ORIGINAL ARTICLE

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Rec. Nat. Prod. 18:1 (2024) 136-142

records of natural products

A New Ascochlorin Glycoside from Brittlestar-Derived Fungus *Acremonium* sp. and Its Biological Activities

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(Received October 25, 2023; Revised January 17, 2024; Accepted January 21, 2024)

Abstract: A new ascochlorin glycoside (**1**) and 8 known ascochlorin derivatives (**2**–**9**), and ilicicolin H (**10**) were purified from brittlestar derived fungus *Acremonium* sp. GXIMD02024. Their structural elucidations were carried out by analyses of 1D and 2D NMR, high resolution mass spectrometry, and CD spectroscopic data and comparison of those with literature data. Compound **1** was a rare fungal polyketide-sesquiterpenoid possessing sugar moiety. The antibacterial and cytotoxicity activities of compounds **1**–**10** have been performed *in vitro*. Compounds **1**–**3**, **7**, **8**, and **10** were measured for their inhibitory effect on α-glucosidase. Compound **1** exhibited poor activity or was inactive, but the detail analysis indicated sugar moiety in **1** maybe play an important role in effect on biological activities.

Keywords: Ascochlorin glycoside; *Acremonium* sp; α-glucosidase inhibitory activity; marine fungus.

1. Introduction

Acremonium fungi were rich sources of novel and bioactive secondary metabolites [1]. It was the most predominant producers of ascochlorin derivatives which are orsellinic acid-sesquiterpene hybrids [2]. The first ascochlorin derivative, ascochlorin, was identified from the fungus *Ascochyta viciae* Libert in 1968 [3], which was re-identified as *Acremonium sclerotigenum* by Hijikawa et al in 2017 [4]. Many ascochlorin derivatives have subsequently been obtained from other genus of filamentous fungi such as *Fusarium*, *Cylindrocarpon*, *Nectria*, *Neonectria*, *Cylindrocladium*, *Verticillium*, *Microcera*, and *Stilbella* [5-12]. They exhibited various biological activities, including antiviral, antitumor, antimicrobial, anti-inflammatory, antibacterial, hypolipidemic, farnesyl-protein transferase inhibitory, hDHODH inhibitory as well as testosterone 5α-reductase inhibitory activities [2]. Due to their promising bioactivities, ascochlorin derivatives had also attracted considerable attention from biologists. Li et al identified the gene cluster of *Stachybotrys bisbyi* PYH05-7 responsible for the biosynthesis of LL-Z1272β in 2016 [13]. The biosynthetic pathway of ascochlorin derivatives,

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The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products January-February 2024 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.438.12310.2940
Available online: January 29, 2024

ascofuranone and ascochlorin produced by *Acremonium egyptiacum*, was completely analyzed in 2019 [14].

Recently, we focused on the secondary metabolites of *Acremonium* sp. GXIMD02024 derived from the *Gymnolophus obscura*. The fungal strain *A.* sp. GXIMD02024 displayed abundant metabolites by analysis of high-performance liquid chromatography. Further chromatographic isolation led to the discovery of 10 compounds, including a new ascochlorin glycoside (**1**), 8 known ascochlorin derivatives (**2**–**9**), and ilicicolin H (**10**). In the present study, we discuss structural elucidation of **1** by analyzing its NMR, MS, and CD spectroscopic data. *In vitro* bioactivity assays of the isolated compounds were also carried out.

2. Materials and Methods

2.1. Microorganism Material

The fungal strain *A.* sp. GXIMD02024 was isolated from the *Gymnolophus obscura* collected at Xisha islands of Hainan Province, China. It was identified as *Acremonium* sp. based on the analysis of ITS rRNA gene sequence. It was deposited in GenBank as accessions OQ430714 and in the culture collection (No. GXIMD02024) at Institute of Marine Drug, Guangxi University of Chinese Medicine.

2.2. Fermentation and Isolation

The strain *A.* sp. GXIMD02024 was inoculated on PDA plate and grown at 25 ℃ for 4 days. The agar plugs containing mycelium were inserted into a flask contained 200 mL of liquid medium (1.5% malt extract, and 3% sea salt). The inoculated medium was incubated by the shaking culture method at 180 rpm at 25 ℃. After 5 days, it was used as seed culture. The seed culture (10 mL) was transferred into a 1000 mL flask containing rice medium (80 g rice, 100 mL distilled water, and 3 g sea salt). All fermentations were performed in 100 flasks at 25 ℃ for 35 days statically.

The *A.* sp. GXIMD02024 were extracted repeatedly with EtOAc (5 × 15 L), and the EtOAc solvent was removed by vacuum to give the metabolite extracts (50.0 g). The extracts were separated by the silica gel column chromatography (CC) using CH_2Cl_2-MeOH gradient elution to obtain four subfractions (Fr.1−Fr.4). Fr. 1 was chromatographed over ODS CC using MeOH-H_2O (80 : 20 ~ 100 : 0, v/v) as mobile phase to obtain six subfractions (Fr.1.1 ~ Fr.1.6). Fr.1.6 was chromatographed over silica gel CC with a gradient elution of petroleum ether-EtOAc (30 : 1 ~ 0 : 1, v/v) and then by semi-preparative HPLC (acetonitrile : H_2O = 75 : 25, v/v) to yield compounds **3** (85.0 mg), **5** (87.0 mg), and **8** (24.5 mg). Fr. 2 was chromatographed on ODS CC eluted by MeOH-H_2O (70 : 30 ~ 0 : 100, v/v) to give four subfractions (Fr. 2.1− Fr. 2. 4). Fr. 2. 2 was subjected to semi-preparative RP-HPLC by elution of acetonitrile-H_2O (55 : 45, v/v) to give **2** (105.3 mg). Fr. 2. 4 was chromatographed by semi-preparative RP-HPLC (acetonitrile : H_2O = 67 : 33, v/v) to yield compounds **4** (20 mg), **6** (43 mg), **7** (4.3 mg), and **9** (46 mg). Subfraction Fr. 3 was chromatographed over ODS CC with a mobile phase of MeOH-H_2O (80 : 20 ~ 100 : 0, v/v) and then semi-preparative RP-HPLC (acetonitrile : H_2O = 56 : 44, v/v) to yield **10** (23.6 mg). ODS CC was used to separated subfraction Fr. 4 with a mobile phase of MeOH-H_2O (20 : 80 ~ 100 : 0, v/v) , and then semi-preparative RP-HPLC using acetonitrile-H_2O(56 : 44, v/v) as mobile phase to afford **1** (2.5 mg).

2.3. α-Glucosidase Inhibitory Assays

The α-glucosidase inhibitory activity of compounds was measured as described in previous reports [15]. Briefly, 20 μL of test compounds (10 mM) and 20 μL of 0.2 U/mL α-glucosidase were transferred into microplates. The mixture was incubated at 37 ℃. After 5 min, 20 μL of p-NPG (2.5 mM) was added and further incubated for 15 min. Acarbose was used as a positive control. The absorbances under 405 nm were recorded using a microplate reader. The assay was performed tree times.

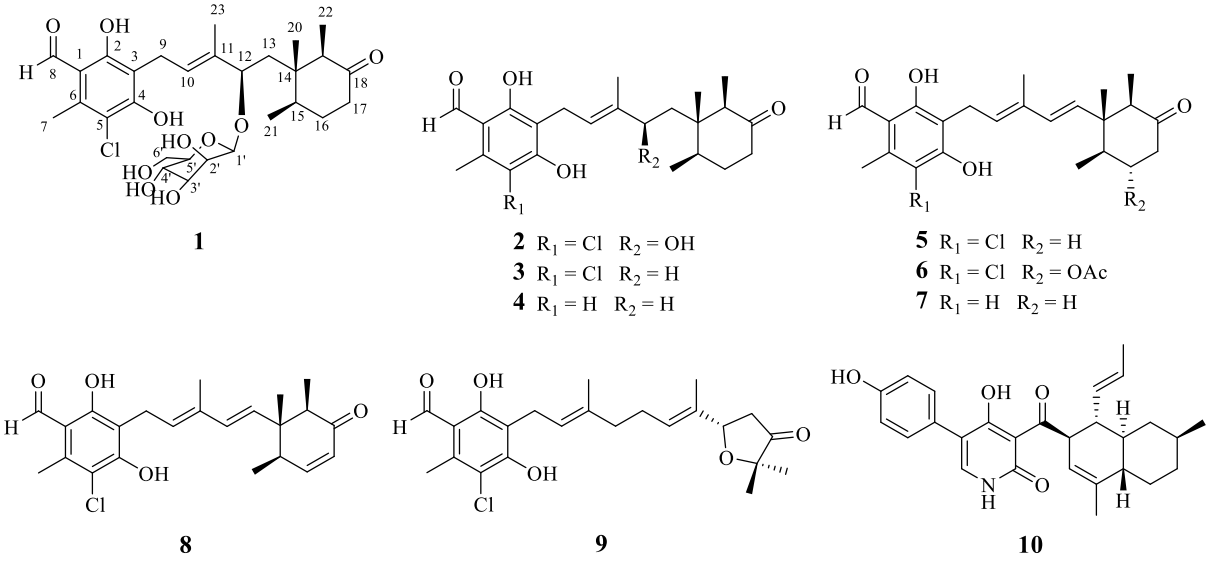

Figure 1. Structures of **1**–**10** isolated from fungus *Acremonium* sp. GXIMD02024

2.4 Cytotoxic Assays

The cytotoxic assay of **1**–**10** was performed by using MTT assays as previously reported protocol [16]. The cytotoxic assays against two pancreatic cancer cell lines (SW1990 and PANC1) and human colorectal carcinoma cells DLD1 was tested.

2.5 Antibacterial Assays

The antibacterial assay was performed as the previously reported method [17]. Briefly, Compounds **1** – **10** was prepared in DMSO, and then were mixed with culture broth to obtain initial concentration. The different concentrations of compounds were prepared by a serial dilution with culture broth. 100 μL/well of bacteria solution (1.0×10^5 CFU/mL) were added. Ciprofloxacin was used as the positive control. LB broths without tested compounds and bacteria and were control groups. Plates were visually read after static incubation at 37 ℃ for 20 h. MIC value was determined to be the minimum antimicrobial concentration with invisible growth of bacteria. The assay was performed in duplicate for each microorganism.

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** was characterized by the white amorphous powder. Its molecular formula, $C_{29}H_{41}ClO_{10}$, was determined based on HR-ESI-MS *m/z* 607.2289 $[M + Na]^+$ (calculated for $C_{29}H_{41}ClNaO_{10}$, 607.2286), indicating 9 degrees of unsaturation. The ^{1}H NMR spectrum of **1** showed signals of five methyl groups at δ_H 0.51, 0.69, 0.97, 1.83 and 2.55, one olefinic triplet (δ_H 5.57), one anomeric proton (δ_H 4.61), and one proton of aldehyde group (δ_H 10.04). The ^{13}C and HSQC spectra of **1** exhibited signals of 29 carbons, including 23 carbons assigned to an ascochlorin moiety and 6 belonged to a sugar moiety. Detail analysis of the ^{1}H and ^{13}C NMR data (Table 1) suggested that the presence of the ascochlorin backbone highly similar to 4', 5'-dihydro-4'-hydroxyascochlorin (**2**), as well as an additional sugar moiety. The connectivity of C-1' to C-6' located at sugar unit was established based on the analyses of HSQC, HMBC, and COSY spectra. It was observed that the sugar moiety was attached to the position of C-12 by the HMBC correlations between H-12 (δ_H 4.16) and C-1' (δ_C 99.4), and between H-1' (δ_H 4.45) and C-12 (δ_C 83.0), which was also supported by the NOESY

correlation of H-1' (δ_H 4.45) with H-12 (δ_H 4.16). The planar structure of **1** was confirmed by detail analysis of HMBC and ^{1}H-^{1}H COSY spectral as showed in figure 1. The double bond between C10 and C-11 was defined as (*E*)-geometry by the key NOESY correlations of H-23 (δ_H 1.83, 3H, s) to H-9 (δ_H 3.40), and H-10 (δ_H 5.57) to H-12 (δ_H 4.16), which was in line with that of **2**. As shown in the figure 2, the relative configuration of the cyclohexanone moiety was unambiguously established by analyses of coupling constants and NOESY correlations. The double quartet (J = 12.5, 5.0 Hz) of the methylene protons located at C-16 (δ_H 1.50) suggested the cyclohexanone ring was chair conformation and the proton was axial orientation. The methyl group suited at C-20 was assigned as axial orientation by NOESY correlation between H-16 (δ_H 1.50) and H-20 (δ_H 0.51). The key NOESY correlations of H-20 (δ_H 0.51) with H-21 (δ_H 0.97) and H-22 (δ_H 0.69) revealed that the methyl groups at C-20, C-21 and C-22 were cofacial and β-orientation. NOESY correlation of H-15 (δ_H 2.21) with H-19 (δ_H 2.59) indicated these protons were coaxial and α-orientation. Based on the above spectroscopic data, the structure of the aglycone of **1** was determined to be **2**. Vicinal coupling constants of $J_{2',3'}$ = 3.0, $J_{3',4'}$ = 9.5, and $J_{4',5'}$ = 9.5 Hz suggested that H-3'/H-4' and H-4'/H-5' was anti diaxial orientation, and H-2'/H-3' was syn equatorial and axial orientation on a pyranose ring, which was also supported by the NOESY correlations of H-1' with H-3' and H-5' (Figure 2). The *β*-configuration of anomeric position was determined based on DEPT-nondecoupling measurement with the $^1J_{CH}$ value of 153.9 Hz at C-1' (δ_C 99.4). Therefore, the sugar moiety was supposed to be *β*-D-mannose. Compound **1** is the third case that monosaccharide was incorporated in the 4', 5'-dihydro-4'-hydroxyascochlorin backbone, with the first being vertihemipterin A attached 4-*O*-methyl-*β*-glucopyranose [10] and the second being ascochlorin N-acetylglucosamine attached N-acetyl-*α*-D-glucosamine [12], representing a rare fungal polyketide-sesquiterpenoid containing sugar moiety.

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for acremonoside (**1**) in CD_3OD

Position	δ_C (type)	δ_H (multiplicity, *J* in Hz)	Position	δ_C (type)	δ_H (multiplicity, *J* in Hz)
1	114.3 (C)		16	30.9 (CH_2)	1.79 (m); 1.50 (dq, 12.5, 5.0)
2	161.5 (C)		17	41.0 (CH_2)	2.18 (m); 2.12 (m)
3	113.6 (C)		18	215.4 (C)	
4	159.3 (C)		19	50.1 (CH)	2.59 (q, 7.0)
5	112.4 (C)		20	14.6 (CH_3)	0.51 (s)
6	138.3 (C)		21	14.7 (CH_3)	0.97 (d, 6.5)
7	13.2 (CH_3)	2.55 (s)	22	7.2 (CH_3)	0.69 (d, 7.0)
8	193.2 (C)		23	10.5 (CH_3)	1.83 (s)
9	21.0 (CH_2)	3.40 (dd, 13.5, 8)	1′	99.4 (CH)	4.45 (brs)
10	125.0 (CH)	5.57 (t, 7.5)	2′	71.4 (CH)	3.82 (d 3.0)
11	137.2 (C)		3′	74.2 (CH)	3.37 (dd, 9.5, 3.0)
12	83.0 (CH)	4.16 (t, 6.0)	4′	66.7 (CH)	3.59 (t, 9.5)
13	39.3 (CH_2)	1.77 (dd, 15.5, 6.0); 1.50 (dd, 15.5, 5.0)	5′	76.6 (CH)	2.97 (ddd, 9.5, 5.0, 3.0)
14	43.7 (C)		6′	61.1 (CH_2)	3.69 (dd, 11.5, 3.0); 3.65 (dd, 11.5, 5.0)
15	36.0 (CH)	2.21 (m)			

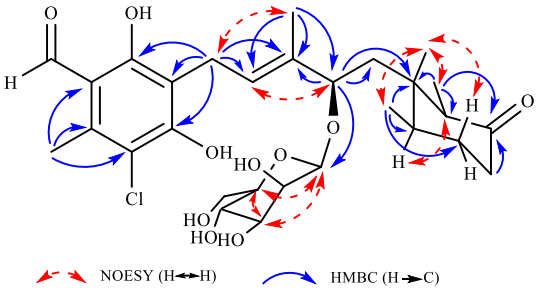

Figure 2. Key HMBC and NOESY correlations of **1**

The absolute configuration of cylindrol A1 and LL-Z1272ε, the closely related ascochlorin derivatives of **1**, was assigned by single-crystal X-ray diffraction method and correlated to that of ascochlorin by CD methods [18, 19]. Cylindrol A1 and LL-Z1272ε displayed a negative Cotton effect at approximately 286 nm due to the absolute configuration of 14*S*, 15*R*, 19*R* in cyclohexanone ring. The CD spectrum of **1** also showed a similar negative effect at 282 nm ($\Delta\varepsilon$ = -0.71), therefore, the absolute configuration of **1** was assigned as 14*S*, 15*R*, 19*R*. Singh et al assumed that Cotton effect at 242 nm ($\Delta\varepsilon$ = +2.03) was due to the chiral center at 12*R* [18]. Compound **1** possessed a negative Cotton effect at 229 ($\Delta\varepsilon$ = -1.65) and maybe be the presence of 12*S*, however, the calculated ECD spectral between 12*R*-**1** and 12*S*-**1** have no significantly difference by WB97XD/def2-TZVP level (Figure 3). The modified Mosher's method was tried to assigned the absolute configuration of C-12. Unfortunately, attempted hydrolysis of **1** had been unsuccessful. Additionally, we tried to discriminate the chiral center at C-12 using theoretical NMR calculations. As shown in Figure S13, the theoretical predictions of 12*R*-**1** and 12*S*-**1** highly matched with the experimental data with great correlation coefficient. Therefore, theoretical NMR calculations had met with failure. In conclusion, the structure of **1** was assigned to be an ascochlorin glycoside derivative and was named acremonoside.

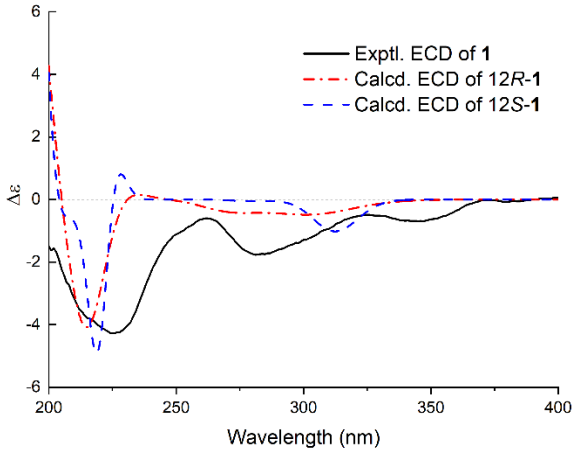

Figure 3. Experimental and calculated CD spectrum of **1**

The known compounds, 4', 5'-dihydro-4'-hydroxyascochlorin (**2**) [20], ilicicolin C (**3**) [7], LL-Z1272ε (**4**) [19], ascochlorin (**5**) [19], ilicicolin F (**6**) [7], cylindrol B (**7**) [18], 8',9'-dehydroascochlorin (**8**) [19], ascofuranone (**9**) [21], and ilicicolin H (**10**) [22], were identified by detail analysis of NMR data and comparison those reported in the literature.

3.2. α-Glucosidase Inhibitory, Cytotoxicity and Antibacterial Activities

In this study, compounds **1**–**3**, **7**, **8**, and **10** were evaluated for inhibitory effect on α-glucosidase. As showed table S1, the tested compounds showed poor inhibitory activity against α-glucosidase in comparison with the positive control acarbose, but compound **1** exhibited stronger inhibitory activity against α-glucosidase than that of **2**, suggesting that sugar moiety of **1** presumably play a key factor in increasing inhibitory effect on α-glucosidase. The cytotoxic activities of compounds **1**–**10** were measured at the concentration of 40 μM by a MTT viability evaluation and recorded in the table S2. Compounds **2**–**10** showed inhibitory activity against two pancreatic cancer cell lines (SW1990 and PANC1) and human colorectal carcinoma cells DLD1, but compound **1** did not. Compounds **2**–**10** display moderate antibacterial activities in comparison with positive control ciprofloxacin (Table S3), which was in line with the previous report [2, 12]. Unfortunately, compound **1** did not exhibit antibacterial activity. In conclusion, the results of the abovementioned bioassays indicated the sugar moiety of **1** maybe play an important effect on biological activity of ascochlorin derivatives.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (U20A20101), Guangxi Natural Science Foundation of Guangxi Province (2020GXNSFGA297002 and AD21075016), the Open Project of Engineering Center of Jiangxi University for Fine Chemicals (JFCEC-KF-2101), and Guangxi University of Chinese Medicine "GuiPai Traditional Chinese Medicine inheritance and innovation team" Project (2022A007).

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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