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Novel Macrolactins as Antibiotic Lactones from a Marine Bacterium

TOMOKAZU NAGAO[†], KYOKO ADACHI*, MIHO SAKAI,
MIYUKI NISHIJIMA and HIROSHI SANO[††]

Shimizu Laboratories, Marine Biotechnology Institute Co. Ltd.,
1900 Sodeshi, Shimizu, Shizuoka 424-0037, Japan

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Seven new macrolactins (named G~M) and known macrolactins A and F were isolated from a culture broth of *Bacillus* sp. PP19-H3. The strain had been isolated from the macroalga, *Schizymenia dubyi*. Macrolactin A, which was 24-membered lactone, had previously been reported to show antibacterial, cytotoxic and antiviral activities. The new macrolactins include 22-membered ring or dicyclic lactone in addition to geometric isomers of known macrolactins A and F. The antibacterial activities of all the macrolactins examined in this study were relatively weak.

There have been few studies of marine microorganisms compared with terrestrial ones. Marine microorganisms live in a quite different environment from their terrestrial counterparts and would thus be expected to have a different metabolic pathway and to produce compounds, which possess unique structures and activities.

We used an analytical chemistry assay as well as antibacterial and antimicroalgal assays to enable us to select a broad range of compounds. The analytical chemistry assay employed HPLC with a photodiode array detector and mass spectrometer, and we developed a method to select substances from HPLC retention time, and UV spectrum and the mass spectrum data[1].

The analytical chemistry assay enabled the isolation of macrolactin A and F and seven novel macrolactins from the cultured broth of *Bacillus* sp. PP19-H3. Macrolactin A and F have previously been reported by GUSTAFSON *et al.* as 24-membered ring lactones that were isolated from an unclassifiable deep-sea bacterium[2]. They reported macrolactin A to show selective antibacterial activities, cytotoxicity against B16-F10 murine melanoma cancer cells, and antiviral activities against Herpes simplex and HIV. In spite of its interesting activities, however, there had been no further study on the activities of macrolactin because of the lack of natural material. This led to investigations into the synthesis of macrolactin A[3~13] based on its estimated three-dimensional architecture[14].

Macrolactin A has also been isolated from a culture broth of *Actinomadura* sp. as neuronal cell-protecting substance by KIM *et al.*[15], while, macrolactin F has also been isolated from the *Bacillus* sp., together with novel 7-*O*-succinyl macrolactin F and 7-*O*-succinyl macrolactin A[16].

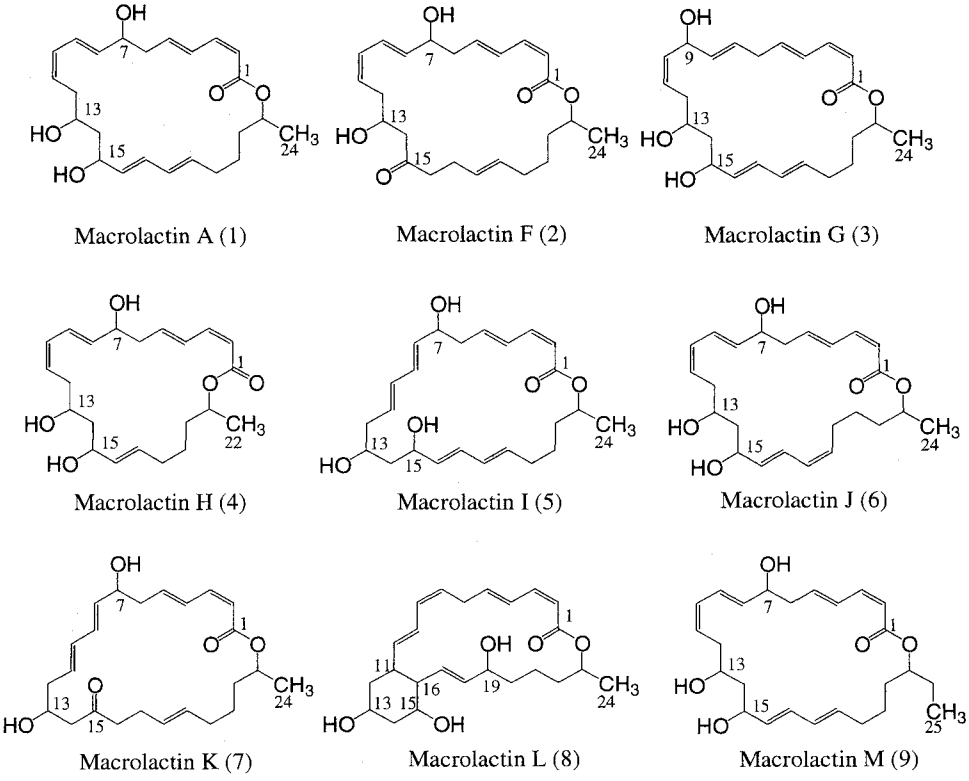
This paper describes the taxonomy of strain PP19-H3, and the fermentation, isolation, physicochemical properties, structural determination and antibacterial activities of novel macrolactins.

Taxonomy of the Producing Organism

The marine bacterium, strain PP19-H3, was isolated from the macroalga *Schizymenia dubyi* that had been collected on the Omaezaki coast of Shizuoka prefecture in Japan. This bacterium was subjected to standard biological and physiological tests. The colony was smooth, entire and opaque milky white. The strain was a facultative anaerobic Gram-positive endospore forming rod (1.4~3.3×0.4~0.9 μm), and it was motile with peritrichous flagella. The major isoprenoid quinone was MK-7. The guanine-plus-cytosine content of the DNA was 47.8 mol%. According to BERGEY's

Present address: [†] Chemical Laboratory, Technical Research Laboratories, Kawasaki-Steel Corporation, 1, Kawasaki-cho, Chuo-ku, Chiba 260-0835, Japan.
[††] Kyowa Hakko Kogyo Co., Ltd., 1-6-1 Otemachi, Chiyoda-ku, Tokyo 100-8185, Japan.
* Corresponding: kyoko.adachi@shimizu.mbio.co.jp

Fig. 1. The macrolactins from PP19-H3 strain culture broth (3~9 novel).



Manual of Systematic Bacteriology[17], the strain was identified as *Bacillus* sp. The 16S rDNA sequence homology searches using the BLAST system[18] corroborated the positioning of strain PP19-H3 within the genus *Bacillus*. The DDBJ accession number for the 16S rDNA sequence of strain PP19-H3 is AB050667.

This strain has been deposited at the National Industrial Science and Technology of the Ministry of International Trade and Industry, Tsukuba-shi in Japan with accession number FERM P-15407.

Fermentation and Isolation

Cultures of strain PP19-H3 were grown for periods of 3~7 days at 30°C and, after centrifugation, the supernatant was extracted with ethyl acetate. The resulting extract was analyzed by reversed phase HPLC with a photo diode array detector. In the analytical HPLC, many peaks appeared which have characteristic UV absorption spectra. On the basis of the results from the analytical HPLC, the extract was fractionated by preparative HPLC. Macrolactins were eluted in the order of H, L, G, I, A, J, M, K and F. Two of the resulting fractions were known macrolactin A (**1**) and F (**2**), which are 24-membered ring lactones, while seven fractions were novel macrolactins G~M (**3~9**) (Fig. 1). The recovery from a 3-liter culture was 9~7 mg of macrolactin A, 4~2 mg of macrolactin F and 0~1 mg of other macrolactins.

Structural Determination

The chemical shifts in the ^{1}H- and ^{13}C-NMR of macrolactins A, F and G~M are shown in Table 1 and 2. Compounds **1** and **2** were identified as macrolactins A and F by ^{1}H-NMR, ^{13}C-NMR, FAB-MS and UV data.

The HR-FAB-MS data for macrolactin G (**3**) revealed its molecular formula to be $C_{24}H_{34}O_5$. The infrared spectrum of **3** (KBr) showed absorbance bands at 3700~3100, 1702 and 1638 cm^{-1}. The absorption at 1702 cm^{-1} indicated the existence of a carbonyl group. The ultraviolet absorbance at 262 nm (ε=13000) and 230 nm (ε=26800) were assigned to a chromospheres with extended conjugation.

The continuity of protons from the 2- to 24-positions was observed by COSY, and the ^{13}C-NMR signals were

Table 1. Chemical shifts in the ^{1}H-NMR of macrolactins (500 MHz).

Carbon	A(1)		F(2)		G(3)		F(4)		I(5)	
No.	δ ppm	J Hz	δ ppm	J Hz	δ ppm	J Hz	δ ppm	J Hz	δ ppm	J Hz
2	5.61	d,11.7	5.59	d,11.2	5.57	d,11.4	5.56	d,11.7	5.49	d,11.5
3	6.17	dd,11.5,11.5	6.16	dd,11.2,12.0	6.16	dd,11.4,11.4	6.11	dd,11.2,11.2	6.09	dd,11.4,11.4
4	7.46	dd,11.5,15.1	7.55	dd,12.2,16.6	7.42	dd,11.2,15.4	7.45	dd,11.2,15.1	7.72	dd,11.2,15.1
5	5.72	ddd,7.3,7.6,14.9	5.68	ddd,6.4,6.4,15.4	5.63	ddd,6.1,6.1,15.1	5.55	ddd,7.1,7.1,15.1	5.54	ddd,7.1,7.6,14.7
6	2.15	m	2.14	m	2.58	m	2.18	m	2.16,2.36	m
7	3.89	m	3.90	m	5.53	ddd,6.6,6.6,15.5	3.91	m	3.81	m
8	5.43	dd,4.9,15.1	5.44	dd,5.9,15.0	5.46	dd,6.1,16.6	5.48	dd,5.1,15.4	5.29	dd,7.1,15.1
9	6.53	dd,11.2,15.4	6.41	dd,12.2,16.4	4.78	dd,7.4,7.4	6.61	dd,11.0,15.1	5.90	dd,10.3,15.4
10	5.94	dd,11.0,11.0	5.99	dd,10.3,11.5	5.49	m	6.04	dd,11.0,11.0	5.64	dd,10.5,15.4
11	5.35	m	5.42	m	5.38	ddd,6.3,10.5,10.5	5.40	m	5.43	m
12	2.28,2.40	m	2.34	m	1.91,2.29	m	2.35	m	1.81,2.09	m
13	3.84	m	3.98	m	3.84	m	3.83	m	3.68	m
14	1.58	m	2.19	m	1.37,1.65	m	1.60	m	1.40,1.58	m
15	4.34	m	-	-	4.29	m	4.35	m	4.26	m
16	5.50	dd,6.1,15.4	2.06,2.14	m	5.46	dd,5.4,15.3	5.40	dd,5.9,15.6	5.41	dd,5.4,15.4
17	6.23	dd,10.3,15.4	2.04,2.14	m	6.28	dd,10.3,15.1	5.55	ddd,6.8,6.8,15.1	6.19	dd,10.7,15.4
18	5.96	dd,10.5,15.1	5.30	m	6.04	dd,10.1,15.4	1.80	m	6.01	dd,10.5,15.4
19	5.55	m	5.30	m	5.50	ddd,6.8,6.8,15.4	1.24	m	5.54	m
20	1.84,1.98	m	1.78,1.90	m	1.93	m	1.25,1.37	m	1.80,1.90	m
21	1.27	m	1.26	m	1.31	m	4.93	m	1.48	m
22	1.11,1.45	m	1.24,1.49	m	1.24,1.49	m	1.03	d,6.5	1.44	m
23	5.04	m	5.05	m	5.05	m	-	-	5.08	m
24	1.14	d,6.5	1.04	d,6.5	1.03	d,6.5	-	-	1.00	d,6.5

Carbon	J(6)		K(7)		L(8)		M(9)	
No.	δ ppm	J Hz	δ ppm	J Hz	δ ppm	J Hz	δ ppm	J Hz
2	5.65	d,11.2	5.53	d,11.5	5.61	d,10.5	5.62	d,11.2
3	6.24	dd,11.2,11.2	6.11	dd,11.2,11.2	6.19	dd,10.7,11.2	6.18	dd,11.2,11.2
4	7.55	dd,11.2,15.4	7.64	dd,11.2,15.4	7.39	dd,11.2,15.4	7.55	dd,11.2,15.4
5	5.56	m	5.56	ddd,5.4,5.4,15.1	5.68	ddd,5.4,5.4,15.9	5.71	ddd,6.1,8.1,15.4
6	2.14	m	2.18,2.31	m	2.55	m	2.09,2.14	m
7	3.93	m	3.86	m	5.43	m	3.84	m
8	5.39	dd,6.7,15.4	5.37	dd,6.8,15.6	5.91	dd,10.5,10.5	5.39	dd,5.9,15.1
9	6.41	dd,11.0,15.4	5.98	dd,10.9,15.4	5.88	dd,10.5,14.4	6.47	dd,11.0,15.1
10	5.94	dd,11.0,11.0	5.83	dd,10.3,15.4	5.09	dd,9.5,14.4	5.94	dd,11.0,11.0
11	5.17	m	5.46	ddd,8.1,8.1,15.1	2.40	m	5.37	m
12	1.94,2.31	m	2.14,2.43	m	1.05,1.53	m	2.25,2.33	m
13	3.69	m	3.89	m	3.75	m	3.82	m
14	1.40,1.72	m	2.10	m	1.32,2.10	m	1.55	m
15	4.44	m	-	-	3.78	m	4.29	m
16	5.57	m	1.95	m	1.59	m	5.47	dd,5.9,15.1
17	6.86	dd,11.0,15.1	1.95,2.10	m	5.13	dd,9.5,16.3	6.19	dd,10.7,15.1
18	6.11	dd,11.0,11.0	5.26	m	5.43	dd,7.3,16.1	6.03	dd,10.7,15.6
19	5.28	m	5.26	m	3.80	m	5.57	m
20	2.01,2.24	m	1.79,1.86	m	1.25,1.57	m	1.85,2.05	m
21	1.25,1.38	m	1.26	m	1.37	m	1.31	m
22	1.28,1.40	m	1.30,1.50	m	1.39,1.51	m	1.37,1.45	m
23	4.93	m	5.08	m	5.12	m	5.02	m
24	1.01	d,6.5	1.04	d,6.5	1.12	d,6.5	1.32,1.49	m
25	-	-	-	-	-	-	0.75	dd,7.3,7.3

Solvent : Benzene-d_6.

Table 2. Chemical shifts in the ^{13}C-NMR of macrolactins (125 MHz).

Carbon No.	A (1)* δ ppm	F (2)** δ ppm	G(3) ** δ ppm	H(4) ** δ ppm	I (5)** δ ppm	J (6)** δ ppm	K (7)** δ ppm	L (8)** δ ppm	M (9)** δ ppm
1	166.2	166.0	166.1	166.3	165.7	166.4	165.8	166.3	166.2
2	117.7	118.0	117.6	117.9	117.4	117.1	117.6	118.2	118.0
3	143.6	143.6	144.0	143.7	144.6	145.0	144.3	143.0	143.5
4	129.3	130.0	128.3	130.1	129.4	130.0	130.0	129.3	130.1
5	142.3	139.7	142.1	139.9	139.8	140.0	139.4	140.1	139.9
6	43.8	41.5	35.5	41.5	42.3	41.4	42.1	34.9	41.9
7	71.1	71.3	134.3	70.6	72.0	71.8	71.6	138.1	71.4
8	138.3	136.5	135.1	135.9	134.4	136.6	134.6	130.7	136.5
9	124.8	125.6	68.6	126.0	131.3	126.5	130.6	132.6	125.4
10	130.5	130.9	127.7	130.7	132.4	131.4	132.8	136.3	130.6
11	128.5	128.0	126.4	134.2	130.9	127.3	130.3	39.7	127.9
12	36.7	35.1	36.5	36.1	41.0	36.5	40.5	39.4	35.9
13	68.7	68.1	68.9	69.8	69.7	69.7	67.7	66.6	69.5
14	42.8	47.8	41.9	41.5	41.7	41.4	47.8	40.5	41.5
15	69.1	210.2	70.3	70.6	70.4	70.4	210.0	67.7	70.2
16	133.6	43.5	134.1	128.1	134.4	136.8	43.4	55.4	134.1
17	131.1	27.1	130.1	130.4	129.9	124.9	26.8	132.6	130.6
18	129.5	129.4	130.6	32.1	130.8	129.2	129.4	128.8	130.6
19	136.6	131.0	134.7	24.7	133.9	131.1	130.9	71.7	134.5
20	32.2	32.1	32.7	35.0	32.4	27.1	32.4	36.6	32.3
21	24.9	25.2	24.5	70.8	25.8	25.2	25.5	20.5	24.9
22	35.2	35.3	35.9	19.6	35.9	34.7	35.6	35.6	27.4
23	70.8	70.3	70.1	-	69.8	70.1	70.0	70.1	74.7
24	19.9	20.0	20.2	-	20.1	20.5	20.1	20.4	33.3
25	-	-	-	-	-	-	-	-	9.9

* Solvent: Pyridine-d_5.
** Solvent: Benzene-d_6.

assigned to each carbon by HSQC. The signals of ethylene and methyl protons were observed between 3.84 and 1.03 ppm. The H-9, H-13, H-15 and H-23 protons between 5.05 and 3.84 ppm were assigned to methine protons bonded with carbons bearing oxygen. One of the hydroxyl groups of macrolactin G is in a different position from that in macrolactin A, having hydroxyl groups at 7-, 13- and 15-positions.

The low-field shift of the H-23 proton at 5.05 ppm and HR-FAB-MS data indicated this compound to be a cyclic ester. This linkage was confirmed by the HMBC data, the olefin protons being observed between 7.42 and 5.38 ppm. The geometric features of the carbon–carbon double bonds were assigned on the basis of their readily measured ^{1}H coupling constants: 10.5~11.4 Hz for *E* and 15.1~15.5 Hz for *Z*.

An analysis of the ^{13}C-NMR spectrum showed an ester carbonyl signal at 166.1 ppm, twelve olefin carbons between 144.0 and 126.4 ppm that were assigned to six double bonds, four methine carbons bonded with oxygen between 70.3 and 68.6 ppm, six ethylene carbons between 41.9 and 24.5 ppm, and a methyl carbon at 20.2 ppm.

The structures of macrolactins H~M were determined in the same manner as the above. Macrolactin H (**4**) was a 22-membered ring lactone, against the other macrolactins that have a 24-membered ring.

Macrolactins I (**5**) and J (**6**) have same molecular formula of $C_{24}H_{34}O_5$. The ^{1}H coupling constant between the 10- and 11-position in **5** was 15.4 Hz, against that of macrolactin A which was 11.0 Hz. The structure established

for **5** at C-10 formed *E*. On the other hand, ^{1}H coupling constant between 18- and 19-position in **6** was 11.0 Hz, against 10.5 Hz in macrolactin A; therefore, C-17 of **6** was of *Z* configuration. Macrolactins I and J were thus geometric isomers of macrolactin A.

Macrolactin K (**7**) showed the formula $C_{24}H_{34}O_5$ by an HR-FAB-MS analysis. Ten olefinic carbons and eight aliphatic methylenes of macrolactin K (**7**) were apparent observed in the ^{13}C-NMR spectrum of **7**. The ^{13}C resonance of a ketone carbonyl appeared at 210.0 ppm. The continuity of protons from H-2 to H-14 and from H-16 to H-24 was confirmed by COSY, although the signals of the H-18 and H-19 olefinic protons could not be shifted far enough apart to measure their coupling constants. The geometry of C-18, C-19 was determined by a NOESY analysis. NOEs between H-18 and H-20, and between H-17 and H-19 were observed, but not between H-17 and H-20, thus confirming the olefin configuration to be *E*. The remaining geometry of the carbon–carbon double bonds was assigned on the basis of their readily measured ^{1}H coupling constants: 11.5 Hz for the *Z* and 15.1~15.4 Hz for the *E* configuration. Macrolactin K (**7**) is geometric isomer of macrolactin F at 10-position.

For macrolactin L (**8**), the DEPT pulse sequence applied to the ^{13}C-NMR data showed ten olefinic carbons between 128.8 and 143.0 ppm, and the proton signals bonding these were observed between 5.09 and 7.39 ppm. Three hydroxyl groups of **8** were present at C-13, C-15 and C-19, since their methine proton signals were between 3.75 and 3.80 ppm.

The C-11 and C-16 carbons were methines from the DEPT analysis. The results of the COSY analysis confirmed continuity from H-2 to H-24, except for the connection between H-11 and H-16, because the signals of one of H-12 protons and the H-16 proton were not far enough apart. Cyclization from C-11 to C-16 was confirmed by the correlation between the H-11 and H-17 protons that was apparent from the TOCSY analysis of **8**. The geometry of carbon–carbon double bonds was assigned from the ^{1}H coupling constants. This is the first evidence of a dicyclic macrolactin.

Macrolactin M (**9**) showed the formula $C_{25}H_{36}O_5$ by its HR-FAB-MS data, which is the largest carbon numbers among the isolated macrolactins. A COSY analysis confirmed the continuity from H-2 to H-25. Hydroxyl groups were confirmed at C-7, C-13 and C-15 by the methine proton signals appearing between 3.84 and 4.29 ppm. The H-23, whose proton appeared at 5.02 ppm, was part of a lactone linkage. The geometry of carbon–carbon double bond was established from the ^{1}H coupling constants. The 24-membered cyclic part of **9** that was established by NMR analysis was the same as that of macrolactin A. The side chain of **9** was constituted an ethyl group by the results of COSY analysis and the triplet proton signal of methyl group at H-25, while the side chain of macrolactin A constituted a methyl group.

Table 3. MIC of macrolactins.

Compounds	MIC (ppm)	
	Sa	Bs
A(**1**)	10	60
F(**2**)	80	>100
G(**3**)	10	60
H(**4**)	10	60
I(**5**)	10	60
J(**6**)	5	30
K(**7**)	>100	>100
L(**8**)	10	60
M(**9**)	10	60

Sa: *Staphylococcus aureus* IFO 12732.
Bs: *Bacillus subtilis* IFO 3134.

Antibacterial Activities

The minimum inhibitory concentration (MIC) of macrolactin A, F and G~M against the bacteria *Staphylococcus aureus* (IFO 12732) and *Bacillus subtilis* (IFO 3134) are shown in Table 3. The macrolactins were more effective against *Staphylococcus aureus* than *Bacillus subtilis* and did not inhibit *Escherichia coli* (IFO 3301) and *Salinivibrio costicola* (ATCC 33508).

Discussion

The macrolactins in our work were produced by *Bacillus* sp., like those in the report by JARUCHOKTAWEECHI *et al.*[16], while other previously reported producers of macrolactin were an unclassifiable marine bacterium that had been isolated from a deep-sea sediment core[2], and *Actinomadura* sp. that had been isolated from a soil[15]. It is interesting that the characteristic antibiotic was produced by different genera of microorganisms.

GUSTAFSON and co-workers reported that macrolactin A inhibited the bacteria *Staphylococcus aureus* and *Bacillus subtilis* in a standard agar plate-disk assay at respective

concentrations of 5 and 20 μg/disk[2]. Macrolactin F and K, in which C-15 was a ketone carbonyl, showed little antibacterial activity, so the presence of a hydroxyl carbon at C-15 was necessary for antibacterial activity. The other macrolactins inhibited *Staphylococcus aureus*, regardless of the presence of hydroxyl group at C-7 or C-9, or of the number of ring members. These results suggest that the hydroxyl group at C-15 may play an important part in the antibacterial activity of macrolactins.

While the antibacterial activity of all the macrolactins examined in this work was relatively weak, the novel macrolactins can be expected to have cytotoxic or antiviral activity.

Experimental

General

UV-visible spectra were obtained on a Shimadzu UV-2100S spectrometer. High performance liquid chromatography was carried out with Shimadzu LC8A system equipped with Shimadzu SPD-M6A photo diode array detector. NMR spectra were measured with a Varian UNITY500 NMR spectrometer. Mass spectra were recorded with a JEOL JMS-SX102 mass spectrometer. Optical rotation was determined with a Horiba SEPA-300 polarimeter. IR spectra were obtained with a JASCO FT-IR 7000 spectrophotometer.

Fermentation and Isolation of Macrolactins

Strain PP19-H3 was cultured in a liquid medium containing distilled water and 37.4 g of Marine Broth (Difco) per liter of solution. Five-liter jar fermenters each containing 3 liters of medium were inoculated with 300 ml of an actively growing bacterial culture and then aerated with filtered compressed air for 3~7 day at 30°C. All the cultures reached maximum cell density, as determined by the OD_{660} value, within 2 days of the original inoculation.

Each culture was centrifuged and the resulting supernatant was extracted with 1.5 times the volume of ethyl acetate. The solvent was evaporated under reduced pressure by a rotary evaporator, yielding approximately 70 mg of a residue. A small amount of the above extract was analyzed by reversed-phase HPLC (Develosil ODS-HG-5 column; 4.6 mm i.d.×250 mm; Nomura Kagaku) with a photo diode array detector, using acetonitrile - water (4 : 6) at a flow rate of 1.0 ml/minute. The residue was fractionated by preparative HPLC (Develosil ODS-HG-5 column; 20 mm i.d.×250 mm; Nomura Kagaku) using acetonitrile - water (4 : 6) at a flow rate of 10 ml/minute.

Macrolactin G

$[\alpha]_D^{25}$ −109.1° (*c* 0.033, MeOH); UV λ_{max}^{MeOH} nm (ε) 231 (36500), 262 (19000); IR (KBr) cm^{-1} 3700~3100, 1702, 1638, 1600; HR-FAB mass spectrum, obsd 425.2291 (M^++Na), $C_{24}H_{34}O_5Na$ requires 425.2304; HPLC retention time, 14.5 minutes (analytical conditions).

Macrolactin H

$[\alpha]_D^{25}$ −92.2° (*c* 0.064, MeOH); UV λ_{max}^{MeOH} nm (ε) 235 (18700), 262 (13100); IR (KBr) cm^{-1} 3700~3100, 1698, 1638, 1600; HR-FAB mass spectrum, obsd 399.2156 (M^++Na), $C_{22}H_{32}O_5Na$ requires 399.2147; HPLC retention time, 10.7 minutes (analytical conditions).

Macrolactin I

$[\alpha]_D^{25}$ −137.7° (*c* 0.167, MeOH); UV λ_{max}^{MeOH} nm (ε) 226 (26000), 262 (9500); IR (KBr) cm^{-1} 3700~3100, 1702, 1638, 1600; HR-FAB mass spectrum, obsd 425.2285 (M^++Na), $C_{24}H_{34}O_5Na$ requires 425.2304; HPLC retention time, 16.0 minutes (analytical conditions).

Macrolactin J

$[\alpha]_D^{25}$ −85.5° (*c* 0.083, MeOH); UV λ_{max}^{MeOH} nm (ε) 231 (20100), 263 (8300); IR (KBr) cm^{-1} 3700~3100, 1702, 1638, 1600; HR-FAB mass spectrum, obsd 425.2310 (M^++Na), $C_{24}H_{34}O_5Na$ requires 425.2304; HPLC retention time, 19.8 minutes (analytical conditions).

Macrolactin K

$[\alpha]_D^{25}$ −169.8° (*c* 0.106, MeOH); UV λ_{max}^{MeOH} nm (ε) 234 (17800), 263 (11500); IR (KBr) cm^{-1} 3700~3100, 1702, 1673, 1638; HR-FAB mass spectrum, obsd 403.2483 $(M+H)^+$, $C_{24}H_{35}O_5$ requires 403.2484; HPLC retention time, 30.4 minutes (analytical conditions).

Macrolactin L

$[\alpha]_D^{25}$ −139.5° (*c* 0.038, MeOH); UV λ_{max}^{MeOH} nm (ε) 230 (26800), 262 (13000); IR (KBr) cm^{-1} 3700~3100, 1702, 1638; HR-FAB mass spectrum, obsd 425.2311 (M^++Na), $C_{24}H_{34}O_5Na$ requires 425.2304; HPLC retention time, 12.0 minutes (analytical conditions).

Macrolactin M

$[\alpha]_D^{25}$ −43.2° (*c* 0.044, MeOH); UV λ_{max}^{MeOH} nm (ε) 228 (33700), 263 (13400); IR (KBr) cm^{-1} 3700~3100, 1698, 1638, 1600; HR-FAB mass spectrum, obsd 439.2449 (M^++Na), $C_{25}H_{36}O_5Na$ requires 439.2460; HPLC retention time, 29.7 minutes (analytical conditions).

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