SUPPLEMENTARY MATERIAL

Chemical constituents of the mangrove-associated fungus *Capnodium* sp. SZ-F22. A new eremophilane sesquiterpene

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Abstract

A new eremophilane sesquiterpene, capnodiumone (1), along with five known eremophilane sesquiterpenes (2-6) and eight other compounds (7-14), have been isolated from a mangrove-associated fungus *Capnodium* sp. SZ-F22. The chemical structures were elucidated on the basis of extensive spectroscopic analysis. The broth extract of the fungus displayed good inhibitory effect on the mycelium growth against *F.graminearum* at 100µg/mL, however, all the 14 compounds showed no expected antifungal activity. The probable reasons were discussed.

Keywords: mangrove-associated fungus; *Capnodium* sp.; chemical constituents; eremophilane sesquiterpenes; *Fusarium graminearum*

1. Experimental

1.1. General

The NMR spectra were recorded at 600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR on Bruker Ascend TM 600 in DMSO- d_6 or CDCl_3 using TMS as internal standard. LC-MS analyses were performed on Agilent 6230 TOF LC/MS system and Zobax SB-C18 (5 μm , 4.6 $\times$ 150 mm) column with gradient elution. IR spectrum was recorded on JASCO FT-IR4100, optical rotation was measured on JASCO P-1010. HPLC analysis was performed on Shimadzu High Performance Liquid Chromatography (DGU-20A5 Degasser, LC-20AT Liquid Chromatograph, SIL-20AC Auto Sampler, SPD-M20A Diode Array Detector, CTO - 20AC Column Oven) using Inerstil ODS-SP (5 μm , 4.6 $\times$ 250 mm) column. UPLC analyses were performed by Acquity UPLC system (Waters, Milford, MS, USA) and a BEH C18 column (2.1 mm $\times$ 100 mm, 1.7 μm). Preparative HPLC was performed on Beijing Chuangxintongheng LC3000 Semi-preparation Gradient HPLC System using Sepax Amethyst C-18(5 μm , 21.2 $\times$ 250 mm) column.

The carbendazim is purchased from Aladdin Industrial Corporation (CAS: 10605-21-7).

1.2. Fungal material

The mangrove-associated fungus *Capnodium* sp. SZ-F22 was isolated from the sample of mangrove plant (Kjer 2010), which was collected from the Guangdong Neilingding-Futian National Nature Reserve in Shenzhen, China. The strain was identified as a *Capnodium* sp. on the basis of the gene sequence data of the 18S rDNA and ITS genes, and the GenBank accession number is KT443921.

The *F.graminearum* strain PH-1, which is kindly provided by Professor Zhonghua Ma in College of Agriculture & Biology, Zhejiang University, China.

1.3. Fermentation and extraction

The small mycelia agar plugs, which were grown on potato dextrose agar at 25 °C for 5 days, were inoculated in 500 mL Erlenmeyer flasks containing 200 mL YGP broth (10 g glucose, 10g yeast extract, 5 g peptone, 25 g crude sea salt in 1 L water) and

incubated at 28 °C, 180 rpm for 3 days as seed culture. A 200 L large scale fermentation was performed using multiple 500 mL Erlenmeyer flasks, each containing 250 mL YGP broth medium. Each flask was inoculated with 4 mL seed culture and incubated at 28 °C, 180 rpm for 10 days.

The flask culture (200 L) was filtered to separate into the filtrate and wet mycelia. The filtrate was extracted twice with ethyl acetate. The organic layer was evaporated to dryness under reduced pressure to afford a dark brown gum (11 g). The mycelia were extracted with MeOH (1000 mL), the MeOH layer was concentrated under reduced pressure, then added H₂O (150 mL) to the MeOH extract, and the mixture was extracted three times with an equal amount of EtOAc. The EtOAc layer was then evaporated to dryness to produce 2 g of a brownish residue.

1.4. Isolation and identification of main compounds (1-14)

The broth extract was separated by CC on silica gel eluting with a gradient of CH₂Cl₂-CH₃OH (100:0; 100:1; 80:1; 50:1; 30:1; 10:1; 0:100) to afford thirteen fractions (21A-21M). The fraction 21C was further purified by CC over silica gel using a gradient of petroleum ether-EtOAc (ethyl acetate) (100:0; 40:1; 30:1; 15:1; 5:1; 0:100) to provide thirteen fractions (22A-22M). The compound **7** was the insoluble part of the fraction 22F in methanol. 22I was subjected to preparative HPLC separation using H₂O-CH₃OH (40:60-v/v, 10 mL/min, λ_{max} = 280 nm) to get compound **3** (8.8 mg, t_{R} = 30 min). The fraction 21E (2.27 g) was purified using silica gel CC with a gradient of petroleum ether - EtOAc (100:0;90:10;70:10;50:1;30:1;0:100) and yielded fourteen major secondary fractions (27A-27N), then fractions 27F and 27G (300 mg totally) were separated using silica gel CC with a gradient of petroleum ether -EtOAc (10:0;8:1; 6:1;4:1;0:10) and yielded six major fractions (28A-27F), fraction 28B submitted to preparative HPLC separation using H₂O-CH₃OH (40:60-v/v, 10 mL/min, λ_{max} = 254 nm), compounds **1** (9.9 mg, t_{R} = 45 min) and **2** (18.6 mg, t_{R} = 60 min) were obtained, fraction 28D submitted to preparative HPLC separation using H₂O-CH₃OH (50:50-v/v, 10 mL/min, λ_{max} = 210 nm) to get compound **9** (16.7 mg, t_{R} = 22 min). Fraction 27H was submitted to preparative HPLC

s-eparation using H₂O–CH₃OH (50:50-v/v, 10 mL/min, λ_{max} = 254 nm) to obtain compounds **8** (13 mg, t_R = 17 min), **4** (8.4 mg, t_R = 35 min) and **5** (13.8mg, t_R = 50 min). Fraction 21F (700 mg) was separated by CC over silica gel using a gradient of petroleum ether-EtOAc (100:0; 60:10; 50:10; 30:10; 20:10; 0:100) to generate seventeen fractions (32A-32Q). Fraction 32I was subjected to preparative HPLC separation using H₂O–CH₃OH (60:40-v/v, 10 mL/min, λ_{max} = 210 nm) to give compounds **10** (9.4 mg, t_R = 12 min) and **11** (11 mg, t_R = 14 min). Fraction 32N was subjected to preparative HPLC separation using H₂O–CH₃OH (50:50-v/v, 10 mL/min, λ_{max} = 254 nm) to give compound **6** (5 mg, t_R = 37 min). 21G, 21H and 21J was submitted to preparative HPLC separation using H₂O–CH₃OH (70:30-v/v, 10 mL/min, λ_{max} = 210 nm) to get compound **12** (7.5 mg, t_R = 31 min), **14** (9.8 mg, t_R = 12 min) and **13** (52.3 mg, t_R = 10 min), respectively.

Figure. S1. Bioactivity of *Capnodium* sp. SZ-F22 against *F.graminearum* in vitro. **S1a**, Antagonistic effect of *Capnodium* sp. SZ-F22 against *F.graminearum*.; **S1b**, Inhibition of *Capnodium* sp. SZ-F22 crude extract against *F.graminearum*.(the inhibition rate of mycelial growth= $(D_a-D_b)/(D_a-D_c)$,where D_a is the diameter of the *F.graminearum* colony in the control plate and D_b is the diameter of the colony in the plate containing a test compound, D_c is the diameter of the *F.graminearum* colony). ($D_a=71$ mm, $D_b=30$ mm, $D_c=13$ mm);**S1c**, Inhibition of *Capnodium* sp. SZ-F22 compounds against *F.graminearum* (compounds **1**, **2**, **3**, **4**, **5**, **6**, **blank**, **DMSO** control and positive control, respectively. The pictures of treatment groups containing compounds **7-14** were not showed).

Table S1. ^1H , ^{13}C , HMBC and HSQC NMR data for compound **1** in CDCl_3

Position	δ_{H} (J in Hz)	δ_{C} ppm,type	HMBC	HSQC
1	3.56 d (3.6)	56.2,CH	C-2,C-5,C-9,C-10	C-1
2	3.87 dd(3.61,4.9)	55.1,CH	C-1,C-3,C-4	C-2
3	5.13 t(5.1)	70.5,CH	C-4,C-5,	C-3
4	1.76 m	42.9,CH	C-10	C-4
5		38.6,C		
6	2.82 d(13.5) 2.05 d(13.5)	42.1,CH ₂	C-4,C-5,C-7,C-8 C-10,C-11,C-14	C-6
7		127.1,C		
8		190.2,C		
9	6.2 s	132.3,CH	C-1,C-5,C-7	C-9
10		158.6,C		
11		146.5,C		
12	2.15 3H d(2.1)	20.9,CH ₃	C-7,C-11,C-13	C-12
13	1.86 3H s	23.2,CH ₃	C-7,C-11,C-12	C-13
14	1.15 3H s	18.4,CH ₃	C-4,C-5,C-6,C-10	C-14
15	1.06 3H d(7.1)	10.2,CH ₃	C-3,C-4,C-5	C-15
16		170.9,CH ₃		
17	2.14 3H s	22.8,CH ₃	C-16	C-17

Figure S2. ^1H NMR spectrum of **1** (CDCl_3 , 600 MHz).

Figure S3. ^{13}C NMR spectrum of **1** (CDCl_3 , 150 MHz)

20150411 EHB-SZ-F22-28E COSY

Figure S4. ^1H - ^1H COSY spectrum of **1** (CDCl_3 , 600 MHz)

20150410 SZ-F22-HH3-28H HMBC

Figure S5. HMBC spectrum of **1** (CDCl₃, 600 MHz)

20150411 HEB-SZ-F22-28H HSQC

Figure S6. HSQC spectrum of **1** (CDCl₃, 600 MHz)

20150411 HMB-SZ-F22-28H NOESY

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NAME      95 F22 28H
EXPNO     280
PROCNO     1
Date_      20150411
Time       2.15
INSTRUM    spect
PROBHD     5 mm PABBO BB/
PULPROG    zgpg30
TD          65536
SOLVENT    CDCl3
NS          32
DS          4
SWH         7911.339 Hz
FIDRES     0.142047 sec
AQ          102.75
RG          69.333 usec
DS          6.50 usec
TS          298.2 K
DO         0.0003363 sec
DI         2.0000000 sec
DS         0.8000001 sec
DI         0.0300000 sec
DI2         0.0002000 sec
DI3         0.0002000 sec
DI4         0.0001880 sec

===== CHANNEL f1 =====
SFO1       600.1324005 MHz
NUC1        1H
P1         10.62 usec
P2          2.64 usec
P17        2500.00 usec
NU0         2
TD          65536
SFO1       600.1324 MHz
FIDRES     0.143011 Hz
SW         12.000 ppm
P17MODE    SL4Leg-TPP1
SI          -0.24
SF         600.1300062 MHz
WDW         GSSIN3
SSB          2
GB          0.00 Hz
G3          0
PC          -1.00
ST          -0.14
NUC2        1H
SFO2       600.1300065 MHz
WDW         GSSIN3
SSB          2
GB          0.00 Hz
G3          0
  
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H₃-14 to H1

H₃-14 to H3

H₃-15 to H2

Figure S7. NOESY spectrum of **1** (CDCl₃, 600 MHz)

Figure S8. Key HMBC, ^1H - ^1H COSY and NOESY correlations of compound **1**

Figure S9. HR-ESI-MS of **1** (Positive-ion)

References

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