VOL. 55 NO. 2, FEB. 2002 THE JOURNAL OF ANTIBIOTICS pp. 147 — 154

Absolute Stereostructures of Cell Adhesion Inhibitors, Macrosphelides H and L, from *Periconia byssoides* OUPS-N133

TAKESHI YAMADA, MASASHI IRITANI, KATSUHIKO MINOURA and ATSUSHI NUMATA*

Osaka University of Pharmaceutical Sciences,
4-20-1, Nasahara, Takatsuki, Osaka 569-1094, Japan

YUICHI KOBAYASHI and YONG-GANG WANG

Department of Biomolecular Engineering, Tokyo Institute of Technology,
4259 Nagatsuta-cho, Midori-ku, Yokohama 226-8501, Japan

(Received for publication September 17, 2001)

Macrosphelide L has been isolated from a strain of *Periconia byssoides* originally separated from the sea hare *Aplysia kurodai*, and the absolute stereostructures of this material and macrosphelide H, previously undetermined, have been elucidated on the basis of spectroscopic analyses using 1D and 2D NMR techniques and some chemical transformation. (*R*)-Methyl 3-*p*-bromobenzoyloxy-5-oxohexanoate has been synthesized for configurational assignments of macrosphelide H. These macrosphelides inhibited the adhesion of human-leukemia HL-60 cells to HUVEC.

Based on the fact that some of the bioactive materials isolated from marine animals have been produced by bacteria, we have focused our attention on new antitumour materials from microorganisms separated from marine organisms.[1~3] As part of this study, we have previously isolated the five 16-membered macrolides, macrosphelides E (**1**)~H (**2**) and I, from a strain of *Periconia byssoides* OUPS-N133 originally separated from the sea hare *Aplysia kurodai*, and reported their absolute stereostructures except for macrosphelide H (**2**).[4,5] Although the planar structure for **2** was previously reported,[4,5] its relative and absolute stereochemistries were undetermined. All of these compounds except for macrosphelide I inhibited the adhesion of human-leukemia HL-60 cells to human-umbilical-vein endothelial cells (HUVEC) more potently than herbimycin A,[6,7] and compound **2** exhibited most potently inhibitory activity.[5] Further investigation for metabolites of this fungal strain has now led to the isolation of one additional new anti-adhesion compound designated macrosphelide L (**3**). It has been reported by OMURA and co-workers that macrosphelides A~D, J and K have been isolated from a strain of *Microsphaeropsis* sp. and the first four compounds potently inhibit cell-cell adhesion.[8~12] We describe herein the absolute stereostructures of macrosphelides H (**2**) and L (**3**), and the inhibition of cell adhesion for the latter and some derivatives (Fig. 1).

Results and Discussion

The fungal strain was cultured at 27°C for 4 weeks in a medium containing malt extract 1%, glucose 1% and peptone 0.05% in artificial seawater adjusted to pH 7.5, as reported previously.[4,5] The AcOEt extract of the culture filtrate was purified by fractionation employing a combination of Sephadex LH-20 and silica gel column chromatographies and HPLC to afford macrosphelide L (**3**) together with macrosphelide H (**2**).

Macrosphelide L (**3**) had the molecular formula $C_{16}H_{22}O_8$ established by HREIMS. Its IR spectrum exhibited bands at 3438, 1718, 1710 and 1651 cm^{-1}, characteristic of an alcohol, an ester, a ketone and a double bond (Table 1). A close inspection of the ^1H and ^{13}C NMR spectra of **3** (Table 2) by DEPT and ^1H-^{13}C COSY

* Corresponding author: numata@gly.oups.ac.jp

Fig. 1. Structures of macrosphelides E (**1**), H (**2**) and L (**3**).

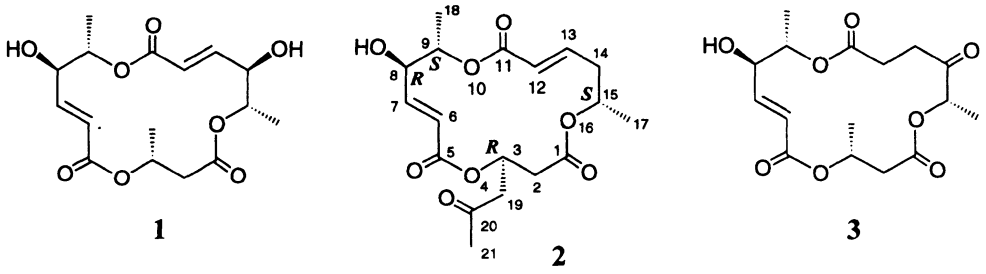


Table 1. Physico-chemical properties of macrosphelide L (**3**).

	3
Appearance	Colorless oil
$[\alpha]_D^{21}$	–24.2° (*c* 0.33, EtOH)
HREIMS	
Found:	342.1310 (M^+)
Calcd:	342.1313 (for $C_{16}H_{22}O_8$)
Molecular formula	$C_{16}H_{22}O_8$
UV λ_{max} (EtOH) nm (log ε)	218 (3.75)
IR ν_{max} (neat) cm^{-1}	3438, 1718, 1710, 1651
CD λ_{max} (EtOH) nm ($\Delta\varepsilon$)	307 (0), 278 (+0.45), 253 (0), 237 (-0.44)
	(*c* 4.85 x 10^{-4} M in EtOH)
TLC Rf[a]	0.18
Solubility	
soluble	DMSO, CH_2Cl_2, MeOH, acetone
insoluble	H_2O

[a] Silica gel (5% MeOH in CH_2Cl_2).

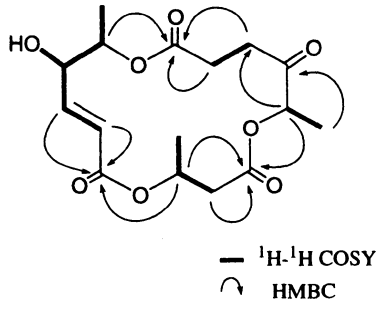
experiments revealed the presence of three secondary methyl (C-17~C-19), three sp^3-hybridized methylene (C-2, C12 and C-13), four oxygen-bearing sp^3-methines (C-3, C-8, C-9 and C-15) including one hydroxymethine (C-8), one 1, 2-disubstituted double bond (C-6 and C-7), and four carbonyl groups of one ketone (C-14) and three esters (C-1, C-5 and C-11). The ^1H-^1H COSY analysis of **3** led to four partial structural units as shown by bold-faced lines in Fig. 2, which were supported by HMBC correlations (Table 2). The *E*-geometry of the Δ^6-double bond was deduced from a coupling constant ($J_{6,7}$ 15.7 Hz) of the olefinic protons. The connection of these three units and the remaining functional groups was determined on the basis of the key HMBC correlations summarized in Fig. 2, and the planar structure of **3** was elucidated.

The stereochemistry of **3** could not be deduced from NOESY experiments. Therefore, the absolute configuration of **3** was determined by a chemical transformation of macrosphelide E (**1**), of which the absolute stereochemistry has already been determined,[4,5] to the hydrogenation product **4** of **3** (Scheme 1).Treatment of **1** with Dess-Martin reagent afforded keto alcohol **5**, which was hydrogenated under the presence of Pd-C catalyst to give the hydrogenation product **4**, identical with that from **3**. The above-summarized evidence led to the absolute stereostructure of **3** for macrosphelide L.

The planar structure of macrosphelide H (**2**) was previously reported, but the stereochemistry was

undetermined. Consequently, in order to determine the absolute configuration of **2**, methanolysis of **2** with sodium methoxide in MeOH was carried out to lead to methyl esters of the three constituent carboxylic acids, which were isolated as *p*-bromobenzoates (**6**, **7** and **8**) by treatment with *p*-bromobenzoyl chloride in pyridine (Scheme 2). The two *p*-bromobenzoates **6** and **7** were respectively identified as (4*R*,5*S*)-methyl 4,5-bis-*p*-bromobenzoyloxy-2*E*-hexenoate and (*S*)-methyl 5-*p*-bromobenzoyloxy-2*E*-hexenoate by comparison of spectral data including CD spectra with authentic samples,[5] implying that the chirality of the 8-, 9- and 15-positions in **2** is *R*, *S* and *S*, respectively.

Fig. 2. Selected ^{1}H-^{1}H COSY and HMBC correlations in macrosphelide L (**3**).



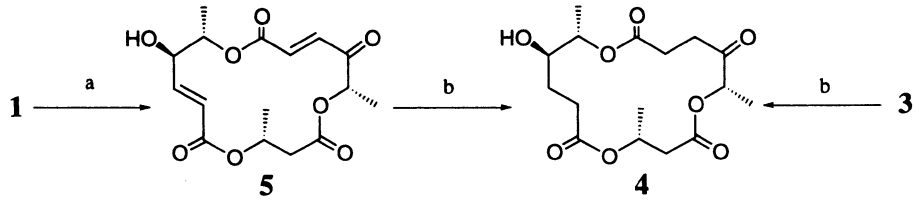
The other *p*-bromobenzoate **8** was identified as (*R*)-methyl 3-*p*-bromobenzoyloxy-5-oxohexanoate by comparison of spectral data including CD spectra with the synthetic compound prepared by the following procedure (Scheme 3). Optically active (*S*)-ethyl 3-hydroxy-4-chlorobutyrate (**9**, 99.3% ee) was used as a starting material for synthesis of **8** (Scheme 3). The *R*-olefin **10** was prepared from **9**, using the method[13] of SATO and co-workers, by which the (*S*)-enatiomer was previously synthesized. Wacher oxidation of **10** followed by

Table 2. NMR spectral data of macrosphelide L (**3**) in $CDCl_3$.

Position	δ_H[a]		J /Hz	^{1}H-^{1}H COSY	δ_C	HMBC (C)[c]
1					169.16 (q)[b]	
2 A	2.68	dd	15.3 (2B), 10.1 (3)	3	41.31 (s)	1, 3, 19
B	2.74	dd	15.3 (2A), 3.2 (3)	3		1, 3, 19
3	5.45	dqd	10.1 (2A), 6.2 (19), 3.2 (2B)	2A, 2B	67.66 (t)	1, 2, 5, 19
5					165.52 (q)	
6	6.11	dd	15.7 (7), 1.8 (8)	7	122.85 (t)	5, 7, 8
7	6.96	dd	15.7 (6), 4.3 (8)	6, 8	144.92 (t)	5, 6, 8, 9
8	4.27	br s		7, 9, 8-OH	75.18 (t)	6, 7, 9, 18
9	4.99	qd	6.6 (18), 2.8 (8)	8, 18	76.38 (t)	7, 8, 18, 11
11					174.26 (q)	
12 A	2.55	m		13A, 13B	33.94 (s)	11, 13, 14
B	2.78	m		13A, 13B		13, 14
13 A	2.43	m		12A, 12B	28.47 (s)	11, 12, 14, 15
B	2.55	m		12A, 12B		11, 12, 14, 15
14					205.23 (q)	
15	5.18	q	7.1 (17)	17	75.11 (t)	1, 13, 14, 17
17	1.39	d	7.1 (15)	15	16.32 (p)	14, 15
18	1.41	d	6.6 (9)	9	18.02 (p)	8, 9
19	1.40	d	6.2 (3)	3	19.97 (p)	2, 3
8-OH	3.61	br s		8		

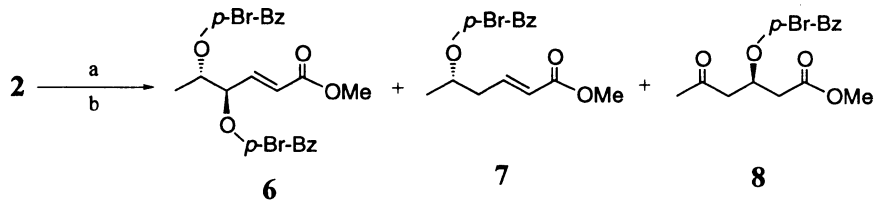
[a] ^{1}H Chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constants (J/Hz). Figures in parentheses indicate the proton coupling with that position. [b] Letters, p, s, t and q, in parentheses indicate respectively primary, secondary, tertiary and quaternary carbons, assigned by DEPT. [c] Long range ^{1}H-^{13}C correlations from H to C.

Scheme 1.



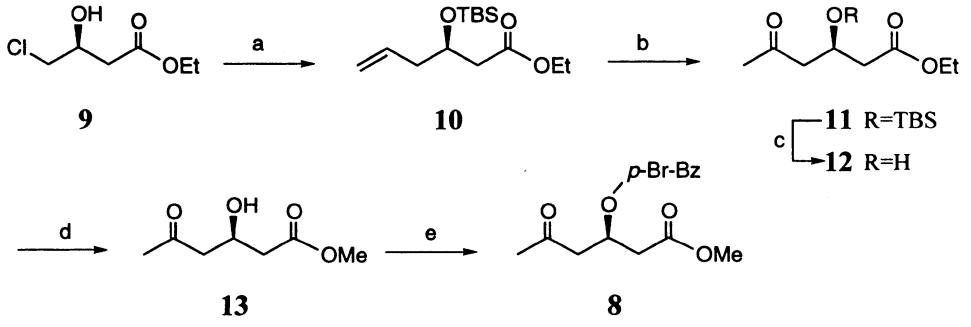
Reagents and conditions: (a) CH_2Cl_2, Dess-Martin reagent; (b) H_2, Pd-C.

Scheme 2.



Reagents and conditions: (a) MeOH, NaOMe; (b) *p*-Br-BzCl, pyridine.

Scheme 3.



Reagents and conditions: (a) i. NaI, ii. TBSCl, iii. CH_2=CHMgBr; (b) O_2, $PdCl_2$, CuCl, 96%; (c) 55% HF, 87%; (d) MeONa, r.t., <30 minutes, 96%; (e) *p*-Br-BzOH, DCC, DMAP, CSA, 81%.

deprotection with HF afforded ketone **12**, of which ester interchange with sodium methoxide gave the *R*-methyl ester **13**. Although this ester and its (*S*)-enantiomer were previously reported, their enatiomeric excesses were moderate (67 and 75% ee for the (*R*)- and (*S*)-enantiomers, respectively).[14] It is most likely that the methyl ester obtained in this experiment is almost enantiopure because of the high enatiometric excess of the starting material (**9**). This was supported by its specific optical rotation value {$[\alpha]_D^{24}$ +13°; lit.[14] $[\alpha]_D^{20}$ −10° for the (*S*)-enantiomer (75% ee)}. Esterification of **13** with *p*-bromobenzoic acid and DCC furnished the desired ester **8**. The above-

Table 3. Inhibitory activity of cell adhesion of macrosphelides.

Compound	IC_{50} (μM)
Macrosphelide L (**3**)	5.6
4	8.3
5	1.2
Herbimycin A[a]	38.0

[a] Standard sample.

summarized evidence allowed assignment of absolute stereostructure **2** for macrosphelide H.

Macrosphelide L (**3**), and two derivatives **4** and **5** were examined using herbimycin A[6,7] as a standard sample in the adhesion assay system using HL-60 cells and HUVEC, according to a modification of the method reported by MIKI and co-workers.[15] As shown in Table 3, all of these compounds inhibited the adhesion of HL-60 cell to HUVEC more potently than herbimycin A. The inhibitory activities of **3** and **4** were comparable to that of macrosphelide H (**2**) (IC_{50} 8.6 μM),[5] while compound **5** exhibited inhibitory activity more potent than compounds **2**~**4**. This evidence suggested that the conjugated ketone at C-14 was important for enhancement of the inhibition of cell adhesion in macrosphelide analogs.

Experimental

General Procedures

UV spectra were recorded on a Shimadzu spectrophotometer and IR spectra on a Perkin Elmer FT-IR spectrometer 1720X. NMR spectra were recorded at 27°C on Varian UNITY INOVA-500 and MERCURY spectrometers with tetramethylsilane (TMS) as an internal reference. EIMS was determined using a Hitachi M-4000H mass spectrometer. ORD and CD spectra were recorded on a JASCO J-820 polarimeters. Liquid chromatography over silica gel (mesh 230~400) was performed in a medium pressure. HPLC was run on a Waters ALC-200 instrument equipped with a differential refractometer (R 401) and Shim-pack PREP-ODS (25 cm×20 mm i.d.). Analytical TLC was performed on precoated Merck aluminium sheets (DC-Alufolien Kieselgel 60 F254, 0.2 mm) with the solvent system CH_2Cl_2-MeOH (19 : 1), and compounds were viewed under UV lamp and sprayed with 10% H_2SO_4 followed by heating.

Production and Isolation of Macrosphelides

A strain of *Periconia byssoides* OUPS-N133, separated from the sea hare *Aplysia kurodai*, was cultured at 27°C for four weeks in a liquid medium (90 liters) containing malt extract 1%, glucose 1% and peptone 0.05% in artificial seawater adjusted to pH 7.5. As reported previously,[5] the AcOEt extract (5.7 g) of the culture filtrate was successively chromatographed on Sephadex LH-20 (CH_2Cl_2-MeOH, 1 : 1) and silica gel (CH_2Cl_2/MeOH). The MeOH-CH_2Cl_2 (2 : 98) eluate (126.6 mg) from silica gel column chromatography was purified by HPLC using MeOH-H_2O (6 : 4) as the eluent to afford **2** (5.7 mg) and **3** (3.8 mg) as a colorless oil.

Oxidation of Macrosphelide E (1)

Dess-Martin reagent (3.2 mg) was added to a solution of **1** (4.5 mg) in CH_2Cl_2 (0.5 ml), and the reaction mixture was stirred for 3 hours at room temperature. The mixture was diluted with ether, the organic layer washed with a 1 : 1 mixture of saturated aq. $NaHCO_3$ and satd aq. $Na_2S_2O_3$ solutions, and then evaporated under reduced pressure. The residue was purified by HPLC [MeOH-H_2O (1 : 1)] to afford the keto alcohol **5** (3.6 mg) as a colorless oil. $[\alpha]_D^{22}$ −4.8° (*c* 0.24, EtOH); UV λ_{max} (EtOH) nm (log ε) 216 (3.73) and 240sh (3.15); IR ν_{max} (neat) cm^{-1} 3448 (OH), 1720 (ester), 1710 (C=O), 1662 and 1651 (C=C); EIMS *m/z* 341 (MH^+, 0.2%), 195 (54.5), 111 (89.6), 82 (41.5) and 69 (100); HREIMS *m/z* for $C_{16}H_{21}O_8$ (MH^+), Calcd: 341.1238; Found: 341.1236; ^1H NMR (500 MHz, $CDCl_3$) δ 1.43 (3H, d, *J*=6.9 Hz, H_3-17), 1.43 (3H, d, *J*=6.6 Hz, H_3-18), 1.45 (3H, d, *J*=6.6 Hz, H_3-19), 2.66 (1H, dd, *J*=15.9, 7.2 Hz, H-2_A), 2.83 (1H, dd, *J*=15.9, 3.6 Hz, H-2_B), 4.42 (1H, br s, H-8), 5.13 (1H, q, *J*=6.9 Hz, H-15), 5.17 (1H, qd, *J*=6.6, 1.9 Hz, H-9), 5.37 (1H, dqd, *J*=7.2, 6.6, 3.6 Hz, H-3), 6.18 (1H, dd, *J*=15.7, 1.8 Hz, H-6), 6.81 (1H, d, *J*=15.8 Hz, H-12), 7.02 (1H, d, *J*=15.8 Hz, H-13) and 7.03 (1H, dd, *J*=15.7, 4.6 Hz, H-7); ^{13}C NMR (125.7, $CDCl_3$) δ 15.95 (C-17), 17.47 (C-18), 19.17 (C-19), 40.07 (C-2), 67.12 (C-3), 74.57 (C-8), 76.05 (C-9), 76.05 (C-15), 122.69 (C-6), 132.68 (C-12), 132.68 (C-13), 144.90 (C-7), 165.21 (C-5), 165.63 (C-11), 169.31 (C-1) and 195.87 (C-14); CD λ (*c* 2.54×10^{-4} M in EtOH) nm (Δε) 399 (0), 358 (−0.50), 250 (−4.66), 229 (0) and 212 (+9.31).

Catalytic Reduction of Compound 5

To a solution of **5** (1.8 mg) in MeOH (1.5 ml) was added

10% Pd-C (4.5 mg), and the reaction mixture was stirred under hydrogen atmosphere (1 atm) for 2 hours at room temperature. The catalyst was filtered off, and the solvent evaporated under reduced pressure. The residue was purified by HPLC using MeOH - H_2O (1 : 1) as the eluent to afford **4** (1.5 mg) as a colorless oil. $[\alpha]_D^{22}$ −2.7° (*c* 0.21, EtOH); IR ν_{max} (neat) cm^{-1} 3448 (OH), 1720 (ester), 1710 (C=O), 1662 (C=C); EIMS *m/z* 345 (MH^+, 3.2%), 197 (30.1) and 113 (100); HREIMS *m/z* for $C_{16}H_{25}O_8$ (MH^+), Calcd: 345.0466; Found: 345.0464; ^1H NMR (500 MHz, $CDCl_3$) δ 1.29 (3H, d, *J*=6.4 Hz, H_3-18), 1.36 (3H, d, *J*=6.4 Hz, H_3-19), 1.43 (3H, d, *J*=7.1 Hz, H_3-17), 1.90 (1H, m, H-7), 1.96 (1H, br s, 8-OH), 2.38 (1H, m, H-6), 2.43 (1H, m, H-12_A), 2.56 (1H, dd, *J*=14.9, 10.1 Hz, H-2_A), 2.62 (1H, m, H-12_B), 2.65 (1H, m, H-13_A), 2.67 (1H, dd, 14.9, 3.0 Hz, H-2_B), 3.04 (1H, m, 13-H_B), 3.68 (1H, m, H-8), 4.91 (1H, quintet, 6.4 Hz, H-9), 5.18 (1H, q, *J*=7.1 Hz, H-15) and 5.27 (1H, dqd, 10.1, 6.4, 3.0 Hz, H-3); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 15.87 (C-17), 16.94 (C-18), 20.03 (C-19), 27.79 (C-7), 28.43 (C-12), 29.61 (C-6), 34.08 (C-13), 40.76 (C-2), 67.93 (C-3), 72.31 (C-9), 73.16 (C-8), 74.04 (C-15), 169.31 (C-1), 171.96 (C-11), 173.21 (C-5) and 205.34 (C-14); CD λ (c 5.67×10^{-4} M in EtOH) nm ($\Delta\varepsilon$) 312 (0), 275 (+10.32) and 212 (+7.81).

Catalytic Reduction of Macrosphelide L (**3**)

Using the same procedure as above with compound **5**, compound **3** (1.2 mg) was hydrogenated in the presence of 10% Pd-C catalyst (3.5 mg) to afford **4** (0.9 mg), identical with an authentic sample derived from compound **1**.

p-Bromobenzoates **6**~**8** of Methanolysis Products from Macrosphelide H (**2**)

A 1 M solution (0.2 ml) of sodium methoxide in MeOH was added to a solution (0.1 ml) of macrosphelide H (**2**) (2.8 mg), and the reaction mixture was left at room temperature for 1.5 hours. The mixture was neutralized with AcOH and concentrated under reduced pressure. The residue was extracted with ether, and the ether layer washed with water and then evaporated under reduced pressure to afford the crude methyl esters (2.5 mg). *p*-Bromobenzoyl chloride (5.3 mg) was added to a pyridine solution (0.7 ml) of the crude methyl esters, and the reaction mixture was left at room temperature overnight. The mixture was concentrated to dryness under reduced pressure, and the residue was purified by HPLC using MeOH - H_2O (9 : 1) as the eluent to afford *p*-bromobenzoates **6** (0.9 mg), **7** (0.7 mg) and **8** (0.5 mg). The benzoates **6** and **7** were identical with authentic samples.[5] *p*-Bromobenzoate **8** was identified as (*R*)-methyl 3-*p*-bromobenzoyloxy-5-oxohexanoate by comparison of spectral data including CD spectra with a synthetic compound described below.

(*R*)-Ethyl 3-(*tert*-Butyldimethylsiloxy)-5-hexenoate (**10**)

This compound, $[\alpha]_D^{28}$ −32° (*c* 0.63, $CHCl_3$), was synthesized by the method of SATO *et al.*[13] Its spectral data were identical with those of the (*S*)-enantiomer reported previouly.[13]

(*R*)-Ethyl 3-(*tert*-Butyldimethylsiloxy)-5-oxohexanoate (**11**)

A suspension of $PdCl_2$ (32 mg, 0.18 mmol) and CuCl (90 mg, 0.91 mmol) in DMF (4 ml) and H_2O (0.5 ml) was stirred at room temperature for 1.5 hours under oxygen. Olefin **10** (247 mg, 0.907 mmol) in DMF (1 ml) was added to the resulting yellow green mixture. The mixture was stirred at room temperature overnight, and diluted with satd aq NH_4Cl. The product was extracted with EtOAc three times. The combined organic layers were dried over $MgSO_4$ and evaporated to leave an oil, which was purified by chromatography (hexane/EtOAc) on silica gel to afford ketone **11** (252 mg, 96%). $[\alpha]_D^{26}$ +2° (*c* 0.52, $CHCl_3$); IR ν_{max} (neat) cm^{-1} 1736, 1719, 1257, 1189, 1151, 1093, 837, 777 cm^{-1}; EIMS *m/z* 289 (MH^+); HREIMS *m/z* for $C_{14}H_{29}O_4Si$ (MH^+), Calcd: 289.1827; Found: 289.1830; ^1H NMR (300 MHz, $CDCl_3$) δ 0.05 (3H, s, SiMe), 0.07 (3H, s, SiMe), 0.84 (9H, s, ^{t}Bu), 1.26 (3H, t, *J*=6.8 Hz, CH_2CH_3), 2.16 (3H, s, H-6), 2.46 (1H, dd, *J*=14.8, 6.0 Hz, H-2_A), 2.50 (1H, dd, *J*=14.8, 6.0 Hz, H-2_B), 2.67 (1H, dd, *J*=16.0, 5.8 Hz, H-4_A), 2.70 (1H, dd, *J*=16.0, 6.3 Hz, H-4_B), 4.11 (1H, dq, *J*=12.0, 6.8 Hz, OCH_2CH_3), 4.12 (1H, dq, *J*=12.0, 6.8 Hz, OCH_2CH_3), 4.55 (1H, dtd, *J*=6.3, 6.0, 5.8 Hz, H-3); ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.7, −4.6, 14.4, 18.1, 25.9, 31.6, 42.6, 50.8, 60.6, 65.8, 171.0, 206.8.

(*R*)-Ethyl 3-Hydroxy-5-oxohexanoate (**12**)

To a solution of **11** (59 mg, 0.205 mmol) in CH_3CN (1 ml) was added 55% aq HF (0.016 ml) in CH_3CN (0.5 ml) at 0°C. After the mixture was stirred at 0°C for 1 hour, the reaction was quenched with satd $NaHCO_3$. The product was extracted with EtOAc three times. The combined organic layers were dried over $MgSO_4$ and concentrated to furnish an oil, which was purified by chromatography (hexane/EtOAc) on silica gel to afford alcohol **12** (31 mg, 86%). $[\alpha]_D^{27}$ +8° (*c* 0.31, $CDCl_3$); ν_{max} (neat) cm^{-1} 3434, 1734, 1717, 1270, 1197, 1157 cm^{-1}; EIMS *m/z* 174 (M^+); HREIMS *m/z* for $C_8H_{14}O_4$ (M^+), Calcd: 174.0891; Found: 174.0883; ^1H NMR (300 MHz, $CDCl_3$) δ 1.28 (3H, t, *J*=7.0 Hz, CH_2CH_3), 2.20 (3H, s, H-6), 2.50 (1H, dd, *J*=16.3, 6.4 Hz, H-2_A), 2.51 (1H, dd, *J*=16.3, 6.4 Hz, H-

2_B), 2.67 (1H, dd, J=17.0, 5.5 Hz, H-4_A), 2.69 (1H, dd, J=17.0, 6.6 Hz, H-4_B), 4.17 (2H, J=7.0 Hz, OCH_2), 4.47 (dtd, J=6.6, 6.4, 5.5 Hz, H-3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.3, 30.9, 40.8, 49.2, 60.8, 64.4, 171.7, 208.1.

(*R*)-Methyl 3-Hydroxy-5-oxohexanoate (**13**)

A solution of NaH (2.4 mg, 55% in mineral oil, 0.053 mmol) in MeOH (1 ml) was stirred at room temperature for 10 minutes, and ethyl ester **12** (31 mg, 0.178 mmol) was added to the solution. After 20 minutes at room temperature, the reaction was quenched by addition of satd $NaHCO_3$, and the product was extracted with EtOAc three times. The combined organic layers were dried over $MgSO_4$ and concentrated to give an oil, which was purified by chromatography (hexane/EtOAc) on silica gel to afford methyl ester **13** (27 mg, 96%). $[\alpha]_D^{24}$ +13° (*c* 0.286, $CDCl_3$); lit.[14] $[\alpha]_D^{20}$ −10° (*c* 1.1, $CDCl_3$) for the (*S*)-enantiomer (75% ee); ν_{max} (neat) cm^{-1} 3430, 1733, 1715, 1213, 1158, 1071 cm^{-1}; Its ^1H NMR and ^{13}C NMR data were identical with those of the (*S*)-enantiomer reported previously.[14]

(*R*)-Methyl 3-*p*-Bromobenzoyloxy-5-oxohexanoate (**8**)

To a solution of *p*-bromobenzoic acid (65 mg, 0.32 mmol) in THF (2 ml) were added DMAP (18 mg, 0.15 mmol), CSA (17 mg, 0.073 mmol), and DCC (62 mg, 0.30 mmol). After 10 minutes at room temperature, alcohol **13** (40 mg, 0.25 mmol) in THF (1 ml) was added to the solution. The solution was stirred at room temperature overnight and the resulting mixture was filtrated through a pad of Celite with EtOAc. The filtrate was concentrated to leave an oil, which was purified by chromatography (hexane/EtOAc) on silica gel to afford ester **8** (72 mg, 81%). $[\alpha]_D^{28}$ −11° (*c* 0.028, $CHCl_3$); ν_{max} (neat) cm^{-1} 1738, 1717, 1590, 1272, 1103, 757 cm^{-1}; EIMS *m/z* 342 (M^+); HREI *m/z* for $C_{14}H_{15}BrO_5$ (M^+), Calcd: 342.0102; Found: 342.0105; ^1H NMR (300 MHz, $CDCl_3$) δ 2.20 (3H, s, H-6), 2.80 (1H, dd, J=16.0, 6.6 Hz, H-2_A), 2.84 (1H, dd, J=16, 5.9 Hz, H-2_B), 2.94 (1H, dd, J=17.0, 6.6 Hz, H-4_A), 3.02 (1H, dd, J=17.0, 6.3 Hz, H-4_B), 3.67 (3H, s, OMe), 5.73 (tdd, J=6.6, 6.3, 5.9 Hz, H-3), 7.56 (2H, d, J=8.5 Hz, Ar-H), 7.83 (d, J=8.5 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 30.6, 38.3, 46.8, 52.0, 67.7, 128.3, 128.7, 131.1, 131.7, 164.8, 170.3, 204.7.

Cell Adhesion Assay

This assay was carried out according to a modification of the Miki's method using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT)-labeled cells.[15] HUVEC (DIA-IATRON Co., Ltd.) were cultured until confluent in a 96-well plate in medium 199 (Gibco) containing 10% fetal calf serum (FCS, Gibco) and washed with phosphate buffered saline (PBS, DIA-IATRON Co., Ltd.) containing 20% FCS. The HUVEC were stimulated with a solution of lipopolysaccharides (LPS, Sigma) in RPMI 1640 medium (Gibco) containing 10% FCS for 4 hours in the presence of various concentrations of macrosphelides, and then MTT-labeled HL-60 cells were added and incuvated for 40 minutes at 37°C in 5% CO_2. Unbound cells were gently washed out with PBS containing 10% FCS, and DMSO was added to lyse the adherent HL-60 cells. Absorbance at 540 nm was measured using a microplate reader (Model 450, BIO-RAD).

Acknowledgements

We thank Dr. K. KAWAI (Fuso Pharmaceutical Industries, Ltd.) for his helpful advice on the cell adhesion assay, respectively. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan.

References

1) AMAGATA, T.; M. DOI, T. OHTA, K. MINOURA & A. NUMATA: Absolute stereostructures of novel cytotoxic metabolites, gymnastatins A~E, from a *Gymnascella* species separated from a *Halichodria* sponge. J. Chem. Soc. Perkin Trans. 1 3585~3599, 1998 and references cited therein.
2) AMAGATA, T.; M. DOI, M. TOHGO, K. MINOURA & A. NUMATA: Dankasterone, a new class of cytotoxic steroid produced by a *Gymnascella* species from a marine sponge. Chem. Commun. 1321~1322, 1999
3) IWAMOTO, C.; T. YAMADA, Y. ITO, K. MINOURA & A. NUMATA: Cytotoxic cytochalasans from a *Penicillium* species separated from a marine alga. Tetrahedron 57: 2997~3004, 2001
4) NUMATA, A.; M. IRITANI, T. YAMADA, K. MINOURA, E. MATSUMURA, T. YAMORI & T. TSURUO; Novel antitumour metabolites produced by a fungal strain from a sea hare. Tetrahedron Lett. 38: 8215~8218, 1997
5) YAMADA, T.; M. IRITANI, M. DOI, K. MINOURA, T. ITO & A. NUMATA: Absolute stereostructures of cell adhesion inhibitors, macrosphelides C, E~G and I, produced by a *Periconia* species separated from an *Aplysia* sea hare. J. Chem. Soc. Perkin Trans. 1 3046~3053, 2001
6) WEBER, C.; E. NEGRESCU, W. ERL, A. PIETSCH, M. FRANKENBERGER, H. W. L. ZIEGLER-HEITBROCK, W. SIESS & P. C. WEBER: Inhibitors of protein tyrosine kinase suppress TNF-stimulated induction of endothelial cell adhesion molecules. J. Immunol. 155: 445~451, 1995
7) MAY, M. J.; C. P. D. WHEELER-JONES & J. D. PEARSON: Effects of protein tyrosine kinase inhibitors on cytokine-induced adhesion molecule expression by human umbilical vein endothelial cells. Br. J. Pharmacol. 118: 1761~1771, 1996
8) HAYASHI, M.; Y.-P. KIM, H. HIRAOKA, M. NATORI, S.

TAKAMATSU, T. KAWAKUBO, R. MASUMA, K. KOMIYAMA & S. OMURA: Macrosphelide, a novel inhibitor of cell-cell adhesion molecule I. Physicochemical properties and structural elucidation. J. Antibiotics 48: 1435~1439, 1995

9) TAKAMATSU, S.; Y.-P. KIM, M. HAYASHI, H. HIRAOKA, M. NATORI, K. KOMIYAMA & S. OMURA: Macrosphelide, a novel inhibitor of cell-cell adhesion molecule II. J. Antibiotics 49: 95~98, 1996

10) SUNAZUKA, T.; T. HIROSE, Y. HARIGAYA, S. TAKAMATSU, M. HAYASHI, K. KOMIYAMA, S. OMURA, P. A. SPRENGLER & A. B. SMITH: Relative and absolute stereochemistries and total synthesis of (+)-macrosphelides A and B, potent, orally bioavailable inhibitors of cell-cell adhesion. J. Am. Chem. Soc. 119: 10247~10248, 1997

11) TAKAMATSU, S.; H. HIRAOKA, Y.-P. KIM, M. HAYASHI, M. NATORI, K. KOMIYAMA & S. OMURA: Macrosphelides C and D, novel inhibitors of cell adhesion. J. Antibiotics 50: 878~880, 1997

12) FUKAMI, A.; Y. TANIGUCHI, T. NAKAMURA, M.-C. RHO, K. KAWAGUCHI, M. HAYASHI, K. KOMIYAMA & S. OMURA: New members of the macrosphelides from *Microsphaeropsis* sp. FO-5050 IV. J. Antibiotics 52: 501~504, 1999

13) HAREAU, G. P.-J.; M. KOIWA, S. HIKICHI & F. SATO: Synthesis of optically active 5-(*tert*-butyldimethyl-siloxy)-2-cyclohexenone and its 6-substituted derivatives as useful chiral building blocks for the synthesis of cyclohexane rings. Syntheses of carvone, penienone, and penihydrone. J. Am. Chem. Soc. 121: 3640~3650, 1999

14) BLANDIN, V.; J.-F. CARPENTIER & A. MORTREUX: One-pot and sequential asymmetric hydrogenation of β,δ-diketoesters into functionalized 1,3-diols: From *anti*- to *syn*-stereoselectivity. Eur. J. Org. Chem. 3421~3427, 1999

15) MIKI, I.; N. ISHIHARA, M. OTOSHI & H. KASE: Simple colorimetric cell-cell adhesion assay using MTT-stained leukemia cells. J. Immunol. Methods 164: 255~261, 1993