Tetrahedron Letters 56 (2015) 1724–1727

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Lycopodiellactone, an unusual δ-lactone-isochromanone from a Hawaiian plant-associated fungus *Paraphaeosphaeria neglecta* FT462

Chunshun Li[a], Baojun Yang[a], Roland Fenstemacher[b], James Turkson[a], Shugeng Cao[a,*]

[a] *Natural Products & Experimental Therapeutics Program, University of Hawaii Cancer Center, 701 Ilalo Street, Honolulu, HI 96813, United States*
[b] *Chemistry Laboratory, Board of Water Supply, City and County of Honolulu, 630 South Beretania Street, Honolulu, HI 96843, United States*

ARTICLE INFO

Article history:
Received 21 January 2015
Revised 14 February 2015
Accepted 16 February 2015
Available online 21 February 2015

Keywords:
Hawaii
Endophytic
NMR
Lactone-isochromanone
Anti-proliferative

ABSTRACT

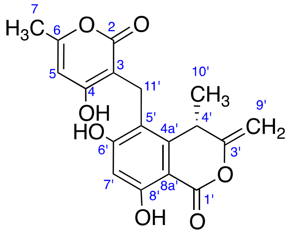
An endophytic fungus *Paraphaeosphaeria neglecta* FT462 from the Hawaiian plant *Lycopodiella cernua* (L.) Pic. Serm produced an unusual δ-lactone-isochromanone with a methylene bridge (**1**). The structure of **1** was determined by NMR and MS spectroscopic analysis.

Many plant-associated fungi produce small-molecule natural products representing a rich source of biologically active compounds with an extensive range of applications in industry, agriculture and medicine.[1] The natural resources of Hawai'i are unique due to its mid-oceanic environment at 2500 miles from any other land with ecologically rich habitats, which present a wide variety of marine life, coastlines, and unique land ecosystems.[2] More than 4500 species including lichens, mosses, liverworts, ferns, gymnosperms, and angiosperms in Hawai'i have been documented, about one third of which are endemic and many of which are endangered or near extinction.[2] A survey conducted in 2012 on endophytic fungal communities in leaves of a single tree species (*Metrosideros polymorpha*) across wide environmental gradients in the Mauna Loa volcano indicated that foliar endophytic fungi in Hawai'i are hyper-diverse.[3] In recent years, new marine and endophytic fungal species from Hawai'i have been described.[4] Despite the fungal diversity, only a handful of interesting compounds have been isolated from Hawaiian fungi, and only a few of which were endophytic.[5]

The fungus, *Paraphaeosphaeria neglecta* FT462, was isolated from a Hawaiian indigenous plant, *Lycopodiella cernua* (L.) Pic. Serm, which was collected in the Mokuleia Forest Reserve.[6] *Lycopodiella cernua* (L.) Pic. Serm usually grows in bogs and in wet to mosaic forests, and the common name for this plant is staghorn club-moss, which is called Wawae'iole in Hawai'i.[7] In some Southeastern Asian countries, this plant has been used in folk medicine for treating central nervous system conditions.[8]

The fungus *Paraphaeosphaeria neglecta* FT462 was identified by analysis of its ITS (internal transcript spacer) sequence.[9a] The strain was cultured in liquid medium under static condition,[9b] and the cultured broth was passed through an HP20 column to afford five fractions, one of which was further separated by C18 preparative HPLC and then phenyl-hexyl semi-preparative HPLC to yield the new compound (**1**, see Fig. 1).[9c] Here, we describe the isolation, structure determination, plausible biosynthesis, and biological activity of compound **1**.

Compound **1**[9d] was obtained as a white powder. Its molecular formula $C_{18}H_{16}O_7$ was deduced from the $[M+H]^+$ ion at *m*/*z* 345.0975 (calcd 345.0974) in the HRESIMS spectrum, which was in accordance with the ^1H and ^{13}C NMR spectroscopic data



1

Figure 1. Structure of compound **1**.

---

* Corresponding author. Tel.: +1 (808) 440 5208; fax: +1 (808) 586 2970.
E-mail address: scao@cc.hawaii.edu (S. Cao).

http://dx.doi.org/10.1016/j.tetlet.2015.02.076
0040-4039/© 2015 Elsevier Ltd. All rights reserved.

(Table 1), corresponding to 11 degrees of unsaturation. The IR spectrum showed the existence of the hydroxyl group (3385 cm^{-1}) and carbonyl groups (1653 and 1603 cm^{-1}). The ^{1}H NMR spectrum demonstrated the presence of one aromatic proton signal at δ_H 6.17 (1H, s); three olefinic proton signals at δ_H 5.75 (1H, s), δ_H 4.67 (1H, br s) and δ_H 4.65 (1H, br s); one methine signal at δ_H 5.15 (1H, q, J = 6.0 Hz); one methylene group at δ_H 3.57 (1H, d, J = 18.0 Hz) and δ_H 3.51 (1H, d, J = 18.0 Hz); and two methyl signals at δ_H 2.10 (1H, s) and δ_H 1.34 (1H, d, J = 6.0 Hz). The ^{13}C NMR spectrum displayed eighteen peaks, indicating the existence of two carbonyl carbons at δ_C 170.1 and δ_C 168.8; five oxygenated and unsaturated quaternary carbons at δ_C 179.0, δ_C 167.9, δ_C 160.7, δ_C 163.9, and δ_C 159.8; four other quaternary carbons at δ_C 145.9, δ_C 119.5, δ_C 100.2, and δ_C 98.6; one aromatic and two olefinic carbons at δ_C 103.8, δ_C 107.2 and δ_C 95.2; one methine, one methylene and two methyl carbons at δ_C 35.4, δ_C 20.8, δ_C 23.6 and δ_C 19.4, respectively.

Detailed interpretation of HMBC correlations (Fig. 2) allowed the construction of fragments A and B. The HMBC correlations from δ_H 2.10 (H_3-7) to δ_C 160.7 (C-6) and δ_C 107.2 (C-5); from δ_H 5.75 (H-5) to δ_C 179.0 (C-4), δ_C 160.7 (C-6), δ_C 100.2 (C-3), and δ_C 19.4 (C-7); and from δ_H 3.51 (Ha-11′) and δ_H 3.57 (Hb-11′) to δ_C 179.0 (C-4), δ_C 170.1 (C-2), δ_C 100.2 (C-3) established fragment A, the 3-substitued 4-hydroxy-6-methyl-2*H*-pyran-2-one moiety (a 2-substituted **2**).[10] The methylene protons at δ_H 3.51 (Ha-11′) and δ_H 3.57 (Hb-11′) also correlated to carbons at δ_C 167.9 (C-6′), δ_C 119.5 (C-5′) and δ_C 145.9 (C-4a′); the aromatic proton at δ_H 6.17 (H-7′) correlated to carbons at δ_C 167.9 (C-6′), δ_C 163.9 (C-8′), δ_C 119.5 (C-5′) and δ_C 98.6 (C-8a′); the methine proton at δ_H 5.15 (H-4′) correlated to carbons at δ_C 145.9 (C-4a′), δ_C 119.5 (C-5′) and δ_C 98.6 (C-8a′), indicating a penta-substituted benzene ring. The methine proton at δ_H 5.15 (H-4′) also showed HMBC correlations to carbons at δ_C 159.8 (C-3′), δ_C 95.2 (C-9′), and δ_C 23.6 (C-10′); both the olefinic methylene at δ_H 4.65/4.67 (H_2-9′) and methyl group at δ_H 1.34 (H_2-10′) exhibited HMBC correlations to carbons at δ_C 159.8 (C-3′) and δ_C 35.4 (C-4′), suggesting the presence of fragment B. Fragments A and B were connected through the methylene (δ_H 3.51 and δ_H 3.57) bridge at 11′-position. The chemical composition of fragments A and B was $C_{17}H_{16}O_6$, which was one carbon and one oxygen (C=O) less than the molecular formula of compound **1**. This carbonyl group must be located between the carbon at 8a′-position and the oxygen at 2′-position, not between the carbon at 8a′-position and any other oxygens at C-8′, C-6′, and C-4. The NMR chemical shifts of the 5-substituted 6′,8′-dihydroxy-4′-methyl-3′-methyleneisochroman-1′-one of **1** matched those of (*S*)-(+)-ascochin (**3**)[11] very well. Hence, the planar structure of **1**, an unusual molecule composed of a δ-lactone and isochromanone, was determined as shown.

Table 1
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of **1** in CD_3OD

No.	**1** (in CD_3OD)		No.	**1** (in CD_3OD)	
	δ_C	δ_H		δ_C	δ_H
2	170.1		4a′	145.9	
3	100.2		5′	119.5	
4	179.0		6′	167.9	
5	107.2	5.75, s	7′	103.8	6.17, s
6	160.7		8′	163.9	
7	19.4	2.10, s	8a′	98.6	
1′	168.8		9′	95.2	4.65, brs; 4.67, brs
3′	159.8		10′	23.6	1.34, d (6.0)
4′	35.4	5.15, q (6.0)	11′	20.8	3.51, d (18.0); 3.57, d (18.0)

Since the optical rotation of (*S*)-(+)-ascochin (**3**, [α] +287.9, *c* 0.28)[11] was recorded in dichloromethane, the optical rotation of **1** was first measured also in the same solvent, which showed the same sign but with big fluctuation, probably due to solubility. Then the optical rotation of **1** was measured in methanol ([α] +20, *c* 0.20), and the positive value suggested the 4*S*-configuration.

Compound **1** possesses an interesting skeleton constructed from 4-hydroxy-6-methyl-2*H*-pyran-2-one (**2**)[10] and (*S*)-6′,8′-dihydroxy-4′-methyl-3′-methylene-1′-oxoisochroman-5-carbaldehyde (**3**).[11] A plausible biosynthetic pathway for compound **1** is proposed as shown in Scheme 1. Both moieties are most likely originated from polyketide biosynthesis. The precursor of **3** could be derived from a combination of acetate and propionate or from acetate followed by methylation. The enolate carbon of compound **2** undergoes nucleophilic addition with the aldehyde carbon of compound **3**, which leads to the formation of the condensation product, **1**.

While δ-lactones are not uncommon, 3-methylene isochromanone moiety in compound **1** is rare in nature. Only a few natural 3-methyleneisochromenone derivatives, including (*S*)-(+)-ascochin

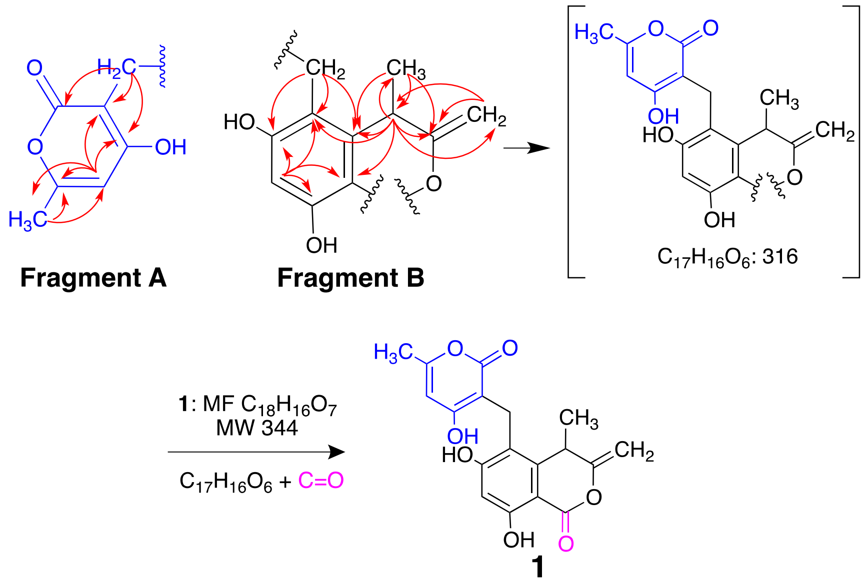


Figure 2. HMBC correlations, fragments A and B in **1**, and the connectivity of the carbonyl group in the isochromanone moiety.