NOTE

Rakicidin F, a new antibacterial cyclic depsipeptide from a marine sponge-derived *Streptomyces* sp.

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A new cyclic depsipeptide, rakicidin F (1), along with the known compound rakicidin C (2), was isolated from the fermentation broth of the marine sponge-derived actinomycete strain *Streptomyces* sp. GKU 220. Their structures were elucidated by interpreting the HRFABMS and NMR spectroscopic data. Rakicidin F (1) showed growth inhibitory activity against bacteria.
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Marine sponges are known to harbor diverse microbial communities.[1] Among these communities, actinomycetes are considered to be one of the most promising resources for new bioactive compounds of value in human/veterinary medicine and agriculture.[2] We previously reported a new rakicidin derivative that was isolated from the marine sediment-derived *Streptomyces* sp. MWW064 and shown to inhibit tumor cell invasion.[3] In the course of our continuing screening program to discover structurally unique secondary metabolites from actinomycetes isolated from different ecosystems in Thailand,[4–7] a new antibacterial cyclic depsipeptide, rakicidin F (**1**), along with the known compound rakicidin C (**2**) (Figure 1a),[8] was isolated from the fermentation broth of the marine sponge-derived actinomycete strain *Streptomyces* sp. GKU 220. Herein, we report the fermentation, isolation, structural elucidation and biological activities of compounds **1** and **2**.

The producing organism, GKU 220, was isolated from a marine sponge sample collected in Andaman sea, Ranong, Thailand. The morphological characteristics and 16 S rRNA gene sequence demonstrated that the strain belongs to the genus *Streptomyces*. Spores of the strain were inoculated into 20 ml of f medium consisting of casitone 0.75%, yeast extract 0.75%, glycerol 1.5% and NaCl 0.25% with no pH adjustment in a 100-ml Erlenmeyer flask, and cultured on a reciprocating shaker at 28 °C for 3 days (120 s.p.m.). The seed culture (1 ml) was inoculated in a 500-ml baffled flask containing A-16 medium (glucose 2%, Pharmamedia 1% and CaCO₃ 0.5% with no pH adjustment) as the production medium. Fermentation was carried out at 28 °C for 7 days under shaking conditions (120 r.p.m.), after which the culture (3 l) was separated by centrifugation. The culture supernatant was extracted with an equal volume of ethyl acetate (EtOAc) three times and the mycelium was extracted with EtOH. The EtOAc and EtOH-soluble portions were concentrated under reduced pressure, and then the two portions were combined to yield 1.1 g crude extract. The crude extract was separated into seven fractions by silica gel column chromatography (CHCl₃-MeOH, gradient 1:0-0:1 (v/v)). Fraction 4 (CHCl₃-MeOH, 4:1 (v/v)) was further purified by a reversed-phase HPLC system on a Capcell-Pak C₁₈ column (UG 80; 5 μm, 10 i.d. x 250 mm; Shiseido, Tokyo, Japan) developed with isocratic elution (38% CH₃CN at 5 ml min⁻¹ and UV detection at 254 nm) to yield pure compounds **1** (5.1 mg) eluting at 20.4 min and **2** (7.9 mg) eluting at 13 min.

Compounds **1** and **2** were obtained as a white amorphous powder. The molecular formula of **1** was deduced to be $C_{27}H_{44}N_4O_6$ by HRFABMS (m/z 521.3345 [M+H]⁺, calcd for $C_{27}H_{45}N_4O_6$, 521.3339). The molecular formula of **2** was also determined to be $C_{26}H_{42}N_4O_6$ in the same manner (m/z 507.3196 [M+H]⁺, calcd for $C_{26}H_{43}N_4O_6$, 507.3183). The IR absorption bands of compound **1** suggested the presence of OH or NH (3372 cm⁻¹) and carbonyl (1655 and 1616 cm⁻¹) functionalities. The ¹H and ¹³C NMR spectra of **1** in conjunction with DEPT-135 and HSQC spectrum presented six methyls at δ_C 37.8, 20.7, 20.7, 16.0 14.4 and 11.7, seven methylenes at δ_C 120.5, 54.1, 46.6, 43.3, 33.1, 31.0 and 30.0, and eight methines at δ_C 141.4, 119.0, 78.1, 53.9, 44.3, 32.9, 32.2 and 28.3 (Table 1). In addition, six quaternary carbon atoms were observed at δ_C 177.3, 175.9, 171.1, 170.3, 168.9 and 139.5, including five carbonyl carbons at δ_C 177.3, 175.9, 171.1, 170.3 and 168.9, and one olefinic quaternary carbon at δ_C 139.5. The ¹H-¹H COSY correlations revealed three partial structures: H-2 to H-4, H-10 to H-11, and H-15 to H-23, including four methyl substitutions (Figure 1b). Two olefinic methylene protons (δ_H 5.54, 5.46, H₂-13) showed HMBC correlations to an olefinic carbon C-11 (δ_C 141.4) and a quaternary carbon C-12 (δ_C 139.5), while a methine proton (δ_H 6.20) at C-10 coupled to a carbonyl C-9 carbon (δ_C 168.9), which revealed the partial structure (C-9 to C-13) in the 2,4-pentadienoate moiety. The geometry of the double bond between C-10 and C-11 was assigned as *E* on the basis of

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a

Rakicidin F (**1**), R= C_2H_5
Rakicidin C (**2**), R= CH_3

Rakicidin A (**3**), R= $(CH_2)_5CH(CH_3)_2$
Rakicidin B (**4**), R= $(CH_2)_6CH(CH_3)_2$
Rakicidin D (**5**), R= H
Rakicidin E (**6**), R= $(CH_2)_7CH(CH_3)_2$

b

— ^{1}H-^{1}H COSY
⤻ HMBC
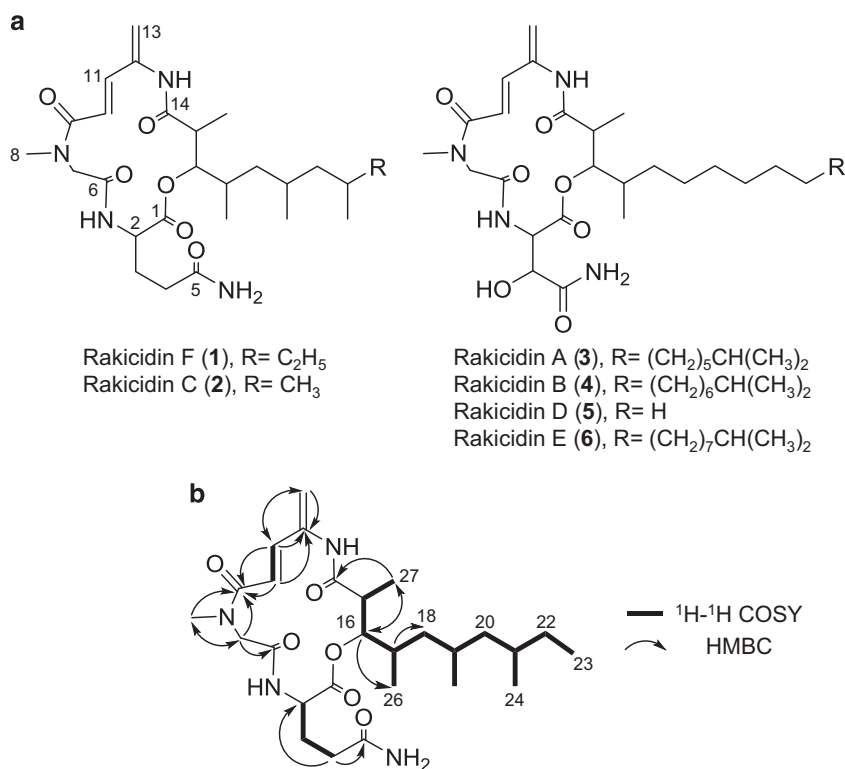
Figure 1 (**a**) Structure of rakicidins F (**1**), C (**2**), A (**3**), B (**4**), D (**5**) and E (**6**). (**b**) ^{1}H-^{1}H COSY and key correlations for **1**.

the vicinal coupling constant ($J_{10,11} = 14.7$ Hz in average). The ^{1}H-^{13}C long-range correlations from H-8 (δ_H 3.13) to C-9 and C-7 (δ_C 54.1), and from H-7 (δ_H 4.47, 3.99) to C-9 and a carbonyl carbon C-6 (δ_C 170.3) confirmed a connection between the 2,4-pentadienoate moiety (C-9 to C-13) and *N*-methylglycine moiety. The HMBC correlations from methylene protons (δ_H 2.29, H_2-4) to a carbonyl carbon C-5 (δ_C 177.3) and from methyl protons (δ_H 1.22, H_3-27) to a carbonyl carbon C-14 (δ_C 175.9) revealed that the carbonyl carbons were located at positions adjacent to C-4 and C-15, respectively. These interpretations indicated that compound **1** has three partial structures, as described above, and the chemical shifts showed high similarity to those of rakicidins A, B, C, D and E.[3,8–10]

The structure of **1** was finally established by comparison with the NMR spectra of **2**. The chemical shifts of **1** and **2** were almost identical except for a methyl and a methylene residue in the acyl group (C-14 to C-27 in **1**) (Supplementary Table 1). The chemical shifts of a methyl (δ_C 11.7 δ_H 0.88) at C-23 and a methylene (δ_C 30.0 δ_H 1.42, 1.07) at C-22 were observed in the NMR spectra of **1**, whereas a new methyl signal (δ_C 24.5 δ_H 0.89) at C-22 came along in the NMR spectra of **2**. Taken together with the difference of the predicted molecular formula between **1** and **2**, these findings suggested that compound **2** is a one-methylene-shorter derivative of **1**. HMBC correlations of **2** from a methine proton (δ_H 4.59, H-2) to a carbonyl carbon C-6 (δ_C 170.4) and a carbonyl carbon C-1 (δ_C 171.1) and from a methine proton (δ_H 5.38, H-16) to C-1 confirmed that there was a connection among three partial fragments: C-1 to C-5, C-6 to C-13, and C-15 to C-26. In addition, the proton and carbon chemical shifts were almost identical with those of rakicidin C as reported in the literature.[8] Thus, we concluded that compound **2** is rakicidin C and that the structure of rakicidin F (**1**) is a one-methylene-longer derivative of rakicidin C (**2**) (Figure 1a).

Table 1 NMR data for rakicidin F (1) in methanol-d_4

	Rakicidin F (**1**)		
Position	δ_C	δ_H mult (J in Hz)	HMBC
1	171.1, qC		
2	53.9, CH	4.59 dd (10.2, 4.2)	
3	31.0, CH_2	2.12 m	
		1.96 m	
4	33.1, CH_2	2.29 t (7.8)	2, 5
5	177.3, qC		
6	170.3, qC		
7	54.1, CH_2	4.47 d (17.4)	
		3.99 d (18.0)	6, 8, 9
8	37.8, CH_3	3.13 s	7, 9
9	168.9, qC		
10	119.0, CH	6.20 d (15.0)	9, 12
11	141.4, CH	7.12 d (14.4)	9, 12
12	139.5, qC		
13	120.5, CH_2	5.54 s	11
		5.46 s	11, 12
14	175.9, qC		
15	44.3, CH	2.92 m	
16	78.1, CH	5.38 dd (10.2, 1.2)	26, 27
17	32.2, CH	2.02 m	18
18	43.3, CH_2	1.28 m	
		0.92 m	
19	28.3, CH	1.79 m	
20	46.6, CH_2	1.18 m	
		0.92 m	
21	32.9, CH	1.42 m	
22	30.0, CH_2	1.42 m	
		1.07 m	
23	11.7, CH_3	0.88 m	
24	20.72^a, CH_3	0.88 m	
25	20.77^a, CH_3	0.88 m	
26	14.4, CH_3	1.04 d (6.6)	
27	16.0, CH_3	1.22 d (7.2)	14, 16

^{a}Interchangeable.

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Compounds **1** and **2** were evaluated for their antimicrobial activity against *Bacillus subtilis*, *Escherichia coli* and *Saccharomyces cerevisiae* by a paper disk diffusion assay. Rakicidin C has been reported to be inactive against Gram-positive and Gram-negative strains and has no cytotoxicity toward various cell lines, although the details of the strains, cell lines and experimental methods were not provided in the literature.[8] Rakicidin F (**1**) showed growth inhibitory activity against *B. subtilis* and *E. coli* at the dosage of 25 μg per disk, while rakicidin C (**2**) showed weak activity only against *B. subtilis* with an MIC value of 50 μg per disk. Rakicidin C (**2**) treated at a dose of 1.25 μM achieved 30% inhibition of invading murine carcinoma colon 26-L5 cells without any cytotoxicity,[11] and rakicidin F (**1**) did not show any anti-invasive effect at non-cytotoxic concentration. Meanwhile, rakicidin C (**2**) is less cytotoxic than rakicidin F (**1**) (IC50 = 17 μM).

In conclusion, marine sponge-derived *Streptomyces* sp. GKU 220 produces a novel antibacterial cyclic depsipeptide rakicidin F (**1**) consisting of three amino acids, including a rare and nonproteinogenic amino acid (4-amino-2,4-pentadienoic acid), and a modified fatty acid. Rakicidins F (**1**) and C (**2**) have a different 3-hydroxy acid moiety from rakicidins A (**3**), B (**4**), D (**5**) and E (**6**), and a glutamine residue in place of the 3-hydroxyasparagine residue in **3**, **4** and **5** (Figure 1a).[3,8–10] Further biological and structure-activity relationship studies could expand the utility of rakicidins in the development of anti-tumor agents.

DEDICATION

The authors dedicate this work to Professor Hamao Umezawa who made significant contributions to the discovery of antibiotics, especially including kanamycin, used in human medicine and agriculture.

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