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BRIEF COMMUNICATION

Curvulaide A, a bicyclic polyketide with anti-anaerobic bacteria activity from marine-derived *Curvularia* sp.

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Abstract

Curvulaide A (**1**), a new bicyclic polyketide, together with its known congener preussilide D, were produced by solid-state fermentation with *Curvularia* sp. IFB-Z10. The structure of **1** was elucidated using a combination of spectral methods, including extensive one-dimensional (1D) and two-dimensional (2D) nuclear magnetic resonance (NMR) data, high resolution electrospray ionization mass spectrometry (HRESIMS), experimental and calculated electronic circular dichroism (ECD) spectra. Curvulaide A (**1**) exhibited moderate anti-anaerobic bacteria activity against periodontal pathogen *Porphyromonas gingivalis* with the MIC value of 62.5 μM, and showed moderate cytotoxicity against human hepatoma cell lines BEL7402/5-Fu with IC_{50} values of 12.46 μM.

Introduction

The rapid development of drugs resistance in human diseases such as cancers and invasive fungal infections calls for a class of structurally unique lead drug compounds and beneficial therapeutic agents with new pharmacological mechanisms[1]. Marine endophytic fungi may play an important role in the discovery of unique secondary metabolites with chemical and biological properties unlike those found in terrestrial ones in recent years[2]. Bicyclic polyketides, a class of compounds with a 4,6-dimethylhepta-2,4,6-trienoic acid substructure and decalin derivative motif from acetyl Co-A and malonyl Co-A interdispersed with units of methylmalonyl Co-A, are moderate inhibitors of angiogenesis, and inhibit proliferation of filamentous fungi and plant pathogens[3]. Bicyclic polyketides had been discovered from terrestrial fungal metabolites, such as the antarones from *Penicillium antarcticum*[4], embellistarin from *Embellisia chlamydospora*[5], and hamigerone from *Hamigera avellenea*[6]. To discover bioactive compounds from marine fungi, a new bicyclic polyketide curvulaide A (**1**) (Fig.1) was isolated from the culture of the marine fungus *Curvularia* sp. IFB-Z10.

Curvularia sp. IFB-Z10 was isolated from the gut flora of fish (white croaker *Argyrosomus argentatus*); it produces novel alkaloids named curvulamine[7] and curindolizine[8], when cultured in shake flask in the Czapek's medium. These compounds exhibited significant antibacterial and anti-inflammatory activities. In this study, we aimed to explore the "new fate" of polyketide analogs from the metabolites of *Curvularia* sp. IFB-Z10 through solid-state fermentation[9]. A new bicyclic polyketide, curvulaide A (**1**), together with its known congener preussilide D[3] were produced by solid-state cultivated *Curvularia* sp. IFB-Z10. Curvulaide A (**1**) was evaluated for antitumor and anti-anaerobic bacteria activities, and showed moderate cytotoxicity against human hepatoma cell lines BEL7402/5-Fu with IC_{50} value of 12.46 μM, and exhibited moderate anti-anaerobic bacteria activity against periodontal pathogen *Porphyromonas gingivalis* with an MIC value of 62.5 μM. Hence, we reported the isolation, structural elucidation, and biological activity evaluation of **1**.

Curvulaide A (**1**) was isolated as a pale-yellow gum, and its molecular formula was determined to be $C_{25}H_{30}O_4$ (11 degrees of unsaturation) based on the HRESIMS ion at *m/z* 417.2035 $[M+Na]^+$ (calcd. 417.2036). In the ^1H NMR

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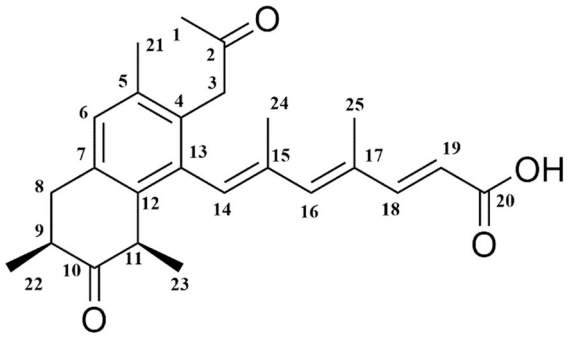

Fig. 1 The chemical structure of **1**

Table 1 The ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) of **1** in CD_3OD

No.	1		No.	1	
	δ_H mult. (J in Hz)	δ_C		δ_H (J in Hz)	δ_C
1	2.08 s	29.9	14	6.47 s	132.0
2		208.8	15		138.6
3	3.88 d (18.0) 3.82 d (18.0)	46.4	16	6.48 d (4.0)	142.5
4		137.7	17		134.6
5		132.5	18	7.41 dd (15.5, 4.0)	151.6
6	7.07 s	130.0	19	5.94 d (15.5)	118.8
7		136.3	20		170.9
8	2.95 m	36.3	21	2.21 s	20.2
9	2.43 m	44.1	22	1.23 d (6.5)	15.7
10		217.9	23	1.23 d (6.5)	19.3
11	3.48 m	47.5	24	1.55 s	18.1
12		136.9	25	2.09 s	14.1
13		136.3			

spectra (Table 1), six methyl groups including four singlet methyl groups (δ_H 2.08, 2.21, 2.09, 1.55, each 3H), two doublet methyl groups (δ_H 1.23, d, $J = 6.5$ Hz, 6H) were observed. It also displayed a pair of characteristic *trans*-coupled olefinic protons (δ_H 7.41, d, $J = 15.5$ Hz; 5.94, d, $J = 15.5$ Hz), a doublet olefinic proton (δ_H 3.82, d, $J = 4.0$ Hz), and two specific singlet olefinic protons (δ_H 7.07, 6.47). The ^{13}C NMR spectrum (Table 1) showed two carbonyl carbons (δ_C 217.9, 208.8), a conjugated carbonyl carbon (δ_C 170.9), and twelve alkenic carbons. Thus, the aforementioned data suggested that compound **1** was a bicyclic polyketide like preussilide C[3].

The ^{1}H NMR data analyses of **1** showed much similarity with those of preussilide C, except for the loss of two specific methine protons (δ_H 1.76, d, $J = 12.5$ Hz; 3.21, d, $J = 10.5$ Hz) in preussilide C, and the presence of two additional alkenic carbons (δ_C 136.3, 136.9) in **1**. The HMBC correlations from H-18 (δ_H 7.41, d, $J = 15.5$ Hz) to C-20 (δ_C 170.9)

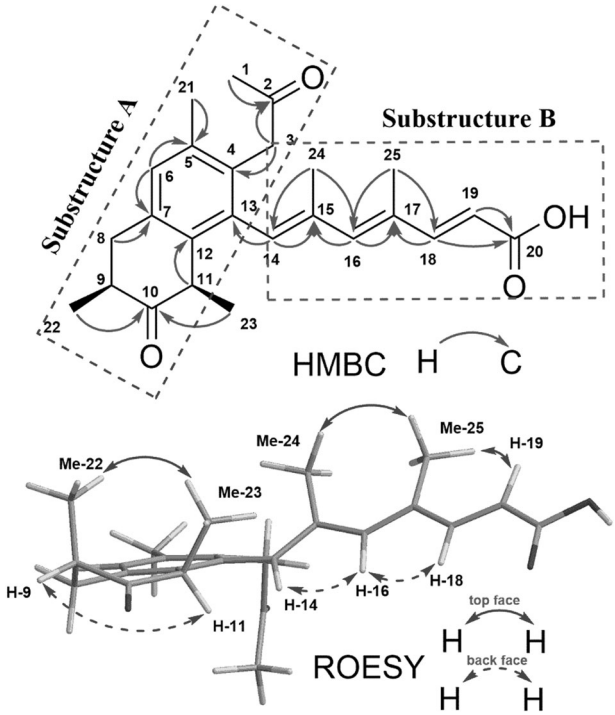

Fig. 2 Selective HMBC and ROESY correlations of **1**

and C-16 (δ_C 142.5), from H-16 (δ_H 3.82, d, $J = 4.0$ Hz) to C-14 (δ_C 132.0) and C-18 (δ_C 151.6), from Me-25 (δ_H 3.09, s) to C-16, C-17 (δ_C 142.5), and C-18 implied a 4,6-dimethylhepta-2,4,6-trienoic acid motif (substructure A) in the structure of **1**. Two specific doublet methyl groups and one singlet methyl group in ^{1}H NMR and the HMBC correlations from H-9 (δ_H 2.43, m) to Me-22 (δ_H 1.23, d, $J = 6.5$ Hz), H-11 (δ_H 3.48, m) to Me-23, H_2-8 (δ_H 2.95, m) to C-7 (δ_C 136.3), C-12 (δ_C 136.9), Me-22 and Me-23 to C-10 (δ_C 217.9) and H-11 to C-12 and C-7 indicated a decalin derivative motif (substructure B) in **1** like in preussilide C. The HMBC correlation between H-14 and C-13 suggested the connection point between substructure A and substructure B located at C-13. The singlet methyl (δ_H 2.08) and two germinal coupled protons (δ_H 3.82, d, $J = 18.0$ Hz; 3.88, d, $J = 18.0$ Hz) showed HMBC correlations to the ketone carbonyl carbon (δ_C 208.8), and this indicated a methyl ketone unit in **1** located at C-4. Thus, the planar structure of **1** was determined as shown in Fig. 2.

The relative configuration of **1** was determined based on vicinal coupling constants and ROESY correlations. The coupling constant $J_{18,19} = 15.5$ Hz indicated an *E*-configuration of this double bond. The trisubstituted double bonds $\Delta^{14,15}$ and $\Delta^{16,17}$ were assigned as *E*-configurations based on the ROESY correlations between H-16 and H-14, H-18 as well as the ROESY correlations between Me-25 and Me-24, H-19. The ROESY correlations between the H-9 and H-11 indicated Me-22 and Me-23 were co-facial, and H-11 and H-9 were on the same face of the decalin motif. Thus, the relative configuration of the decalin moiety was determined

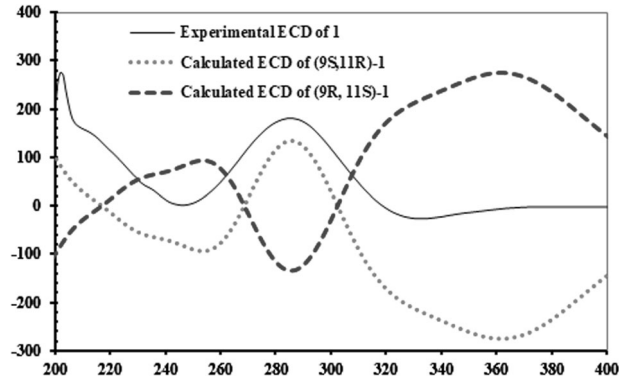

Fig. 3 Experimental and calculated ECD spectra of **1**

as shown in Fig. 1, which was consistent with the configuration of the revised preussilide A[10].

Unfortunately, the curvulaide A (**1**) failed to crystallize in different solvent. To determine the absolute configuration of curvulaide (**1**), the theoretical calculation of ECD spectra was performed with the Gaussian 09 program package, which was conducted in MeOH using time-dependent density functional theory at B3LYP/6–311G** (Fig. 3), and led us to determine the absolute configuration of **1** (9*S*, 11*R*) by comparing experimental and calculated ECD spectra.

The anti-anaerobic bacteria activity was evaluated against periodontal pathogen *Porphyromonas gingivalis* by broth microdilution method in a 96-well plate[11]. Compound **1** exhibited moderate activity with the MIC value of 62.5 μM (tinidazole was added as the positive control with MIC value of 7.8 μM). The cytotoxic activity of **1** was assessed against human hepatoma cell line BEL7402 and the drug-resistant cell line BEL7402/5-Fu[12], and showed effective cytotoxicity with IC_{50} values of 19.85 μM and 12.46 μM, respectively[13] [the 5-Fu was used as the positive control (BEL7402, IC_{50}: 14 μM; BEL7402/5-FU, IC_{50}: 1630 μM)].

In conclusion, a new bicyclic polyketide, curvulaide A (**1**), with a 4,6-dimenthyhepta-2,4,6-trienoic acid substructure and decalin derivative motif, was produced by solid-state fermentation with *Curvularia* sp. IFB-Z10 using rice as substrate. Compound **1** showed moderate anti-anaerobic bacteria activity and cytotoxic activities. Therefore, the isolation of the new bicyclic polyketide **1** suggested that more unique bioactive compounds can be isolated from *Curvularia* sp. IFB-Z10, and these novel compounds could serve as lead compounds for new drug discovery.

Curvulaide A (**1**): pale-yellow resin, $[\alpha]^{23}_{D} + 10.3$ (*c*, 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 207 nm (4.56) 280 nm (4.02); ^{1}H and ^{13}C NMR (CD_3OD, 600 MHz), see Table 1; negative ESIMS *m/z* 393.2 $[M-H]^-$; positive ESIMS *m/z* 417.2 $[M+Na]^+$; HRESIMS *m/z* 417.2035 $[M+Na]^+$ (calcd for $C_{25}H_{30}O_4Na$, 417.2036).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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