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H1-A, a compound isolated from *Fusarium oxysporum* inhibits hepatitis C virus (HCV) NS3 serine protease

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[ABSTRACT] The present study was aimed to isolate the active compounds from the fermentation products of *Fusarium oxysporum*, which had hepatitis C virus (HCV) NS3 protease inhibitory activity. A bioactive compound was isolated by reverse-phase silica-gel column chromatography, silica-gel column chromatography, semi-preparative reverse-phase High Performance Liquid Chromatography (HPLC), and then its molecular structure was elucidated based on the spectrosopic analysis. As a result, the compound (H1-A, **1**) Ergosta-5, 8 (14), 22-trien-7-one, 3-hydroxy-(3β, 22E) was isolated and identified. To the best of our knowledge, this was the first report on the isolation of H1-A from microorganisms with the inhibitory activity of NS3 protease.

[KEY WORDS] H1-A; Bioactive compound; *Fusarium oxysporum*

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Introduction

Hepatitis C virus (HCV) infection is one of the main causes of chronic liver disease. HCV infected approximately 130–200 million persons worldwide, leading to liver fibrosis, liver cirrhosis, and hepatocellular carcinoma [1]. HCV has a positive-strand RNA genome that contains a single large open reading frame encoding a polyprotein. During the replication of HCV, the protease NS3 (a 70 kDa protein) possesses serine protease activity at its amino terminal and helicase function at its carboxyl terminal [2]. Due to its bifunctional role, NS3 protease becomes an attractive target for antiviral therapy [3-4].

Based on the screening model of HCV proteinase inhibitors, a crude extract of SIPI-4004 was found to be active; a novel compound (**1**) was isolated as the active component. Compound **1** was identified as Ergosta-5, 8 (14), 22-trien-7-one, 3-hydroxy-(3β, 22E) [5]. Herein, we report the isolation and structural elucidation of compound **1** as well as its NS3 protease inhibitor activity.

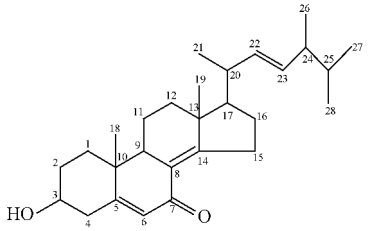

Fig. 1 Chemical structure of compound 1

Results and Discussion

In our previous study，a series of microorganisms were isolated from soil [6]. Furthermore, the fermentation of the crude extract of these microorganisms were made for the screening of bioactive compounds [6]. As a result, several samples were identified with NS3 protease inhibitory activity . Based on the comparison of strain stability and inhibitory activity, strain numbered SIPI-4004 was used in the following studies. SIPI- 4004 was isolated from a soil sample collected

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Phylogenetic analysis of the strain SIPI-4004 using the ITS sequence

The internal transcribed spacer (ITS) sequence of the strain SIPI-4004 was obtained by PCR amplification and then was analyzed using Nucleotide BLAST software of NCBI. The results of sequence alignment showed that strain SIPI-4004 had the identical ITS sequence with the strain *Fusarium oxysporum* (GenBank accession number KR051413). The phylogenetic analysis of the strain SIPI-4004 using the ITS gene sequence (Data not shown) suggested that the strain could be *F. oxysporum* [7] (Fig. 2)

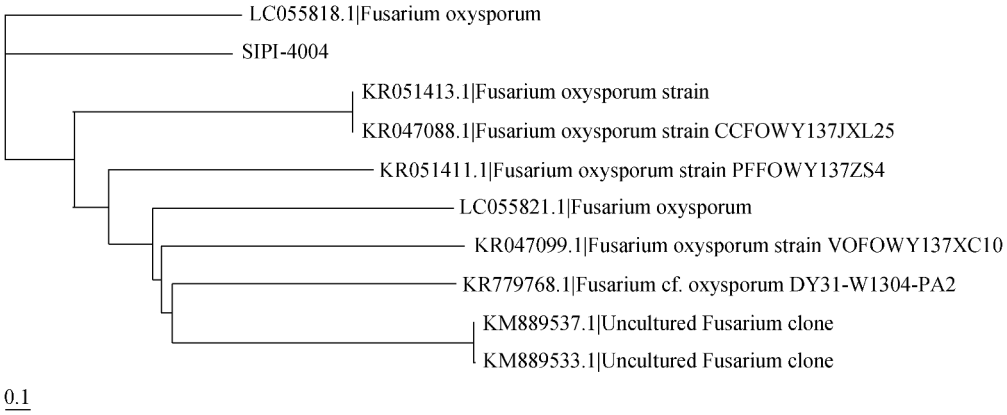

Fig. 2 Phylogenetic tree of strain *F. oxysporum*

Identification of antibiotic active against NS3 protease

Compound **1** was obtained in the form of white powder; its structure was identified mainly by spectral analysis. Briefly, the positive ESI-MS showed molecular ion peak at m/z 411.20 $[M + H]^+$, the formulae is $C_{28}H_{42}O_2$. The NMR spectrum was shown in Table 1. δ_H 6.06 (δ_C126.81) ppm which was attributed to H(C)-6, δ_H 5.18–5.29 (δ_C132.22–134.10) ppm which were attributed to H(C)-22, 23, δ_H3.64–3.71 (δ_C 71.92) ppm to H(C)-3, and δ_C186.17 (C=O) ppm. The rest ^{1}H and ^{13}C NMR chemical shifts and other spectral data of compound **1** was consistent with those in the literature [5, 8], suggesting the antibiotic to be Ergosta-5, 8(14), 22-trien-7-one, 3-hydroxy-(3*β*, 22E) (Fig. 1), which was a derivative of ergosterol. By the NS3 protease inhibitory assay, Ergosta-5, 8 (14), 22-trien-7-one, 3-hydroxy-(3*β*, 22E) inhibited HCV protease with an Ki value being 99.7 μmol·L^{-1}; VX950 Ki value was 3.5 μmol·L^{-1}.

Ergosta-5, 8 (14), 22-trien-7-one, 3-hydroxy-(3*β*, 22E) was already reported as H1-A. H1-A is also an active compound in the fruiting body of Cordyceps sinensis (Brek) *Sacc*(CS) [5, 8]. H1-A may be potentially useful for the treatment of systemic lupus erythematosus [9]. Before this, H1-A was only found in CS. CS, a traditional Chinese herb medicine, has been used to hasten recovery from exhaustion for hundreds of years [10]. It is a parasitic fungus that feeds on the larvae of Lepidoptera. Wild CS is extremely rare and difficult to be obtained [11]. A solid state fermentation (SSF) is provided which is effective for both small-and large-scale fungal cultivation [12]. However, the major drawbacks of SSF are the high fermentation cost, long extraction time, and low extraction efficiency [12-13]. In the present study, H1-A was first isolated from *F. oxysporum*. Different from the culture of CS extract，the method to isolate H1-A from *F. oxysporum* described herein was more convenient and with high efficiency.

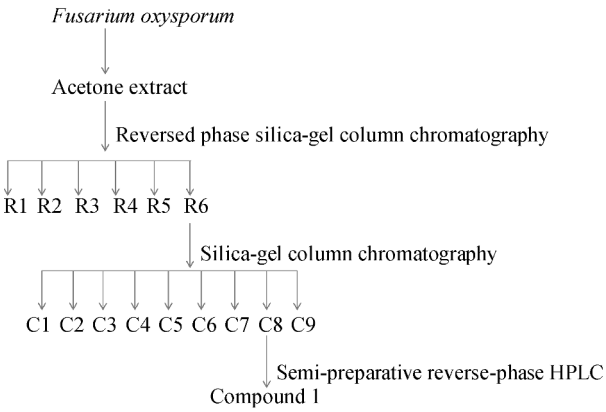

Fig. 3 Flow chart for the isolation of active compound 1 from *F. oxysporum*

Conclusion

In the present study, a fungus SIPI-4004 was isolated from the soil, and identified as *F. oxysporum* by the ITS sequence analysis. The column chromatography, semi-preparative reverse- phase HPLC methods were used to extract the bioactive compounds from the fermentation product of SIPI-4004. As a result, one active metabolite was isolated. The ^{1}H, ^{13}C NMR and mass data of the compound was compared with that of the reported for natural compounds in data bases. The results indicated that the compound found in the present study was already reported as H1-A. However, H1-A was first isolated from microorganisms and showed inhibitory activity against HCV NS3 protease.