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SECONDARY METABOLITES FROM A MARINE-DERIVED FUNGUS *Penicillium chrysogenum* 045-357-2

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ABSTRACT

Marine fungi represent a potential source for natural products in the future due to the incredible diversity of chemical compounds. In our previous investigation to search new antimicrobial agents from marine-derived fungi, we isolated fungal strain *Penicillium chrysogenum* 045-357-2 from a soft coral sample collected from Ca Na Bay, Ninh Thuan, Vietnam. The fungus showed high antibacterial activity and was selected for further study. By various chromatography separations, two compounds including andrastinA (**1**) and citreohybridonol (**2**) were obtained from the ethyl acetate extract of culture medium of this strain. Their chemical structures were determined by analysis of 1D and 2D NMR spectra and high-resolution mass spectroscopic data, as well as by comparison of the corresponding data to those previously reported in the literature. The compound **2** exhibited antibacterial activity towards *Bacillus cereus* ATCC 11778 and *Streptoccocus faecalis* ATCC 19433 with Minimal Inhibitory Concentration (MIC) values of 32 and 64 µg/ml, respectively; however, antibacterial activity was not detected in the compound **1**. This is the first report on these compounds of marine fungal strain *P. chrysogenum* isolated in Vietnam.

Keywords: Penicillium chrysogenum, andrastin A, antibacterial activity, citreohybridonol, marine fungi.

1. INTRODUCTION

The marine environment is a promising source for the isolation of new marine microbes including bacteria, fungi, actinomycetes, cyanobacteria and diatoms that are potential producers of bioactive natural products [1]. Among marine microorganisms, particularly fungi have been

shown to be a source of biologically active secondary metabolites [2]. Because of their characteristic properties with reference to temperature, nutrients, competition and salinity, the ways that they produce secondary metabolites are specific compared with terrestrial fungi [3].

Marine fungi associated with marine algae, sponges, corals and invertebrates are considered to be a rich source for secondary metabolites with antibiotic, antiviral, and antifungal activities [4].

In the previous experiment, we isolated and screened for antimicrobial activity of 100 fungal strains from various marine habitats at 3 different sites in Vietnam including Con Son Island, Tho Chu Island and Ca Na Bay. Among them, the strain *P. chrysogenum* 045-357-2 was isolated from an unidentified soft coral at Ca Na Bay exhibited high activity against pathogens [5]. There fore, the strain was analyzed further for causative secondary metabolites. As a result, andrastin A (**1**) [6] and citreohybridonol (**2**) [7] were obtained and identified from this fungus. Furthermore, these compounds were examined for antimicrobial activity.

2. MATERIAL AND METHODS

2.1. Fungal material

The fungus *P. chrysogenum* 045-357-2 was originally isolated from an unidentified soft coral at Ca Na Bay, Vietnam. The fungus was identified by Internal Transcribed Spacer (ITS) rDNA molecular methods (GenBank accession number EF200090). A BLAST search results indicated that the sequence is similar 100 % to the sequence of *P. chrysogenum*. The strain was currently preserved in the Marine Microorganism Collection, Nhatrang Institute of Technology Research and Application (NITRA).

2.2. General experimental procedures

1D and 2D spectroscopic data were recorded on a Varian Unity 500 NMR spectrometer (MCKinley, Sparta, NJ). HRESIMS data were obtained on a Shimadzu hybrid ion-trap time-of-flight mass spectrometer (Shimadzu, Kyoto, Japan). HPLC was conducted on a column 250 mm x 10 mm i.d., S-5 μm, 12 nm, YMC-Pack-ODS-A, with a PrimeLine Binary pump with RI-101 Shodex, RI detector (Shoko Scientific Co., Yokohama, Japan).

2.3. Fermentation, extraction and isolation

The fungal strain was grown stationary at 28 ^{o}C for 20 days in 45 Erlenmeyer flasks (500 ml), each flask containing 20 g of rice, 20 mg of yeast extract, 10 mg of KH_2PO_4, and 40 ml of natural seawater. At the end of the incubation period, the mycelia and medium were homogenized and extracted with ethyl acetate (EtOAc). The extract of the fungus was concentrated to dryness using rotary evaporators at 40 ^{o}C. The crude EtOAc extract (8.9 g) obtained was subjected to ODS open column chromatography (200 mm x 50 mm i.d., C18) followed by stepwise gradient elution with MeOH in H_2O (v/v) (10-100 %, 2L each) as the eluent.

The fraction eluted with MeOH in H_2O 70 % - 1 (271.2 mg) was utilized to purify compounds by analytical ODS HPLC (column: YMC-Pack-ODS-A, 250 mm x 4.6 mm i.d., 5

μm, flow rate: 1 ml/min; RI detector) using isocratic program with MeOH-H_2O (63:27, v/v) to yield compound **1** (7.6 mg) (Figure 1).

The white crystal from fraction eluted with MeOH in H_2O 50 % - 3 was washed many times with MeOH and collected one pure compound **2** (17.6 mg) (Figure 1).

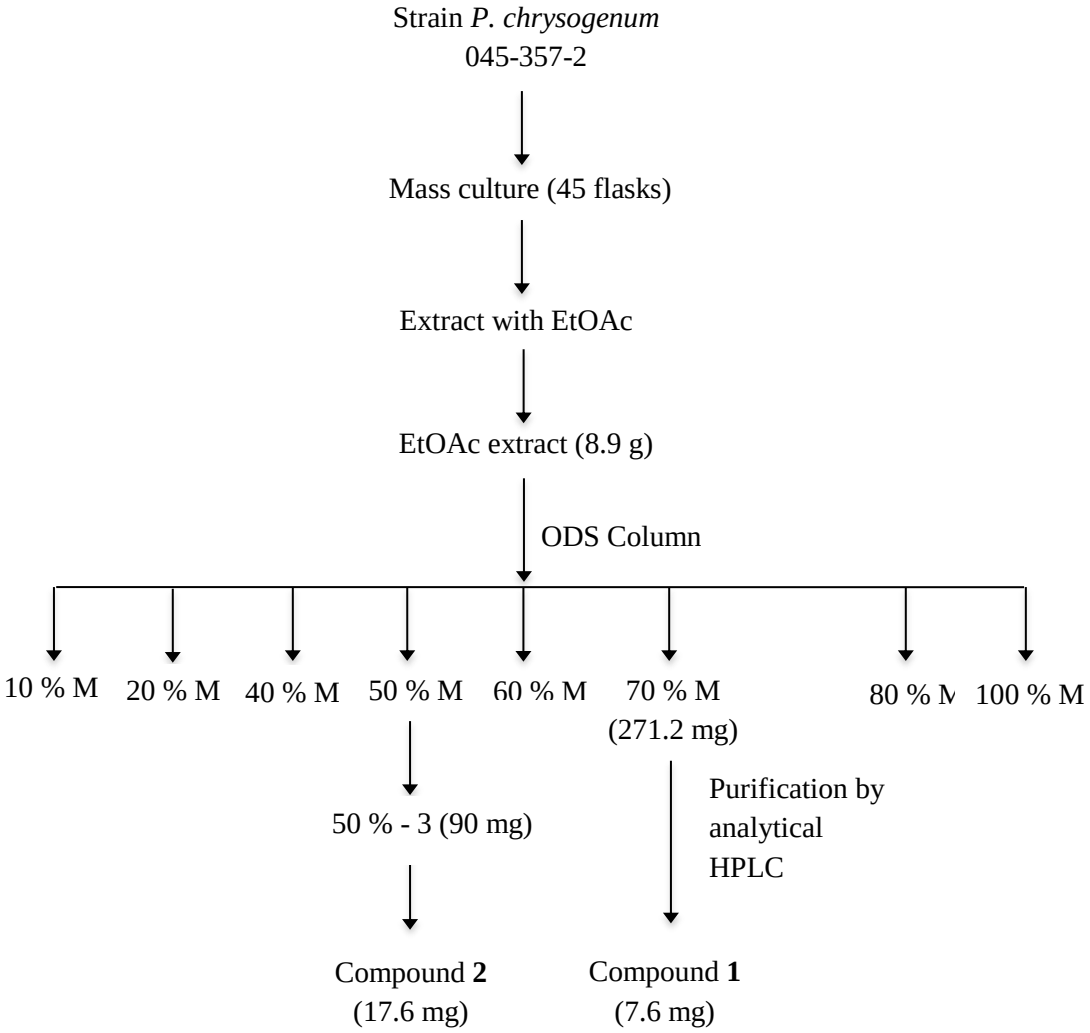

Figure 1. Isolation scheme of metabolites from the strain *P. chrysogenum* 045-357-2.

*2.3.1. Andrastin A (**1**)*

Brown amorphous solid; $[\alpha]_D$ – 116 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 262 (1.55), 206 (1.95) nm; IR (MeOH) ν_{max} 3457, 2954, 2883, 1713 cm^{-1};^{1}H and ^{13}C NMR (see Table 1); HRESIMS *m/z* 487.10 $[M + H]^+$, calcd. for $C_{28}H_{38}O_7$.

*2.3.2. Citreohybridonol (**2**)*

White amorphous solid; $[\alpha]_D$ +150 (*c* 0.2, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 258 (1.42), 232 (0.66) nm; IR ($CHCl_3$) ν_{max} 3472, 3209, 2954, 1624 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); HRESIMS *m/z* 501.26 $[M + H]^+$, calcd. for $C_{28}H_{36}O_8$.

2.4. Antibacterial assay

MIC values of the compounds were determined by the modified 0.5 McFarland standard method. Two-fold dilutions of the compounds in the range of (128 - 0.5 µg/ml) were prepared in DMSO. Streptomycin (Himedia, India) was used as a positive control. The turbidity of the bacterial suspensions (*B. cereus* and *S. faecalis*) was measured at 630 nm and adjusted with a medium to match the 0.5 McFarland standard (10^5-10^6 colony forming units/ml). Subsequently, 100 µl of bacterial culture was inoculated into each well, and the test solutions (100 µl) were added to 96-well plates. Finally, the plates were incubated at 36 ^oC for 24 h, and the MIC values were inspected as the lowest concentrations in which no microorganism growth could be observed [8].

3. RESULTS AND DISCUSSION

The crude EtOAc extract of mycelia and culture medium of the marine fungus *P. chrysogenum* 045-357-2 was subjected to ODS open column chromatography and HPLC to obtain two purified compounds. The structures of these compounds were determined by means of spectroscopic methods and comparison with literature data.

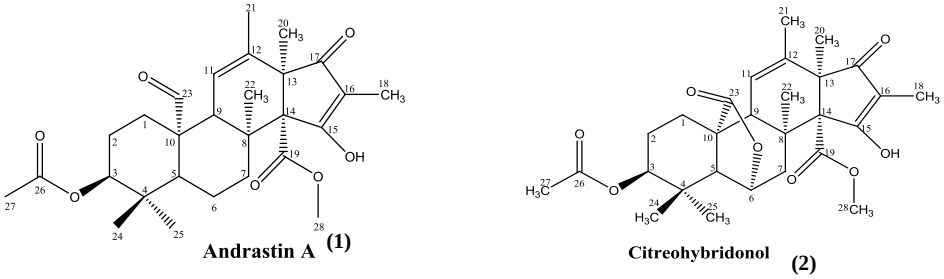

Figure 2. Chemical structures of compound **1** and **2**.

3.1. Andrastin A (1)

The ^1H-NMR spectrum of **1** showed 7 methyl protons singlets at δ_H 1.59 (3H, *s*, H-18), δ_H 1.15 (3H, *s*, H-20), δ_H 1.74 (3H, *s*, H-21), δ_H 1.23 (3H, *s*, H-22), δ_H 0.94 (3H, *s*, H-24), δ_H 0.87 (3H, *s*, H-25), 1 acetoxy group at δ_H 1.59 (3H, *s*, H-18) and 1 methoxy group at δ_H 3.58 (3H, *s*, H-28). In addition, the ^1H-NMR spectrum of this compound exhibited one proton olefinic at δ_H 5.38 (1H, *s*, H-11) (Table 1).

The ^{13}C NMR spectrum of **1** showed 28 carbon signals, including 1 aldehyde at δ_C 205.5 (C23), 1 ketone at δ_C 200.0 (C17), 02 ester at δ_C 170.8 (C19) and δ_C 170.6 (C26), 04 signals of double carbon at δ_C 122.0 (C11), δ_C 135.9 (C12), δ_C 185.0 (C15) and δ_C 112.7 (C16); an acetoxy methyl δ_C 19.7 (C27), a methyl ester δ_C 50.7 (C28), 2 signals of methyl geminal group δ_C 25.7 (C24) and δ_C 20.1 (C25), 02 signals of methyl vinyl δ_C 5.1 (C18) and δ_C 18.5 (C21) and 02 methyl groups attached to quaternary carbon at δ_C 14.6 (C20) and δ_C 18.4 (C22).

The HMBC spectrum of **1** indicated the correlations between the proton H-1 with C-5, C-9, C-23; between the proton H-3 with C-1, C-4; between the proton H-5 with C-4, C-6, C-7, C-10, C-23, C-24 và C-25; between the proton H-7 with C-6, C-8, C-9 and C-14; H-9 with C-8, C-10, C-11, C-12 and C-23; H-11 with C-8, C-9 and C13. Further, the HMBC spectrum indicated one –CHO group by the correlations between the protons H-1, H-5, and H-9 with C-23.

According to the ^{1}H, ^{13}C, HSQC and HMBC spectra (Figures 3, 4) and comparison these data in references [6], compound 1 was therefore identified as andrastin A and its structure was shown in Figure 2.

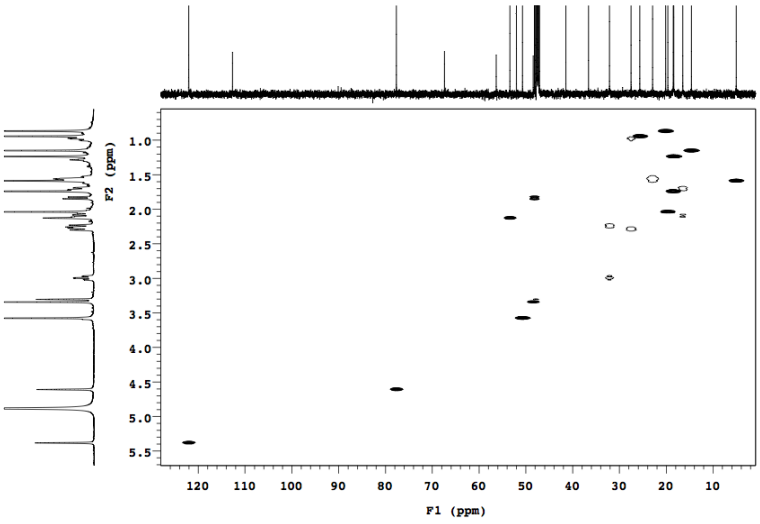

Figure 3. HSQC spectrum of compound **1** in CD_3OD.

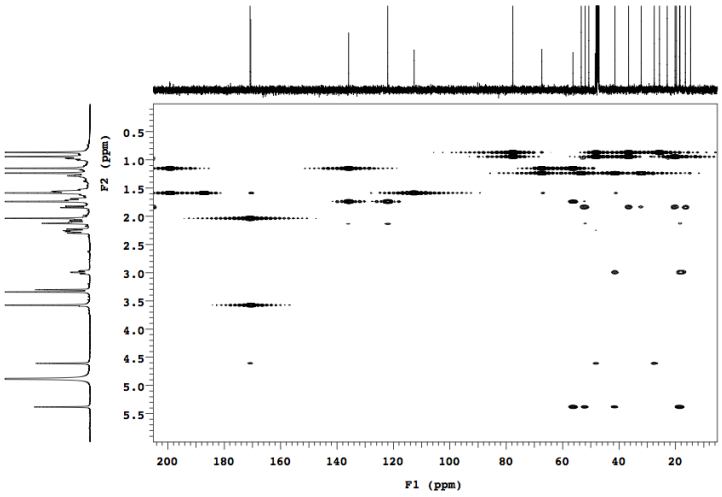

Figure 4. HMBC spectrum of compound **1** in CD_3OD.

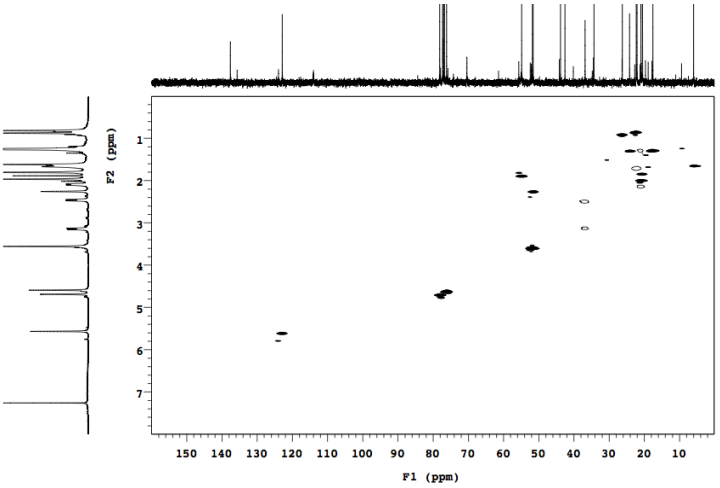

Figure 5. HSQC spectrum of compound **2** in $CDCl_3$.

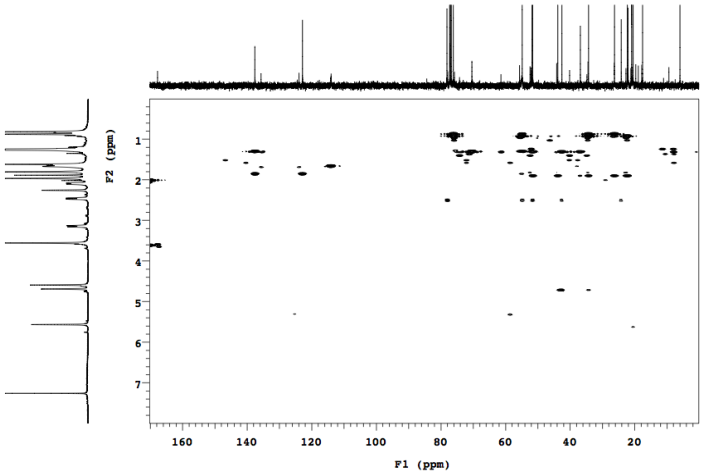

Figure 6. HMBC spectrum of compound **2** in $CDCl_3$.

3.2. Citreohybridonol (2)

The ^{1}H and ^{13}C NMR spectra of the compound **2** also showed the characteristic signals of the meroterpenoid frame and indicated many similar signals with compound **1**. However, aldehyde group of **1** at C-10 was replaced by lactone ring of **2**. According to the ^{1}H, ^{13}C, HSQC and HMBC spectra (Figures 5, 6) and the comparison of spectral data of **2** with the reported data [7] confirmed that **2** is citrohybridonol (Table 1).

Furthermore, compounds **1** and **2** were tested for their antibacterial activity on *B. cereus* and *S. faecalis*. The only compound **2** exhibited the antibacterial activities against *B. cereus* and *S. faecalis* with MIC values being 32 and 64 μg/ml, respectively; comparable to that of streptomycin (MIC 16 μg/ml) as positive control.

Table 1. ^{1}H and ^{13}C NMR data of compound **1** in CD_3OD and **2** in $CDCl_3$ (at 500 MHz for ^{1}H and 125 for MHz for ^{13}C).

Pos.	**1**		**2**	
	δ_H, J (Hz)	δ_C	δ_H, J (Hz)	δ_C
1	0.99 (1H, *s*) 2.28 (1H, *s*)	27.5	2.09 (1H, *d*, 11.5) 1.20 (1H, *d*, 6.5)	20.9
2	1.56 (2H, *s*)	22.9	1.65 (2H, *d*, 13.0)	22.1
3	4.61 (1H, *br*)	77.7	4.69 (1H, *br*)	78.1
4	-	36.6	-	34.3
5	1.83 (1H, *d*, 10.0)	48.4	1.87 (1H, *s*)	54.8
6	1.70 (1H, *m*) 2.08 (1H, *m*)	16.5	4.59 (1H, *br*)	76.2
7	2.24 (1H, *m*) 2.99 (1H, *m*)	32.1	2.46 (1H, *d*, 11.0) 3.14 (1H, *d*, 13.5)	36.8
8	-	41.5	-	42.5
9	2.11 (1H, *m*)	53.4	2.26 (1H, *s*)	51.6
10	-	52.0	-	43.8
11	5.38 (1H, *s*)	122.0	5.56 (1H, *s*)	122.1
12	-	135.9	-	137.6
13	-	56.3	-	61.9
14	-	67.4	-	70.0
15	-	178.0	-	179.0
16	-	112.7	-	114.0
17		200	-	208.0
18	1.59 (3H, *s*)	5.1	1.62 (3H, *s*)	6.0
19	-	170.8	-	170.4
20	1.15 (3H, *s*)	14.6	1.24 (3H, *s*)	17.5
21	1.74 (3H, *s*)	18.5	1.80 (3H, *s*)	20.5
22	1.23 (3H, *s*)	18.4	1.25 (3H, *s*)	24.2
23	10.18 (1H, s)	205.5	-	179.9
24	0.94 (3H, *s*)	25.7	0.87 (3H, *s*)	26.3
25	0.87 (3H, *s*)	20.1	0.82 (3H, *s*)	22.3
26	-	170.6	-	170.2
27	2.04 (3H, *s*)	19.7	1.96 (3H, *s*)	20.9
28	3.58 (3H, *s*)	50.7	3.56 (3H, *s*)	51.8

4. CONCLUSION

From the ethyl acetate extract of culture medium of a marine-derived fungus *P. chrysogenum* 045-357-2, we obtained andrastin A (**1**) and citreohybridonol (**2**). The chemical structure of the isolates was elucidated based on MS and NMR spectroscopy and compared with literature data. Compound **2** showed antibacterial activity towards *B. cereus* and *S. faecalis* with MIC values of 32 and 64 μg/ml, respectively. The other bioactivities of these compounds are going on.

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REFERENCES

1. Coleman J. J., Ghosh S., Okoli I. and Mylonakis E. - Antifungal Activity of Microbial Secondary Metabolites. PLoS ONE **6** (9) (2011) e25321. doi:10.1371/ journal.pone.0025321.
2. Gamal-Eldeen A. M., Abdel-Lateff A. and Okino T. - Modulation of carcinogen metabolizing enzymes by chromanone A; a new chromone derivative from algicolous marine fungus *Penicillium* sp. Environ Toxicol Pharmacol **28** (3) (2009) 317–322.
3. Liberra K. and Lindequist U. - Marine fungi-a prolific resource of biologically active natural products?, *Pharmazie* **50** (1995) 583–588.
4. Blunt J. W., Copp B. R., Munro M. H., Northcote P. T. and Prinsep M. R. - Marine natural products, Nat Prod Rep **23** (1) (2006) 26–78.
5. Ngoc N. T. D., Trinh P. T. H., Hang C. T. T., Thuan N. T., Trang V. T. D., Khanh H. H. N., Nhut N. D. and Van T. T. T. - Potential marine fungi from Viet Nam sea for antimicrobial activity, Journal of Science and Technology **54** (2B) (2016) 125–130.
6. Shiomi K., Uchida R., Inokoshi J., Tanaka H., Iwai Y. and Omura, S. - Andrastin A – C, new protein farnesyltransferase inhibitors, produced by *Penicillium* sp. FO-3929, Tetrahedron Letters **37** (1996) 1265–1268.
7. Kosemura S., Matsunaga K.,Yamamura S., Kubota M. and Ohba S. – The structures of citreohybridones A and B novel sesterterpenoid-type metabolites of a hybrid strain KO 0031 derived from *Penicillium citreo-viride* B. IFO 6200 and 4692, Tetrahedron Letters **32** (1992) 3543 – 3546.
8. Al-Burtamani S. K. S., Fatope M. O., Marwah R. G., Onifade A. K. and Al-Saidi S. H. - Chemical composition, antibacterial and antifungal activities of the essential oil of *Haplophyllum tuberculatum* from Oman, J Ethnopharmacol **96** (2005) 107–112.