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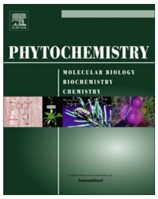

Isochromenones, isobenzofuranone, and tetrahydronaphthalenes produced by *Paraphoma radicina*, a fungus isolated from a freshwater habitat

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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.

1. Introduction

In search of structurally diverse scaffolds from ecologically unique fungi, our group has initiated investigations of freshwater fungi (Raja et al., 2013a,b), 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; 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; 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; 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; 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-dimethylisochroman-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(3*H*)-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

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Abbreviations: BLAST, basic local alignment search tool; $CHCl_3$, chloroform; CH_3CN, 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.

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to have various degrees of antifungal activity (Gerea et al., 2012; Tayone et al., 2011a,b) 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.

2. 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,b). 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).

Compound **3** (7.5 mg) was obtained as a colorless oil with a molecular formula of $C_{13}H_{16}O_6$ 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 $C_{13}H_{16}O_5$ 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(3*H*)-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 $C_{13}H_{16}O_6$ 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 (δ_C 103.3) in **3a** into a methine (δ_H/δ_C 4.36/82.4) in **5**. Additionally, C-4 changed from a methine in **3a** (δ_H/δ_C 3.27/36.1) into an oxygenated quaternary carbon (δ_C 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 ^{13}C NMR data). An HMBC correlation was observed from 12-OCH_3 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-CH_3 to C-4a and C-3, from 4-OH to 10-CH_3, from H-3 to C-4a, and from 9-CH_2 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; Stephens et al., 2007). Similar studies have used calculated

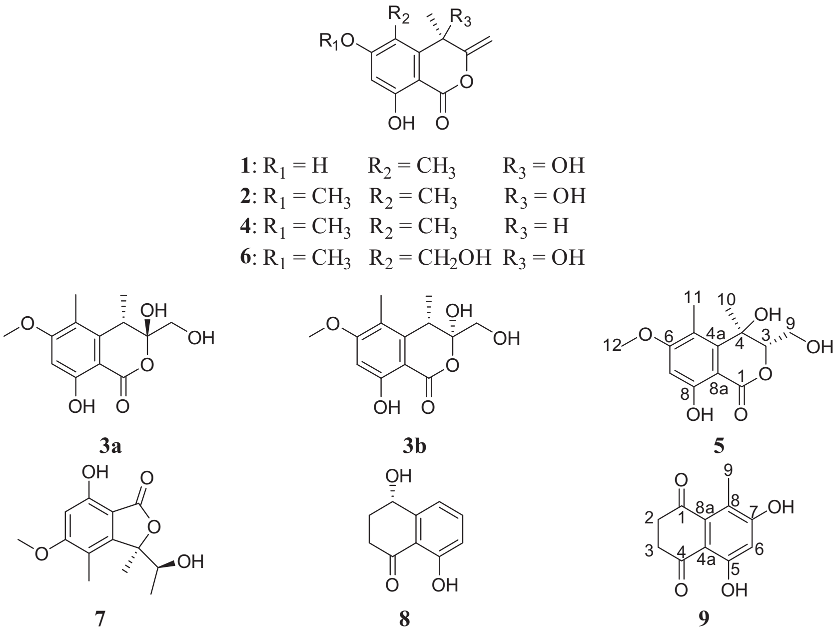

Fig. 1. Structures of compounds **1**–**9**.

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