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Cladospolide B, an Antifungal Polyketide Isolated from the Fungus, Lambertella brunneola

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Abstract

A polyketide known as cladospolide B, (Z)-4,5-dihydroxy-2-dodecen-11-olide was isolated from the culture broth of the fungus, *Lambertella brunneola*. The structure elucidation of this compound was based on Nuclear Magnetic Resonance (NMR) spectroscopic data and Mass Spectroscopy (MS) analyses. Cladospolide B exhibited a seemingly weak antifungal activity and inhibited the hyphalgrowth of the phyto-pathogenicfungus, *Helminthosporium oryzae(=Cochliobolus miyabeanus)* by 50 % at more than 1 mg/mL but less than 5 mg/mL (1 < IC50 <5). This is the first report on the isolation of cladospolide B from L. *brunneola*.

Keywords: cladospolide B antifungal, chromatography, Nuclear Magnetic Resonance (NMR), structure elucidation

Introduction

Polyketides are a large family of structurally diverse natural products possessing a broad range of biological activities, including antibiotic and pharmacological properties (Fu and others 1994, p 9321; Pfeifer and others 2001, p 1790). In our continuing search for fungal metabolites with attractive chemical structures and/or biological activities (Hashimoto and others 2008, p 4228; Kudo and others 2009, p 203; Murakami and others 2009, p 492; Tayone and others 2009, p 7464; Tayone and others 2011, p 425), cladospolide B was isolated from the culture broth of *L. brunneola*. The planar structure of this compound is depicted as shown in Fig. 1. This interesting 12membered lactone ring was originally
isolated by Hirota's group from the culture filtrate of Cladosporium cladosporioides and is known to promote root growth of lettuce seedling (1985, p 731). Although the compound is already known, none has been published about the isolation of cladospolide B from L. brunneola. Moreover, the antifungal assay of cladospolide B

is not tested yet.

The objectives therefore of this research were to establish and elucidate the two-dimensional structure of cladospolide B as well as to determine its antifungal property against the phytopathogenic fungus, Helminthosporium oryzae (= Cochliobolus miyabeanus).

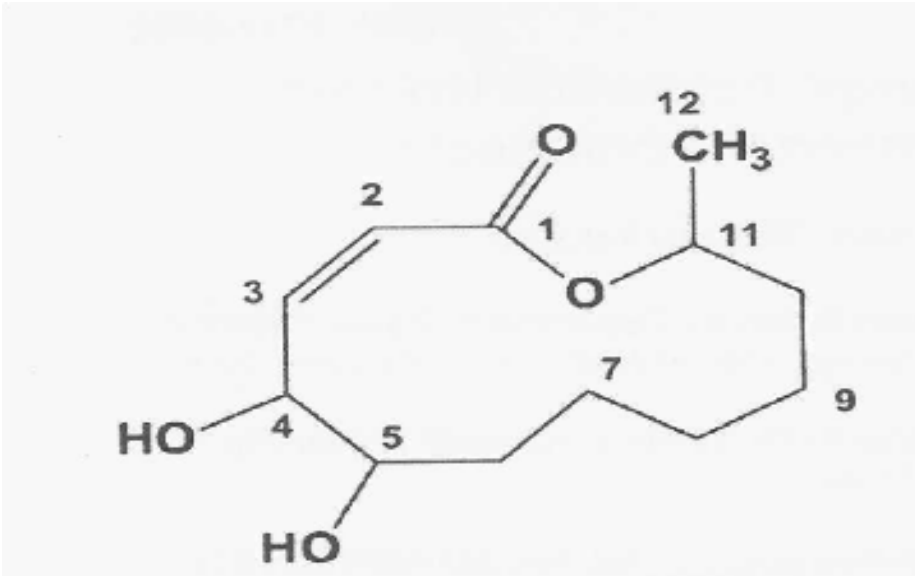

Figure 1. Planar structure of cladospolide B.

Materials and Methods

General Experimental Procedures

After concentrating in vacuo, the methanolic extract of the culture broth of *L. brunneola* was partitioned by ethyl acetate (EtOAc). The water layer was discarded while the EtOAc layer was concentrated in vacuo. The concentrated organic fraction was then subjected to a series of silica gel column chromatography to give 4.5 mg of cladospolide B, a known compound. The I H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on a JEOL JNMECA500 spectrometer. In $CDCI_3$, the signal due to 7.24 ppm was used as the standard. Electrospray ionization (ESI) MS spectra were obtained from a Hitachi NanoFrontier LD spectrometer. Measurements of IR spectra were performed with a Horiba FT720 spectrometer on KBr cell. The optical rotation values were measured on a Horiba SEP-700 spectrometer. Chemicals used in these experiments were obtained from Wako Pure Chemical Industries Ltd. and Nacalai Tesque Inc.

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Fungal Material, Fermentation and Isolation

The *L. brunneola* that was collected from northern Japan was cultured with 200mL potato-sucrose medium [prepared from a potato extract (40 g potato), 4 g of sucrose, and H_20] in 500 mL Erlenmeyer flasks (x5) at 25^0C for 30 days in stationary condition. The combined culture broth was then extracted with c.a. 1.0 L of methanol and the filtrate was concentrated in vacuo. The extract was then partitioned with EtOAc (1.0 Lx3) and the organic layer was concentrated in vacuo followed by series of silica gel column chromatography (7.5 g, 12 mmIDx150 mm) using hexane/ethyl acetate solvent system to afford 4.5 mg of colorless cladospolide B.

Antifungal Test

Solutions of *H. oryzae* (*C miyabeanus*) spores provided by Mitsubishi Chemical Corporation were treated with 0.5, 1.0, 5.0 and 10 mg/mL of cladospolide B isolated from *L. brunneola*. Sucrose at 2% in dimethyl sulfoxide (DMSO) was added to the suspension. After 36 h at 25^0C, germination and the shapes of the spores were observed under a compound microscope. The IC_{50} value was determined by the concentration that inhibited 50% of the spores from germination.

Results and Discussion

Structural Elucidation

Cladospolide B, was found to have an R*f* value of 0.3 using hexane/EtOAc (1:1) as developing solvent and an optical rotation of $[a]^{27}_D = +20^0$ (c = 0.45, CH_30H). The molecular formula of cladospolide B was established to be $C_{12}H_{20}0_4$ by High Resolution Electron Spray Ionization Mass Spectroscopy (HRESIMS) at m/z 229.1440 $[M+H]^+$. The $_1H$ ^{13}C, and Heteronuclear Multiple Quantum Coherence (HMQC) NMR spectra indicated the presence of one ester carbonyl carbon (δ_c 165.88 ppm) at carbon position C_1, one methyl group (δ_H1.27 ppm) at C_{12}, two oxygenated

methines (δ_H 3.76, 5.25 ppm), respectively at C_5 and C_4, an acyloxy methine (δ_H 4.87 ppm) at C_{11}, Wvo olefinic protons (δ_H 5.76, 6.23 ppm) at C_2 and C_3, and five methylene hydrogens (δ_H 1.38 - 1.80 ppm) for positions C_6 to C_{10}.

The above findings were further confirmed by Infrared (IR) absorptions at 1704 cm-I and 3413 cm-I suggesting the presence of an a, ß-□- unsaturated ester and a hydroxy group, respectively. The connectivity from C-1 to C-12 was addressed by Correlation Spectroscopy (COSY), HMQC, and Heteronuclear Multiple Bond Coherence (HMBC) spectra (Fig. 2). The ^1H NMR data of this dodecacyclic lactone was identical to those of the literature values reported by Hirota's group (1985, p 731). Thus, it was identified as cladospolide B. The coupling constant between two olefinic

proton nuclei was 12.2 Hz which could not indicate the geometry. However, initial result of molecular modeling suggested a cis configuration (Fig. 3). Although the absolute configuration was not established, this is the first study to report the isolation of cladospolide B from *L. brunneola*.

As described, cladospolide B was isolated from the culture broth ofL brunneola. The planar structure was established through spectral data and MS analyses.

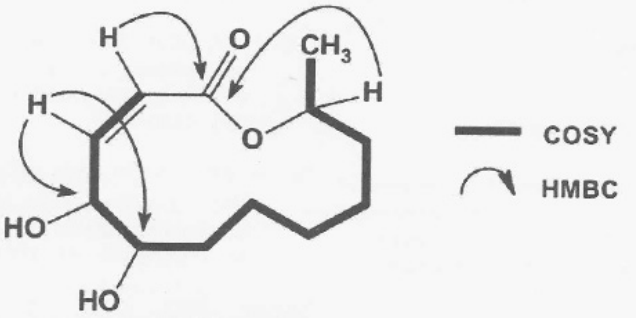

Figure 2. Selected COSY and HMBC correlations of cladospolide B.

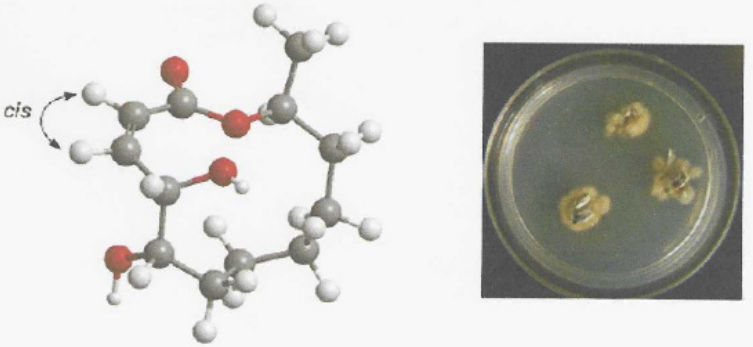

Figure 3. Molecular model of cladospolide B and culture of *Lambertella brunneola*

Antifungal Activity

The cladospolide B at 5 and 10 mg/ mL inhibited the spore germination and growth of H. oryzae hyphae by >50% after 36 h treatment at 25 oc. Lower concentrations at 0.5 and 1 mg cladospolide B per mL did not attain the desired antifungal effect on the pathogenic fungus. Apparently, this polyketide has a weak antifungal activity with a half minimal inhibitory concentration (1%) value of mg/mL but less than 5 mg/ ml- (1 < IC <5).

Acknowledgment

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