Isochromenones, isobenzofuranone, and tetrahydronaphthalenes produced by Paraphoma radicina, a fungus isolated from a freshwater habitat

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El-Elimat T., Raja H.A., Figueroa M., Falkinham III J.O., and Oberlies N.H. 2014. Isochromenones, isobenzofuranone, and tetrahydronaphthalenes produced by Paraphoma radicina, a fungus isolated from a freshwater habitat. Phytochemistry 104: 114–120.

Abstract:

Six isochromenones (1–6), clearanols F (5) and G (6), one isobenzofuranone (7), and two tetrahydronaphthalene derivatives (8 and radinaphthalenone (9)), were isolated and identified from a culture of the fungus Paraphoma radicina, which was isolated from submerged wood in a freshwater lake. Compounds 5, 6 and 9 were previously unknown. The structures were elucidated using a set of spectroscopic and spectrometric techniques; the absolute configurations of compounds 5 and 6 were determined by comparison of their experimental ECD measurements with values predicted by TDDFT calculations. Compounds 1–9 were evaluated for antimicrobial activity against an array of bacteria and fungi. The inhibitory activity of compound 4 against Staphylococcus aureus biofilm formation was evaluated.

Abbreviations: BLAST, basic local alignment search tool; CHCl3, chloroform; CH3CN, acetonitrile; COSMO, conductor-like screening model; DFT, density functional theory; ECD, electronic circular dichroism; HPLC, high performance liquid chromatography; HRESIMS, high-resolution electrospray ionization mass spectrometry; ITS, internal transcribed spacer; LS, large scale; LSU, large subunit; MeOH, methanol; MICs, minimal inhibitory concentrations; NMR, nuclear magnetic resonance; nrDNA, nuclear ribosomal deoxyribonucleic acid; PDA, potato dextrose agar; rRNA, ribosomal ribonucleic acid; SCRF, self-consistent reaction field; TDDFT, time-dependent density functional theory; UPLC, ultra-performance liquid chromatography; UV, ultraviolet; UV–Vis, ultraviolet/visible

Keywords: *Paraphoma radicina* | Isochromenones | Isobenzofuranone | Tetrahydronaphthalenes

Article:

Introduction

In search of structurally diverse scaffolds from ecologically unique fungi, our group has initiated investigations of freshwater fungi (Raja et al., 2013a and Raja et al., 2013b), specifically ascomycetes that inhabit submerged woody and herbaceous organic matter in lakes and streams (Shearer et al., 2007). Freshwater fungi represent an ecologically important, though poorly studied, class of fungi in terms of chemistry (Dong et al., 2011 and Hernández-Carlos and Gamboa-Angulo, 2011) and mycology (Shearer et al., 2007). Only fragmentary knowledge, at best, exists regarding habitat and substrate distribution patterns, species identities, and role(s) that these fungi play in freshwater ecosystems. Of the 1.5–5.1 M estimated fungi in the world (Blackwell, 2011 and Hawksworth, 1991), only about 3000 species have been characterized from freshwater habitats. This is somewhat surprising given that less than 2% of the planet is covered by freshwater (Grant and Gross, 1996 and Shearer et al., 2007). Similarly, of the 14,000 secondary metabolites that have been isolated from fungi (Dictionary of Natural Products online 21.2., 2013), only about 125 compounds, i.e. less than 1%, have been from freshwater fungi (Dong et al., 2011 and Hernández-Carlos and Gamboa-Angulo, 2011).

A fungus, which was accessioned as G104, was isolated from submerged wood in a freshwater lake and was identified as Paraphoma radicina. This fungus produced six isochromenones (1–6), of which two were new [clearanol F (5), and clearanol G (6)], and four were known [(R)-3,4-dihydro-4,6,8-trihydroxy-4,5-dimethyl-3-methyleneisochromen-1-one (1), (R)-3,4-dihydro-4,8-dihydroxy-6-methoxy-4,5-dimethyl-3-methyleneisochromen-1-one (2), 3,8-dihydroxy-3-hydroxymethyl-6-methoxy-4,5-dimethyl-isochroman-1-one (3), and clearanol C (4)]. Also isolated were one known isobenzofuranone (R)-7-hydroxy-3-((S)-1-hydroxyethyl)-5-methoxy-3,4-dimethylisobenzofuran-1(3H)-one (7) and two tetrahydronaphthalene derivatives [isosclerone (8) and radinaphthalenone (9)], the latter having not been reported previously. Herein, details of the isolation, structural elucidation, and determination of the absolute configuration of these compounds are presented. Structurally related compounds have been reported to have various degrees of antifungal activity (Gerea et al., 2012, Tayone et al., 2011a and Tayone et al., 2011b) and mild antimycobacterial activity (Chinworrungsee et al., 2002). As such, compounds 1–9 were evaluated against a panel of microorganisms; the most promising (4) was also tested in a Staphylococcus aureus biofilm assay.

Results and discussion

Two large-scale solid-phase cultures (LS1 and LS2) of the fungus (G104) were extracted with 1:1 $CHCl_3$–MeOH and partitioned with organic solvents. The organic extracts were purified using flash chromatography to yield 3 and 4 fractions, respectively, which were then subjected to further purifications using preparative and semipreparative HPLC to yield nine compounds (1–9). The purity of the isolated compounds was evaluated via UPLC (Fig. S1). Compounds 1–6 and 7 belong to isochromenone and isobenzofuranone classes of natural products, respectively, while compounds 8 and 9 were tetrahydronaphthalene derivatives.

Compounds 1 (5.53 mg), 2 (34.16 mg), and 4 (9.19 mg) had molecular formulas of $C_{12}H_{12}O_5$, $C_{13}H_{14}O_5$, and $C_{13}H_{14}O_4$, respectively, as determined by HRESIMS. The NMR and HRMS

data of these were similar, indicative of analogous structures, with key differences being a methoxy moiety in 2 relative to 1 and an OH moiety in 2 relative to 4. The NMR, HRMS, CD and optical rotation data identified 1, 2, and 4 as the known compounds, (R)-3,4-dihydro-4,6,8-trihydroxy-4,5-dimethyl-3-methyleneisochromen-1-one, (R)-3,4-dihydro-4,8-dihydroxy-6-methoxy-4,5-dimethyl-3-methyleneisochromen-1-one, and (S)-8-hydroxy-6-methoxy-4,5-dimethyl-3-methyleneisochromen-1-one (clearanol C), respectively (Fig. 1 and Table S1). Compound 2 was first isolated in 2002 from the EtOAc extract of a broth of the marine fungus Halorosellinia oceanica (Chinworrungsee et al., 2002), and compounds 1, 2 and 4 were isolated in 2011 from the culture broth of Leptosphaeria sp., which was collected from woody debris (Tayone et al., 2011a and Tayone et al., 2011b). Compound 4 was re-isolated in 2012 from a complex microbial mat that occupied an iron-rich freshwater spring and was ascribed the trivial name clearanol C (Gerea et al., 2012).

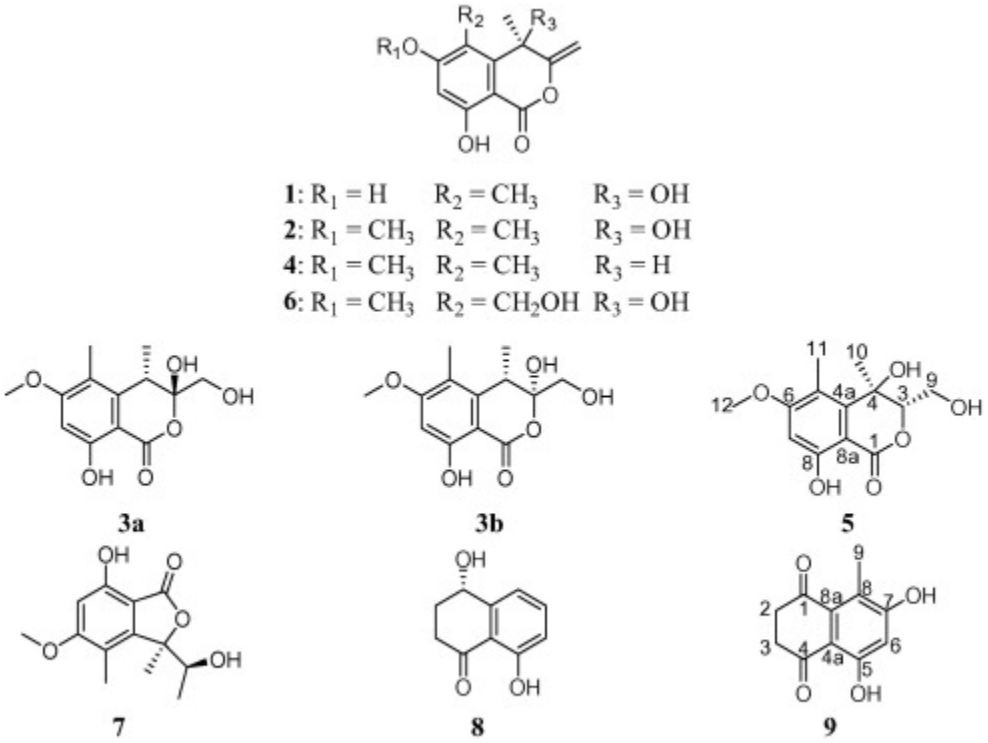


Fig. 1.
Structures of compounds 1–9.

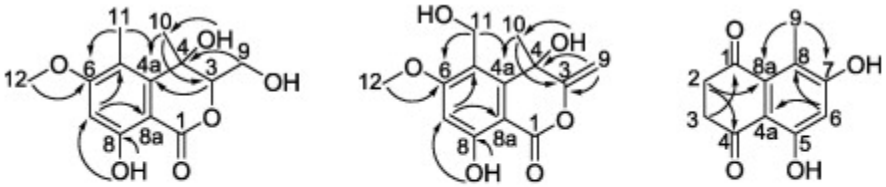
Compound 3 (7.5 mg) was obtained as a colorless oil with a molecular formula of C13H16O6 as determined by HRESIMS. The NMR spectroscopic data indicated the presence of an inseparable pair of compounds existing in 7:1 ratio, with the major one identified as the known compound 3,8-dihydroxy-3-hydroxymethyl-6-methoxy-4,5-dimethyl-isochroman-1-one (See Table S1 for CD and optical rotation data) (Tayone et al., 2011b). Tayone et al. suggested the minor compound as a stereogenic tautomer of the major compound around the hemiacetal moiety at C3 (Fig. 1) (Tayone et al., 2011b). These were first isolated in 2011 from an Allantophomopsis sp. (Schuffler et al., 2011) and later in the same year from a Leptosphaeria sp. (Tayone et al., 2011b).

Compound 7 (3.5 mg), which was obtained as a colorless oil, had a molecular formula of C13H16O5 as determined by HRESIMS. The NMR, HRMS, and optical rotation data identified 7 as the known compound (R)-7-hydroxy-3-((S)-1-hydroxyethyl)-5-methoxy-3,4-dimethylisobenzofuran-1(3H)-one (Fig. 1 and Table S1),(Tayone et al., 2011a) which was first isolated in 2011 from the culture broth of Leptosphaeria sp. (Tayone et al., 2011a).

Compound 5 (0.85 mg) was also obtained as a colorless oil. The molecular formula was determined as C13H16O6 via HRESIMS, establishing an index of hydrogen deficiency of 6. The NMR spectroscopic data suggested structural similarity with 3a, both having the same molecular formula. Key differences were noticed in the chemical shift and splitting of the C-10 methyl group. It changed from a doublet that resonated at 1.14 ppm in 3a into a singlet resonating at 1.53 ppm in 5. Moreover, C-3 changed from a quaternary carbon (δC 103.3) in 3a into a methine (δH/δC 4.36/82.4) in 5. Additionally, C-4 changed from a methine in 3a (δH/δC 3.27/36.1) into an oxygenated quaternary carbon (δC 72.4) in 5. These data suggested a switch in the hydroxyl group position from C-3 in 3a to C-4 in 5 (Table 1 and Figs. S2 and S3 for the 1H and 13C NMR data). An HMBC correlation was observed from 12-OCH3 to C-6, indicating the connectivity of the methoxy group. HMBC correlations from H-11 to C-6 and C-4a, from H-7 to C-5 and C-8a, from 8-OH to C-7 and C-8, from 10-CH3 to C-4a and C-3, from 4-OH to 10-CH3, from H-3 to C-4a, and from 9-CH2 to C-4 were observed (Fig. 2). These data suggested the structure of 5 (Fig. 1), which was ascribed the trivial name clearanol F. The absolute configuration of 5 was determined by comparing experimental and calculated ECD spectra predicted by the time-dependent density functional theory (TDDFT) method (Acuña et al., 2010, Bringmann et al., 2009, Stephens and Harada, 2010 and Stephens et al., 2007). Similar studies have used calculated ECD values for determination of the absolute configuration of a series of chromone derivatives from Penicillium sp. (Gan et al., 2013) and isochromenones from Leptosphaeria sp. (Tayone et al., 2011a and Tayone et al., 2011b). Theoretical ECD spectra calculated for the four possible diastereomers 5a–5d were compared with the experimental spectra (Fig. S8). A detailed conformational search was performed for each diastereomer using molecular mechanics followed by geometry optimization with DFT at the B3LYP/DGDZVP2 level for the most stable conformers (Acuña et al., 2010 and Figueroa et al., 2009). The relative free energies and the Boltzmann distribution for the most relevant conformers of each diastereomer (4, 7, 4, and 5 conformers for 5a–5d, respectively), each with a ΔG0 range of between 0.0 and 3.0 kcal mol−1, were taken into account to obtain population-weighted averaged calculated ECD spectra (Figs. 3 and S8, and Table S2). The results for 5c (3S,4R) were in excellent agreement with the experimental ECD spectrum where two negative low amplitude Cotton effects were observed at approximately 225 and 255 nm, along with two positive effects at approximately 235 and 275 nm (Fig. 4), confirming the S and R configurations at C-3 and C-4, respectively.

Table 1.
NMR spectroscopic data for **5** and **6** [700 MHz for ^{1}H, 175 MHz for ^{13}C; chemical shifts in δ, coupling constants in Hz, $CDCl_3$].

	5		**6**	
Position	δ_C, type	δ_H, mult, *J* in Hz	δ_C, type	δ_H, mult, *J* in Hz
1	169.5, C		166.5, C	
3	82.4, CH	4.36, dd, 6.3, 5.7	159.6, C	
4	72.4, C		72.3, C	
4a	144.6, C		146.1, C	
5	116.2, C		117.6, C	
6	165.8, C		165.4, C	
7	98.8, CH	6.42, s	99.2, CH	6.46, s
8	163.7, C		164.8, C	
8a	98.9, C		99.2, C	
9	61.2, CH_2	4.06, dd, 11.5, 6.3	95.9, CH_2	4.94, d, 1.7
		4.12, dd, 11.5, 5.7		5.12, d, 1.7
10	21.1, CH_3	1.53, s	31.3, CH_3	1.72, s
11	12.2, CH_3	2.33, s	55.3, CH_2	4.98, d, 9.2
12	56.2, CH_3	3.85, s	56.4, CH_3	3.88, s
4-OH		2.51, s		
8-OH		11.45, s		11.40, s
9-OH		2.17, br s		2.28, br



5 **6** **9**

Fig. 2.
Key HMBC correlations of compounds **5**, **6**, and **9**.

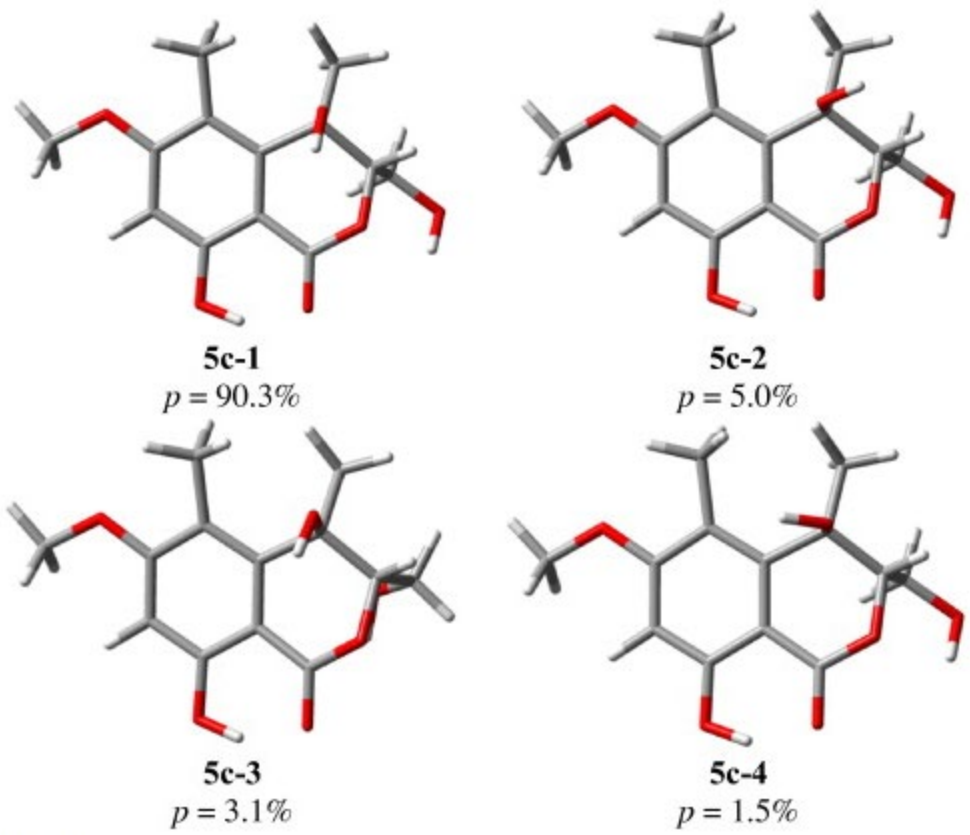


Fig. 3.
DFT B3LYP/DGDZVP2 geometry optimized conformers of **5c** [(3*S*,4*R*)-**5**)] at 298 K and 1 atm, accounting for ca. 99% of the conformational population.

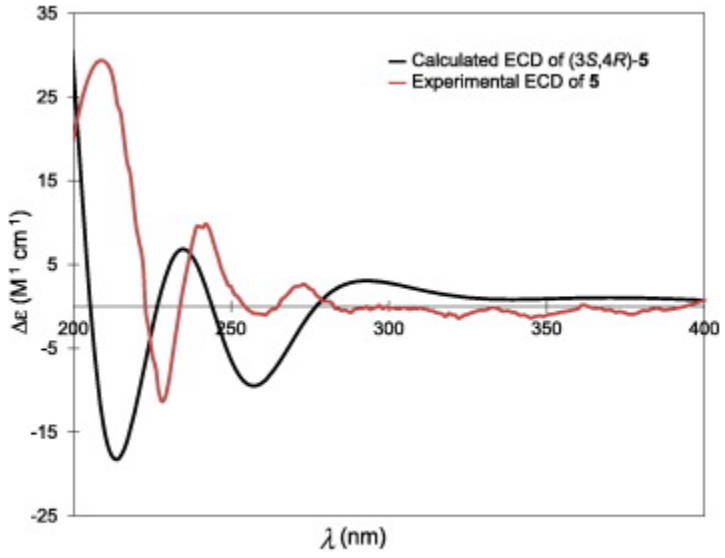


Fig. 4.
Experimental and calculated CD spectra for **5** in CH_3CN.

Compound 6 (0.65 mg) was obtained as a colorless oil. The molecular formula was determined as C13H14O6 via HRESIMS. The NMR spectroscopic data suggested an isochromenone with structural similarity to 2. However, compound 6 had an extra oxygen atom as evidenced by a 16 amu difference in the HRMS between 2 and 6. Examination of the 1- and 2-D NMR data indicated that the C-11 methyl group in 2 (δH/δC 2.36/29.0) was hydroxylated in 6 (δH/δC 4.98/55.3). Key HMBC correlations were observed from H2-11 to C-6 and C-4a, confirming the connectivity of the methylene hydroxyl group to C-4 and establishing the structure of 6 (Fig. 1 and Fig. 2, S4, and S5). The trivial name, clearanol G, was ascribed to this compound. The absolute configuration at C-4 was established by comparing the ECD data with those obtained through molecular modeling calculations following the same protocol described for compound 5 (Table S3). The agreement between the observed and calculated ECD (Fig. 5) confirmed the R configuration at C-4.

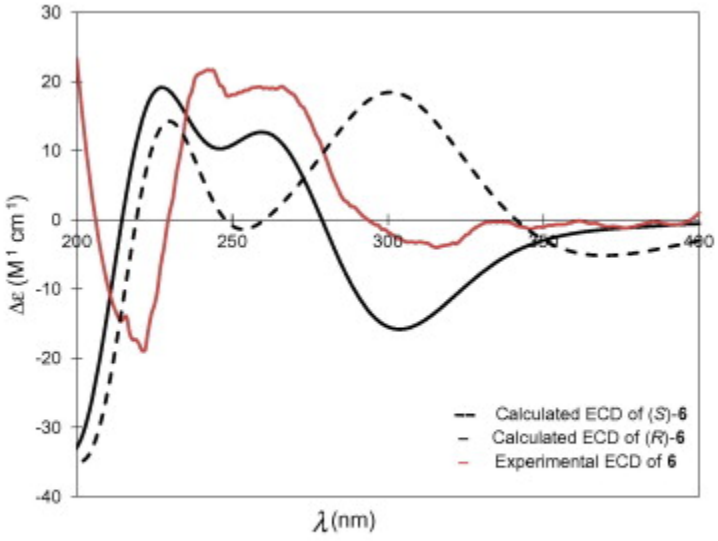


Fig. 5.
Experimental and calculated CD spectra for **6** in CH_3CN.

In addition to compounds 1–7, two tetrahydronaphthalene derivatives, one known (8) and one new (9), were isolated and identified as well. The known compound 8 was identified as isosclerone by means of NMR, HRMS, and optical rotation data, which all compared favorably to the literature (Evidente et al., 2011). Compound 9 (0.81 mg) was isolated as an off-white powder. The molecular formula was determined as C11H10O4 by HRESIMS, indicative of an index of hydrogen deficiency of 7. The 1H NMR data indicated the presence of one aromatic proton (δH 6.57, s), two methylenes (δH 2.98, m, and 3.02 m), one methyl (δH 2.38, s), and two hydroxyl protons (δH 11.36, s, and 5.91, broad peak, for 5-OH and 7-OH, respectively) (Table 2 and Fig. S6). The 13C NMR data suggested 11 carbons, consistent with the HRMS data and indicative of two carbonyls (δC 198.8 and 201.5 for C-1 and C-4, respectively), six aromatic carbons (δC 113.4, 162.1, 107.3, 163.0, 118.5, and 135.7 for C-4a, C-5, C-6, C-7, C-8, and C-8a, respectively), indicator of a penta substituted benzene ring, two methylenes (δC 40.0 and 37.2 for C-2 and C-3, respectively), and one methyl (δC 11.9 for C-9) (Table 2 and Fig. S7). The benzene ring and the two carbonyl groups accounted for six degrees of unsaturation, leaving the

remaining one accommodated by a ring. HMBC correlations were observed from CH3-9 to C-7 and C-8a from H-6 to C-8 and C-4a, from CH2-2 to C-8a, and C-4, and from CH2-3 to C-1 (Fig. 2). These spectroscopic and spectrometric data suggested the structure of 9 (Fig. 1), which was ascribed the trivial name radinaphthalenone.

Table 2.
NMR spectroscopic data for **9** (500 MHz for ^{1}H, 100 MHz for ^{13}C; chemical shifts in δ, coupling constants in Hz, $CDCl_3$).

Position	δ_C, type	δ_H, mult
1	198.8, C	
2	40.0, CH_2	2.98, m
3	37.2, CH_2	3.02, m
4	201.5, C	
4a	113.4, C	
5	162.1, C	
6	107.3, CH	6.57, s
7	163.0, C	
8	118.5, C	
8a	135.7, C	
9	11.9, CH_3	2.38, s
5-OH		11.36, s
7-OH		5.91, broad

Compounds 1–9 were tested for antimicrobial activity against an array of bacteria and fungi (Table S4). Compound 4 showed promising activity against S. aureus with an MIC value of 33 μg/mL. It was previously reported by Gerea et al. (2012) to have weak inhibitory activity against Candida albicans biofilm formation and modest inhibition against polyene-resistant C. albicans. As such, 4 was evaluated for inhibitory activity against S. aureus biofilm formation and was found inactive. Compound 7 was reported to have strong antifungal activity against Cochliobolus miyabeanus at 0.5 μg/mL (Tayone et al., 2011a). When tested against Aspergillus niger, compound 7 was inactive (Table S4).

Conclusions

From a fungus that was isolated from a freshwater niche, nine compounds (1–9) belonging to three structural classes, isochromenones, isobenzofuranones, and tetrahydronaphthalenes, were isolated and identified. The absolute configuration of the new isochromenones, 5 and 6, was elucidated by means of TDDFT ECD spectra calculations. While most of the compounds were inactive, compound 4 had activity against S. aureus. Coupled with literature data on 4 and 7 regarding activity against biofilm formation in C. albicans and antifungal activity against C. miyabeanus, these compounds may confer a competitive advantage for this fungus, which was collected from a freshwater habitat. The current study demonstrated how structurally diverse

compounds can be obtained from ecologically unique environments such as freshwater habitat, a highly underexplored source of bioactive natural products.

P. radicina is a plurivorous species, and it was originally reported from young cysts of nematodes from root surfaces of soybean in different counties in North Carolina, USA (Morganjones and White, 1983). It was interesting to isolate the same species of fungus from the same state but a different environment. Its occurrence in both terrestrial and freshwater habitats suggested that it may be an immigrant species sensu (Park et al., 2007).

Experimental

4.1. General experimental procedures

UV and CD spectra were acquired on a Varian Cary 100 Bio UV–Vis spectrophotometer and an Olis DSM 17 CD spectrophotometer (Olis), respectively. NMR experiments were conducted using either a JEOL ECA-500 NMR spectrometer operating at 500 MHz for 1H and 125 MHz for 13C, or a JEOL ECS-400 NMR spectrometer equipped with a high sensitivity JEOL Royal probe operating at 400 MHz for 1H and 100 MHz for 13C, or an Agilent 700 MHz NMR spectrometer, equipped with a cryoprobe, operating at 700 MHz for 1H and 175 MHz for 13C. Residual solvent signals were utilized for referencing. HRESIMS was performed on a Thermo LTQ Orbitrap XL mass spectrometer equipped with an electrospray ionization source. UPLC was carried out on a Waters Acquity system with data collected and analyzed using Empower 3 software. HPLC was carried out using a Varian ProStar HPLC system equipped with ProStar 210 pumps and a ProStar 335 photodiode array detector (PDA), with data collected and analyzed using Galaxie Chromatography Workstation software (version 1.9.3.2). For preparative HPLC, a Phenomenex Gemini-NX C18 (5 μm; 250 × 21.2 mm) column was used at a 21 mL/min flow rate. For UPLC, a Waters BEH C18 column (1.7 μm; 50 × 2.1 mm) was used with a 0.6 mL/min flow rate. Flash chromatography was performed on a Teledyne ISCO CombiFlash Rf 200 using a 4 g Silica Gold column and monitored by UV and evaporative light-scattering detectors. All other reagents and solvents were obtained from Fisher Scientific and were used without further purification. The HTP MBEC Assay Biofilm Incubator (Innovotech Inc., Edmonton, Alberta, Canada) was used for the S. aurous biofilm assay.

4.2. Fungal strain isolation and identification

The fungal strain, G104, was isolated from submerged wood sampled in October of 2011 from a freshwater lake in Greensboro, North Carolina, USA (Lake Brandt; 36°10′ 1″N, 79°50′ 18″W). Collection methods and culturing conditions were based on earlier work by Shearer et al. (Raja et al., 2009 and Shearer et al., 2004). Strain G104 was identified using molecular techniques by sequencing the internal transcribed spacer regions 1 & 2 and 5.8S nrDNA (ITS) (Schoch et al., 2012) along with the D1 and D2 regions of the 28S nuclear ribosomal large subunit rRNA gene (LSU) (Liu et al., 2012). Methodology for DNA extraction, PCR amplification, sequencing, and phylogenetic analyses were performed as described previously (El-Elimat et al., 2013a, El-Elimat et al., 2013b, Figueroa et al., 2013, Raja et al., 2013a and Raja et al., 2013b). BLAST search in GenBank using ITS rDNA sequences and phylogenetic analysis using partial LSU sequence data suggested that strain G104 had phylogenetic affinities to P. radicina (McAlpine)

Morgan-Jones & White belonging to the section Phoma (Fig. S9) (de Gruyter et al., 2010 and Morganjones and White, 1983). Therefore, strain G104 was identified as P. radicina (Ascomycota). The combined ITS and LSU sequence was deposited in the GenBank (accession no. KF938577). A voucher culture of strain G104 is maintained in the Department of Chemistry and Biochemistry culture collection at the University of North Carolina at Greensboro.

4.3. Fermentation and isolation

The culture of strain G104 was stored on a malt extract slant and was transferred periodically. A fresh culture was prepared in a similar slant and subsequently grown on 2% MEA, potato dextrose agar (PDA, Difco), and YESD media. After 14–21 d, agar plugs of the fungus were used to inoculate 30 mL of autoclaved rice medium, prepared using 10 g of rice and twice the volume of rice with H2O in a 250 mL Erlenmeyer flask. Large scale cultures were prepared by the parallel processing of four such cultures in quadruplicates, which were incubated at 22 °C until showing good growth (approximately 14 d).

To a large scale culture, MeOH–CHCl3 (500 mL, 1:1) were added. The culture was chopped with a spatula and shaken overnight (~16 h) at ~100 rpm at rt. The sample was filtered with vacuum, and the remaining residues were washed with MeOH–CHCl3 (100 mL, 1:1). To the filtrate, CHCl3 (900 mL) and H2O (1500 mL) were added; the mixture was stirred for 30 min and then transferred into a separatory funnel. The bottom layer was drawn off and evaporated to dryness. The dried organic extract was re-constituted in MeOH–CH3CN (100 mL 1:1) and hexanes (100 mL). The biphasic solution was shaken vigorously and then transferred to a separatory funnel. The MeOH–CH3CN layer was drawn off and evaporated to dryness under vacuum. The defatted material (231 mg) was dissolved in a mixture of CHCl3–MeOH, adsorbed onto Celite 545, and fractionated via flash chromatography using a gradient solvent system of hexane–CHCl3–MeOH at a 18 mL/min flow rate and 68.1 column volumes over 18.2 min to afford three fractions. Fraction 1 (78.87 mg) was subjected to preparative HPLC using a gradient system of 20:80 to 80:20 of CH3CN–H2O (acidified with 0.1% HCO2H) over 20 min at a flow rate of 21 mL/min to yield six sub-fractions. Sub-fractions 1, 3, 4, and 6 yielded compounds 8 (0.71 mg), 7 (1.61 mg), 2 (16.05 mg), and 4 (9.19 mg), which eluted at ~11.3, 12.0, 17.6, and 21.2 min, respectively. Fraction 2 (26.11 mg) was subjected to preparative HPLC using a gradient system of 30:70 to 40:60 of CH3CN–H2O (acidified with 0.1% HCO2H) over 20 min at a flow rate of 21 mL/min to yield four sub-fractions. Sub-fractions 1 and 2 yielded compounds 6 (0.65 mg) and 3 (5.64 mg), which eluted at 10.7 and 12.6 min, respectively. Fraction 3 (~84.99 mg) was subjected to preparative HPLC using a gradient system of 20:80 to 40:60 of CH3CN–H2O (acidified with 0.1% HCO2H) over 20 min at a flow rate of 21 mL/min to yield four sub-fractions. Sub-fractions 3 and 4 yielded compounds 5 (0.85 mg) and 3 (1.88 mg), which eluted at ~17.1 and 19.6 min, respectively.

To generate more material for biological evaluation, another large scale culture of strain G104 was extracted and fractionated as described above. The MeOH–CH3CN fraction (242 mg) was subjected to fractionation using flash chromatography using a gradient solvent system of hexane–CHCl3–MeOH at a 30 mL/min flow rate and 61.0 column volumes over 34.1 min to afford four fractions. Fraction 2 (145.95 mg) was subjected to preparative HPLC using a gradient system of 40:60 to 80:20 of CH3CN–H2O (acidified with 0.1% HCO2H) over 15 min at a flow

rate of 21 mL/min to yield six sub-fractions. Sub-fractions 1, 2, and 3 yielded compounds 7 (1.89 mg), 8 (0.82 mg), and 2 (18.11 mg), which eluted at 4.5, 7.6, and 13.1 min, respectively. Fraction 3 (35.69 mg) was subjected to preparative HPLC using a gradient system of 20:80 to 60:40 of CH3CN–H2O (acidified with 0.1% HCO2H) over 15 min at a flow rate of 21 mL/min to yield four sub-fractions. Sub-fraction 3 yielded compound 1 (5.53 mg), which eluted at ∼16.0 min.

4.3.1. Clearanol F (5)

Colorless oil;View the MathML source = +18° (c = 0.05, CHCl3); UV (MeOH) λmax (log ε) 313 (3.20), 269 (3.36), 228 (3.36), 217 (3.75) nm; CD (c 7.46 × 10−5 M, CH3CN) λ (Δε) 209 (+29.4) nm, 228 (−11.3) nm, 242 (+9.8) nm; 261 (−1.0) nm, 273 (+2.6) nm; For 1H NMR (CDCl3, 700 MHz) and 13C NMR (CDCl3, 175 MHz) spectroscopic data, see Table 1; HRESIMS m/z 269.1013 [M+H]+ (calcd for C13H17O6 269.1020).

4.3.2. Clearanol G (6)

Colorless oil;View the MathML source = +43° (c = 0.03, Chloroform); UV (MeOH) λmax (log ε) 308 (3.18), 268 (3.36), 228 (3.41) nm; CD (c 7.51 × 10−5 M, CH3CN) λ (Δε) 221 (−18.9) nm, 242 (+21.7) nm, 266 (+19.3) nm, 316 (−4.0) nm; For 1H NMR (CDCl3, 700 MHz) and 13C NMR (CDCl3, 175 MHz) spectroscopic data, see Table 1; HRESIMS m/z 267.0861 [M+H]+ (calcd for C13H15O6 267.0863).

4.3.3. Radinaphthalenone (9)

Off-white powder; UV (MeOH) λmax (log ε) 352 (3.28), 293 (3.24), 244 (3.35), 211 (3.18) nm; For 1H NMR (CDCl3, 500 MHz) and 13C NMR (CDCl3, 100 MHz) spectroscopic data, see Table 2; HRESIMS m/z 207.0647 [M+H]+ (calcd for C11H11O4 207.0652).

4.4 Computational methods

Theoretical calculations of ECD spectra for compounds 5 and 6 were performed with the Gaussian 09 (Gaussian Inc., Pittsburgh, PA, USA) program package (Frisch et al., 2003). Geometry optimizations for both compounds were carried out using the PM3 semi-empirical force field calculations as implemented in the Spartan 08 program (Wavefunction Inc. Irvine, CA, USA) (Kong et al., 2000). A Monte Carlo search protocol (Chang et al., 1989) was carried out considering an energy cutoff of 5 kcal/mol. In each case, the minimum energy structures were filtered and checked for duplicity. Each conformer was geometrically optimized using hybrid DFT method B3LYP and basis set DGDZVP2 (B3LYP/DGDZVP2), and thermochemical parameters and the frequencies at 298 K and 1 atm. The self-consistent reaction field method (SCRF) with the conductor-like screening model (COSMO) was employed to perform the ECD calculation of major conformers of compounds 5 and 6 in CH3CN solution with the same basis set. The calculated excitation energy (in nm) and rotatory strength R, in dipole velocity (Rvel) and dipole length (Rlen) forms, were simulated into an ECD curve by using the following Gaussian function:

$$\triangle\epsilon(E) = \sum_{i=1}^{n} \triangle\epsilon_i(E) = \sum_{i=1}^{n} \left(\frac{R_i E_i}{2.29 \times 10^{-39} \sqrt{\pi\sigma}} exp\left[-\left(\frac{E - E_i}{\sigma} \right)^2 \right] \right)$$

where σ is the width of the band at 1/e height, and Ei and Ri are the excitation energies and rotatory strengths for transition i, respectively. σ = 0.40 eV and Rvel were used. All quantum calculations were carried out on a Linux operating system in the KanBalam cluster from a Hewlett-Packard HP CP 4000, which includes 1368 AMD Opteron processors at 2.6 GHz and a RAM memory of 3 terabytes (KanBalam, Dirección General de Cómputo y de Tecnologías de Información y Comunicación, UNAM).

4.5. Antimicrobial assay

Minimal inhibitory concentrations (MICs) of compounds 1–9 were measured as described previously (Ayers et al., 2012, Falkinham et al., 2012 and Williams et al., 2007). All measurements were made in duplicate. The positive controls were ampicillin, rifamycin, and amphotericin B.

4.6. Biofilm-Grown cells inhibition by a 96-well plate assay

The inhibition of biofilm-grown cells of S. aureus strain ATCC 6358 was measured as described by Nett et al. (2011) with the only change being the growth of the S. aureus strain in Mueller-Hinton broth at 37 °C. The MIC was defined as the lowest concentration that resulted in 50% (IC50) and 90% (IC90) reduction in reduced XTT absorbance. Vancomycin was used as a positive control and showed an IC90 of 0.5 μg/mL.

Acknowledgments

The authors thank the City of Greensboro for permission to collect samples. The high resolution mass spectrometry data were acquired at the Triad Mass Spectrometry Laboratory at the University of North Carolina at Greensboro. Sequence data were generated at the Mycology laboratory of Dr. Andrew N. Miller, Illinois Natural History Survey, University of Illinois at Urbana-Champaign. We are indebted to Dirección General de Cómputo y de Tecnologías de Información y Comunicación (DGTIC), UNAM, for providing the resources to carry out computational calculations through the KanBalam system.

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