

Rec. Nat. Prod. 6:4 (2012) 334 -338

records of natural products

# Three New Compounds from the Marine Fungal Strain Aspergillus sp. AF119

# Shaosong Liu, Chunhua Lu<sup>\*</sup>, Jingjing Huang and Yuemao Shen

Key Laboratory of the Ministry of Education for Cell Biology and Tumor Cell Engineering, Xiamen Engineering Research Center of Marine Microbial Drug Discovery, Fujian Engineering Laboratory for Pharmaceuticals; School of Life Sciences, Xiamen University, No.422 South Siming Road, Xiamen, Fujian 361005, P. R. China

(Received Octobert 11, 2010; Revised Octobert 9, 2011; Accepted January 2, 2012)

Abstract: Three new compounds, namely barceloneic lactone B (1) and barceloneic acid C (2) and 5'hydroxychlorflavonin (3), together with one known compound chlorflavonin (4), were isolated from the marine fungal strain *Aspergillus* sp. AF119. Their structures were elucidated by spectroscopic analyses including 1D- and 2D NMR experiments, and HR Q-TOF mass spectrometry. The antifungal activities against *Candida albicans* of these compounds were evaluated.

Keywords: Barceloneic lactone; barceloneic acid; chlorflavonin; Aspergillus sp. AF119.

# **1. Introduction**

Marine microorganisms are widely recognized as rich sources of novel natural products [1-3]. In recent years, numerous novel compounds discovered from marine fungi have been reported [4-6]. During the course of our search for biologically active substances from marine fungi, a strain of the genus *Aspergillus* was selected because of its remarkable antifungal activity. Herein, we report the isolation and structure determination of three new metabolites (1-3).

# 2. Materials and Methods

# 2.1. Microorganism Material

The fungus strain AF119 was isolated from the soil of Xiamen beach. It was identified as *Aspergillus* sp. by traditional morphology and ITS sequencing. The sequencing of the strain AF119

<sup>&</sup>lt;sup>\*</sup> Corresponding author: E-Mail: <u>ahua0966@xmu.edu.cn</u>; Phone:086-592-2184180 *Fax*:086-592-2181722

was cloned and sequenced (GenBank accession number: AY373842.1). The blast search result showed that the sequence of strain AF119 was 99% homologous to species of *Aspergillus candidus* ATCC 1002.

#### 2.2 Fermentation and Isolation

The fermentation was cultured in *petri dishes* laid with ca. 20 ml half sea water PDA medium for 15 d at 28°C. The culture material (total 7 L) was extracted with EtOAc thrice. The solvent was evaporated under reduced pressure to afford 7.2 g of crude extract. The crude extract was subjected to MPLC over RP-18 (170 g) using a stepwise gradient of 30, 50, 70, and 100% (v/v) acetone in H<sub>2</sub>O to afford Fr.1 (750 mg), Fr.2 (1.08 g), Fr.3 (320 mg) and Fr.4 (220 mg), obtained from 30% acetone, and Fr.5 (490 mg) and Fr.6 (65 mg), from 50% acetone. Fr.1 was subjected to *Sephadex LH-20* column twice, and eluted with MeOH and acetone, respectively, and then it was fractionated on silica gel CC (200mg, CHCl<sub>3</sub>/MeOH) to yield **1** (5 mg). Fr.3 was dissolved in MeOH and filtered. Filtrate was subjected to *Sephadex LH-20* eluted with acetone, then purified by CC (silica gel, 500mg, CHCl<sub>3</sub>/MeOH) to yield **2** (10 mg). Fr.5 was subjected to *Sephadex LH-20* eluted with acetone, then purified by CC (silica gel, 150mg, petroleum ether/EtOAc) to yield **4** (3 mg). Fr.6 was subjected to *Sephadex LH-20* column twice, and eluted with MeOH and eluted with MeOH and acetone, respectively, and then it was fractionated on silica gel CC (150mg, petroleum ether/AcOEt) to yield **3** (3 mg).



Figure 1. The structures of compounds 1-4.

#### 3. Results and Discussion

#### 3.1 Structure elucidation

Compound **1** was obtained as white amorphous powder. The molecular formula was determined to be  $C_{16}H_{14}O_6$  according to the HR Q-TOF MS data (m/z 325.0704 [M + Na]<sup>+</sup>) and NMR data (*Table 1*). The IR spectra showed the absorptions for hydroxyl (3341 cm<sup>-1</sup>) and ester carbonyl (1731 cm<sup>-1</sup>) groups. Inspection of the <sup>1</sup>H and <sup>13</sup>C NMR (DEPT) spectra of **1** (*Table 1*) revealed the presence of one OMe, two oxygenated CH<sub>2</sub>, five CH and eight quaternary C-atoms including a carbonyl carbon atom at  $\delta$  166.8 [C(1)]. The <sup>1</sup>H NMR and HSQC spectra indicated the presence of two hydroxyl groups at  $\delta$  9.71 (br s) and  $\delta$  5.15 (t, J = 5.7), which suggested the presence of one free hydroxyl and one hydroxymethyl, and were assigned to C(9) and C(15) on the basis of the HMBC correlations.

The presence of a trisubstituted benzene ring was indicated by the <sup>1</sup>H and <sup>13</sup>C long - range correlations from the protons at  $\delta$  6.79 (d, J = 8.3), 7.09 (d, J = 8.3) and 7.57 (t, J = 8.3) to corresponding carbons (What do you mean with corresponding carbons on HMBC – The coupling

constants directly prove 1,2,3-trisubstituted benzene ring). The methoxyl substitution at C(3) was indicated by the HMBC correlations between Me protons (3a) and C(3).

The presence of another tetrasubstituted benzene ring was indicated by two singlets at  $\delta$  6.56 and 6.92 in the <sup>1</sup>H NMR, and six aromatic carbons at  $\delta$  142.8 [C(8)], 149.1 [C(9)], 116.2 [C(10)], 139.7 [C(11)], 117.8 [C(12)] and 127.7 [C(13)] in the <sup>13</sup>C NMR spectra. The protons of another oxymethylene H-C(14) at  $\delta$  5.05 (s, 2H) showed long range correlations to three quarternary carbons at  $\delta$  127.7 [C(12)], 142.8 [C(8)] and 166.8 [C(1)].

The oxygen bridge between C(8) and C(7) positions was deduced based on the downfield shifts of C(8) ( $\delta$  142.8) and C(7) ( $\delta$  152.8) carbons.

Thus, the structure of **1** was established to be 4-methoxy-11-hydroxy-9-hydroxymethyl-5H,7H-dibenzo[b,g][1,5]dioxocin-5-one [7], known as barceloneic lactone B.

Compound **2** was obtained as white amorphous powder. Mass spectral analysis of **2** HR Q-TOF MS gave a molecular weight of 302 daltons implying same molecular formula of  $C_{I6}H_{I4}O_6$  (*m/z*: 325.0780 [*M* + Na]<sup>+</sup>) as compound **1**. The presence of a carboxylic acid group was further supported by a <sup>13</sup>C-NMR signal at  $\delta$  166.3 and IR band at 1692 cm<sup>-1</sup>. The <sup>13</sup>C NMR (DEPT) spectrum of **2** (*Table 1*) showed 16 signals: one Me, one OMe, six olefinic CH, and eight quaternary C-atoms. The <sup>1</sup>H NMR spectrum demonstrated the presence of one aromatic Me at  $\delta$  2.31 (s), one methoxyl at  $\delta$  3.81 (s), five aromatic protons at  $\delta$  7.22 (t, *J* = 8.4), 7.12 (s), 7.09 (s), 6.77 (d, *J* = 8.4) and 6.09 (d, *J* = 8.4), and an aldehyde proton at  $\delta$  10.11 (s).

No	1		2	2	
INO. –	$\delta_{\rm H}$ (mult., J in Hz)	$\delta_{\rm C}$	$\delta_{\rm H}$ (mult., J in Hz)	$\delta_{\rm C}$	
1		166.8 (C)		166.3 (C)	
2		115.6 (C)		114.1 (C)	
3		157.2 (C)		156.5 (C)	
4	7.09 (d, J = 8.3)	110.0 (CH)	6.77 (d, J = 8.4)	105.4 (CH)	
5	7.57 $(t, J = 8.3)$	134.3 (CH)	7.22 (t, J = 8.4)	130.4 (CH)	
6	6.79 (d, J = 8.3)	114.7 (CH)	6.09 (d, J = 8.4)	106.5 (CH)	
7		152.8 (C)		155.3 (C)	
8		142.8 (C)		141.9 (C)	
9		149.1 (C)		150.5 (C)	
10	6.92 (s)	116.2 (CH)	7.12 (s)	123.8 (CH)	
11		139.7 (C)		136.2 (C)	
12	6.56 (s)	117.8 (CH)	7.09(s)	117.8 (CH)	
13		127.7 (C)		129.4 (C)	
14	5.05 (s)	68.9 (CH <sub>2</sub> )	10.11 (s)	189.4 (CH)	
15	4.35 (d, J = 5.7)	62.6 (CH <sub>2</sub> )	2.31 (s, 3H)	20.5 (CH <sub>3</sub> )	
3a	3.86 (s, 3H)	56.7 (CH <sub>3</sub> )	3.81 (s, 3H)	56.0 (CH <sub>3</sub> )	
OH-9	9.71 (br <i>s</i> )				
OH-15	5.15 ( <i>t</i> , <i>J</i> = 5.7)				

**Table 1.** <sup>1</sup>H and <sup>13</sup>C NMR data of **1** and **2** (recorded at 600 / 150MHz, respectively, in DMSO-d<sub>6</sub>;  $\delta$  in ppm).

The structure of **2** was established on the basis of HMQC and HMBC experiments similar to those described for **1**. The presence of a trisubstituted benzene ring was indicated by two doublets and a triplet with the equal *J* values of 8.4 Hz at  $\delta$  6.09, 6.77 and 7.22 in the <sup>1</sup>H NMR, and HMBC correlations from H-C(4) to C(2), C(6), H-C(5) to C(3), C(7), and H-C(6) to C(2), C(4),. The positions of OMe at C(3), the COOH at C(2), and an ether linkage at C(7) on the benzene ring were deduced from abovementioned data together with the observed downfield shifts.

The presence of another tetrasubstituted benzene ring was indicated by two singlets at  $\delta$  7.09 and 7.12 in the <sup>1</sup>H NMR, and six carbon signals from C(8) to C(13). The HMBC correlations from Me(15) to C(10), C(11) and C(12) suggested that Me was at C(11). The aldehyde group was located at

C(13) according to the HMBC correlations from H-C(14) to C(12) and C(13). Detailed inspection of the 2D-NMR spectra, and the abovementioned data indicated that the skeleton of compound **2** was similar to that of compound **1**, and a linkage between C-7 and C-8 through an ether bond was evident.

Thus, the structure of **2** was established to be 6-methoxyl-2-(4-methyl-2-hydroxy-6-formyl)-benzoic acid, previously reported as barceloneic acids C [7].

Compound **3** was obtained as yellow amorphous powder. The molecular formula was determined as  $C_{18}H_{15}ClO_8$  according to the HR Q-TOF MS ( $[M + H]^+$  at m/z 395.0948) and NMR data (*Table 2*). The structure of **3** was determined by comparison of its spectral properties with those of CJ-19784 [8] and chlorflavonin [9]. The <sup>13</sup>C NMR (DEPT) spectrum of **3** (*Table 2*) showed 18 signals: three methoxyl groups, three olefinic CH, twelve quaternary C-atoms. A comparison of the NMR data of **3** with those of chlorflavonin indicated that **3** was the 5'-hydroxyl derivative of chlorflavonin. Therefore, we determined the structure of **3** to be 5'-hydroxychlorflavonin.

Compound 4 was determined as chlorflavonin by comparison of its spectral properties with those reported [8-9] and its HR Q-TOF MS ( $[M + H]^+$  at m/z 379.0853).

**Table 2.** <sup>1</sup>H and <sup>13</sup>C NMR data of **3** and **4**. Recorded at 600 /150 MHz in acetone– $d_6$ (**3**) and DMSO- $d_6$ (**4**);  $\delta$  in ppm, *J* in Hz.

No	4		3	
	$\delta_{\rm H}$ (mult., J in Hz)	$\delta_{\rm C}$	$\delta_{\rm H}$ (mult., J in Hz)	$\delta_{\rm C}$
2		156.1 (C)		155.4 (C)
3		139.9 (C)		139.3 (C)
4		179.1 (C)		178.9 (C)
5		157.0 (C)		157.4 (C)
6	6.64 ( <i>s</i> )	96.3 (CH)	6.54 ( <i>s</i> )	95.6 (CH)
7		158.7 (C)		158.9 (C)
8		128.9 (C)		129.0 (C)
9		149.2 (C)		149.2 (C)
10		105.7 (C)		105.4 (C)
1'		120.3 (C)		122.4 (C)
2'		151.4 (C)		144.1 (C)
3'		122.1 (C)		120.4 (C)
4'	7.59 (d, J = 8.0)	119.2 (CH)	7.11 ( <i>d</i> , <i>J</i> = 2.8)	119.2 (CH)
5'	7.02 (t, J = 8.0)	120.7 (CH)		150.5 (C)
6'	7.42 (d, J = 8.0)	130.0 (CH)	7.05 ( $d$ , $J$ = 2.8)	115.6 (CH)
3a	3.71 (s, 3H)	57.1 (CH <sub>3</sub> )	3.84 ( <i>s</i> , 3H)	60.3 (CH <sub>3</sub> )
7a	3.92 (s, 3H)	60.6 (CH <sub>3</sub> )	3.98 (s, 3H)	56.0 (CH <sub>3</sub> )
8a	3.70 (s, 3H)	61.4 (CH <sub>3</sub> )	3.78 (s, 3H)	60.7 (CH <sub>3</sub> )
HO-5	12.42 (br <i>s</i> )		12.43 (br <i>s</i> )	
HO-2'	10.08 (br <i>s</i> )		8.19 (br <i>s</i> )	
HO-5'			8.63 (br <i>s</i> )	

#### 3.2 Antifungal activity

Barceloneic acids A and B, and barceloneic lactone were isolated from a fungus of genus *Phoma* and reported as FPTase inhibitor [7]. Chlorflavonin exhibits a remarkable degree of specificity in its antimicrobial activity [9]. In our antifungal activity tests, compounds 1-4 exhibited no evident antifungal activities against *Candida albicans* at 30  $\mu$ g/disc in agar diffusion assay.

# Acknowledgments

This work was financially supported by the Key Grant of the Chinese Ministry of Education (No.

306010), and the National Ocean 863 Foundation of China (No. 2006AA092410).

#### **Supporting Information**

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

# References

- [1] J. W. Blunt, B. R. Copp, W. P. Hu, M. H. Munro, P. T. Northcote and M. R. Prinsep (2009). Marine natural products. *Nat. Prod. Rep.* 26, 170-244.
- [2] T. S. Bugni and C. M. Ireland (2004).Marine-derived fungi: a chemically and biologically diverse group of microorganisms. *Nat. Prod. Rep.* **21**, 143-163.
- [3] M. Saleem, M. S. Ali, S. Hussain and W. S. Zheng (2005). Cytotoxic and antimicrobial metabolites from marine lignicolous fungi, Diaporthe sp. *FEMS Microbiol Lett.* **251**, 53-58.
- [5] Q. Y. Xu (2004). Metabolites from mangrove endophytic fungus *Dothiorella sp. Acta Oceanologica Sinica.* 23, 541-547.
- [6] Y. Zhan, X. Du, H. Chen, J. Liu, B. Zhao, D. Huang, G. Li, Q. Xu, M. Zhang, B. C. Weimer, D. Chen, Z. Cheng, L. Zhang, Q. Li, S. Li, Z. Zheng, S. Song, Y. Huang, Z. Ye, W. Su, S. C. Lin, Y. Shen and Q. Wu (2008). Cytosporone B is an agonist for nuclear orphan receptor Nur77, *Nat Chem Biol.* 4, 548-556.
- H. Jayasuriya, R. G. Ball, D. L. Zink, J. L. Smith, M. A. Goetz, R. G. Jenkins, M. Nallin-Omstead, K. C. Silverman, G. F. Bills and R. B. Lingham (1995). Barceloneic acid A, a new farnesyl-protein transferase inhibitor from a Phoma species. *J Nat Prod.* 58, 986-991.
- [8] S. Watanabe, H. Hirai, Y. Kato, H. Nishida, T. Saito, N. Yoshikawa, T. Parkinson and Y. Kojima (2001). CJ-19,784, a new antifungal agent from a fungus, *Acanthostigmella sp. J Antibiot.* **54**, 1031-1035.
- [9] M. Richards, A. E. Bird and J. E. Munden (1996). Chlorflavonin, a new antifungal antibiotic, *J Antibiot*, **22**, 388-389.



© 2012 Reproduction is free for scientific studies