Steroids xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Three new highly oxygenated sterols and one new dihydroisocoumarin from the marine sponge-derived fungus *Cladosporium* sp. SCSIO41007

Xiaoyan Pang[a,b], Xiuping Lin[a], Junfeng Wang[a,*], Rui Liang[c], Yongqi Tian[a,b], Limbadri Salendra[a,b], Xiaowei Luo[a,b], Xuefeng Zhou[a], Bin Yang[a], Zhengchao Tu[c], Yonghong Liu[a,b,d,*]

[a] CAS Key Laboratory of Tropical Marine Bio-resources and Ecology/Guangdong Key Laboratory of Marine Materia Medica/RNAM Center for Marine Microbiology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, China
[b] University of Chinese Academy of Sciences, Beijing, China
[c] Laboratory of Molecular Engineering and Laboratory of Natural Product Synthesis, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China
[d] South China Sea Bio-Resource Exploitation and Utilization Collaborative Innovation Center, China

ARTICLE INFO

Keywords:
Sponge-derived fungus
Cladosporium sp.
Antiviral
Sterols

ABSTRACT

Three new highly oxygenated sterols (**1–3**) and a new dihydroisocoumarin (**7**) together with six known compounds were isolated from the extracts of the culture of a sponge-derived fungus *Cladosporium* sp. SCSIO41007. The structures of all new compounds (**1–3, 7**) were determined by the extensive spectroscopic analysis including NMR, MS, IR, and UV. Their absolute configurations were determined by X-ray single-crystal and CD data analysis. Compound **2** exhibited weak inhibitory activity against H3N2 with the IC_{50} value of 16.2 μM.

1. Introduction

In recent years, marine-derived fungi have proven to be a prolific source of structurally unique and possess interesting biological and pharmacological properties, and gained considerable attention [1,2]. Several of new sterols have been found in marine organisms, mainly in marine sponges and corals, while fewer new sterols from marine-derived fungi have been reported [3,4]. In our previous research, compounds which exhibited significant antiviral activities were isolated from the sponge-derived fungi *Aspergillus* sp. SCSIOXWS02F40 [5], *Aspergillus sydowii* ZSDS1-F6 [6], and *Trichoderma* sp. SCSIO41004 [7]. In our ongoing effort to discover antiviral metabolites, the fungus *Cladosporium* sp. SCSIO41007 isolated from a *Callyspongia* sp. sponge collected from the sea area near Xuwen County, Guangdong Province, China, was studied. Three new sterols cladosporisteroid A (**1**), cladosporisteroid B (**2**), cladosporisteroid C (**3**) and a new dihydroisocoumarin (3*R*)-3-(2-hydroxypropyl)-6,8-dihydroxy-3,4-dihydroiso-coumarin (**7**) together with six known compounds including pregn-7-dien-3,6,20-trione (**4**) [8,9], 3β,5α,9α-trihydroxy-(22*E*,24*R*)-ergosta-7,22-diene-6-one (**5**) [10], cerevisterol (**6**) [11], 2′-deoxythymidine (**8**) [12], cyclo-(Gly-Leu) (**9**) [13], 3-carboxylic acid (**10**) [14] were isolated from the extracts of the culture of the fungus *Cladosporium* sp. SCSIO41007. In this paper we report the isolation and structure elucidation of the new compounds as well as their biological activities.

2. Experimental

2.1. General experimental procedures

1D and 2D NMR spectra were measured on a Bruker AV 500 MHz or AVANCE III HD 700 MHz NMR spectrometer (Bruker, Fällanden, Switzerland) with TMS as an internal standard. Chemical shifts were given as δ values, with J values reported in Hz. UV spectra were recorded on a UV-2600 UV–Vis spectrophotometer (Shimadzu, Japan). Optical rotations were measured using a MCP-500 Polarimeter (Anton, Austria). CD spectrum was measured with a Chirascan circular dichroism spectrometer (Applied Photophysics, Surrey, UK). HRESIMS data were recorded on a Bruker maXis Q-TOF in positive/negative ion mode. (Bruker, Fällanden, Switzerland). X-ray diffraction intensity data were collected on Agilent Xcalibur Nova single-crystal diffractometer using Cu Kα radiation. HPLC was carried on shimadzu LC-10ATvp with YMC ODS SERIES (YMC-Pack ODS-A, 250 × 10 mm I.D., S-5 μm, 12 nm). The TLC plates with silica gel GF254 (0.4–0.5 mm, Qingdao Marine Chemical Factory, Qingdao, China) were used for analysis and preparative. Column chromatography was carried out on silica gel

* Corresponding authors at: CAS Key Laboratory of Tropical Marine Bio-Resources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China.
E-mail addresses: wangjunfeng@scsio.ac.cn (J. Wang), yonghongliu@scsio.ac.cn (Y. Liu).

https://doi.org/10.1016/j.steroids.2017.12.001
Received 31 July 2017; Received in revised form 21 September 2017; Accepted 2 December 2017

Table 1
NMR data for compound **1** (700/175 MHz, TMS, δ ppm) in DMSO-d_6.

Position	δ_C	δ_H mult (*J* in Hz)	^{1}H-^{1}H COSY	HMBC	NOESY
1a	33.3	1.91–1.95 m	H-1b	C-2, 3, 5, 10, 19	H-11a, 11b, 1b
1b		1.62 td (14.0, 5.6)		C-2, 3, 5, 9, 10, 19	
2a	33.6	2.54 ddd (17.5, 14.7, 5.6)	H-1a, 1b, 2b	C-1, 3, 10	
2b		2.24 brdd (16.8, 4.2)	H-1b, 1a	C-1, 3, 4, 10	H-1b, 1a
3	198.1				
4	122.9	5.60 s		C-2, 6, 10, 19	
5	163.4				
6	127.5	6.17 m		C-4, 5, 8	H-4, 12b, 14
7	141.5	6.17 m	H-8	C-5, 8, 9, 14	H-15a, 16b, 15b,
8	36.7	2.15 t (10.5)	H-9, 14	C-4, 5, 6, 7, 9, 13, 14	H-18, 19,
9	50.3	1.11 td (9.8, 4.2)		C-5, 7, 14, 10, 19	
10	35.5				
11a	20.2	1.44–1.49 m	H-9, 12b	C-9, 10, 12, 13	
11b		1.39 qd (13.3, 4.2)	H-9, 12b	C-9, 10, 12, 13	
12a	39.5	2.10 dt (12.6, 3.5)	H-12b, 11b	C-9, 11, 13, 14, 18	H-11b, 11a, 21, 27
11b		1.20 td (12.6, 3.5)		C-9, 11, 13, 14, 18	
13	43.6				
14	53.2	1.09 td (12.6, 7.0)		C-18, 13, 9, 12	
15a	23.2	1.73–1.78 m	H-14, 15b	C-13, 14, 16	
15b		1.23 qd (12.6, 6.3)		C-8, 14, 16, 17	
16a	22.1	1.89–1.96 *m*	H-15b, 16b	C-15, 17, 20	H-15b, 16b
16b		1.68 m	H-15b	C-13, 14, 15	
17	53.5	2.13 t (9.8)	H-16a, 16b	C-12, 13, 16, 18, 20	H-19, 14, 16a
18	13.5	0.89 s		C-12, 13, 14	
19	16.0	1.06 s		C-1, 5, 9, 10	
20	76.2				
21	23.1	1.22 s		C-17, 20, 22	
22	74.7	3.20 dd (9.1, 2.8)		C-17, 20, 21, 23, 24	H-18, 28, 16b, 16a, 17
23	73.2	3.73 dt (8.4, 3.5)	H-22, 24	C-20, 22, 24, 25, 28	H-22, 17, 24, 21, 27
24	48.3	1.65 qd (7.0, 3.5)	H-28	C-22, 23, 25, 26, 28	
25	71.5				
26	28.1	1.14 s		C-24, 25, 27	
27	29.8	1.21 s		C-24, 25, 26	H-26
28	10.6	0.94 d (7.0)		C-23, 24, 25	H-18
OH-20		4.15 s		C-17, 20, 21, 22	H-21, 28
OH-22		5.49 d (3.5)	H-22	C-20, 23	OH-23, 20, H-26, 27
OH-23		5.03 d (4.2)	H-23	C-22, 24	OH-20, H-24, 26, 27,
OH-25		5.41 s		C-24, 25, 26	OH-23, 20, H-26, 27

(200–300 mesh, Jiangyou Silica Gel Development Co., Yantai, China), Sephadex LH-20 (40–70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden) and YMC Gel ODS-A (12 nm, S-50 μm YMC, MA, USA). Spots were detected on TLC under UV light or by heating after spraying with the mixed solvent of saturated vanillin and 5% H_2SO_4 in H_2O.

2.2. Fungus material

The fungal strain SCSIO41007 was isolated from a *Callyspongia* sp. sponge collected from the sea area near Xuwen County, Guangdong Province, China. The producing strain was stored on MB agar (malt extract 15 g, sea salt 10 g, agar 16 g, water 1 L, pH 7.4–7.8) slants at 4 °C and then deposited at Key Laboratory of Tropical Marine Bio-resources and Ecology, Chinese Academy of Science. The ITS1-5.8S-ITS2 sequence region (496 base pairs (bp), accession number MF188197) of strain SCSIO41007 was amplified by PCR and DNA sequencing showed it shared significant homology to several species of *Cladosporium*. A phylogenetic tree was procured by the neighbor-joining method based on similarity of a 371-bp consensus length of the ITS1-5.8S-ITS2 sequence (Fig. S42). It revealed that strain SCSIO41007 has close proximity to *C. halotolerans* DTO 109-D3 (KP701911), and was designated as *Cladosporium* sp. SCSIO41007.

2.3. Fermentation and extraction

The strain SCSIO41007 was cultured in 100 mL flasks (×41) each containing 10 mL seed medium (malt extract: 15 g, sea salt: 2.5 g, distilled water: 1 L, pH 7.4–7.8) at 27 °C on a rotary shaker (172 r.p.m.) for 48 h. The mass fermentation of the strain was carried out at 25 °C for 50 days using a rice solid medium (rice: 200 g/flask, salt: 2.5 g/flask, distilled water: 200 mL/flask) in the 1 L flask (×41). The flasks were incubated stationary at 25 °C under normal day night cycle. After 50 days, cultures were soaked in acetone and mashed into small pieces and vibrated with ultrasound for 20 mins. Then the acetone solution was evaporated under reduced pressure to afford an aqueous solution, which was extracted three times with EtOAc. Meanwhile, the rice solid residue was extracted with EtOAc to give another EtOAc solution. Both of the EtOAc solution was combined and concentrated under reduced pressure to afford a crude extract. The extract was suspended in MeOH and then partitioned with equivoluminal petroleum ether (PE) to remove the oil. Finally, the MeOH solution was concentrated under reduced pressure to yield a black extractive (91.9 g).

2.4. Isolation and purification

The black extractive was subjected to silica gel column chromatography (CC) eluted with CH_2Cl_2 and MeOH mixed solvent in a step gradient (100:0–1:1, V/V) to give seven fractions (Fr-1 ~ Fr-7). Fr-3 (6.6 g) was subjected to CC eluted with PE and EtOAc mixed solvent in a step gradient (10:1–0:1, V/V) to gain three fractions (Fr-3–1 ~ Fr-3–3). Fr-3–1 (308.7 mg) was subjected to a Sephadex LH-20 column eluted with CH_2Cl_2/MeOH (1:1, V/V), then followed with semi preparative HPLC (40% CH_3CN/H_2O, 1.5 mL/min) to afford **2** (51.9 mg, t_R = 34.0 min). Fr-3–2 (489.7 mg) was subjected to a Sephadex LH-20 column eluted with CH_2Cl_2/MeOH (1:1, V/V), then followed with semi