D-1

Novel Antifungal Diketopiperazine from Marine Fungus Metabolites

Hee-Guk Byun and Se-Kwon Kim
Department of Chemistry, Pukyong National University, Busan 608-737, Korea

Introduction

Rice blast, caused by *Pyricularia oryzae* (*P. oryzae*), is generally considered to be the most serious fungal disease of rice by its widespread distribution and destructiveness (Manandhar *et al.*, 1998). The pathogenic fungus directly penetrates into the rice plant from a cellular structure called an appressorium that is formed at the tip of the germ tube. And the fungus can attack any aerial part of the rice plant, including seeds, in which the fungus may overwinter for several years. Recently, pollution problems in the environment and the toxic effects of synthetic chemicals on non-target organisms have prompted investigations on exploiting pesticides of plant origin. Therefore, an environment-friendly method with biocontrol agents is recognized as an alternative approach to control this disease.

In this study, marine fungi producing antifungal substances were screened and the antifungal substance against *P. oryzae* was purified from culture broths of the marine fungus M-3 isolated from laver. Then the chemical structure of purified antifungal compound was elucidated by the spectroscopic method.

Materials and Methods

Marine fungus M-3 was isolated the laver (*Porphyra yezoensis*) taken from the coast at Huttsu city, Chiba, Japan. The seed culture of marine fungus was preformed by inoculating mycelial fragments of strain M-3 into 100-ml Erlenmeyer flasks containing 30 ml of 1/2 potato dextrose medium at 20℃ for 3 days. Five ml of this seed culture was transferred into each 1-liter Erlenmeyer flasks containing 300 ml of 1/2 potato dextrose medium (total 3 liters) at 20℃ for 21 days. Ethyl acetate extract isolated from the culture broth was purified using TLC glass plates. The active fraction obtained from the TLC was further purified by HPLC. Antifungal assay was performed by Kobayashi *et al.* (1996) method. IR spectra were obtained with a JASCO FT-IR 7000 spectrophotometer. NMR spectra

were measured with a Varian UNITY500 NMR spectrometer. Mass spectra were recorded with a JEOL JMS-SX102 mass spectrometer.

Results

A preliminary antifungal screening was carried out to isolate 70 strains of marine fungi from several lavers and sponges. Only marine fungus M-3 showed strong antifungal activity over this pathogenic fungus. The ethyl acetate extract from the culture broth of M-3 was concentrated in vaccum to obtain the dry yield (86 mg). The extract was fractionated with TLC glass plates. The TLC fractions were divided into fourteen sections. Among the antifungal activity of the each TLC fractions against *P. oryzae*, fraction 10 (Rf, 0.19) showed the highest activity at minimum inhibitory concentration (MIC) values 1.3 μg/ml, and yield was 3.9 mg. The active fraction 10 obtained using TLC, was fractionated by HPLC.

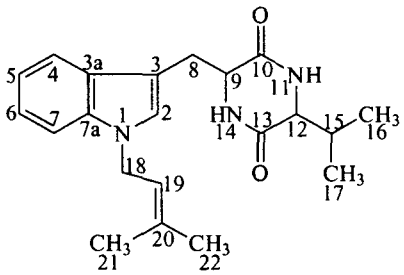

Fig. 1. The chemical structure of compound **1** purified from M-3 metabolite.

The chemical structure of compound **1** (Fig. 1) purified from M-3 culture broth was determined using IR, FAB-MS and NMR. In the IR spectrum, compound **1** showed multiple absorptions at 1670 and 1660 cm^{-1}, which are these of amide groups. These findings in which absence of the amide II band near 1550 cm^{-1} suggest the presence of diketopiperazine system. Positive FAB-MS data for the compound gave a molecular ion at m/z 354 ($[M+H]^+$). High resolution FAB-MS ($[M+H]^+$: found, 354.2183; calculated, 354.2182) established the molecular formula as $C_{21}H_{27}N_3O_2$. The antifungal activity of the compound **1** was 0.13 μg/ml as MIC values, and yield was 0.8 mg.

Novel diketopiperazine **1** was isolated from the M-3 metabolite, which structure was elucidated by spectroscopic methods. Antifungal activity of **1** was 0.36 μM as MIC values.

References

Manandhar, H. K., H. J. Lyngs Jorgensen, S. B. Mathur and V. Smedegaard-Petersen. 1998. Suppression of rice blast by preinoculation with avirulent *Pyricularia oryzae* and the nonrice pathogen, *Bipolaris sorokiniana*. Phytopathology 88: 735~739.

Kobayashi, H., M. Namikoshi, T. Yoshimoto and T. Yokochi. 1996. A screening method for antimitotic and antifungal substances using conidia of *Pyricularia oryzae*, modification and application to tropical marine fungi. J. Antibiotics 49: 873~879.