Macrosphelides C and D, Novel Inhibitors of Cell Adhesion

SATOSHI TAKAMATSU, HIDEMI HIRAOKA†, YONG-PIL KIM, MASAHIKO HAYASHI, MASAHIKO NATORI†, KANKI KOMIYAMA and SATOSHI ŌMURA*

The Kitasato Institute,
5-9-1 Shirokane, Minato-ku, Tokyo 108, Japan
† Department of Applied Biological Sciences, Nihon University, 1866 Kameino, Fujisawa-shi, Kanagawa 252, Japan

(Received for publication July 14, 1997)

In our continuing of a search for novel anti-cell adherence compounds of microbial origin, we have reported fermentation, isolation and biological activities of macrosphelides A and B from the culture broth of *Microsphaeropsis* sp. FO-5050 in a previous paper[1,2]. The absolute configuration of macrosphelides A and B were clarified by single crystal X-ray analysis and chemical conversion including a modified MOSHER's method as 3*S*, 8*R*, 9*S*, 14*R*, 15*S* and 3*S*, 8*R*, 9*S*, 15*S*, respectively, further, the total synthesis of macrosphelide A has also been done *via* asymmetric dihydroxylation in eleven steps[3].

During the purification of macrosphelides A and B in the cultured broth of FO-5050, we discovered two new 16-membered macrocyclic compounds, macrosphelides C (**1**) and D (**2**) (Fig. 1). This paper describes physicochemical properties structural determination and biological activities of **1** and **2**.

The fermentation of strain FO-5050 was carried out in the same way as reported previously[1]. Compound **1** was purified by silica gel chromatography ($CHCl_3$-CH_3OH, 50:1 v/v) and HPLC (Senshu Pac Pegasil ODS; i.d. 2×25 cm, detection, UV at 210 nm; flow rate, 7 ml/miniute; solv. sys., CH_3CN-H_2O, 4:6 v/v and CH_3OH-H_2O, 4:6 v/v) from the EtOAc extract of culture broth. On the other hand, purification of the EtOAc extract from mycelium by silica gel chromatography ($CHCl_3$-CH_3OH, 50:1 v/v) and HPLC (solv. sys., CH_3CN-H_2O, 4:6 v/v) gave **2**. Finally, compounds **1** and **2** were obtained in the yield of 1.8 mg and 8.0 mg, rspectively, together with macrosphelides A (580 mg) and B (16.1 mg).

Physico-chemical properties of **1** and **2** are summarized in Table 1. Compound **1** was isolated as hygroscopic needles. The molecular formula of **1** was determined as $C_{16}H_{22}O_7$ by HR-FAB-MS. The IR absorptions at 3462 cm^{-1} and 1732 cm^{-1} of **1** showed the presence of hydroxy group and ester functions, respectively. In the ^1H NMR spectrum of **1** (Table 2), the signals at δ 2.36 (dddd, J=13.9, 10.1, 9.5, 1.5 Hz, H-8a) and δ 2.55 (m, H-8b) were newly observed compared with those of macrosphelide A (**3**). In the ^{13}C NMR spectrum of **1** (Table 3), methylene carbon signal was also appeared at δ 38.8 (t, C-8) compared with that of **3**. These signals suggest the presence of methylene carbon in the molecule of **1**. In addition, the multiplicity of methine (δ 5.10, m, H-9) and olefinic proton (δ 6.85, ddd, J=15.5, 9.5, 6.5 Hz, H-7) adjacent to the methylene carbon at C-8 position were changed. Thus, compound **1** is assumed to be a 8-deoxy derivative of **3**. The ^1H-^1H COSY of **1** showed the connectivity between C-6 and 9-Me *via* C-8 methylene (data not shown). Final confirmation of the structure of **1** was undertaken using the HMBC (8 Hz) experiment as shown in Fig. 1. These results clearly indicated that the structure of macrosphelide C (**1**) is

Table 1. Physico-chemical data of **1** and **2**.

	1	**2**
Appearance	Hygroscopic needles	Colorless oil
MP	80~84°C	—
$[\alpha]_D^{20}$	+29.5° (*c* 0.10, MeOH)	+65.3° (*c* 0.34, MeOH)
Molecular weight	326	342
Molecular formula	$C_{16}H_{22}O_7$	$C_{16}H_{22}O_8$
Pos. FAB-MS (*m/z*)	327 $(M+H)^+$	343 $(M+H)^+$
HR Pos. FAB-MS (*m/z*)	Found 327.1451 ($C_{16}H_{23}O_7$)	Found 365.1237 ($C_{16}H_{22}O_8Na$)
	Calcd 327.1443	Calcd 365.1212
UV λ_{max}^{MeOH} nm (log ε)	207.5 (4.22)	207 (4.21)
IR ν_{max}^{KBr} cm^{-1}	3462, 2924, 1732, 1701, 1643, 1452, 1384, 1186, 1053	3400, 1716, 1660, 1367, 1188, 1055, 985
Color reaction		
Positive	50% $H_2SO_4+\Delta$, iodine	50% $H_2SO_4+\Delta$, iodine
Negative	DRAGENDORFF's reagent, EHRLICH's reagent+Δ, ninhydrin reagent	DRAGENDORFF's regaent, EHRLICH's reagent+Δ, ninhydrin reagent

Table 2. ^{1}H NMR chemical shifts of **1**, **2** and **3** in $CDCl_3$.

H	**1**	(*M*, *J* value in Hz)	**2**	(*M*, *J* value in Hz)	**3**[a]	(*M*, *J* value in Hz)
2a	2.63	(1H, dd, 14.5, 3.0)	2.65	(1H, dd, 13.5, 11.5)	2.60	(2H, dd, 8.3, 4.3)
2b	2.51	(1H, dd, 14.5, 8.5)	2.52	(1H, dd, 13.5, 3.3)	—	
3	5.30	(1H, m)	5.35	(1H, m)	5.38	(1H, m)
6	5.80	(1H, ddd, 15.5, 1.5, 1.5)	5.96	(1H, d, 15.8)	6.03	(1H, dd, 15.5, 1.8)
7	6.85	(1H, ddd, 15.5, 9.5, 6.5)	6.64	(1H, dd, 15.8, 7.9)	6.88	(1H, dd, 15.5, 3.3)
8a	2.36	(1H, dddd, 13.9, 10.1, 9.5, 1.5)	4.16	(1H, dd, 8.6, 4.0)	4.25	(1H, br.s)
8b	2.55	(1H, m)	—		—	
9	5.10	(1H, m)	4.76	(1H, dq, 7.9, 6.3)	4.97	(1H, q, 6.6)
12	6.06	(1H, dd, 15.5, 2.0)	5.96	(1H, d, 15.8)	6.04	(1H, dd, 15.5, 1.5)
13	6.89	(1H, dd, 15.5, 4.8)	6.59	(1H, dd, 15.8, 8.6)	6.87	(1H, dd, 15.5, 3.3)
14	4.16	(1H, dd, 4.8, 4.8)	5.05	(1H, dd, 8.6, 4.0)	4.13	(1H, br.s)
15	4.92	(1H, dq, 6.5, 4.8)	4.06	(1H, dq, 4.0, 6.6)	4.86	(1H, q, 6.6)
3*Me*	1.33	(3H, d, 5.5)	1.35	(3H, d, 6.3)	1.33	(3H, d, 6.3)
9*Me*	1.38	(3H, d, 5.5)	1.47	(3H, d, 6.3)	1.45	(3H, d, 6.6)
15*Me*	1.36	(3H, d, 6.3)	1.22	(3H, d, 6.6)	1.37	(3H, d, 6.6)

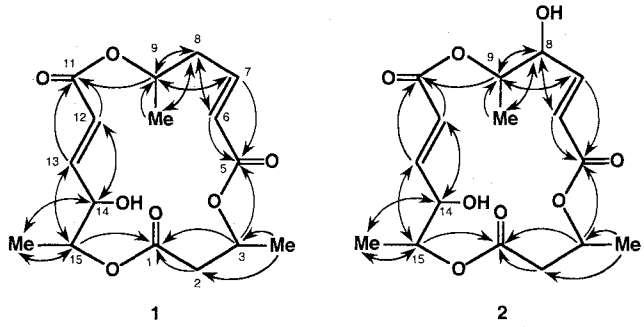
M: Multiplicity. [a] Macrosphelide A (**3**); ref. 2.

Table 3. ^{13}C NMR chemical shifts of **1**, **2** and **3** in $CDCl_3$.

C	**1**	*M*	(*Δ*1-3)	**2**	*M*	(*Δ*2-3)	**3**[a]	*M*
1	170.0	s	−0.2	169.7	s	−0.5	170.2	s
2	40.9	t	±0	41.5	t	+0.6	40.9	t
3	67.4	d	−0.3	69.2	d	+1.5	67.7	d
5	164.8	s	+0.1	164.4	s	−0.3	164.7	s
6	124.7	d	+2.0	124.3	d	+1.6	122.7	d
7	143.8	d	−1.4	145.8	d	+0.6	145.2	d
8	38.8	t	−35.9	75.9	d	+1.2	74.7	d
9	69.0	d	−5.8	72.5	d	−2.3	74.8	d
11	165.0	s	−0.8	164.1	s	−1.7	165.8	s
12	123.0	d	+0.8	126.9	d	+4.7	122.2	d
13	144.9	d	−1.3	140.8	d	−5.7	146.2	d
14	72.9	d	−0.1	77.7	d	+4.7	73.0	d
15	73.7	d	−0.2	68.1	d	−5.8	73.9	d
3*Me*	19.5	q	−0.1	20.2	q	+0.6	19.6	q
9*Me*	20.5	q	+2.6	17.8	q	−0.1	17.9	q
15*Me*	17.5	q	−0.3	18.3	q	+0.5	17.8	q

M: Multiplicity. [a] Macrosphelide A (**3**); ref. 2.

Fig. 1. Structure of **1** and **2**.



Arrows show key ^{1}H-^{13}C long range couplings detected by HMBC experiments (J=4.0 Hz).

8-deoxymacrosphelide A as shown in Fig. 1.

Compound **2** was obtained as colorless oil. The molecular formula ($C_{16}H_{22}O_8$) assigned based on the HR-FAB-MS of **2** gave the same as that of **3**, but **2** did not show similar pattern to that of **3** on ^{1}H NMR spectra (Table 2). The ^{1}H-^{1}H COSY (data not shown) and HMBC experiment of **2** support that compound **2** possesses the same planar structure of **3** (Fig.1). The NOE experiments (400 MHz) of **2** did not show any information about the stereochemistry. The proton signals at H-14 (δ 5.05) shifted downfield of 0.92 ppm compared with that of **3**. On the other hand, the proton signals adjacent to the methyl carbon at H-15 (δ 4.06) shifted upfield of 0.8 ppm. In the ^{13}C NMR spectrum of **2** (Table 3), the chemical shifts of carbon signals showed similarly to those of **3**, except for signals of C-12, C-13, C-14 and C-15. In the ^{1}H NMR spectrum of **2** (Table 2), the coupling constants between δ 5.96 (d, J=15.8, H-12) and δ 6.59, (d, J=15.8, 8.6 Hz, H-13) showed the same *trans* configuration as that of **3**. Therefore, macrosphelide D (**2**) is presumed to be a stereoisomer of macrosphelide A (**3**) at C-14 or C-15 positions. The stereochemistry of **1** and **2** are under study using organic synthesis approach.

Biological activities of new macrospherides were examined according to the previous methods[1]. Macrosphelides were assayed in an adhesion assay system using HL-60 cells and HUVEC cells. The IC_{50} values of **1** and **2** were 67.5 μM and 25.0 μM, respectively. Compounds **1** and **2** had no effect on cell growth against B16 melanoma, HeLa S3 carcinoma, P388 leukemia, L929 fibroblast, Shionogi carcinoma (SC-115), human prostate tumor

(LNCap, PC-3), human leukemia (CEM, THP-1) and calf pulmonary artery endothelial cell (CPAE) at concentration of 307 μM and 292 μM, respectively (data not shown). They also showed no antimicrobial activity at a concentration of 1000 μg/ml (data not shown).

Thus we isolated two new inhibitors of cell adhesion molecule, macrosphelides C (**1**) and D (**2**), as derivatives of macrosphelides A and B from the fermentation broth of *Microsphaeropsis* sp. FO-5050. Recently, arthritis[4] and metastasis[5] were reported to be associated with adhesion molecules, and anti-adhesion compounds were expected to be effective in the treatment of inflammation and metastasis[6]. Therefore, we are interested in relationships between the structure and the activity of these macrosphelides, and the results will be reported in elsewhere.

Acknowledgments

We express our thanks to Dr. T. SUNAZUKA, School of Pharmaceutical Sciences, Kitasato University, for useful comments and discussion. This work was supported in part by Grants-in Aid from the Ministry of Education, Science and Culture, Japan and funds from the Japan Keirin Association.

References

1) HAYASHI, M.; Y.-P. KIM, H. HIRAOKA, M. NATORI, S. TAKAMATSU, T. KAWAKUBO, R. MASUMA, K. KOMIYAMA & S. ŌMURA: Macrosphelide, a novel inhibitor of cell-cell adhesion molecule. I. Taxonomy, fermentation, isolation and biological activities. J. Antibiotics 48: 1435~1439, 1995

2) TAKAMATSU, S.; Y.-P. KIM, M. HAYASHI, H. HIRAOKA, M. NATORI, K. KOMIYAMA & S. ŌMURA: Macrosphelide, a novel inhibitor of cell-cell adhesion molecule. II. Physicochemical properties and structural elucidation. J. Antibiotics 49: 95~98, 1996

3) SUNAZUKA, T.; T. HIROSE, Y. HARIGAYA, S. TAKAMATSU, Y.-P. KIM, M. HAYASHI, K. KOMIYAMA, S. ŌMURA & A. B. SMITH, III: The relative and stereochemistries and total synthesis of (+)-macrosphelides A and B, potent orally bioavailable inhibitors of cell-cell adhesion molecule. J. Am. Chem. Soc., (acceptable)

4) IIGO, Y.; T. TAKAHASHI, T. TAMATANI, M. MIYASAKA, T. HIGASHIDA, H. YAGITA, K. OKUMURA & W. TSUKUDA: ICAM-1-dependent-pathway is critically involved in pathologenesis of adjuvant arthritis in rats. J. Immunol. 147: 4167~4171, 1991

5) LAURI, D.; L. NEEDHAM, I. MARTIN-PADURA & E. DEJANA: Tumor cell adhesion to endothelial cells: endothelial leukocyte adhesion molecule-1 as an inducible adhesive receptor specific for colon carcinoma cells. J. Natl. Cancer Inst. 83: 1321~1324, 1991

6) NAKAMORI, S.; M. KAMEYAMA, S. IMAOKA, H. FURUKAWA, O. ISHIKAWA, Y. SASAKI, Y. IZUMI & T. IRIMURA: Involvement of carbohydrate antigen sialyl Lewis^x in colorectal cancer metastasis. Dis. Colon Rectum. 40: 420~431, 1997