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Micromonospolides A–C, new macrolides from *Micromonospora* sp.

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Abstract—Three new macrolides, micromonospolides A–C, have been isolated from a new species of the genus *Micromonospora* and their structures were elucidated to be bafilomycin-type macrolides which have a 16-membered lactone ring on the basis of spectroscopic data. Micromonospolides A–C inhibited gastrulation of starfish embryos at minimum inhibitory concentrations of 0.010, 0.011 and 1.6 μg mL^{-1}, respectively.

1. Introduction

Gastrulation is one of the fundamental events in embryogenesis, and is the first process closely related to cellular differentiation.[1] In the starfish embryo, the early cleavage stage (1–128-cell stages) is followed by blastulation in which loosely arranged embryonic cells enwrapped in the fertilization envelope pack themselves into a sheet and further into a hollow blastula.[2] After the blastula hatches from the fertilization envelope, the stage is set for the coordinated movements of gastrulation the process which transforms the simple hollow ball of epithelial cells into a multilayered structure with a mesendodermal archenteron produced by tucking cells from the exterior into the interior.[3] Meanwhile, cells escape from the tip of the archenteron and move into the blastocoel to form motile mesenchyme cells.

During our search for inhibitors of starfish (*Asterina pectinifera*) embryonic development,[4–12] we found that the *n*-BuOH extract of a new species of the genus *Micromonospora* inhibited gastrulation of *A. pectinifera* embryos. A preliminary examination led to the isolation of a new bioactive macrolide designated micromonospolide A (**1**, Fig. 1).[13] Further investigation of the extract led to the isolation of two new macrolides, micromonospolides B and C (**2** and **3**, Fig. 1). In this paper we report isolation, structure elucidation, and biological activities of **1**–**3**.

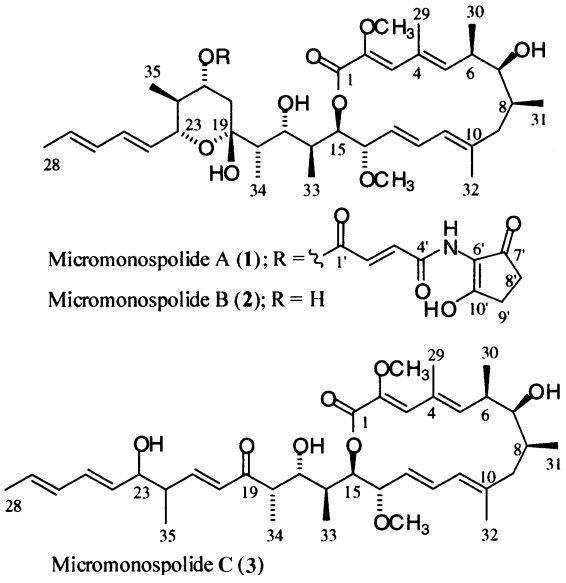

Figure 1. Structures of **1**–**3**.

2. Results and discussion

The fermentation broth filtrate of *Micromonospora* sp. was extracted with *n*-BuOH. The extract was subjected to chromatography on ODS using a gradient of 80–100% MeOH in H_2O as eluent. The bioactive fractions were subsequently chromatographed on ODS using CH_3CN as eluent to afford **1**–**3**.

Micromonospolide A (**1**) was obtained as pale yellow

Keywords: biologically active compounds; macrolides; microorganisms; natural products.

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Table 1. ^{1}H NMR data (500 MHz, CD_3OD) for **1**–**3**

H#	**1**		**2**		**3**	
	δ_H mult.	J (Hz)	δ_H mult.	J (Hz)	δ_H mult.	J (Hz)
3	6.63 s		6.63 s		6.68 s	
5	5.87 br d	8.8	5.86 br d	8.5	5.88 br d	9.0
6	2.50 ddq	8.8, 7.1, 1.9	2.50 ddq	8.5, 7.0, 1.8	2.51 ddq	9.0, 7.1, 1.7
7	3.23 dd	6.4, 1.9	3.23 dd	6.0, 1.8	3.27 dd	6.0, 1.7
8	1.83 m		1.84 m		1.86 m	
9a	2.00–2.05 m		2.00–2.05 m		2.08 dd	14.3, 11.1
9b	2.00–2.05 m		2.00–2.05 m		2.01 m	
11	5.76 br d	10.7	5.76 br d	11.0	5.78 br d	10.9
12	6.60 dd	15.1, 10.7	6.60 dd	14.9, 11.0	6.56 dd	15.1, 10.9
13	5.09 dd	15.1, 8.8	5.09 dd	14.9, 8.8	5.16 dd	15.1, 8.3
14	4.00 dd	8.8, 7.8	3.99 dd	8.8, 7.9	3.94 dd	8.3, 6.7
15	5.04 dd	7.8, 1.5	5.03 dd	7.9, 1.5	5.18 dd	6.7, 2.0
16	2.07 m		2.06 m		1.98 m	
17	4.09 dd	10.5, 1.7	4.09 dd	10.7, 1.8	3.80 dd	8.9, 3.8
18	1.81 dq	7.1, 1.7	1.75 dq	7.3, 1.8	3.06 dq	6.9, 3.8
20a	2.34 dd	11.7, 4.9	2.21 dd	12.2, 4.6	6.23 d	16.0
20b	1.38 t	11.7	1.22 dd	12.2, 11.6		
21	5.06 ddd	11.7, 10.6, 4.9	3.57 ddd	11.6, 10.4, 4.6	6.87 dd	16.0, 8.0
22	1.52 tq	10.6, 6.6	1.17 tq	10.4, 6.7	2.43 ddq	8.0, 6.9, 6.6
23	4.13 dd	10.6, 7.7	3.98 dd	10.4, 7.9	3.97 dd	7.1, 6.6
24	5.46 dd	15.1, 7.7	5.43 dd	15.2, 7.9	5.50 dd	15.1, 7.1
25	6.16 dd	15.1, 10.5	6.11 dd	15.2, 10.4	6.17 dd	15.1, 10.3
26	5.99 ddd	15.1, 10.5, 1.5	5.98 ddd	14.9, 10.4, 1.5	6.05 ddd	14.9, 10.3, 1.4
27	5.66 dq	15.1, 6.8	5.63 dq	14.9, 6.7	5.69 dq	14.9, 6.9
28	1.72 dd, 3H	6.8, 1.5	1.71 dd, 3H	6.7, 1.5	1.73 dd, 3H	6.9, 1.4
29	1.95 br s, 3H		1.94 d, 3H	1.5	1.96 br s, 3H	
30	1.05 d, 3H	7.1	1.05 d, 3H	7.0	1.05 d, 3H	7.1
31	0.92 d, 3H	6.8	0.91 d, 3H	6.7	0.93 d, 3H	6.9
32	1.90 br s, 3H		1.90 br s, 3H		1.84 br s, 3H	
33	0.86 d, 3H	6.8	0.85 d, 3H	7.7	0.97 d, 3H	6.9
34	1.00 d, 3H	7.1	1.00 d, 3H	7.3	1.10 d, 3H	6.9
35	0.83 d, 3H	6.6	0.90 d, 3H	6.7	1.03 d, 3H	6.9
2′	6.73 d	15.1				
3′	7.17 d	15.1				
8′	2.34 br s, 2H					
9′	2.34 br s, 2H					
2-OMe	3.49 s, 3H		3.48 s, 3H		3.62 s, 3H	
14-OMe	3.24 s, 3H		3.24 s, 3H		3.23 s, 3H	

needles from a diethyl ether–hexane solution. It was soluble in MeOH and $CHCl_3$, and has R_f values of 0.43 on ODS TLC (MeOH–H_2O, 9:1) and 0.30 on silica gel TLC ($CHCl_3$–MeOH, 19:1). The molecular formula, $C_{46}H_{65}NO_{13}$, which requires 15 degrees of unsaturation, was determined by an HRFAB mass measurement of the $[M+Na]^+$ ion at *m/z* 862.4390. The IR and UV spectra showed the presence of OH groups and conjugated carbonyl groups. The ^{1}H NMR (Table 1), ^{13}C NMR (Table 2), ^{1}H–^{1}H COSY, HMQC and HMBC spectra allowed the gross structure of **1**. The partial structures from C-1 to C-23 and from C-1′ to C-10′ were supported by comparison of the ^{1}H- and ^{13}C NMR data ($CDCl_3$)[13] of **1** with those of the known compounds bafilomycin B_1.[14] Comparison of ^{1}H- and ^{13}C NMR chemical shifts and the magnitude of the coupling constants of **1** with those of bafilomycin A_1[15] revealed that the relative stereochemistry of **1** is the same as for bafilomycin A_1 (**4**), which was supported by the NOESY correlations, as shown in Fig. 2. Consequently, the structure of micromonospolide A was elucidated as **1**.

The absolute configuration of bafilomycin A_1 (**4**) has been unambiguously determined by X-ray crystallographic analyses of bafilomycin A_1 (**4**) and its 21-(2′,2′,2′-trichloroethylcarbonate) derivative.[16] However, the stereochemistry at C-8 of bafilomycin A_1 (**4**) has not been shown exactly in the structural formula.[15–17] The structural formula of bafilomycin A_1 (**4**) should be given as shown in Fig. 3, with the 8*S* stereochemistry.

Micromonospolide B (**2**) was obtained as colorless needles from a diethyl ether–hexane solution. It was soluble in MeOH and $CHCl_3$, and has R_f values of 0.43 on ODS TLC (MeOH–H_2O, 9:1) and 0.31 on silica gel TLC ($CHCl_3$–MeOH, 19:1). The molecular formula, $C_{37}H_{58}O_9$, which requires nine degrees of unsaturation, was determined by an HRESI mass measurement of the $[M+Na]^+$ ion at *m/z* 669.4025. The IR spectrum contained a strong hydroxyl band at 3450 cm^{-1} and bands at 1709, 1688, 1636 and 1618 cm^{-1}, which must be due to the conjugated lactone carbonyl group and the C–C double bonds. The presence of the conjugated lactone carbonyl group was supported by UV maxima at 230 (ϵ 39,300), 237 (ϵ 39,000), 250 (sh, ϵ 30,700) and 283 nm (ϵ 13,200). The ^{1}H NMR (Table 1) and ^{13}C NMR (Table 2) spectra of **2** were almost identical to those of micromonospolide A (**1**) except that they lacked signals due to an *N*-(3-hydroxy-2-cyclopentenone-2-yl)-fumarylester monoamide. The ^{13}C NMR spectrum of **2** revealed the presence of 37 carbon atoms. The C-21 signal at δ_C 71.6 is shifted upfield by

Table 2. ^{13}C NMR data (125 MHz, CD_3OD) for **1**–**3**

C#	δ_C, mult.[a]		
	1	**2**	**3**
1	168.8 s	168.8 s	168.0 s
2	143.2 s	143.2 s	143.4 s
3	135.0 d	135.0 d	135.1 d
4	134.0 s	134.0 s	133.8 s
5	146.4 d	146.4 d	145.9 d
6	39.2 d	39.2 d	39.6 d
7	81.9 d	82.0 d	82.0 d
8	42.6 d	42.6 d	41.9 d
9	43.4 t	43.4 t	43.4 t
10	146.1 s	146.1 s	145.2 s
11	126.3 d	126.4 d	126.5 d
12	135.5 d	135.6 d	134.9 d
13	127.7 d	127.7 d	127.6 d
14	85.0 d	85.0 d	85.9 d
15	78.3 d	78.3 d	78.2 d
16	40.1 d	40.1 d	41.3 d
17	72.5 d	72.5 d	74.6 d
18	44.0 d	44.0 d	48.4 d
19	101.8 s	101.9 s	205.7 s
20	41.0 t	44.4 t	130.7 d
21	76.2 d	71.6 d	151.7 d
22	43.1 d	45.9 d	45.3 d
23	76.9 d	77.2 d	77.5 d
24	131.3 d	131.9 d	133.0 d
25	135.1 d	134.5 d	134.1 d
26	133.0 d	133.2 d	133.0 d
27	131.6 d	131.2 d	131.5 d
28	19.0 q	18.9 q	19.0 q
29	15.0 q	14.9 q	14.8 q
30	18.6 q	18.6 q	19.0 q
31	23.3 q	23.3 q	23.7 q
32	20.9 q	20.9 q	20.4 q
33	11.1 q	11.1 q	12.2 q
34	8.0 q	7.9 q	10.6 q
35	13.2 q	14.3 q	16.8 q
1′	167.5 s		
2′	131.2 d		
3′	139.4 d		
4′	166.1 s		
6′	112.6 s		
7′	200.2 s		
8′	32.6 t		
9′	32.6 t		
10′	200.2 s		
2-OMe	61.2 q	61.2 q	61.4 q
14-OMe	56.8 q	56.8 q	56.9 q

[a] Multiplicities were determined by DEPT experiments.

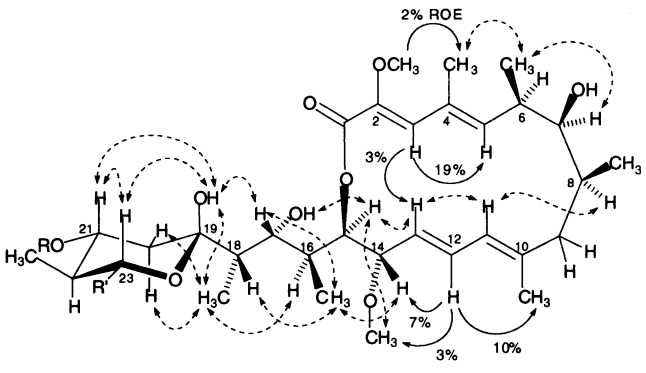

Figure 2. Selected ROEs (⟶) and NOESY correlations (⟵–⟶) of **1** in $CDCl_3$.

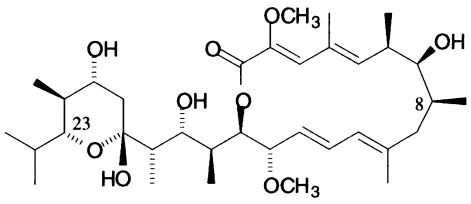

Figure 3. Structure of bafilomycin A_1 (**4**).

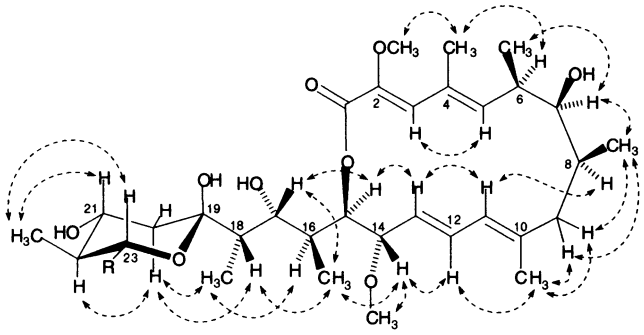

Figure 4. Selected NOESY correlations of **2** in CD_3OD.

4.6 ppm relative to the C-21 signal in **1**, suggesting the replacement of the *N*-(3-hydroxy-2-cyclopentenone-2-yl)-fumarylester monoamide group in **1** by a hydroxyl group. The 2D NMR spectral data including ^1H–^1H COSY, HMQC and HMBC were compatible with the gross structure proposed for micromonospolide B (**2**). Comparison of ^1H and ^{13}C chemical shifts and the magnitude of the coupling constants of **2** with those of **1** revealed that the relative stereochemistry of **2** is the same as for **1**, which was supported by the NOESY correlations (Fig. 4).

Micromonospolide C (**3**) was isolated as a white amorphous solid. It was soluble in MeOH and $CHCl_3$, and has R_f values of 0.52 on ODS TLC (MeOH–H_2O, 9:1) and 0.31 on silica gel TLC ($CHCl_3$–MeOH, 19:1). Since compound **3** was unstable in $CHCl_3$, MeOH or CD_3OD was used as the solvent for spectral measurements. The molecular formula, $C_{37}H_{56}O_8$, which was determined by an HRESI mass measurement of the $[M+Na]^+$ ion at *m/z* 651.3874, suggested that **3** was the dehydrated analog of **2**. The ^{13}C NMR spectrum (Table 2) of **3** revealed the presence of 37 carbon atoms and was similar to that of **2** except for the presence of a carbonyl signal at δ_C 205.7 (C-19) and two olefinic carbon signals at δ_C 130.7 (C-20) and 151.7 (C-21) in **3** instead of the signals at δ_C 101.9 (C-19), 44.4 (C-20) and 71.6 (C-21) in **2**. The ^1H NMR spectrum (Table 1) together with ^{13}C NMR spectrum revealed that the partial structures from C-24 to C-28 and from C-1 to C-18 including a 16-membered lactone ring were the same as those of **2**. The ^1H–^1H COSY and HMQC spectra disclosed the presence of a C-20/C-28 unit. The olefinic proton signal at δ_H 6.23 (H-20) showed an HMBC correlation to the carbonyl carbon signal at δ_C 205.7 (C-19), which in turn showed a two-bond correlation to a methine signal at δ_H 3.06 (H-18) and a three-bond correlation to a methyl signal at δ_H 1.10 (Me-34), thereby allowing the carbonyl group to be unambiguously placed between C-18 and C-20. The geometry of the disubstituted olefins out of the

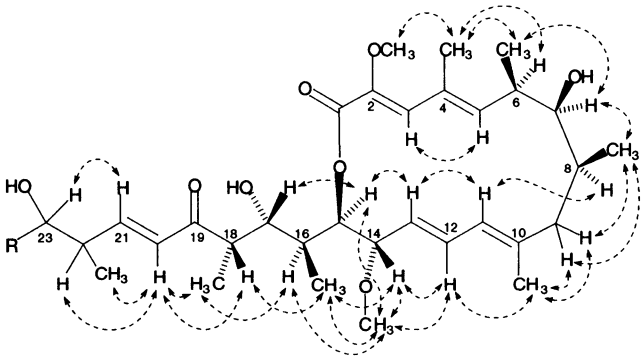

Figure 5. Selected NOESY correlations of **3** in CD_3OD.

Table 3. Inhibitory effects of **1**–**4** on gastrulation of starfish embryos

Compound	Minimum inhibitory concentration ($\mu g\ mL^{-1}$)
1	0.010
2	0.011
3	1.6
4	0.10

16-membered ring was determined to be 20*E*,24*E*,26*E* from the large vicinal ^1H coupling constants ($J_{20,21}$=16.0, $J_{24,25}$=15.1, $J_{26,27}$=14.9 Hz). The relative stereochemistry of C-1 to C-18 including the 16-membered lactone ring was confirmed to be the same as for **2** by comparison of ^1H and ^{13}C chemical shifts and the magnitude of the coupling constants of **3** with those of **2**, which was supported by the NOESY correlations (Fig. 5). Consequently, the structure of micromonospolide C was elucidated as **3**.

When 8-hour-old embryos at the early blastula stage of *A. pectinifera* were cultured in the presence of **1**–**3** and bafilomycin A_1 (**4**), the progression of embryonic development was arrested at the late blastula stage just prior to gastrulation. Micromonospolide B (**2**) showed nearly the same inhibitory activity as that of **1** (Table 3), indicating that the presence of an *N*-(3-hydroxy-2-cyclopentenone-2-yl)-fumarylester monoamide functionality at C-21 in **1** does not affect the activity. Micromonospolide C (**3**) was also active with a 100-fold decrease in potency compared to **1** and **2**, indicating that the opening of the tetrahydropyran ring (C-19–C-23) and the subsequent formation of the α,β-unsaturated ketone functionality resulted in the decrease in potency of the inhibitory activity. On the other hand, the minimum inibitory concentration of bafilomycin A_1 (**4**), which has an isopropyl group attached to a tetrahydropyran ring, was 0.10 $\mu g\ mL^{-1}$. The potent inhibitory activity of **1** and **2** against gastrulation of the starfish embryo seems to be due to the replacement of the isopropyl group in bafilomycin A_1 (**4**) by the (*E*,*E*)-1,3-pentadienyl group.

3. Experimental

3.1. General

^1H- and ^{13}C NMR spectra were recorded on a JEOL GSX500 spectrometer (500 MHz for ^1H, 125 MHz for ^{13}C). ^1H- and ^{13}C NMR chemical shifts were referenced to solvent peaks: δ_H 3.30 (residual CHD_2OD) and δ_C 49.8 for CD_3OD. FABMS, HR-FABMS, ESIMS and HR-ESIMS were measured on a JEOL SX102A spectrometer. UV and IR spectra were recorded on a Shimadzu UV-160A and a JASCO FT/IR-5300 spectrometer, respectively. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. CD spectra were recorded with a JASCO J-600 circular dichroic spectropolarimeter. Bafilomycin A_1 and 1-methyladenine were purchased from Sigma, St. Louis, Missouri, USA.

3.2. Fermentation

The fermentation of the micromonospolide-producing strain *Micromonospora* sp. was carried out at 28°C for 5 days under agitation at 220 rpm in a 300 mL Erlenmeyer flask containing 50 mL of a production medium (soluble starch 4.0%, soybean meal 1.0%, corn steep liquor 0.5%, dry yeast 0.5%, KH_2PO_4 0.5%, $ZnSO_4{\cdot}7H_2O$ 0.001%, $CoCl_2{\cdot}6H_2O$ 0.0001%, $NiSO_4$ 0.0001%, and $Mg_3(PO_4)_2{\cdot}8H_2O$ 0.05% adjusted to pH 7.0). The medium was inoculated with 5.0% of the volume of a seed culture prepared as follows. The organism was first cultured for 3 days at 28°C with vigorous shaking in a test tube (21 mm i.d.×200 mm) containing 10 mL of a seed medium (glucose 1.0%, soluble starch 2.0%, beef extract 0.3%, yeast extract 0.3%, difco tryptone 0.5%, KH_2PO_4 0.1%, $Mg_3(PO_4)_2{\cdot}8H_2O$ 0.05% adjusted to pH 7.0), and the culture (3.0%) was then inoculated into 50 mL of a seed medium in a 300 mL Erlenmeyer flask and cultured for 3 days at 28°C on a rotary shaker.

3.3. Bioassays

Specimens of *A. pectinifera* were collected from the coastal waters off Japan during their breeding season and kept in seawater at 15°C in laboratory aquaria. Oocytes and sperm were removed from ovarian and testicular fragments, respectively. Experiments were performed at 20°C and artificial seawater (Jamarin Laboratory, Osaka, Japan) was used throughout. Oocytes were induced to mature by the treatment with 1 μM 1-methyladenine.[18] Maturing oocytes were fertilized at 40 min after the start of 1-methyladenine treatment. The fertilized eggs were cultured for 8 h until they would achieve at the early blastula stage. Compounds to be tested were serially diluted in MeOH, and added to the suspensions of eight-hour-old embryos to give final concentrations of MeOH less than 0.2% in seawater. MeOH at the concentrations used had no effect on embryonic development. To assay for the developmental arrest of embryos, small number of embryos (ca. 20–30) were cultured in 200 μL of seawater containing a sample solution. The embryos were periodically observed for any cytological changes.

3.4. Isolation

The fermentation broth (2 L) of *Micromonospora* sp. was separated to the mycelial cake and supernatant by centrifugation. The supernatant was filtered, and the filtrate was extracted twice with 2 L of *n*-BuOH. The *n*-BuOH layer

was concentrated to a small volume (20 mL) under reduced pressure below 30°C, and subjected to reversed phase column chromatography on ODS (10 g, Wakogel LP-40C18) using a gradient of 80–100% MeOH in H_2O as eluent. The biologically active fractions had the spots detected by UV light as 254 nm and by spraying with 1% vanillin in sulfuric acid and then heating at 120°C. On the basis of TLC analysis, the bioactive fractions which had the same R_f value spot were combined and concentrated to give three crude fractions A (120 mg), B (20 mg) and C (15 mg). Fractions A–C were rechromatographed separately on ODS (5 g, Wakogel LP-40C18) using 100% CH_3CN as eluent to afford **1** (57 mg), **2** (10 mg) and **3** (11 mg), respectively.

3.4.1. Micromonospolide A (1). Pale yellow needles; mp 141–142°C (diethyl ether–hexane); $[\alpha]_D^{25}=+14.3°$ (*c* 0.63, MeOH); UV (MeOH) λ_{max} 230 (ϵ 75,800), 240 (sh, ϵ 73,300), 252 (ϵ 61,100), 290 (sh, ϵ 24,400), 350 nm (ϵ 4,700); IR (KBr) 3400, 1717, 1703, 1688, 1651 (sh), 1618 cm^{-1}; ^1H NMR data: see Table 1; ^{13}C NMR data: see Table 2; HR-FABMS (positive) *m/z* 862.4390 $[M+Na]^+$ (calcd for $C_{46}H_{65}NO_{13}Na$, 862.4353); FABMS (positive) *m/z* 862 $[M+Na]^+$; FABMS (negative) *m/z* 838 $[M-H]^-$; ESIMS (positive) *m/z* 1701 $[2M+Na]^+$, 862 $[M+Na]^+$.

3.4.2. Micromonospolide B (2). Colorless needles; mp 111–114°C (diethyl ether–hexane); $[\alpha]_D^{25}=+22°$ (*c* 0.06, MeOH); UV (MeOH) λ_{max} 230 (ϵ 39,300), 237 (ϵ 39,000), 250 (sh, ϵ 30,700), 283 nm (ϵ 13,200); IR (KBr) 3450, 1709, 1688, 1636, 1618 cm^{-1}; ^1H NMR data: see Table 1; ^{13}C NMR data: see Table 2; HR-ESIMS (positive) *m/z* 669.4025 $[M+Na]^+$ (calcd for $C_{37}H_{58}O_9Na$, 669.3978); ESIMS (positive) *m/z* 1315 $[2M+Na]^+$, 669 $[M+Na]^+$, 685 $[M+K]^+$.

3.4.3. Micromonospolide C (3). A white amorphous solid; mp 54–58°C; $[\alpha]_D^{25}=-23°$ (*c* 0.1, MeOH); UV (MeOH) λ_{max} 238 (ϵ 70,900), 283 nm (ϵ 20,300); IR (KBr) 3420, 1696, 1649, 1626 cm^{-1}; ^1H NMR data: see Table 1; ^{13}C NMR data: see Table 2; HR-ESIMS (positive) *m/z* 651.3874 $[M+Na]^+$ (calcd for $C_{37}H_{56}O_8Na$, 651.3873); ESIMS (positive) *m/z* 1279 $[2M+Na]^+$, 651 $[M+Na]^+$.

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References

1. Gilbert, S. F. *Developmental Biology*; 3rd ed.; Sinauer: Sunderland, 1991.
2. Dan-Sohkawa, M.; Fujisawa, H. *Dev. Biol.* **1980**, *77*, 328–339.
3. Kuraishi, R.; Osanai, K. *Biol. Bull.* **1992**, *183*, 258–268.
4. Ohta, S.; Okada, H.; Kobayashi, H.; Oclarit, J. M.; Ikegami, S. *Tetrahedron Lett.* **1993**, *34*, 5935–5938.
5. Ohta, S.; Kobayashi, H.; Ikegami, S. *Tetrahedron Lett.* **1994**, *35*, 4579–4580.
6. Ohta, S.; Kobayashi, H.; Ikegami, S. *Biosci. Biotech. Biochem.* **1994**, *58*, 1752–1753.
7. Ikegami, S.; Kobayashi, H.; Myotoishi, Y.; Ohta, S.; Kato, K. H. *J. Biol. Chem.* **1994**, *269*, 23262–23267.
8. Ohta, S.; Uno, M.; Yoshimura, M.; Hiraga, Y.; Ikegami, S. *Tetrahedron Lett.* **1996**, *37*, 2265–2266.
9. Uno, M.; Ohta, S.; Ohta, E.; Ikegami, S. *J. Nat. Prod.* **1996**, *59*, 1146–1148.
10. Yanai, M.; Ohta, S.; Ohta, E.; Ikegami, S. *Tetrahedron* **1998**, *54*, 15607–15612.
11. Ohta, S.; Ohta, E.; Ikegami, S. *J. Org. Chem.* **1997**, *62*, 6452–6453.
12. Ohta, E.; Ohta, S.; Ikegami, S. *Tetrahedron* **2001**, *57*, 4699–4703.
13. Ohta, E.; Ohta, S.; Kubota, N. K.; Suzuki, M.; Ogawa, T.; Yamasaki, A.; Ikegami, S. *Tetrahedron Lett.* **2001**, *42*, 4179–4181.
14. Werner, G.; Hagenmaier, H.; Albert, K.; Kohlshorn, H.; Drautz, H. *Tetrahedron Lett.* **1983**, *24*, 5193–5196.
15. Baker, G. H.; Brown, P. J.; Dorgan, R. J. J.; Everett, J. R. *J. Chem. Soc., Perkin Trans. 2* **1989**, 1073–1079.
16. Baker, G. H.; Brown, P. J.; Dorgan, R. J. J.; Everett, J. R.; Ley, S. V.; Slawin, A. M. Z.; Williams, D. J. *Tetrahedron Lett.* **1987**, *28*, 5565–5568.
17. Everett, J. R.; Baker, G. H.; Dorgan, R. J. J. *J. Chem. Soc., Perkin Trans. 2* **1990**, 717–724.
18. Tsuchimori, N.; Miyashiro, S.; Shibai, H.; Ikegami, S. *Development* **1988**, *103*, 345–351.