

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

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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

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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 δ 6.56 and 6.92 in the ^1H NMR, and six aromatic carbons at δ 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 ^{13}C NMR spectra. The protons of another oxymethylene H-C(14) at δ 5.05 (s, 2H) showed long range correlations to three quaternary carbons at δ 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) (δ 142.8) and C(7) (δ 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 $\text{C}_{16}\text{H}_{14}\text{O}_6$ (m/z : 325.0780 [$M + \text{Na}$] $^+$) as compound **1**. The presence of a carboxylic acid group was further supported by a ^{13}C -NMR signal at δ 166.3 and IR band at 1692 cm^{-1} . The ^{13}C NMR (DEPT) spectrum of **2** (Table 1) showed 16 signals: one Me, one OMe, six olefinic CH, and eight quaternary C-atoms. The ^1H NMR spectrum demonstrated the presence of one aromatic Me at δ 2.31 (s), one methoxyl at δ 3.81 (s), five aromatic protons at δ 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 δ 10.11 (s).

Table 1. ^1H and ^{13}C NMR data of **1** and **2** (recorded at 600 / 150MHz, respectively, in $\text{DMSO-}d_6$; δ in ppm).

No.	1		2	
	δ_{H} (mult., J in Hz)	δ_{C}	δ_{H} (mult., J in Hz)	δ_{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_2)	10.11 (s)	189.4 (CH)
15	4.35 (d, $J = 5.7$)	62.6 (CH_2)	2.31 (s, 3H)	20.5 (CH_3)
3a	3.86 (s, 3H)	56.7 (CH_3)	3.81 (s, 3H)	56.0 (CH_3)
OH-9	9.71 (br s)			
OH-15	5.15 (t, $J = 5.7$)			

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 δ 6.09, 6.77 and 7.22 in the ^1H 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 δ 7.09 and 7.12 in the ^1H 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 C₁₈H₁₅ClO₈ 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 ¹³C 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. ¹H and ¹³C NMR data of **3** and **4**. Recorded at 600 /150 MHz in acetone-*d*₆(**3**) and DMSO-*d*₆(**4**); δ in ppm, J in Hz.

No.	4		3	
	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_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 (s)	96.3 (CH)	6.54 (s)	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 (<i>d</i> , $J = 8.0$)	119.2 (CH)	7.11 (<i>d</i> , $J = 2.8$)	119.2 (CH)
5'	7.02 (<i>t</i> , $J = 8.0$)	120.7 (CH)		150.5 (C)
6'	7.42 (<i>d</i> , $J = 8.0$)	130.0 (CH)	7.05 (<i>d</i> , $J = 2.8$)	115.6 (CH)
3a	3.71 (<i>s</i> , 3H)	57.1 (CH ₃)	3.84 (<i>s</i> , 3H)	60.3 (CH ₃)
7a	3.92 (<i>s</i> , 3H)	60.6 (CH ₃)	3.98 (<i>s</i> , 3H)	56.0 (CH ₃)
8a	3.70 (<i>s</i> , 3H)	61.4 (CH ₃)	3.78 (<i>s</i> , 3H)	60.7 (CH ₃)
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 μ g/disc in agar diffusion assay.

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Supporting Information

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

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