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Acetophenone derivatives from a freshwater fungal isolate of recently described *Lindgomyces madisonensis* (G416)

Noemi D. Paguigan [a], Huzefa A. Raja [a], Cynthia S. Day [b], Nicholas H. Oberlies [a,*]

[a] Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, 435 Patricia A. Sullivan Science Building, PO Box 26170, Greensboro, NC 27402-6170, USA
[b] Department of Chemistry, Wake Forest University, Winston-Salem, NC 27109, USA

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ABSTRACT

The exploration of freshwater ascomycetes, which have undergone only limited investigation, may provide opportunities both to characterize new genera/species of fungi and to uncover new chemical diversity. In this study, seven acetophenone derivatives, madisone, 4′-methoxymadisone, dehydromadisone, 2″-methoxymadisone, dihydroallovisnaginone, dimadisone, and 4′-methoxydimadisone were characterized from an organic extract of a recently described *Lindgomyces madisonensis* (G416) culture, which was isolated from submerged wood collected in a stream in North Carolina. Madisone, dehydromadisone, 2″-methoxymadisone, dimadisone and 4′-demethoxydimadisone have not been reported previously, while 4′-methoxymadisone and dihydroallovisnaginone were previously unknown as natural products. Their structures were assigned on the basis of NMR and HRESIMS data, with the structure of madisone supported by X-ray crystallography. The antimicrobial activities of madisone, 4′-methoxymadisone and dihydroallovisnaginone were evaluated against a panel of bacteria and fungi. A heat map analysis of the surface of a G416 culture showed that most of the isolated compounds concentrated in the guttate compared with the vegetative mycelium of the fungus.

1. Introduction

Fungi represent a highly diverse group of organisms, and even though there exists an estimated 5.1 million species, less than 100 thousand have been cultivated and described (Blackwell, 2011), and only a fraction of those have been studied with respect to their chemistry (Aly et al., 2011). Freshwater ascomycetes are an ecological group that occur on submerged substrates in fresh water and play an important role as decomposers in these habitats (Shearer, 1993, 2007; Shearer and Raja, 2015). The number of described ascomycetes has increased dramatically over the past 25 years. Shearer (1993) reported about 200 species from freshwater habitats worldwide; that number doubled to 414 in a 2001 review (Shearer, 2001). Currently, about 640 freshwater ascomycetes have been described (Shearer and Raja, 2015). While knowledge regarding the distribution patterns and taxonomy has increased for this ecological group of fungi due to intensive collection over the last two decades, their chemistry, particularly regarding secondary metabolites, has had limited investigation (Hosoe et al., 2010; Jiao et al., 2006; Li et al., 2003; Mudur et al., 2006; Oh et al., 1999; Reategui et al., 2005). As of 2011, approximately 127 chemical structures had been reported from about 30–40 freshwater fungal species (El-Elimat et al., 2014a, 2014b; Hernández-Carlos and Gamboa-Angulo, 2011). To ameliorate this knowledge gap, studies were initiated on the chemical mycology of freshwater ascomycetes in North Carolina, USA (El-Elimat et al., 2014a,b; Raja et al., 2015; Raja et al., 2013a, 2013b), representing the first systematic study of freshwater ascomycetes from this region of North America. Ongoing investigations led to the isolation of five new acetophenones (**1**, **3**, **4**, **6**, and **7**), along with 4′-methoxymadisone (**2**) and dihydroallovisnaginone (**5**), from a fungal isolate recently described as *Lindgomyces madisonensis* (G416) Raja & Oberlies (Crous et al., 2015). A heat map analysis by *in situ* sampling via droplet-liquid microjunction-surface

Abbreviations: $CHCl_3$, chloroform; CH_3OH, methanol; CH_3CN, acetonitrile; NMR, nuclear magnetic resonance; HRESIMS, high-resolution electrospray ionization mass spectrometry; HPLC, high-performance liquid chromatography; HMBC, heteronuclear multiple bond coherence; HSQC, heteronuclear single quantum coherence; CD_3OD, deuterated methanol; $CDCl_3$, deuterated chloroform; ITS rDNA, internal transcribed spacer region of ribosomal deoxyribonucleic acid; DNA, deoxyribonucleic acid; PCR, polymerase chain reaction; BLAST, basic local alignment search tool; PDA, potato dextrose agar; YESD, yeast extract soy dextrose; ELSD, evaporative light scattering detector; UV, ultraviolet; amu, atomic mass unit; Droplet-LMJ-SSP, droplet-liquid microjunction-surface sampling probe.

* Corresponding author.
E-mail address: nicholas_oberlies@uncg.edu (N.H. Oberlies).

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sampling probe (droplet-LMJ-SSP) of a *L. madisonensis* (G416) culture showed that the isolated compounds were more abundant in the guttate compared to the fungal mycelium.

2. Results and discussion

2.1. Structural characterization and bioactivity of compounds 1–7

A culture of *L. madisonensis* (G416) was isolated from decomposing wood collected in the central Piedmont region of North Carolina. The fungus was cultured by solid-substrate fermentation on rice, and this material was extracted with 1:1 $CHCl_3$–CH_3OH. The resulting extract was subjected to partitioning with organic solvents followed by purification using flash chromatography yielding five fractions. These fractions were further purified using preparative HPLC, leading to the isolation of compounds **1**–**7** (Fig. 1). The structures of these were established by analysis of spectroscopic (NMR) and spectrometric (HRESIMS) data.

On the basis of HRESIMS and NMR data (Table 1 and Fig. S1), compound **1** was found to have the molecular formula $C_{11}H_{14}O_5$ (five degrees of unsaturation). The IR spectrum of **1** showed absorption bands at 2972 and 1610 cm^{-1}, indicative of an aromatic ring with chelated hydroxy and carbonyl moieties; these functionalities were supported by the observed UV absorption maxima at 306 and 237 nm. ^{13}C NMR and HSQC data, indicated 11 carbon signals, which were attributed to six aromatic carbons (five nonprotonated and one protonated), one ketone carbonyl, and four carbon signals located in the aliphatic region of the spectrum. Furthermore, chemical shift data indicated that three of the aromatic carbons were oxygenated (δ_C 163.3, 166.1, 164.7 for C-6′, C-2′, and C-4′, respectively). The downfield region of the ^1H NMR spectrum of **1** exhibited a singlet at δ_H 5.98 that integrated for one aromatic proton, as expected for a penta-substituted benzene ring. Based on the ^1H NMR data (Table 1), the structure of **1** had one isolated methyl group (δ_H 2.55, singlet), one methoxy, and a hydroxyethyl group. The connections between the subunits were deduced from key HMBC correlations (Fig. 2), including: H-5′ to C-6′, C-1′, C-3′, and C-4′; the methoxy to C-6′; the isolated methyl H_3-2 to C-1′; and H_2-1″ and H_2-2″ of the hydroxyethyl group to C-2′, C-3′, and C-4′, and to C-3′, respectively. In further efforts to verify the locations of subunits on the aromatic ring, ^1H NMR data for **1** were recorded in $CDCl_3$, revealing a singlet resonance at δ_H 14.58 that was not observed when recorded in CD_3OD (Fig. S8). This result was consistent with the downfield shift associated with intramolecular H-bonding between the proton of the C-2′ hydroxy group and the oxygen of the C-1 carbonyl. The structure of **1**, recrystallized from CH_3OH, was unambiguously assigned by X-ray crystallography (Fig. S23) and ascribed the trivial name, madisone.

Compound **2** was assigned the molecular formula $C_{12}H_{16}O_5$ (five degrees of unsaturation) on the basis of HRESIMS and NMR data. The NMR spectroscopic data for **2** (Table 1) were nearly identical to that of compound **1**, but with additional signals for a second methoxy group (δ_C/δ_H 56.1/3.93), which were consistent with a 14 amu mass difference. A key HMBC correlation was observed from the methoxy group to C-4′, confirming its connectivity and establishing the structure of **2** (Figs. 1 and 2 and S10). Compound **2** was first described as one of the intermediates in an attempted synthetic oxidative approach to LL-D253α (Wootton, 2000). Their data were acquired in $CDCl_3$; the data herein (Fig. S8) were consistent with the literature with one caveat. It is considered that there was an error in the previous assignment of the C-3′ chemical shift value (Wootton, 2000). Thus, we have included the NMR shift assignments of **2**. As this is the first report of the isolation of **2** from a natural source, it was given the trivial name 4′-methoxymadisone.

The HRESIMS and NMR data established the molecular formula for compound **3** as $C_{11}H_{14}O_4$ (five degrees of unsaturation). Comparison of these data to those of compound **1** indicated the loss of the hydroxy moiety at C-2″, which was consistent with the associated changes in the shifts and the multiplicities of H_2-1″ (δ_H 2.54, quartet, 7.3 Hz) and H_3-2″ (δ_H 1.04, triplet, 7.3 Hz); it also accounted for the 16 amu mass difference between these compounds. As such, compound **3** was identified as shown (Fig. 1) and assigned the trivial name dehydromadisone.

The molecular formula of compound **4** was deduced as $C_{12}H_{16}O_5$ (five degrees of unsaturation) based on HRESIMS and NMR data. The NMR and HRMS of **4** suggested structural similarities to **1**. However, **4** had an additional methoxy unit (δ_C/δ_H 58.5/3.36) as evidenced by a 14 amu difference in the HRMS between **1** and **4**. Further analysis of the 1- and 2-D NMR data indicated that the 2″-OH in **1** was replaced with a methoxy unit in **4**. This substitution was confirmed by the observed more downfield C-2″ shift in **4** (δ_C 72.6) compared to the C-2″ shift in **1** (δ_C 62.0). Conversely, C1″ appeared more upfield in **4** compared to **1** at δ_C 23.4 and δ_C 26.7, respectively. Moreover, an HMBC correlation observed for the methoxy protons to C-2″ (Fig. 2 and Fig. S12) established its connectivity. Thus, the structure of compound **4** was assigned (Fig. 1) and ascribed the trivial name 2″-methoxymadisone.

Compound **5** was found to have the molecular formula $C_{11}H_{12}O_4$ (six degrees of unsaturation) on the basis of HRESIMS and NMR data. The ^1H NMR spectrum of **5** exhibited signals that were almost identical to those of **1** but with more deshielded H_2-1″ and H_2-2″ resonances at δ_H 3.05 (triplet, 7.6 Hz) and δ_H 4.65 (triplet, 7.6 Hz), respectively. This distinct change in the chemical shift values suggested a heterocycle, which also accounted for the additional unsaturation and the 18 amu mass difference between **1** and **5** (Fig. 1). Although this is the first report of the isolation of **5** from a natural source, Geissman and Hinreiner described it (and named it dihydroallovisnaginone) as one of the derivatives in the monomethylation of 4,6-dihydroxy-5-acetylcoumarane in an attempt to synthesize visnaginone (Geissman and Hinreiner, 1951). Although NMR and HRMS data were not available in 1951, the UV data were consistent with the literature.

The ^1H and ^{13}C NMR data of **6** (Table 1) showed signals that were nearly identical to those of **1**. However, the HRMS data established the molecular formula to be $C_{22}H_{26}O_9$ (ten degrees of unsaturation), which indicated twice as many carbons, along with 12 additional hydrogen and four more oxygen atoms relative to the

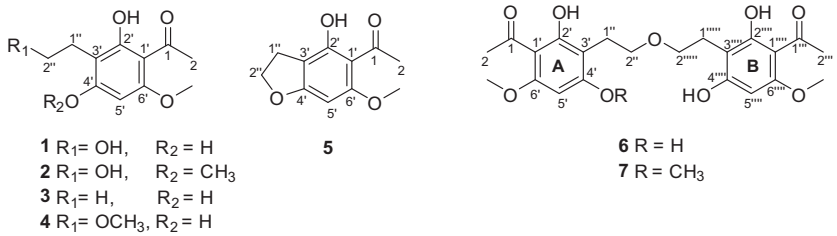

Fig. 1. Structures of compounds **1**–**7**.