Planar Structure Elucidation of Iso-cladospolide B Isolated from *Lambertella brunneola* fungus

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Abstract

A compound known as iso-cladospolide B was isolated from fungus Lambertella brunneola. The compound was isolated and purified using SiO_2 column chromatography while the structure elucidation was based on Nuclear Magnetic Resonance (NMR) spectroscopic data and Mass Spectroscopy (MS) analyses. Iso-cladospolide B exhibited a weak antifungal property against pathogenic fungus Cochliobolus miyabeanus.

Keywords: column chromatography, iso-cladospolide B, *Lambertella brunneola*, Nuclear Magnetic Resonance (NMR), structure elucidation

1. Introduction

In recent years, the microscopic world especially fungi have always been a source of unique compounds with various biological potential such as anticancer [1], antihypertensive[2], antitumor [3], biosynthetic congener [4] and antifungal([5], [6]). In the course of investigations involving natural products from fungi ([7], [8] and [9]), *Lambertella brunneola* fungus was found to produce iso-cladospolide B. This secondary metabolite was first isolated by Smith's group [10] from an unidentified marine source collected in Indonesia last October 1996. Detailed structural analyses revealed that this molecule has five membered-lactone moiety as shown in figure 1, which was confirmed by Nuclear Magnetic Resonance (NMR) and Mass Spectroscopy (MS) spectral analyses. Its absolute stereochemistry and total synthesis were also reported by several groups ([11], [12] and [13]). Although the compound is already known, none has been published yet about the isolation of iso-cladospolide B from the fungus *L. brunneola*.

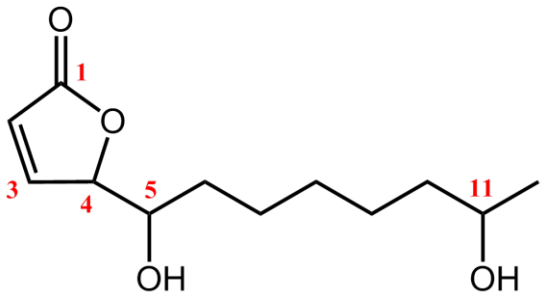

Figure 1. Planar structure of iso-cladospolide B

2. Material and Methods

2.1 Structure Elucidation

The ^{1}H (500 MHz), ^{13}C (125 MHz), Correlation Spectroscopy (COSY), Heteronuclear Multiple Quantum Coherence (HMQC) and Heteronuclear Multiple Bond Coherence (HMBC) NMR spectra were recorded on a JEOL JNM-ECA500 spectrometer. In $CDCl_3$, the signal due to 7.24 ppm was used as the standard. Electrospray ionization (ESI) MS spectrum was obtained from a HITACHI NanoFrontier LD spectrometer. Measurements of IR spectrum were performed with a HORIBA FT-720 spectrometer on KBr cell. Chemicals used in these experiments were obtained from Wako Pure Chemical Industries Ltd. and Nacalai Tesque Inc.

2.2 Fungus

The *L. brunneola* fungus was collected from Tsugaru region in northern part of Japan. The species was identified by Professor Harada and the fungal isolate was deposited at the mycology laboratory of Professors Harada and Tanaka, Faculty of Agriculture and Life Sciences, Hirosaki University, Japan.

2.3 Fermentation and Isolation

L. brunneola was cultured in potato-sucrose medium (200 mL in 500 mL Erlenmeyer flask × 5) in stationary condition at 25.0 °C for 2 months. After the media was filtered by suction, the filtrate was extracted with about 200 mL (× 5) methanol (MeOH). The combined extract was then partitioned with ethyl acetate (EtOAc) (1.0 L × 3) and the organic layer was concentrated *in vacuo*. Purification then followed through a series of silica gel column chromatography using hexane/ethyl acetate solvent system to afford 8.7 mg of colorless iso-cladospolide B.

2.4 Biological Assay

Solutions of pathogenic fungus *Cochliobolus miyabeanus* spores were prepared containing 2,000, 1,000, 500, 100, 50, 10, 5.0 1.0 and 0.5 μg/mL in two replicates with 2% sucrose in DMSO. After 36 h at 25 °C, germination and the shapes of the spores were observed under a microscope. The IC_{50} values were determined by the concentration which showed 50% inhibition of germination.

3. Result and Discussions

3.1 Purification

A 1.0 L culture broth of *L. brunneola* was extracted with methanol and further partitioned with EtOAc. The solvent was filtered and then removed *in vacuo* to afford a brown crude extract. The extract was then fractionated through a series of silica gel column chromatography to give iso-cladospolide B with an R_f value of 0.2 using hexane/EtOAc (1:2) as solvent.

3.2 Structure elucidation

Iso-cladospolide B was isolated as colorless oil and showed an optical rotation of -74^{o} (c = 0.67, $CHCl_3$). Its molecular formula was determined to be $C_{12}H_{20}O_4$by HREIMS m/z229.1440 $[M+H]^{+}$. The presence of a hydroxyl group and an α,β-unsaturated ester were confirmed by an IR signals at 3394 cm^{-1} and 1747 cm^{-1} respectively. The ^{1}H NMR spectrum shown in figure 2 indicated the presence of two doublets of doublets olefinic protons (δ_H7.43 and 6.16), a doublets of triplets acyloxy proton (δ_H4.96), two oxygenated methine (δ_H3.73 and 3.77), ten methylenes (δ_H1.57, 1.57, 1.52, 1.51,1.45, 1.41, 1.40, 1.39, 1.33 and 1.32) and one doublet methyl group (δ_H1.17).

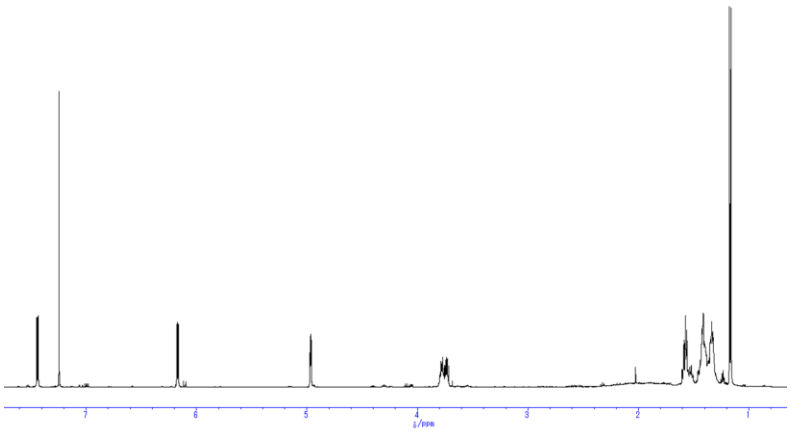

Figure 2. Proton NMR spectrum of iso-cladospolide B

The presence of an ester functionality was further confirmed by a 172.9 ppm ^{13}C NMR signal. Other ^{13}C resonances include δ_C 153.7 and 122.8 suggesting the presence of an olefinic carbon and three oxygenated carbons (δ_C 86.1, 71.8 and 68.1). The ^{13}C and ^{1}H NMR data including their multiplicities and coupling constants are summarized in Table 1. The connectivities of hydrogen and carbon atoms were further established from the HMQC spectra. The HMBC correlations detected for the two olefinic protons to the carbonyl carbon and acyloxy hydrogen to the alkene carbons proved the presence of a five membered lactone ring moiety. The calculated coupling constant of 5.8 Hz for both olefinic protons indicated a cis orientation. The connections from C2 to C12 were addressed on the basis of COSY and HMBC correlations which are shown in figure 3. Comparison of the ^{1}H and ^{13}C NMR spectra with those of the literature suggested that this compound should be an iso-cladospolide B.

Table 1. ^{13}C and ^{1}H NMR data of iso-cladospolide B in $CDCl_3$

Position	δ_C	multiplicity	$\delta_H$mult. (*J* in Hz)
1	172.9	s	
2	122.8	d	6.16 dd(2.05, 5.8)
3	153.7	d	7.43 dd(1.5, 5.8)
4	86.1	d	4.96 dt(1.85, 4.65)
5	71.8	d	3.73 q(6.1)
6	33.0	t	1.51 m, 1.57 m
7	25.4	t	1.52 m, 1.57 m
8	29.3	t	1.33 m, 1.41 m
9	25.5	t	1.32 m, 1.39 m
10	39.0	t	1.40 m, 1.45 m
11	68.1	d	3.77 m
12	23.0	q	1.17 d(6.15)

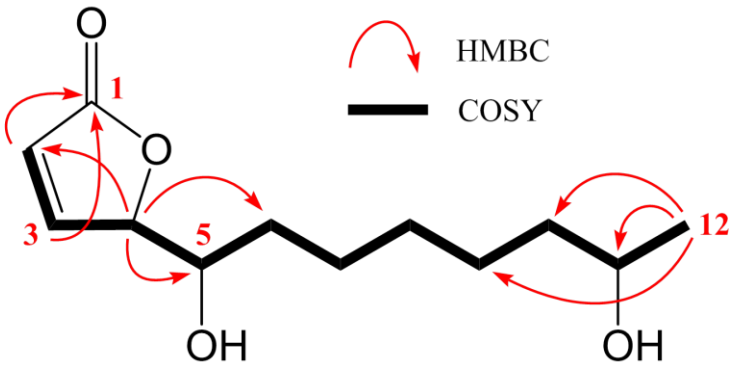

Figure 3. Key COSY and HMBC correlations of iso-cladospolide B

Finally, iso-cladospolide B showed a seemingly weak antifungal property against disease causing fungus *Cochliobolus miyabeanus* with IC_{50} value of 2 mg/mL. As described, iso-cladospolide B was isolated from the culture broth of *L. brunneola*. The planar structure was established through NMR spectroscopic data and MS analyses.

Acknowledgement

The author is grateful to Professor Noburu Takada of Hirosaki University, Japan for the guidance and pointers that were essential to the completion of this study.

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