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Structure Elucidation of Pandangolide 1 Isolated From *Lambertella Brunneola*

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Abstract : *A macrolide compound known as pandangolide 1 was isolated from fungus Lambertella brunneola. The compound was purified using SiO_2 column chromatography while the elucidation of its planar structure was based on the analyses of Nuclear Magnetic Resonance (NMR) spectroscopic data and Mass Spectroscopy (MS). Antifungal assay disclosed that pandangolide 1 weakly showed hyphal growth inhibition against pathogenic fungus Cochliobolus miyabeanus at 2,000 μg/mL. This is the first paper to report the isolation of Pandangolide 1 from L. brunneola.*

Keywords: *Lambertella brunneola, Nuclear Magnetic Resonance (NMR), pandangolide 1, structure elucidation*

I. Introduction

Natural products are naturally occurring compounds that can be tapped from plants, animals, marine source and microorganisms. Several studies particularly of Newman and Cragg further demonstrated that natural products are significant rich source of new drugs [1]. In fact, fungi are abundant bioresource of new molecules that exhibit various biological properties such as antitumor [2,3,4], antibiotic [5,6,7], anticancer [8,9,10], antiviral [11,12], antihypertensive [13], biosynthetic congener [14] and antifungal [15,16]. In the course of investigation involving *Lambertella brunneola* fungus, pandangolide 1 was found. This secondary metabolite was first isolated by Smith's group [17] from an unidentified marine source collected in Indonesia. Detailed structural analyses via Nuclear Magnetic Resonance (NMR) and Mass Spectroscopy (MS) revealed that this molecule is a twelve membered-lactone compound as shown in Fig. 1. Although the compound is already known, this is the first report on the isolation of pandangolide 1 from fungus *L. brunneola*

Fig 1: Planar structure of pandangolide 1

II. 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. Splitting patterns are designated as singlet (*s*), doublet (*d*), triplet (*t*), quartet (*q*), multiplet (*m*), doublet of doublets (*dd*), doublet of doublet of doublets (*ddd*) and broad (*br*). 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 1 L potato-sucrose medium in stationary condition at 25.0 °C for 2 months. The medium was filtered by suction and the filtrate was extracted with about 1 L methanol (MeOH). The combined extract was then partitioned with 1 L ethyl acetate (EtOAc) 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 3.8 mg of colorless pandangolide 1.

2.4. Biological Assay

Solutions of disease causing fungus *Cochliobolus miyabeanus* spores provided by Mitsubishi Chemical Corporation 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 the hyphal growth of *C. miyabeanus.*

III. Result And Discussions

3.1 Purification

The culture broth of *L. brunneola* was extracted with methanol and further partitioned with EtOAc. It was then filtered and the solvent removed *in vacuo* to give an 86.6 mg of brown crude extract. The extract was then fractionated through a series of silica gel column chromatography to afford pandangolide 1 with an R_f value of 0.3 using hexane/EtOAc (1:2) as solvent.

3.2 Structure elucidation

Pandangolide 1 was isolated as colorless oil. Its molecular formula was determined to be $C_{12}H_{20}O_5$ by HREIMS *m/z* 245.1389 $[M+H]^+$. The presence of a hydroxyl group, an ester and ketone moieties were confirmed by an IR signals at 3444 cm^{-1}(broad), 1731 cm^{-1} and 1716 cm^{-1} respectively. Prominent signals of the ^1H NMR spectrum shown in Fig. 2 gave a downfield resonance at δ_H 4.85 (*ddd*) suggesting an acyloxy proton in C_{11}, two oxygenated protons at δ_H 4.66 (*ddd*) and δ_H 4.29 (*br s*) for C_3 and C_5 respectively, two methylene protons at δ_H 3.17 (*dd*) and δ_H 2.84 (*dd*) for C_2 position, a doublet hydroxyl proton at δ_H 3.15 (*d*) that couples with C_3 hydrogen, ten methylenes (δ_H 1.92, 1.70, 1.62, 1.45, 1.37, 1.32, 1.25, 1.22, 1.14, and 1.10), and an upfield doublet methyl group at δ_H 1.18 for C_{12} position.

The presence of an ester and ketone functionalities were further confirmed by 174.5 and 209.1 ppm ^{13}C NMR signals respectively for C_1 and C_4. Other ^{13}C resonances include δ_C 76.0, 75.0 and 65.4 ppm suggesting the presence of three oxygenated carbons for C_5, C_{11} and C_3 respectively. The ^{13}C and ^1H NMR data including their multiplicities and coupling constants (*J*) are summarized in Table 1. The connectivities of hydrogen and carbon atoms were further established from the HMQC spectra. The connections from C_1 to C_{12} were addressed on the basis of COSY and HMBC correlations which are shown in Fig. 3. Comparison of the ^1H and ^{13}C NMR spectra with those of the literature suggested that this compound should be pandangolide 1.

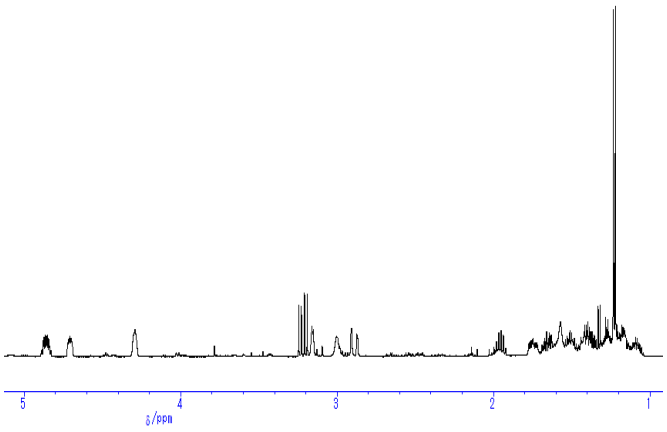

Fig 2: Proton NMR spectrum of pandangolide 1

Table 1: ^{13}C and ^{1}H NMR data of pandangolide 1 in $CDCl_3$

Position	δ_C	multiplicity	δ_H mult. (*J* in Hz)
1	174.5	*s*	
2	42.7	*t*	3.17 *dd*(8.30, 18.6) 2.84 *dd*(2.30, 18.6)
3	65.4	*d*	4.66 *ddd*(2.30, 4.52, 8.30) 3.15 *d*(4.52) for OH
4	209.1	*s*	
5	76.0	*d*	4.29 *br. s*
6	30.4	*t*	1.92 *m*, 1.70 *m*
7	19.0	*t*	1.37 *m*, 1.25 *m*
8	26.6	*t*	1.22 *m*, 1.14 *m*
9	22.4	*t*	1.45 *m*, 1.10 *m*
10	33.2	*t*	1.62 *m*, 1.32 *m*
11	75.0	*d*	4.85 *ddd*(2.10, 6.20, 8.30)
12	20.5	*q*	1.18 *d*(6.20)

Lastly, two replicates of each suspension of spores of *C. miyabeanus* and 2 % sucrose containing 2,000, 1,000, 500, 100, 50, 10, 5.0 1.0 and 0.5 µg/mL of samples in petri dish were prepared and incubated at 25°C for 36 h. These were observed under the microscope. Pandangolide 1 showed a seemingly weak antifungal property at IC_{50} value of 2,000 µg/mL against disease causing fungus *C. miyabeanus*.

Fig 3: Key COSY and HMBC correlations of pandangolide 1

IV. Conclusion

As described, pandangolide 1 was isolated from the culture broth of *L. brunneola*. The planar structure was established through NMR spectral data and MS analyses. Although the compound was originally reported by Smith *et. al.* [17] in 2000 and Hartanti *et. al.* [18] in 2014, this is the first report on the isolation of pandangolide 1 from *L. brunneola* fungus.

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