Macrosphelide, a Novel Inhibitor of Cell-cell Adhesion Molecule

II. Physicochemical Properties and Structural Elucidation

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

The Kitasato Institute,
5-9-1 Shirokane, Minato-ku, Tokyo 108, Japan
†College of Agriculture and Veterinary Medicine, Nihon University,
1866, Kameino, Fujisawa-shi, Kanagawa 252, Japan

(Received for publication August 16, 1995)

New anticell-adherence compounds, macrosphelides A and B, were isolated from the fermentation broth of *Microsphaeropsis* sp. FO-5050, and their structures were elucidated by spectroscopic methods and by chemical transformations. Macrosphelides A (M.W. 342, $C_{16}H_{22}O_8$) and B (M.W. 340, $C_{16}H_{20}O_8$) with three esters in their molecules were classified as 16-membered macrocyclic compounds. Macrosphelide B was found to be a corresponding oxidative product of macrosphelide A at the C-14 position.

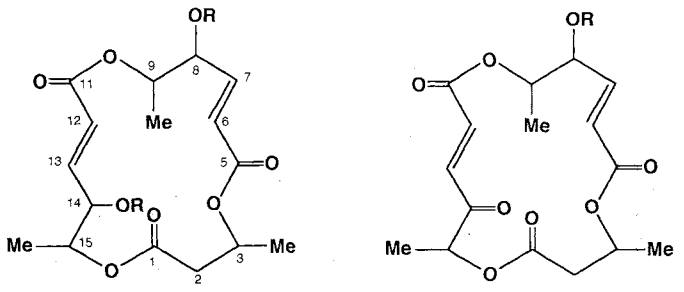
Leukocyte-endothelial cell interactions are early and critical events in inflammation and tumor metastasis. Cytokines and chemical mediators control leukocyte adhesion and invasion through regulating expression of adhesion molecules[1,2]. Recently, arthritis[3] and metastasis[4] were reported to be associated with adhesion molecules, and anti-adhesion compounds are therefore expected to be effective in the treatment of inflammation and metastasis. In the course of a search for novel anticell-adherence compounds of microbial origin, we isolated macrosphelides A (**1**) and B (**2**) from the culture broth of *Microsphaeropsis* sp. FO-5050, which had been isolated from a soil sample collected in Shizuoka prefecture (Fig. 1). Taxonomic studies of the producing strain, the isolation procedure and the biological characteristics of macrosphelides A (**1**) and B (**2**) were reported in a previous paper[5]. This paper describes determination of the structure of macrosphelides A (**1**) and B (**2**).

Results and Discussion

Structure of 1

Physicochemical properties of **1** and **2** are summarized in Table 1. Compound **1** was isolated as colorless needles. The molecular formula of **1** was determined as $C_{16}H_{22}O_8$ by HR-FAB mass spectrum. The IR absorptions at 3428 cm^{-1} and 1713 cm^{-1} of **1** showed the presence of hydroxy groups and ester functions, respectively. Acetylation of **1** with acetic anhydride in pyridine at room temperature gave a diacetate of **1** (**1'**), which showed that **1** has two hydroxy groups in the molecule. In the ^1H-^1H COSY (Fig. 2) and the ^1H NMR spectrum of **1** (Table 2), two sets of olefinic proton signals were observed at δ 6.03 (dd, J=15.5, 1.8 Hz, H-6) and δ 6.88 (dd, J=15.5, 3.3 Hz, H-7), and δ 6.04 (dd, J=15.5,

Fig. 1. Structures of macrosphelide A (**1**) and B (**2**).



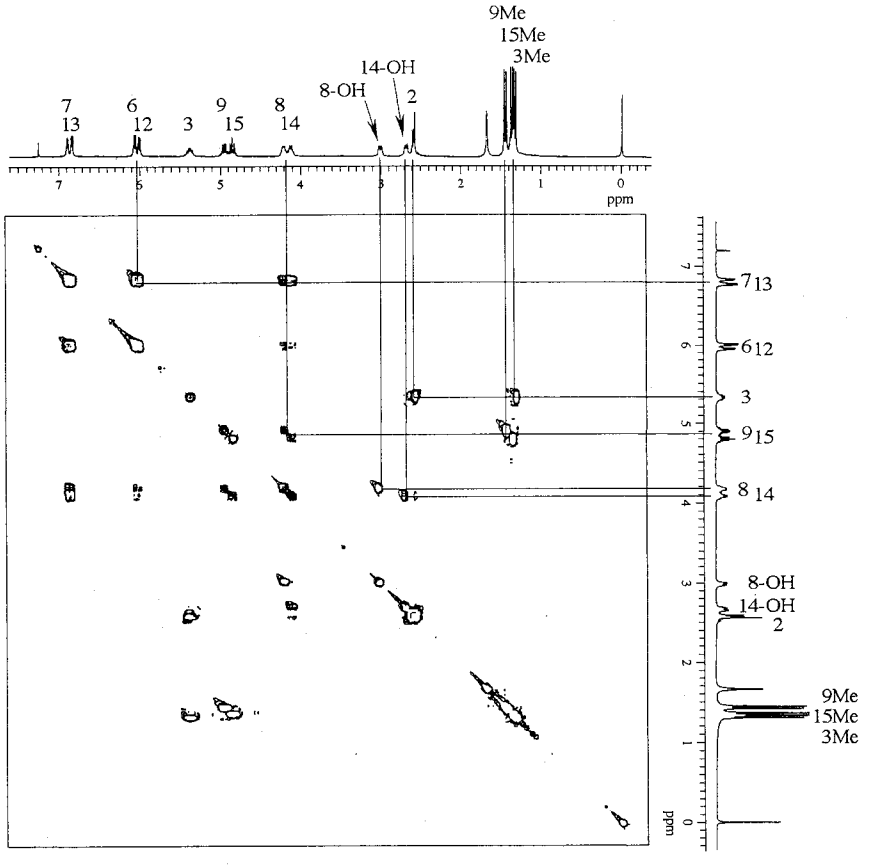
Macrosphelide A (**1**): R = H
Diacetate of **1** (**1'**) : R = –COCH3

Macrosphelide B (**2**): R = H
Acetate of **2** (**2'**) : R = –COCH3

Table 1. Physicochemical properties of macrosphelide A (**1**) and B (**2**).

	1	**2**
Appearance:	Colorless needles	Colorless plates
MP:	141~142°C	148~150°C
$[\alpha]_D^{23}$ (MeOH):	+84.1 (*c* 0.59)	+4.10 (*c* 0.99)
UV λ_{max}^{MeOH} nm:	207 ($\log\varepsilon$ 4.12)	210 ($\log\varepsilon$ 4.15)
IR ν_{max}^{KBr} cm^{-1}:	3428 (OH), 1713 (ester), 1461, 1384, 1269, 1192	3458 (OH), 1743 (ester), 1726 (ester), 1705 (ester)
Molecular weight:	342	340
Molecular formula:	$C_{16}H_{22}O_8$	$C_{16}H_{20}O_8$
Pos. FAB-MS (*m/z*):	343 $(M+H)^+$	341 $(M+H)^+$
HR Pos. FAB-MS (*m/z*):	Obsd. 343.1408 ($C_{16}H_{23}O_8$) Calcd. 343.1393	Obsd. 341.1235 ($C_{16}H_{21}O_8$) Calcd. 341.1237
Color reation		
50% $H_2SO_4+\Delta$	Positive	Positive
p-Anisaldehyde	Positive	Positive
DRAGENDORFF's reagent	Negative	Negative
Ninhydrin reagent	Negative	Negative

Fig. 2. ^1H-^1H COSY spectrum of **1** in $CDCl_3$.



1.5 Hz, H-12) and δ 6.87 (dd, J=15.5, 3.3 Hz, H-13). This coupling constant (J=15.5 Hz) showed *trans* configuration. In the ^{13}C NMR spectrum of **1** (Table 3), these corresponding olefinic carbon signals were also observed at δ 122.7 (d, C-6) and δ 145.2 (d, C-7), and δ 122.2 (d, C-12) and δ 146.2 (d, C-13), respectively. In the ^1H-^1H COSY (Fig. 2) and H-C COSY spectrum of **1**, the presence of partial structures (2→3→3Me), (6→7→8→9→9Me), (12→13→14→15→15Me) of **1** were demonstrated (Fig. 3). Finally the structure of **1** was determined by the HMBC (8 Hz) experiments as shown in Fig. 3.

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

H	1	(M, *J* value)	2	(M, *J* value)
2a	2.60	(2H, dd, 8.3, 4.3)	2.83	(1H, dd, 16.2, 10.9)
2b	—		2.64	(1H, dd, 16.2, 3.0)
3	5.38	(1H, m)	5.45	(1H, m)
6	6.03	(1H, dd, 15.5, 1.8)	6.08	(1H, dd, 15.8, 3.0)
7	6.88	(1H, dd, 15.5, 3.3)	6.94	(1H, dd, 15.8, 3.6)
8	4.25	(1H, br s)	4.32	(1H, br s)
9	4.97	(1H, q, 6.6)	5.08	(1H, m)
12	6.04	(1H, dd, 15.5, 1.5)	7.03	(1H, d, 15.8)
13	6.87	(1H, dd, 15.5, 3.3)	6.73	(1H, d, 15.8)
14	4.13	(1H, br s)	—	
15	4.86	(1H, q, 6.6)	5.08	(1H, m)
3Me	1.33	(3H, d, 6.3)	1.36	(3H, d, 6.6)
9Me	1.45	(3H, d, 6.6)	1.49	(3H, d, 6.6)
15Me	1.37	(3H, d, 6.6)	1.43	(3H, d, 6.9)
8OH	3.15	(1H, br s)	3.44	(1H, br s)
14OH	2.85	(1H, br s)	—	

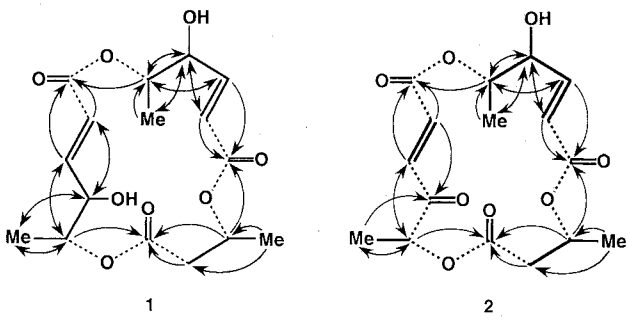
M: Multiplicity.

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

C	1 (M)	2 (M)
1	170.2 (s)	170.4 (s)
2	40.9 (t)	40.6 (t)
3	67.7 (d)	67.7 (d)
5	164.7 (s)	164.3 (s)
6	122.7 (d)	122.4 (d)
7	145.2 (d)	144.5 (d)
8	74.7 (d)	74.6 (d)
9	74.8 (d)	75.7 (d)
11	165.8 (s)	165.2 (s)
12	122.2 (d)	132.1 (d)
13	146.2 (d)	132.6 (d)
14	73.0 (d)	196.2 (s)
15	73.9 (d)	76.5 (d)
3Me	19.6 (q)	19.7 (q)
9Me	17.9 (q)	17.8 (q)
15Me	17.8 (q)	16.0 (q)

M: Multiplicity.

Fig. 3. Key ^{1}H-^{13}C long range couplings detected by HMBC experiments on **1** and **2**.



Connections represented by a solid line were elucidated by ^{1}H-^{1}H COSY NMR. Arrows show ^{1}H → ^{13}C.

Structure of 2

Compound **2** was obtained as colorless plates. The molecular formula of **2** was assigned as $C_{16}H_{20}O_8$ based on the HR-FAB mass spectrum. From physicochemical properties of **2** (Table 1), **2** was assumed to be a derivative of **1**. Compound **2** reacted with acetic anhydride in pyridine to give a monoacetate of **2** (**2'**). In the ^{13}C NMR spectrum of **2** (Table 3), the signal of C-14 (δ 196.2) was observed as a singlet in the downfield region unlike that of **1**. Other chemical carbon shifts of **2** were very similar to those of **1** except at the C-12, C-13 and C-15 positions. In the ^{1}H NMR spectrum of **2** (Table 2), the methine and hydroxy proton signals corresponding to those of **1** at the C-14 position had disappeared. These results revealed that the carbon at C-14 of **2** was a ketonic group. The partial structures of **2** were elucidated by ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY spectroscopy (Fig. 3). Final confirmation of the structure of **2** was undertaken using the HMBC experiments (8 Hz) as shown in Fig. 3. These results clearly indicated that the structure of **2** is as shown in Fig. 1.

We isolated two new anticell-adherence compounds, macrosphelides A (**1**) and B (**2**), with three lactone moieties in the molecule from the fermentation broth of *Microsphaeropsis* sp. FO-5050. The new compounds were classified in the 16-membered macrolide group. Macrosphelide B (**2**) seems to be a corresponding oxidative product of macrosphelide A (**1**) at the C-14 position. To the best of our knowledge, these are the first natural products bearing three lactone groups in the molecule among the 16-membered macrocyclic antibiotics[6,7]. It is of interest to examine the action mechanisms of the metabolites, since 14- and 16-membered macrolide aglycones (albocycline and related substances, leucono-

lides and protylonolide)[8~10] did not show anticell-adherence activity (private communication). Therefore, we are investigating relationships between the structure and the activity of these compounds.

Experimental

Spectroscopic Studies

UV spectra were recorded on a Shimadzu model UV-160A spectrophotometer. IR spectra were taken with a Horiba model Fourier transform infrared spectrophotometer FT-210. MS were obtained with a JEOL model JMS DX-300 mass spectrometer. ^1H (270 MHz) and ^{13}C (67.8 MHz) NMR spectra were recorded on a JEOL JNM-EX 270.

Acetylation **1** and **2**

Compound **1** (6.3 mg) and **2** (4.1 mg) were each dissolved in 1 ml of pyridine. Acetic anhydride (2 ml) was added gradually to the solution. After stirring for 24 hours at room temperature, the products were evaporated *in vacuo* and purified by column chromatography on silica gel with $CHCl_3$ - acetone (9 : 1). Compound **1'** and **2'** were obtained with a yield of 89% (6.3 mg) and 77% (3.5 mg), respectively.

Compound **1'**: UV λ_{max}^{MeOH} nm: End abs.; $[\alpha]_D^{27}$ $-18.1°$ (*c* 0.37, MeOH); HR Pos. FAB-mass: $C_{20}H_{27}O_{10}$ (M^+, *m/z* 427.1615, Calc. 427.1604); ^{13}C NMR ($CDCl_3$) δ ppm: 169.8 (s, C-1), 169.5 (s, acetyl ketone), 169.5 (s, acetyl ketone), 164.1 (s, C-7), 164.0 (s, C-11), 141.7 (d, C-13), 141.5 (d, C-7), 124.1 (d, C-12), 123.4 (d, C-6), 74.8 (d, C-8), 73.0 (d, C-3), 70.9 (d, C-14), 70.4 (d, C-9), 67.9 (d, C-15), 40.9 (t, C-2), 20.8 (q, acetyl methyl), 20.8 (q, acetyl methyl), 19.6 (q, C-3 Me), 17.6 (q, C-9 Me), 17.4 (q, C-15 Me); ^1H NMR ($CDCl_3$) δ ppm: 6.78 (1H, dd, $J=15.8$ and 5.3 Hz, H-13), 6.75 (1H, dd, $J=15.8$ and 6.3 Hz, H-7), 5.95 (1H, dd, $J=15.8$ and 1.3 Hz, H-12), 5.94 (1H, dd, $J=15.8$ and 1.3 Hz, H-6), 5.36 (1H, m, H-14), 5.32 (1H, m, H-3), 5.27 (1H, m, H-8), 5.08 (1H, m, H-15), 5.07 (1H, m, H-9), 2.60 (2H, dd, $J=5.3$ and 1.0 Hz, H-2), 2.12 (6H, s, acetyl methyl), 1.36 (3H, d, $J=10.5$ Hz, 9-Me), 1.34 (3H, d, $J=10.9$ Hz, 3-Me), 1.28 (3H, d, $J=10.2$ Hz, 15-Me).

Compound **2'**: UV λ_{max}^{MeOH} nm: 231 sh.; $[\alpha]_D^{27}$ $-2.6°$ (*c* 0.15, MeOH); HR Pos. FAB-mass: $C_{18}H_{22}O_9Na$ ($M^+ + Na$, *m/z* 405.1168, Calc. 405.1161); ^1H NMR ($CDCl_3$) δ ppm: 7.06 (1H, d, $J=15.8$ Hz, H-12), 6.87 (1H, dd, $J=15.8$ and 4.6 Hz, H-7), 6.85 (1H, d, $J=15.8$ Hz, H-13), 6.00 (1H, dd, $J=15.8$ and 2.0 Hz, H-6), 5.46 (1H, m, H-15), 5.38 (1H, m, H-9), 5.18 (1H, m, H-3), 2.83 (1H, m, H-2a), 2.65 (1H, dd, $J=16.2$ and 2.4 Hz, H-2b), 2.12 (3H, s, acetyl methyl), 1.46 (3H, d, $J=6.6$ Hz, 9-Me), 1.42 (3H, d, $J=5.6$ Hz, 15-Me), 1.38 (3H, d, $J=6.3$ Hz, 3-Me).

Acknowledgments

We express our thanks to Mr. K. MATSUZAKI, 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) SPRINGER, T. A.: Adhesion receptors of the immune system. Nature 346: 425~434, 1990
2) BUTCHER, E.: Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. Cell 67: 1033~1036, 1991
3) IIGO, Y.; T. TAKASHI, 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
4) 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
5) 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
6) ŌMURA, S. & H. TANAKA: Production and antimicrobial activity of macrolides. *In* Macrolide antibiotics. Chemistry, biology, and practice. *Ed.*, S. ŌMURA, pp. 3~35, Academic Press, Inc, 1984
7) ŌMURA, S.: Production, structure, and biological properties of macrolide-like antibiotics. *In* Macrolide antibiotics. Chemistry, biology, and practice. *Ed.*, S. ŌMURA, pp. 509~552, Academic Press, Inc, 1984
8) KOMIYAMA, K.; S. TAKAMATSU, Y. TAKAHASHI, M. SHINOSE, M. HAYASHI, H. TANAKA, Y. IWAI, S. ŌMURA & G. IMOKAWA: New inhibitors of melanogenesis, OH-3984 K1 and K2: I. Taxonomy, fermentation, isolation and biological characteristics. J. Antibiotics 46: 1520~1525, 1993
9) TAKAMATSU, S.; M-C. RHO, M. HAYASHI, K. KOMIYAMA, H. TANAKA, S. ŌMURA & G. IMOKAWA: New inhibitors of melanogenesis, OH-3984 K1 and K2: II. Physicochemical properties and structural elucidation. J. Antibiotics 46: 1526~1529, 1993
10) ŌMURA, S.: Studies on macrolides and new antibiotics. Yakugaku Zasshi 106: 729~757, 1986