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# Natural Fungicides from *Ruta graveolens* L. Leaves, Including a New Quinolone Alkaloid

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Bioassay-directed isolation of antifungal compounds from an ethyl acetate extract of *Ruta graveolens* leaves yielded two furanocoumarins, one quinoline alkaloid, and four quinolone alkaloids, including a novel compound, 1-methyl-2-[6′-(3″,4″-methylenedioxyphenyl)hexyl]-4-quinolone. The $^{1}$H and $^{13}$C NMR assignments of the new compound are reported. Antifungal activities of the isolated compounds, together with 7-hydroxycoumarin, 4-hydroxycoumarin, and 7-methoxycoumarin, which are known to occur in Rutaceae species, were evaluated by bioautography and microbioassay. Four of the alkaloids had moderate activity against *Colletotrichum* species, including a benomyl-resistant *C. acutatum*. These compounds and the furanocoumarins 5- and 8-methoxypsoralen had moderate activity against *Fusarium oxysporum*. The novel quinolone alkaloid was highly active against *Botrytis cinerea*. *Phomopsis* species were much more sensitive to most of the compounds, with *P. viticola* being highly sensitive to all of the compounds.

**KEYWORDS:** ***Botrytis cinerea*; *Colletotrichum acutatum*; *C. fragariae*; *C. gloeosporioides*; fungicide; furanocoumarin; *Fusarium oxysporum*; *Phomopsis viticola*; *P. obscurans*; psoralen; quinoline alkaloid; quinolone alkaloid; rue; *Ruta graveolens***

## INTRODUCTION

Discovery of new antimicrobial substances is an important research objective, due to the continuing evolution of microbial resistance in medicine and agriculture. In addition, the desire for safer agrochemicals with less environmental and mammalian toxicity is a major concern. Particularly attractive is the discovery of novel prototype antimicrobial agents representing new chemical classes that operate by modes of action different from those of existing antifungal agents and, consequently, lack cross-resistance to chemicals currently used (e.g., refs *1* and *2*). Following natural product leads offers an efficient approach to discovering and optimizing new agrochemicals for plant disease control.

Filamentous fungi of the genera *Botrytis*, *Colletotrichum*, *Fusarium*, and *Phomopsis* species are all considered to be major plant pathogens worldwide (*3*). Failure to control these fungi can result in serious economic losses to both U.S. and global agriculture. Anthracnose (caused by *Colletotrichum* spp.) and Phomopsis stem and leaf blight (caused by *Phomopsis* spp.) are serious problems for fruit production in many areas of the world (e.g., ref *4*).

Many *Ruta* species contain diverse classes of secondary metabolites, including coumarins, flavanoids, furanocoumarins, and alkaloids. Ethnobotanical lore indicates that *Ruta graveolens* (rue) has potent antimicrobial activity. Extracts of rue have been proposed as topical pharmaceutical fungicides (*5*, *6*). Antifungal compounds were found in callus cultures of *R. graveolens* (*7*). Rue extracts and two constituents of rue, 5- and 8-methoxypsoralen (**3** and **4**, **Figure 1**), are active against fungal plant pathogens (*Rhizoctonia solanii*, *Fusarium* spp., *Pyrenochaeta lycopersici*, *Trichoderma viride*, *Penicillium* spp., *Thielaviopsis basicola*, and *Verticillium dahliae*) (*8*−*10*). However, some extracts of rue were reported to stimulate the growth of some plant pathogens (*9*). The current study investigates the fungicidal activity against *Botrytis*, *Colletotrichum*, *Fusarium*, and *Phomopsis* species of some coumarins known to be constituents of *R. graveolens* (rue), as well as other compounds found in this study by bioassay-directed isolation of the leaves of rue.

## MATERIALS AND METHODS

**Plant Material.** Fresh leaves of *R. graveolens* were purchased from Elixir Farm Botanicals, Brixey, MO. The plant material was kept at 4 °C until use.

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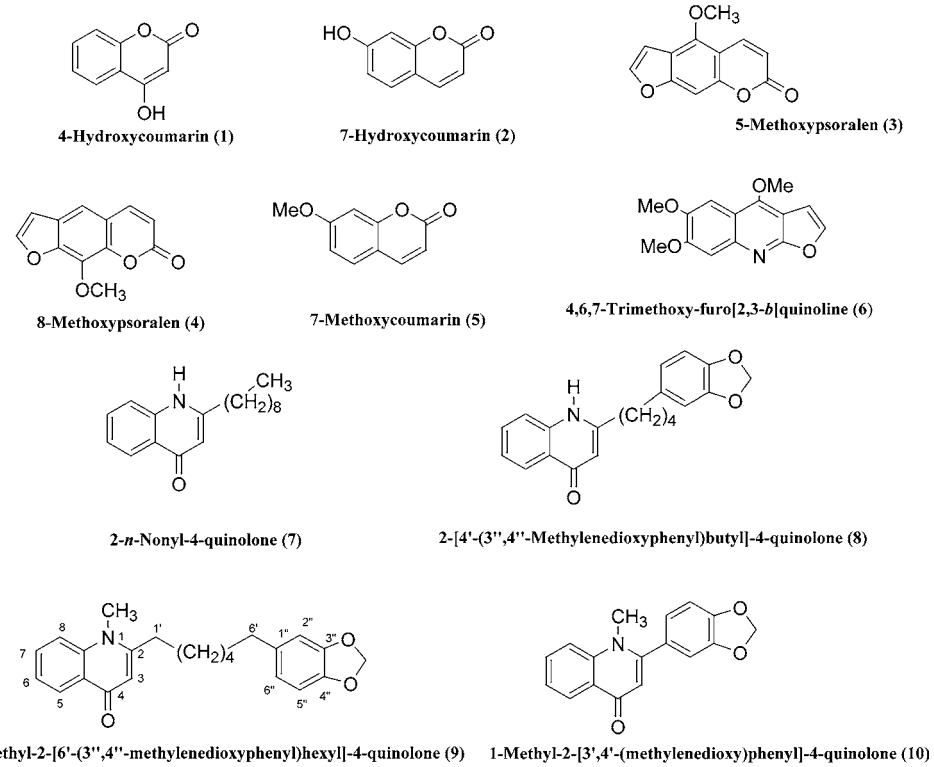


**Figure 1.** Structures of antifungal compounds isolated from *R. graveolens* leaves.

**Test Chemicals.** Fungicide standards benomyl and captan were purchased from Chem Service, West Chester, PA. 4-Hydroxycoumarin (**1**), 7- hydroxycoumarin (**2**), **3**, **4**, and 7-methoxycoumarin (**5**) were purchased from Aldrich Chemical Co., West St. Paul, Milwaukee, WI.

**General Chemical Methods.** Extracts were analyzed on 250 $\mu$m silica gel TLC plates GF with fluorescent indicator (Analtech, Newark, DE). Iodine vapor, UV light (at 254 and 365 nm), and Dragendorff and anisaldehyde spray reagents were used for the detection of compounds. Column chromatography was carried out with kieselgel 60, particle size = 0.063−0.2 mm (Merck, Darmstadt, Germany) with hexane, ethyl acetate, and methanol in various amounts. All solvents were of reagent grade and used without further purification. $^1$H and $^{13}$C NMR spectra were recorded either on a Bruker AMX NMR spectrometer operating at 500 MHz for $^1$H NMR and at 125 MHz for $^{13}$C NMR or on a Bruker Avance DPX 300 spectrometer operating at 300 MHz for $^1$H NMR and at 75 MHz for $^{13}$C NMR. Unambiguous proton and carbon NMR assignments were made by gradient DQF-COSY, gradient HMQC, and gradient HMBC experiments. The HR-ESIMS was measured using a Bruker Bioapex FTMS with ESI source in positive mode. GC-MS analysis was carried out on an HP5790 MSD spectrometer (Hewlett-Packard, Palo Alto, CA) equipped with a GC 5890 using a 20 m × 0.2 mm i.d., 0.18 $\mu$m DB-1 column (J&W Scientific Inc., Folsom, CA). The oven was temperature programmed from 60 °C (5 min) to 280 °C (20 min) at 5 °C/min with helium as the carrier gas.

**Extraction and Isolation of Compounds.** Fresh *R. graveolens* leaves (500 g) were homogenized with hexane in a commercial blender at ambient temperature and extracted with hexane (1.5 L × 2) by sonication for 2 h and steeping overnight at room temperature. Following filtration through filter paper (Whatman no. 1), the combined hexane extract was concentrated by evaporation under reduced pressure at 40 °C. The residue was further extracted with ethyl acetate (1.5 L × 2), methanol (1.5 L × 2), and 70% aqueous acetone (1.5 L × 2), successively, with sonication and steeping overnight in the same manner as with hexane. All extracts were filtered and then concentrated to afford 12.5, 8.3, 9.3, and 6.7 g of hexane, ethyl acetate, methanol, and 70% acetone extracts, respectively. The antifungal activity of each extract was evaluated by bioautography with methods described in detail below.

The ethyl acetate extract was further fractionated, due to the fact that it possessed the highest antifungal activity with bioautography. A portion of this extract (5 g) was subjected to column chromatography, using hexane (2 L) and succesively increasing ethyl acetate by 10, 20, 30, 50, and 80% in hexane (2 L each) up to 100% and then with increasing amounts of methanol in ethyl acetate (1, 5, 10, and 30%) (2 L each). Fractions of 300 mL were collected and concentrated at 40 °C, and the fractions with similar TLC profiles were combined to produce 62 fractions. Each fraction was tested with TLC bioautography to identify fractions with antifungal activity.

Fractions with antifungal activity with the same $R_f$ profile on TLC bioautography were pooled and further purified by crystallization and preparative thin-layer chromatography (TLC). Seven active compounds were isolated and then identified by $^1$H and $^{13}$C NMR spectroscopy and mass spectrometry.

5-Methoxypsoralen (**3**): crystals formed in fraction 17 were separated by decanting the solvent and recrystallized with methanol and $CH_2Cl_2$ to obtain white needle-like crystals of 5-MOP (49 mg). The identity was confirmed by $^1$H and $^{13}$C NMR and mass spectrometric data of an authentic sample.

8-Methoxypsoralen (**4**): crystals formed in fraction 29 were separated by decanting the solvent and recrystallized with ethyl acetate and hexane to obtain white needle-like crystals of 8-MOP (88 mg). The identity was confirmed by $^1$H and $^{13}$C NMR and mass spectrometric data of an authentic sample.

Fractions 32−37 were combined and purified by repeated preparative layer chromatography, eluting twice with 45% acetone in hexane to afford compounds **8** (43.4 mg), **9** (32.4 mg), and **10** (24 mg).

2-[4′-(3′,4′-Methylenedioxyphenyl)butyl]-4-quinolone (**8**): $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 1.35−1.50 (4H, m, 2H-2′ and 2H-3′), 2.29 (2H, t, $J$ = 7.0 Hz, 2H-4′), 2.40 (2H, t, $J$ = 7.6 Hz, 2H-1′), 5.59 (2H, s, −O−$CH_2$−O), 5.92 (1H, s, H-3), 6.30 (1H, dd, $J$ = 7.8, 1.5 Hz, H-6″), 6.35 (1H, d, $J$ = 1.5 Hz, H-2″), 6.39 (1H, d, $J$ = 7.8 Hz, H-5″), 7.07 (1H, ddd, $J$ = 8.2, 6.9, 1.0 Hz, H-6), 7.21 (1H, br d, $J$ = 8.1 Hz, H-8), 7.34 (1H, ddd, $J$ = 8.1, 6.9, 1.4 Hz, H-7), 7.92 (1H, dd, $J$ = 8.2, 1.0 Hz, H-5); ESI-MS, $m/z$ $(M + 1)^+$ 322.

1-Methyl-2-[6′-(3′,4′-methylenedioxyphenyl]hexyl]-4-quinolone (**9**): white crystals; mp 167−168 °C; $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$ 1.37 (2H, m, H-4′), 1.45 (2H, m, H-3′), 1.58 (2H, m, H-5), 1.65 (2H, m, H-2), 2.51 (2H, t, $J$ = 7.25 Hz, H-6′), 2.66 (2H, m, H-1′), 3.70 (3H,s, $CH_3$-1), 5.89 (2H, s, −$OCH_2$O−), 6.20 (1H, s, H-3), 6.59 (1H, dd, $J$ = 7.8, 1.4 Hz, H-6″), 6.64 (1H, d, $J$ = 1.4, H-2″), 6.70 (1H, d,

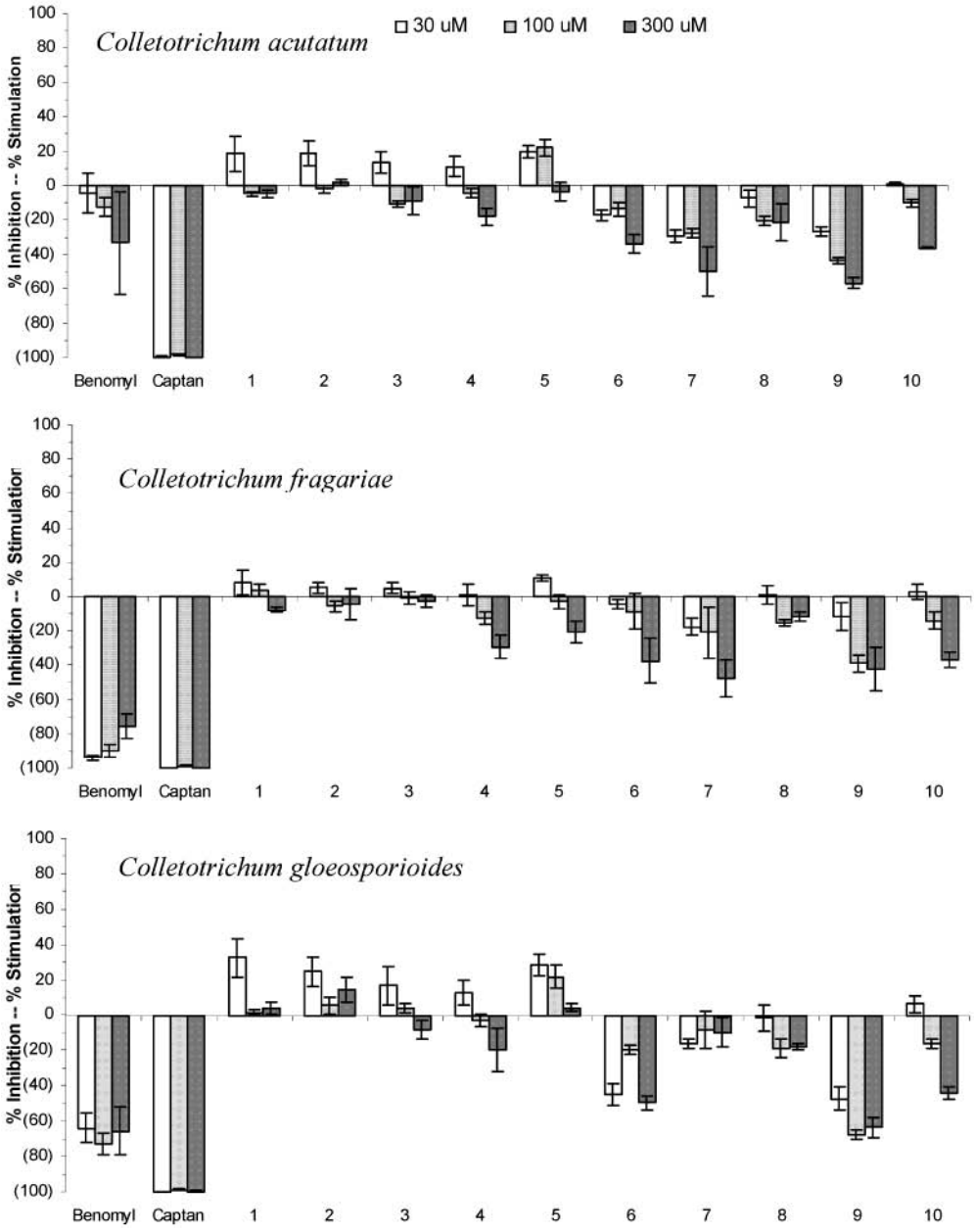


**Figure 2.** Concentration-dependent effects of known *R. graveolens* compounds (**1**, **2**, and **5**) and isolated compounds **3**, **4**, and **6**–**10** using a 96-well microtiter assay on the rate of growth of *C. acutatum*, *C. fragariae*, and *C. gloeosporioides* after 48 h incubation in media supplemented with 30, 100, and 300 $\mu$M of tested compounds. Fungal growth was determined by absorbance measurements at 620 nm. Bars represent the standard error of each mean.

$J$ = 7.8, H-5″), 7.35 (1H, t, $J$ = 7.7 Hz, H-6), 7.49 (1H, d, $J$ = 8.6 Hz, H-8), 7.65 (1H, ddd, $J$ = 8.6, 7.8, 1.6 Hz, H-7), 8.43 (1H, dd $J$ = 7.9, 1.6 Hz, H-5); $^{13}$C NMR ($CDCl_3$, 125 MHz) $\delta$ 28.8 (C-2′), 29.11 (C-4′), 29.5 (C-3′), 31.8 (C-5′), 34.6 (1-$CH_3$), 35.1 (C-1′), 35.9 (C-6′), 101.1 (−$OCH_2O$−), 108.4 (C-5″), 109.2 (C-2″), 111.3 (C-3), 115.9 (C-8), 121.4 (C-6″), 124.0 (C-6), 126.8 (C-5, C-10), 132.5 (C-7), 136.7 (C-1″), 142.3 (C-10), 145.9 (C-4″), 147.9 (C-3″), 155.4 (C-2), 178.1 (C-4); HR-ESIMS, $m/z$ [M + H] $^+$ calcd for $C_{23}H_{25}NO_3$, 364.19068; found, 364.1898.

1-Methyl-2-[3′,4′-(methylenedioxy)phenyl]-4-quinolone (**10**). This compound was identified as graveoline by comparing the spectroscopic data with those reported in the literature (*11*).

2-*n*-Nonyl-4-quinolone (**7**). Fraction 38 was crystallized with ethyl acetate and hexane to yield off-white crystals (7.7 mg). The identity of this compound was confirmed as 2-*n*-nonyl-4-quinoline by comparison of NMR and mass spectroscopic data with those reported in the literature (*12*).

4,6,7-Trimethoxyfuro[2,3-*b*]quinoline (**6**). Fractions 54−62 were combined and further purified by repeated preparative layer chromatography (1000 and 250 $\mu$m) to yield a compound as a pale yellow solid (24 mg). The identity was confirmed as 4,6,7-trimethoxyfuro-[2,3-*b*]quinoline by comparison of spectroscopic data with those reported in the literature (*13*).

**Fungal Isolates and Media.** Isolates of *Colletotrichum acutatum* Simmonds, *Colletotrichum fragariae* Brooks, and *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. were obtained from B. J. Smith, USDA, ARS, Small Fruit Research Station, Poplarville, MS, whereas *Phomopsis viticola* and *Phomopsis obscurans* were obtained from Mike Ellis, The Ohio State University, Columbus, OH, and *Botrytis cinerea* Pers. and *Fusarium oxysporum* Schlechtend were isolated in our laboratory. The three *Colletotrichum* species and the *P. obscurans* strain were isolated from strawberry (*Fragaria* × *ananassa* Duchesne). *P. viticola* and *B. cinerea* Pers were isolated from commercial grape (*Vitis vinifera* L.), and *F. oxysporum* Schlechtend Fr was isolated from orchid (*Cynoches* sp.). Fungi were grown on potato dextrose agar (PDA, Difco, Detroit, MI) in 9-cm Petri dishes and incubated in a growth chamber at 24 ± 2 °C under cool-white fluorescent lights (55 ± 5 $\mu$mol/m$^2$/s) with a 12-h photoperiod.

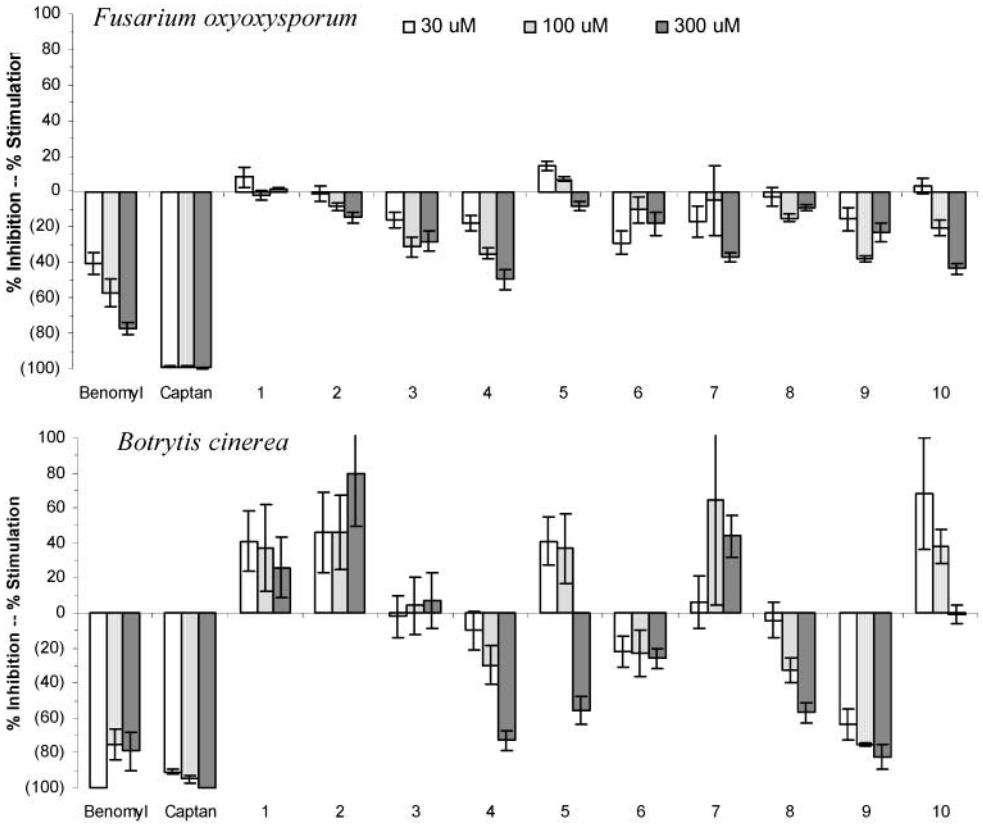


**Figure 3.** Concentration-dependent effects of known *R. graveolens* compounds (**1**, **2**, and **5**) and isolated compounds **3**, **4**, and **6**–**10** using a 96-well microtiter assay on the rate of growth of *F. oxysporum* and *B. cinerea* after 48 h of incubation in media supplemented with 30, 100, and 300 $\mu$M of tested compounds. Fungal growth was determined by absorbance measurements at 620 nm. Bars represent the standard error of each mean.

**Conidia Preparation.** Conidia were harvested from 7−10-day-old cultures by flooding plates with 5 mL of sterile distilled water and dislodging conidia by softly brushing the colonies with an L-shaped glass rod. Aqueous conidial suspensions were filtered through sterile Miracloth (Calbiochem-Novabiochem Corp., La Jolla, CA) to remove mycelia. Conidia concentrations were determined photometrically (*14*, *15*) from a standard curve based on the percent of transmittance (%T) at 625 nm, and suspensions were then adjusted with sterile distilled water to a concentration of $1.0 \times 10^6$ conidia/mL.

Standard conidial concentrations were determined from a standard curve for each fungal species. Standard turbidity curves are periodically validated using both a Bechman/Coulter Z1 particle counter and hemocytometer counts. Conidial and mycelial growth were evaluated using a Packard Spectra Count. Conidial growth and germ tube development were evaluated using an Olympus IX 70 inverted microscope and recorded with a DP12 digital camera as appropriate for compounds that affect spore germination and early germ tube development.

**Bioautography.** Bioautography on silica gel TLC plates with *Colletotrichum* spp. was used to identify the antifungal activity according to the previously published method (*16*). Crude hexane, ethyl acetate, and methanol extracts of *R. graveolens* were separated on silica TLC plates using ethyl acetate and hexane and various amounts of methanol and ethyl acetate and air-dried. Each plate was subsequently sprayed with a spore suspension ($10^5$ spores/mL) of the fungus of interest and was incubated in a moisture chamber for 3 days at 26 °C with a 12-h photoperiod. Clear zones of fungal growth inhibition on the TLC plate indicated the presence of antifungal constituents in each extract. The presence and size of zones of inhibited fungal growth were evaluated 4 days after treatment.

**Microtiter Fungal Assay.** A standardized 96-well microtiter plate (Nunc MicroWell, untreated; Roskilde, Denmark) assay developed for discovery of natural product fungicidal agents (*15*, *17*) was used to evaluate purified, naturally occurring antifungal constituents from *R. graveolens* leaves. The sensitivity of *C. acutatum*, *C. fragariae*, *C. gloeosporioides*, *F. oxysporum*, *B. cinerea*, *P. obscurans*, and *P. viticola* to the various antifungal agents, in comparison with the commercial fungicides benomyl and captan, was determined. Each fungus was challenged in a dose−response format using test compounds where the final treatment concentrations were 30, 100, and 300 $\mu$M. Each microtiter test well received 80 $\mu$L of RPMI broth (Roswell Park Memorial Institute mycological mediym, 16.2 g/L; Life Technologies, Grand Island, NY) buffered with 0.165 M 3-[*N*-morpholino]propanesulfonic acid (Sigma Chemical Co., St. Louis, MO) (pH 7), 100 $\mu$L of conidia at $1.0 \times 10^4$, and 20 $\mu$L of antifungal solution from the parent plate. Growth was then evaluated by measuring the absorbance of each well at 620 nm using a microplate photometer (Packard Spectra Count, Packard Instrument Co., Downers Grove, IL). Conidial solutions were agitated and read immediately thereafter. Mean absorbance values and standard errors were used to evaluate fungal growth at 24, 48, and 72 h (48 h was selected as the most representative time point) except for *P. obscurans* and *P. viticola*, for which the data were recorded at 120 h because their conidia germinated more slowly than those of the other species.

Three known coumarins, 4-hydroxycoumarin (**1**), 7-hydroxycoumarin (**2**), and 7-methoxycoumarin (**5**), as well as the seven compounds isolated by guided bioautography were dissolved in 95% ethanol at 120 mM and then diluted in a 96-well dilution plate (parent plate) with RPMI-buffered broth to achieve 300, 1000, and 3000 $\mu$M concentrations. Test compounds and standards were then transferred to a 96-well test plate (daughter plate) and subsequently diluted 1:10 with buffered RPMI broth to obtain the final treatment concentrations of 30, 100, and 300 $\mu$M. Each test compound was evaluated in duplicate against a well without condia (reagent blank) containing test compound and RPMI at each concentration. Sixteen wells containing broth and conidia preparation served as positive controls, and eight wells containing broth without conidia were used as negative controls. Microtiter plates were covered with a plastic lid and incubated in a growth chamber as described previously for fungal growth. Growth

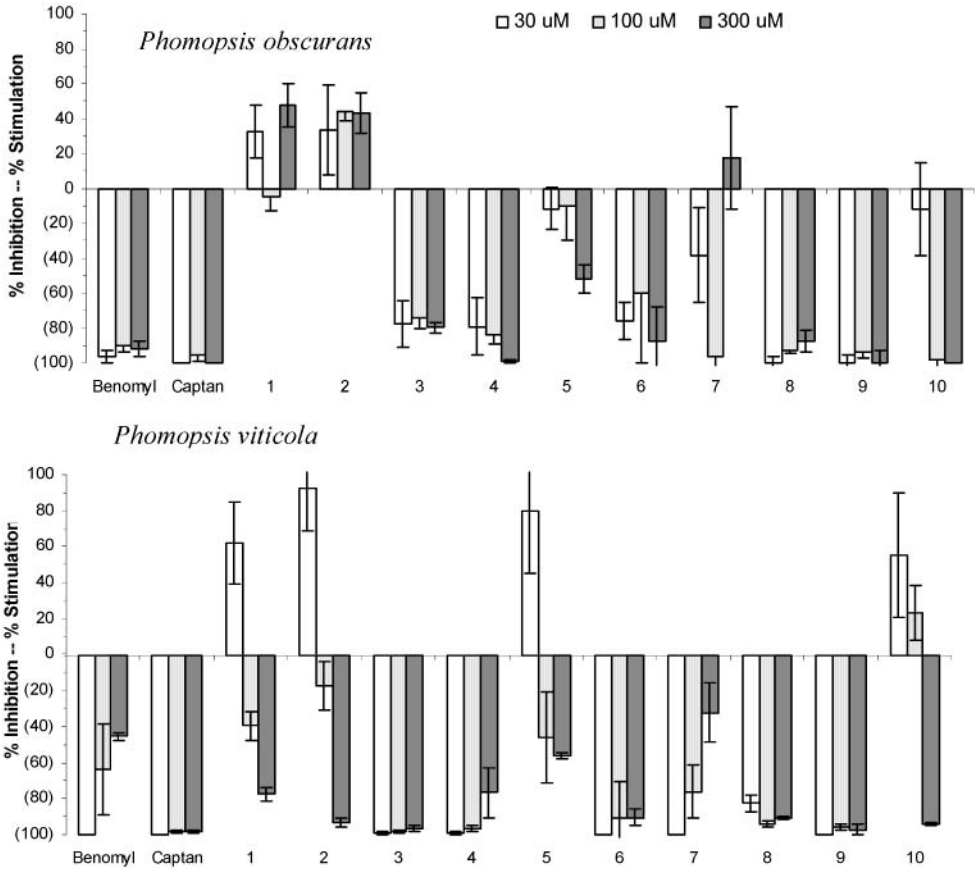


**Figure 4.** Concentration-dependent effects of known rue compounds (**1**, **2**, and **5**) and isolated compounds **3**, **4**, and **6**–**10** using a 96-well microtiter assay on the rate of growth of *P. obscurans* and *P. viticola* after 120 h of incubation in media supplemented with 30, 100, and 300 $\mu$M of tested compounds. Fungal growth was determined by absorbance measurements at 620 nm. Bars represent the standard error of each mean.

was then evaluated by measuring mean absorbance values of each well at 620 nm using a microplate photometer.

**Experimental Design and Statistical Analysis.** Treatments were arranged as a split-plot design replicated twice in time. Whole plots were fungal isolates, and subplots were chemicals. Mean percent inhibition and standard errors for each fungus at each dose of test compound ($n = 4$) relative to the untreated positive growth controls ($n = 32$) were used to evaluate fungal growth inhibition. Results for each dose level and time point were analyzed separately using Statistical Analysis Systems (SAS) software, ver. 8 (SAS Institute, Inc., Cary, NC).

## RESULTS AND DISCUSSION

**Isolation and Identification of Active Compounds.** The hexane, ethyl acetate, methanol, and 70% acetone/water extracts of rue leaves were chromatographed on precoated silica gel plates with various amounts of hexane/ethyl acetate and methanol/ethyl acetate for bioautography assay against *C. fragariaea*. Antifungal activity was observed in each extract, but the largest and most well-defined inhibitory zones were associated with the ethyl acetate extract (data not shown). Hence, this extract was fractionated by silica gel column chromatography in conjunction with crystallization and preparative layer chromatography and guided by bioautography, yielding two methoxypsoralens, four quinolone alkaloids, and one quinoline alkaloid as antifungal constituents (**Figure 1**). The psoralens were 5- and 8-methoxypsoralen (**3** and **4**), and the alkaloids were identified as 4,6,7-trimethoxyfuro[2,3-*b*]quinoline (**6**) (*13*), 2-*n*-nonyl-4-quinolone (**7**) (*12*), 2-[4′-(3″,4″-methylenedioxyphenyl)butyl]-4-quinolone (**8**) (*18*, *19*), and 1-methyl-2-[3′,4′-(methylenedioxy)phenyl]-4-quinolone (**10**) (*11*) by comparison with previously reported spectroscopic data. The trivial names for **6** and **10** are kokusaginin and graveoline, respectively. A positive test of **9** with Dragendorff spray reagent suggested the compound to be an alkaloid. The HR-ESIMS of this compound displayed a molecular ion peak for $[M + H]^+$ corresponding to the molecular formula $C_{23}H_{25}NO_3$. The NMR showed the presence of 2-substituted 1-methyl-4-quinolone (*20*) and 3,4-methylenedioxyphenyl (*21*) moieties and a hexyl side chain. The NMR characteristics of this compound were similar to those reported for 2-[6′-[3″,4″-(methylenedioxy)phenyl]hexyl]-4-quinolone (*21*) except for an additional signal due to an *N*-methyl signal at $\delta$ 3.70. These data suggested that this compound is 1-methyl-2-[6′-(3″,4″-methylenedioxyphenyl]hexyl]-4-quinolone. The COSY and HMQC data in combination with HMBC data allowed us to confirm the structure and unambiguously assign the NMR signals for this compound. In the HMBC spectrum, the *N*-methyl signal at $\delta$ 3.70 showed cross-peaks with carbons at $\delta$ 142.3 and 155.4, which were assigned to C-9 and C-2, respectively. Additionally, the 1′ methylene signal at $\delta$ 2.66 showed cross-peaks with C-2 ($\delta$ 155.4) and C-3 ($\delta$ 11.3), and the 6′ methylene signal at $\delta$ 2.51 showed cross-peaks with C-1″ ($\delta$ 136.7), C-2″ ($\delta$ 109.2), and C-6″ ($\delta$ 121.4). The yield of seven of the compounds from 0.5 kg of rue leaves was similar, ranging from 24 to 88 mg, whereas only 8 mg of **7** was obtained.

**Antifungal Activity of Compounds.** The antifungal activity of the isolated and known rue compounds was evaluated against seven fungal species. Activity against conidia of *Colletotrichum* spp. is shown in **Figure 2**. Of the alkaloids, **7** and **9** had the highest activity, inhibiting growth of *C. acutatum* by 50 and

57%, respectively, at 300 $\mu$M, which was comparable to benomyl activity (33%) at the same concentration. Compounds **6**, **7**, **9**, and **10** had similar activity on *C. fragariae* but were less active than benomyl (76%). Compound **9** had the most potent activity against *C. gloeosporioides*, with 67.7% inhibition at 100 $\mu$M. Compound **6** had the next highest activity, with 50% inhibition at 300 $\mu$M, but was less effective than benomyl (66% at 300 $\mu$M). The known rue compounds that were not isolated from the tissue by bioassay (**1**, **2**, and **5**) caused various degrees of growth stimulation instead of growth inhibition. Compounds **1**−**5** caused ∼15% stimulation of *C. acutatum* growth at the lowest concentration (30 $\mu$M) tested (**Figure 2**). The stimulatory effect was less with *C. fragariae*, and at 300 $\mu$M **4** and **5** there was 29 and 21% growth inhibition, respectively. With *C. gloeosporioides*, the stimulatory activity was more pronounced for all of the non-alkaloid compounds. Growth stimulation caused by low levels of potentially toxic agents is termed "hormesis" (*22*). Several examples from the literature show that this phenomenon has been observed during experiments exposing target organisms to toxic growth inhibitors (*23*−*25*). All of the alkaloids in this study were less active than captan.

At 300 $\mu$M, **4**, **7**, and **10** were the most active compounds against *F. oxysporum*, inhibiting growth by 50, 37, and 44%, respectively, but they were less fungitoxic than benomyl and captan (77 and 100%, respectively) (**Figure 3**). Similar to our findings, Wolters and Eilert (*7*) found skimmiane, a close analogue of **6**, to have no effect on *F. oxysporum*. Similar to the effects on *Colletotrichum* spp., **5** caused a slight stimulatory effect on *F. oxysporum* when tested at 30 $\mu$M. These results are in agreement with those of Oliva et al. (*10*), who reported no antifungal activity on different pathogenic fungi including *Fusarium* spp., when **1** was tested at concentrations ranging from 2 to 16 $\mu$M, whereas **3** and **4** inhibited the mycelial growth of *F. solani* with $IC_{50}$ values of 27.7 and 125 $\mu$M, respectively.

Compounds **8** and **9**, as well as **4** and **5**, had high activity on *B. cinerea* at 300 $\mu$M (**Figure 3**), but only compound **9** had high activity at 30 $\mu$M. The activity of compound **9** was similar to that of benomyl on *B. cinerea*. At low concentrations, **5**, **7**, and **10** stimulated the growth of this fungus, and **1** and **2** stimulated growth at all concentrations.

At a concentration of 30 $\mu$M, **8** and **9** inhibited *P. obscurans* growth 100% after 120 h of exposure (**Figure 4**). At the same concentration, inhibition for **3**, **4**, and **6** was ∼80%. At 100 $\mu$M, compounds **7** and **10** gave 100% inhibition, and at 300 $\mu$M **4** and **6** were 100% effective. Compound **7** precipitated at 300 $\mu$M in both *Phomopsis* studies, reducing its effectiveness. The commercial fungicide standards had similar fungitoxicity to **8** and **9**. Compounds **1** and **2** were both stimulatory at all tested concentrations.

Compounds **6**−**9** were the most active isolated compounds against *P. viticola* at 30 $\mu$M. Compounds **3** and **4** had similar inhibitory profiles, whereas **1**, **2**, **5**, and **10** at 30 $\mu$M had slight stimulatory activity (**Figure 4**). All of the alkaloids mentioned above were, however, more active than benomyl, the standard fungicide (57% inhibition at 300 $\mu$M), but had the same inhibitory activity as captan.

In summary, seven fungitoxic compounds were isolated from the leaves of *R. graveolens* by bioassay-directed isolation against plant pathogenic fungi. One of the quinolones (**9**) is new. The two methoxypsoralens are generally cytotoxic and had previously been reported to be fungitoxic (*10*). However, the quinoline and quinolone alkaloids were all generally as active or more active than these compounds and were present in similar amounts in the tissue, except for **7**. These data suggest that the alkaloids are important to the rue plant in defense against plant pathogens. Our results also indicate that the quinoline and quinolone alkaloids are potential leads for natural product based fungicides for use against plant pathogens of economic significance in the horticultural industry. None of the compounds were strongly active against all fungi, and the hormesis effect could be of concern if it is also seen with in vivo studies. Further studies with these compounds with in vivo systems (fungi on plant tissue) are needed to further determine their potential as commercial fungicides or fungicide leads.

## ACKNOWLEDGMENT

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