. and Nemec, S. (1981) *Phytopathology* **71**, 951.

5. Tatum, J. H. and Baker, R. A. (1983) *Phytochemistry* **22**, 543.
6. Baker, R. A. and Tatum, J. H. (1983) *Proc. Fla. State Hortic. Soc.* **96**, 53.
7. Tatum, J. H., Baker, R. A. and Berry, R. E. (1985) *Phytochemistry* **24**, 457.
8. Tatum, J. H., Baker, R. A. and Berry, R. E. (1985) *Phytochemistry* **24**, 3019.
9. Moore, R. E., Singh, H., Chang, C. W. J. and Scheuer, P. J. (1967) *Tetrahedron* **23**, 3271.
10. Moore, R. E. and Scheuer, P. J. (1966) *J. Org. Chem.* **31**, 3272.
11. Steyn, P. S., Wessels, P. L. and Marasas, W. F. O. (1979) *Tetrahedron* **35**, 1551.
12. DeBoer, T. J. and Backer, H. J. (1954) *Rec. Trav. Chim.* **73**, 232.