New Macrolactins from a Marine *Bacillus* sp. Sc026

Chutima Jaruchoktaweechai,[†,⊥] Khanit Suwanborirux,*[,†] Somboon Tanasupawatt,[†] Prasat Kittakoop,[‡] and Piamsak Menasveta[§]

Departments of Pharmacognosy and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand, National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, 73/1 Rama VI Road, Rajdhevee, Bangkok 10400, Thailand, and Marine Biotechnology Research Unit, Aquatic Resource Research Institute, Chulalongkorn University, Bangkok 10330, Thailand

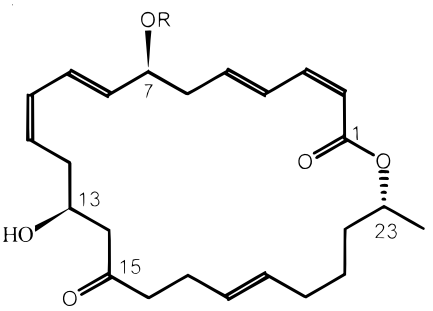
Received December 1, 1999

Bioassay-guided fractionation of the EtOAc extract of a marine *Bacillus* sp. Sc026 culture broth has led to the isolation of the known compound, macrolactin F (**1**), together with two new compounds, 7-*O*-succinyl macrolactin F (**2**) and 7-*O*-succinyl macrolactin A (**3**). The chemical structures of **1**−**3** were elucidated on the basis of spectral data analyses and literature data comparison. Compounds **1**−**3** exhibited antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*.

As part of our interest in exploring the biologically active secondary metabolites of marine microorganisms obtained from the Thai marine environment, we have investigated several *Bacillus* strains isolated from sediments around Sichang Island (an island on the east coast in the Gulf of Thailand). We found antibacterial activity in the crude EtOAc extract of the marine *Bacillus* sp. Sc026 culture broth. Herein, we report the isolation, antibacterial activity, and structures of macrolactins from the marine *Bacillus* sp. Sc026, including the known compound, macrolactin F (**1**), together with two new compounds, 7-*O*-succinyl macrolactin F (**2**) and 7-*O*-succinyl macrolactin A (**3**). The macrolactins are polyene macrolides containing a 24-membered lactone ring previously isolated from a taxonomically unidentified deep sea bacterium.[1,2] So far, six macrolactins (A−F) have been chemically characterized, and only macrolactins A and E have been studied for total synthesis.[3−6] Macrolactin A showed selective antibacterial activity, inhibited B16−F10 murine melanoma cancer cells in vitro assays, showed significant inhibition of mammalian Herpes simplex viruses (types I and II), and protected T-lymphoblast cells against human HIV viral replication.[1]

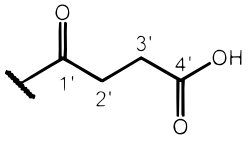
The EtOAc extract from the 90-L culture broth of the marine *Bacillus* sp. Sc026 was subjected to repeated Si gel column chromatography to afford macrolactin F (**1**). Fractions possessing antibacterial activity were subsequently chromatographed by Sephadex LH-20, Si gel, and C_{18} reversed-phase chromatography and preparative C_{18} reversed-phase TLC, to yield two new macrolactins, 7-*O*-succinyl macrolactin F (**2**) and 7-*O*-succinyl macrolactin A (**3**). The chemical structures of **1**−**3** were elucidated by extensive analyses of spectral data and comparison with known macrolactins.

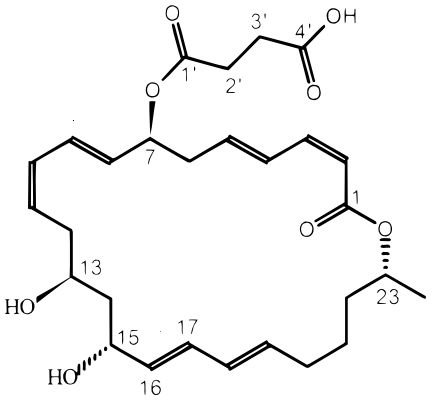
The HRFABMS established the molecular formula of **1** as $C_{24}H_{34}O_5$ by showing a molecular ion adduct peak at *m/z* 425.2326 $[M + Na]^+$. The ^1H and ^{13}C NMR data of **1** were identical with those of the known compound, macrolactin F, hence the identity of **1** was established.[1]



(1) R = H

(2) R =





(3)

The molecular formula of $C_{28}H_{38}O_8$ for **2** was determined by the HRFABMS showing a pseudomolecular ion peak at *m/z* 525.2490 $[M + Na]^+$. Compound **2** has an extra $C_4H_4O_3$ unit compared with the molecular formula of **1**. Extensive analyses of ^1H NMR, 2D $^1H-^1H$ COSY, and 2D TOCSY spectra of **2** indicated the similarity of the chemical structure to **1.** The downfield shift of H-7 (from δ 4.34 of **1**

* To whom correspondence should be addressed. Tel.: +66 2 218 8363. Fax: +66 2 255 8227. E-mail: skhanit@chula.ac.th.
† Faculty of Pharmaceutical Sciences.
‡ National Center for Genetic Engineering and Biotechnology.
§ Marine Biotechnology Research Unit, Aquatic Resource Research Institute.
⊥ Present address: Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Silpakorn University, Nakornpathom 73000, Thailand.

10.1021/np990605c CCC: $19.00
Published on Web 06/27/2000

Table 1. ^{1}H and ^{13}C NMR Data of Compounds **2** and **3** in $CDCl_3$

	2[a]		**3**[b]	
carbon	δ_C	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)
1	166.2		166.3	
2	117.9	5.53, d (11.6)	118.5	5.54, d (11.4)
3	143.0	6.45, dd (10.9, 11.6)	141.8	6.48, dd (11.4, 11.6)
4	130.0	7.19, dd (10.9, 15.5)	129.8	7.08, dd (11.6, 15.1)
5	137.7	5.88, dt (7.5, 15.5)	137.7	5.59, dt (7.1, 15.1)
6	38.4	2.48, 2H, m	39.4	2.55, 2H, dd (7.0, 7.1)
7	72.5	5.41, m	72.3	5.54, m[c]
8	130.8	5.62, dd (5.2, 15.2)	130.8	5.68, dd (3.4, 15.0)
9	126.5	6.41, tdd (1.2, 10.9, 15.2)	124.9	6.59, dd (11.6, 15.0)
10	130.5	6.04, dd (10.8, 10.9)	130.1	6.02[c]
11	127.8	5.44, dt (8.2, 10.8)	127.3	5.36, dt (5.5, 10.7)
12	34.7	2.34, 2H, m[c]	34.5	2.40, m
				2.92, dt (10.7, 13.0)
13	68.0	4.01, m	70.6	4.09, m
14	47.6	2.49, dd (7.9, 17.4)	39.1	1.58, m[c]
		2.55, dd (4.0, 17.4)		1.82, ddd (2.9, 9.5, 14.4)
15	211.9		70.2	4.54, br t (7.1)
16	43.6	2.40, 2H, t (7.3)	131.9	5.53[c]
17	27.0	2.20, 2H, m[c]	131.3	6.09[c]
18	128.5	5.32, m[c]	130.0	5.99[c]
19	131.0	5.35, m[c]	135.2	5.59[c]
20	31.7	1.91, m	32.0	2.08, m
		1.97, m		2.20, m
21	24.9	1.34, 2H, m	24.5	1.45, 2H, m[c]
22	35.0	1.44, m	34.9	1.55, 2H, m[c]
		1.57, m		
23	70.9	4.95, m	71.5	4.98, m
23-Me	20.0	1.18, 3H, d (6.4)	20.0	1.27, 3H, d (6.2)
1′	171.0		170.8	
2′	28.9[d]		28.8[d]	2.65, 2H, m[d]
3′	29.6[d]	} 2.58, 4H, br s	29.7[d]	2.70, 2H, m[d]
4′	174.3		174.8	

[a] Data were recorded at 500 MHz (^{1}H) and 75 MHz (^{13}C). [b] Data were recorded at 300 MHz (^{1}H) and 75 MHz (^{13}C). [c] Overlapping signals. [d] Assignments may be interchangeable.

to δ 5.41 of **2**) and the additional four equivalent protons (H_2-2′, H_2-3′) at δ 2.58 were the main spectral differences. The ^{13}C NMR spectrum of **2** was also similar to that of **1**, with additional carbon signals at δ 28.9 (d), 29.6 (d), 171.0 (s), and 174.3 (s). The HMQC spectrum of **2** revealed connections of the four protons (H-2′ and H-3′) to the methylene carbons at δ 28.9 and 29.6. The HMBC spectrum of **2** demonstrated long-range correlations of the methylene protons (H-2′ and H-3′) to the carbonyl carbons at δ 171.0 (C-1′) and 174.3 (C-4′). These observed spectral data were indicative of the presence of the succinic acid half-ester moiety in **2**, which was supported by the IR spectrum of **2** exhibiting intensely broad hydroxyl absorption from 3500 to 2500 cm^{-1}, carboxylic carbonyl absorption at 1730 cm^{-1}, and ester carbonyl absorption at 1710 cm^{-1}. Placement of the succinic acid group at the C-7 hydroxyl group was confirmed by the downfield shift of H-7 (δ 5.41) in **2** compared to the shift of H-7 (δ 4.34) in **1** and also by the long-range correlation of H-7 to the carbonyl carbon at δ 171.0 (C-1′) in the HMBC spectrum of **2**. Compound **2** was therefore 7-*O*-succinyl macrolactin F, a new derivative of the macrolactins.

As determined by the HRFABMS showing a pseudomolecular ion peak at 525.2421 [M + Na]$^+$, the molecular formula of **3**, $C_{28}H_{38}O_8$, was the same as that of **2**. The appearance of the succinic acid half-ester fragment in **3** was defined by 2D NMR studies of ^{1}H−^{1}H COSY, HMQC, and HMBC spectra. The ^{1}H NMR resonances at δ 2.65 and 2.75 and the ^{13}C NMR resonances at δ 28.8, 29.7, 170.8, and 174.8 were assigned to the succinyl group. The attachment of the succinyl group at the C-7 hydroxyl group was also secured by the downfield shift of H-7 at δ 5.54. Further 2D NMR analyses suggested that the macrolide ring of **3** was identical with that of macrolactin A.[1] The absence of the ketone carbon at δ 211.9 (C-15) and the presence of the oxymethine carbon at δ 70.2 (C-15), the olefinic carbons at δ 131.9 (C-16) and 131.3 (C-17), the oxymethine proton at δ 5.53 (H-15), and the olefinic protons at δ 6.09 (H-16) and 5.99 (H-17) revealed the characteristic allylic alcohol functionality at C-15 as in macrolactin A. Compound **3** was then assigned as a new macrolactin derivative, 7-*O*-succinyl macrolactin A.

Compounds **2** and **3** showed optical rotations similar to those of their parent macrolactins, F and A, respectively.[2] It can therefore, be assumed that both new macrolactins have the same stereochemistry as their respective parent molecules. Compounds **1**−**3** exhibited antibacterial activity against *B. subtilis* and *S. aureus* (inhibition zones of 8−28 mm at 50−100 μg/disk).

Experimental Section

General Experimental Procedures. The 300-MHz NMR experiments were performed on a Bruker AVANCE DPX-300 FT-NMR spectrometer, while the 500-MHz NMR data were obtained on a JEOL JNM-A500 NMR spectrometer, and chemical shifts (ppm) were referenced relative to the residual undeuterated solvent signals. The number of attached protons for the ^{13}C NMR signals was determined from DEPT experiments.[7] Proton-detected heteronuclear correlations were measured using HMQC[8] (optimized for $^1J_{HC} = 145$ Hz) and HMBC[9] (optimized for $^nJ_{HC} = 8$ Hz) pulse sequences. The HRFABMS spectra were obtained with JEOL JMS−HX110 mass spectrometer, operating at 10 kV ionization voltage and 3 kV acceleration voltage, using a mixture of glycerol and *m*-nitrobenzyl alcohol as a matrix and NaCl as an alkali metal cation source. Optical rotations were measured on a Perkin-Elmer 341 polarimeter using a sodium lamp operating at 589 nm. IR and UV spectra were obtained on a Perkin-Elmer 2000

FT-IR spectrometer and a Spectronic 3000 UV spectrometer, respectively.

Microorganism. The bacterium (Sc026) was isolated from marine sediment collected nearby Sichang Island (at a 15-m depth), Chonburi, Thailand. The isolation medium was marine agar (MA). The characteristics of strain Sc026 were determined by the method described by Barrow and Feltham.[10] The bacterium was Gram-positive, rod-shaped with subterminal spores, and motiled by peritrichous flagella. Colonies on a MA plate were opaque, circular, raised along the entire edge, and white. The bacterium showed the following characteristics: positive reaction to catalase and oxidase; reduction of nitrate; positive Voges−Proskauer reaction; hydrolysis of casein and starch, but exhibited negative reaction to urease, indole; utilization of citrate; and hydrolysis of hippurate. Growth occurred in media containing 0−8% NaCl (wt/vol). The bacterium could grow at temperatures ranging from 30 to 42 °C; however, the optimal temperature for growth was at 30 °C. Acid was produced from D-cellobiose, D-fructose, D-glucose, inositol, raffinose, salicin, sorbitol, sucrose, and D-trehalose, but not from L-arabinose, D-galactose, lactose, maltose, d-mannitol, D-mannose, melibiose, melizitose, L-rhamnose, D-ribose, sorbose, and D-xylose. On the basis of these available characteristics, this bacterium was identified as of the genus *Bacillus*.[11,12] A specimen of *Bacillus* sp. Sc026 was deposited at the Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

Cultivation of *Bacillus* sp. Sc026. The bacterium was grown on MA plates and incubated at 30 °C for a day. A loopful of the bacterium was inoculated into a 500-mL Erlenmeyer flask containing 250 mL of marine broth and incubated on a rotatory shaker at 200 rpm, 30 °C, for 3 days.

Extraction and Isolation. The culture broth (90 L) of the marine *Bacillus* sp. Sc026 was extracted with EtOAc. The EtOAc layer was concentrated under reduced pressure to yield the crude extract (8 g), which was subjected to Si gel flash column chromatography using a gradient elution of $CHCl_3$−MeOH. Elution with 10% MeOH in $CHCl_3$ gave a 444-mg fraction, which was rechromatographed on a Si gel column using a gradient elution of $CHCl_3$−EtOAc−MeOH to yield macrolactin F (**1**) (65 mg). The fraction (1.4 g) eluted with 30−40% MeOH in $CHCl_3$ was repeatedly subjected to gel filtration on a Sephadex LH-20 column using an isocratic elution of $CHCl_3$−MeOH−hexane (75:20:5) to give an antibacterial active fraction (616 mg). The fraction was subsequently chromatographed on a Si gel column using a gradient elution of $CHCl_3$-MeOH, a C_{18} reversed-phase column using a gradient elution of MeOH−H_2O, and finally by preparative C_{18} reversed-phase TLC developed with a solvent system of MeOH−H_2O (4:1), yielding two new macrolactins, 7-*O*-succinyl macrolactin F (**2**) (7 mg) and 7-*O*-succinyl macrolactin A (**3**) (28 mg).

Antibacterial Assay. Antibacterial activity of the isolated compounds was performed using the agar diffusion assay against *B. subtilis* ATCC 6633 and *S. aureus* ATCC 25923. Compounds **1** and **2** were tested at a concentration of 100 μg/disk, while **3** was tested at 50 μg/disk. The inhibition zones of **1**−**3** against *B. subtilis* were 8, 9, and 10 mm, respectively; those of **1**−**3** against *S. aureus* were 28, 8, and 24 mm, respectively.

Macrolactin F (1): amorphous solid; $[\alpha]^{25}_D$ −27.9° (*c* 1.31, MeOH) (lit.[1] $[\alpha]_D$ −30.1°); ^{1}H and ^{13}C NMR data in accordance with those of macrolactin F;[1] HRFABMS *m*/*z* 425.2326 [M + Na]$^+$ (calcd for $C_{24}H_{34}O_5Na$, 425.2304).

7-*O*-Succinyl macrolactin F (2): amorphous solid; $[\alpha]^{25}_D$ −24.4° (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ϵ) 235 (4.17), 261 (4.23) nm; IR ($CHCl_3$) ν_{max} 3439, 1730, 1710, 1639, 1406 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; HRFABMS *m*/*z* 525.2490 [M + Na]$^+$ (calcd for $C_{28}H_{38}O_8Na$, 525.2464).

7-*O*-Succinyl macrolactin A (3): amorphous solid; $[\alpha]^{25}_D$ −9.6° (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ϵ) 229 (4.57), 261 (4.18), nm; IR ($CHCl_3$) ν_{max} 3295, 1730, 1700, 1667, 1557, 1410 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; HRFABMS *m*/*z* 525.2421 [M + Na]$^+$ (calcd for $C_{28}H_{38}O_8Na$, 525.2464).

Acknowledgment. We thank the National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Thailand, for financial support. We thank Dr. H. Kobayashi, Institute of Molecular and Cellular Biosciences, the University of Tokyo, Japan, for HRFABMS data. The Scientific and Technological Research Equipment Center of Chulalongkorn University and the Pharmaceutical Research Instrument Center of the Faculty of Pharmaceutical Sciences are gratefully acknowledged.

References and Notes

(1) Gustafson, K.; Roman, M.; Fenical, W. *J. Am. Chem. Soc.* **1989**, *111*, 7519−7524.
(2) Rychnovsky, S. D.; Skalitzky, D. J.; Pathirana, C.; Jensen, P. R.; Fenical, W. *J. Am. Chem. Soc.* **1992**, *114*, 671−677.
(3) Donaldson, W. A.; Bell, P. T.; Wang, Z.; Bennett, D. W. *Tetrahedron Lett.* **1994**, *35*, 5829−5832.
(4) Smith, A. B.; Ott, G. R. *J. Am. Chem. Soc.* **1996**, *118*, 13095−13096.
(5) Boyce, R. J.; Pattenden, G. *Tetrahedron Lett.* **1996**, *37*, 3501−3504.
(6) Smith, A. B.; Ott, G. R. *J. Am. Chem. Soc.* **1998**, *120*, 3935−3948.
(7) Doddrell, D. M.; Pegg, D. T.; Bendall, M. R. *J. Magn. Reson.* **1982**, *48*, 323−327.
(8) Bax, A.; Griffey, R. H.; Hawkins, B. L. *J. Magn. Reson.* **1983**, *55*, 301−315.
(9) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093−2096.
(10) Barrow, G. I.; Feltham, R. K. A. *Cowan and Steel's Manual for the Identification of Medicinal Bacteria*, 3rd ed.; Cambridge University Press: Cambridge, 1993; pp 50−90.
(11) Berkeley, R. C. W.; Claus, D. In *Bergey's Manual of Systematic Bacteriology*; Sneath, P. H. A., Mair, N. S., Sharpe, M. E., Holt, J. G., Eds.; Williams & Wilkins: Baltimore, 1989; Vol. 2, Section 13, pp 1104−1139.
(12) Shida, O.; Takagi, H.; Kadowaki, K.; Nakamura, K. L.; Komagata, K. *Int. J. Syst. Bacteriol.* **1997**, *47*, 289−298.

NP990605C