Relative and Absolute Stereochemistries and Total Synthesis of (+)-Macrosphelides A and B, Potent, Orally Bioavailable Inhibitors of Cell−Cell Adhesion

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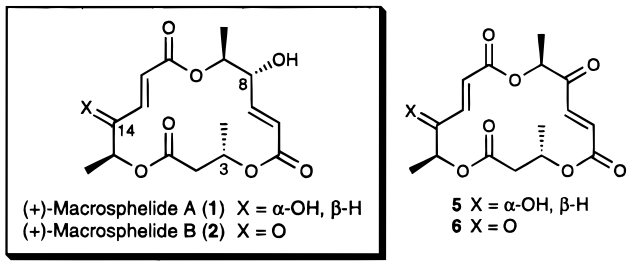
Received May 21, 1997

Critical early events in inflammation,[1−3] the allergic response,[4−6] and tumor metastasis[7−9] involve interactions between leukocytes and endothelial cells. A variety of cytokinins and related chemical mediators control both leukocyte adhesion and subsequent intercellular invasion by regulating the expression of cellular adhesion molecules.[10,11] Inhibition of cell−cell adhesion thus holds promise for the treatment of diverse pathologies.

Recently we reported the isolation, planar structures, and preliminary biological evaluation of (+)-macrosphelides A and B (**1** and **2**).[12] These novel macrolides, produced by *Microsphaeropsis* sp. FO-5050, are the first 16-membered-ring antibiotics embodying three lactone linkages (i.e., macrotriolides). The macrosphelides strongly inhibit the adhesion of human-leukemia HL-60 cells to human-umbilical-vein endothelial cells (HUVEC) in dose-dependent fashion (IC_{50} 3.5 and 36 μM, respectively).[12] Preliminary studies suggest that **1** and **2** prevent cell−cell adhesion by inhibiting the binding of sialyl Lewis x to E-selectin.[13] Macrosphelide A also proved to be orally active against lung metastasis of B16/BL6 melanoma in mice (50 mg/kg). Importantly, **1** did not inhibit the growth of various mammalian cell lines (0.2 mg/mL) or microorganisms (1 mg/mL) in vitro. No acute toxicity was observed upon intraperitoneal injection into BDF1 mice (200 mg/kg for 5 days).[13] The macrosphelides also display significant activity against the rodent-ear edema reaction induced by arachidonic acid and, thus, may serve as valuable leads for the development of lipoxygenase inhibitors.[13] In conjunction with our continuing program directed toward the structure elucidation and synthesis of important bioregulatory natural products, we describe here the determination of the complete relative and absolute stereochemistries of (+)-macrosphelides A and B (**1** and **2**) and the first total synthesis of these materials.



Initially we deduced the connectivity of **1** and **2** via a series of NMR studies, including $^1H-^1H$ and $^1H-^{13}C$ COSY and HMBC experiments, in conjunction with FAB MS and IR data and chemical characterization of the derived di- and monoacetates, respectively.[12] Single-crystal X-ray diffraction has now been employed to elucidate the relative stereochemistry of **1** and verify the planar structure (Figure 1).[14]

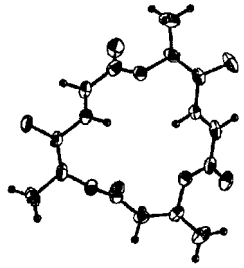


Figure 1. ORTEP plot for (+)-macrosphelide A (**1**).

We next sought to determine the absolute configuration via the Kakisawa−Kashman modification[15] of the Mosher NMR method.[16] To this end, the bis(Mosher ester) derivatives (−)-**3** and (+)-**4** were prepared by treatment of **1** with (*S*)-(−)- and (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) in the presence of dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) (THF, room temperature).[15] The ^1H NMR spectra of **3** and **4** were completely assigned via selective ^1H decoupling. Application of the Kakisawa−Kashman test[15] to the ^1H $\Delta\delta$ values for **3** and **4** (Figure 2) indicated that the absolute configurations at C(8) and C(14) are *R*; thus, (+)-macrosphelide A (**1**) contains two (4*R*,5*S*)-4,5-dihydroxypentenoic acid moieties and a (3*S*)-3-hydroxybutanoic acid unit. The larger $\Delta\delta$ shifts observed for the protons β to the secondary

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(14) Compound (+)-**1**, $C_{16}H_{22}O_8$, crystallizes in the monoclinic space group $P2_1$ with a = 10.387(4), b = 5.656(5), and c = 16.392(4) Å, β = 106.49(2)°, V = 923.4(9) Å^3, Z = 2 and d_{calcd} = 1.231 g/cm^3. The cell constants were determined from a least-squares fit of the setting angles for 15 accurately centered reflections. X-ray intensity data were collected on a Rigaku AFC5S diffractometer employing Cu K_α radiation (λ = 1.541 78 Å) and the $\omega-2\theta$ scan technique. A total of 1904 reflections were measured with $2\theta_{max}$ = 140.3°. The intensity data were corrected for Lorentz and polarization effects but not for absorption. The structure was solved by direct methods. For refinement, 1522 unique reflections with $F^2 > 3\sigma(F^2)$ were used. Full-matrix least-squares refinement based on F, minimizing the quantity $\sum w(|F_o| - |F_c|)^2$ with $w = 4F_o^2/\sigma^2(F_o^2)$, converged to R = 0.082 and R_w = 0.092.

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S0002-7863(97)01657-0 CCC: $14.00

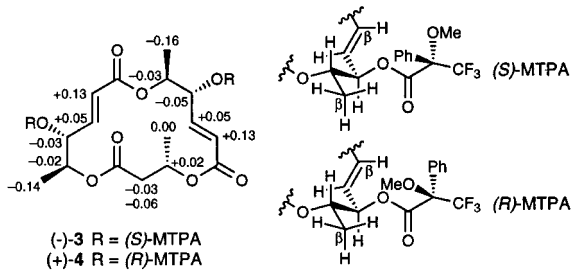


Figure 2. Absolute stereochemistry determination: $\Delta\delta$ values for the bis(Mosher ester) derivatives **3** and **4** (ppm, 500 MHz; $\Delta\delta = \delta_S - \delta_R = \delta_3 - \delta_4$).

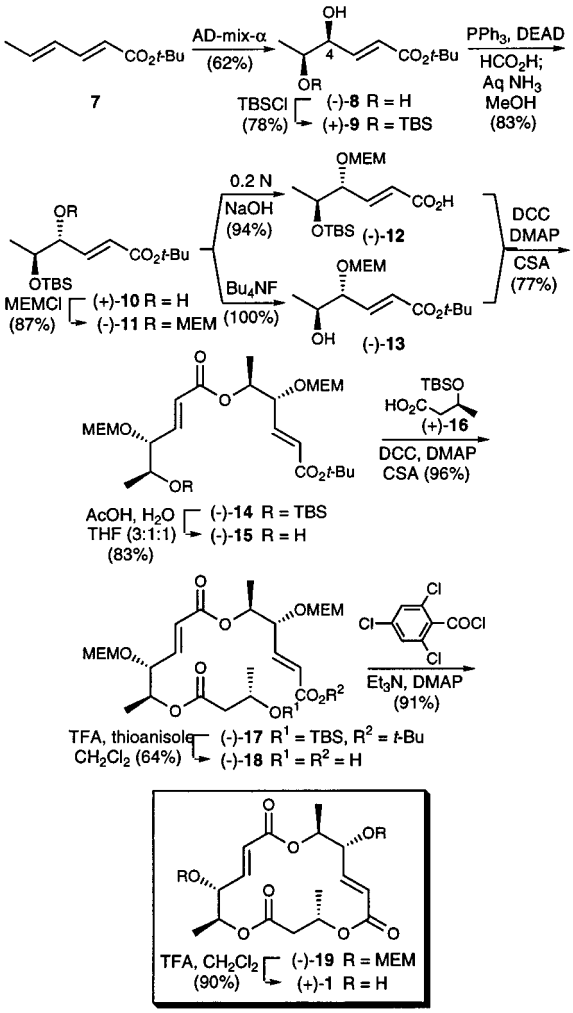
hydroxyls [i.e., the C(6) and C(13) vinyl and C(9) and C(15) methyl protons] are consistent with the proposed solution conformations, wherein the MTPA aromatic rings lie closer to the β hydrogens than to the α hydrogens.

To secure the relative and absolute stereochemistries of **2**, we subjected (+)-macrosphelide A to pyridinium dichromate (PDC) oxidation in CH_2Cl_2 (room temperature, 3 h). Preparative TLC gave a mixture of the 14- and 8-monoketones **2**[17] and **5**[17] as well as pure 8,14-diketone **6**[17] (16% yield) and recovered **1** (45%). HPLC separation then afforded **2** and **5** in 21 and 18% yields. Synthetic **2** proved to be indistinguishable from the natural product (^{1}H and ^{13}C NMR, IR, high-resolution mass spectrometry, and optical rotation). Accordingly, the configurations of (+)-macrosphelides A (**1**) and B (**2**) are (3*S*, 8*R*, 9*S*, 14*R*, 15*S*) and (3*S*, 8*R*, 9*S*, 15*S*), respectively. These assignments were confirmed by total synthesis.

Our approach to the construction of **1** and **2** entailed the enantioselective preparation of two differentially protected derivatives of *trans*-(4*R*,5*S*)-4,5-dihydroxy-2-hexenoic acid. The third building block, (3*S*)-3-hydroxybutyric acid, is commercially available. As our point of departure, we selected the asymmetric dihydroxylation[18] of (*E*,*E*)-hexa-2,4-dienoic acid *tert*-butyl ester (**7**),[19] which afforded the (4*S*,5*S*)-diol (−)-**8**[17] in 62% yield (Scheme 1). Selective monosilylation of (−)-**8** [*tert*-butyldimethylsilyl chloride (TBSCl), DMAP, CH_2Cl_2] provided the desired ether (+)-**9**[17] (56% yield; 78% based on recovered **8**) plus the 4-silyloxy isomer (11%, not shown). Mitsunobu inversion [PPh_3, diethyl azodicarboxylate (DEAD), HCO_2H; dilute NH_3/MeOH] at C(4) of **9** furnished (+)-**10**[17] (83% yield); the enantiomeric purities of both **9** and **10** were 85% ee as determined by Mosher analysis.[16] After protection of (+)-**10** as the (methoxyethoxy)methyl (MEM) ether (−)-**11**[17] (87% yield), saponification (0.2 N NaOH, MeOH/THF/H_2O) gave (−)-**12**[17] in 94% yield, whereas desilylation generated the second building block (−)-**13**[17] (Bu_4NF, THF, 100%).

Condensation of carboxylic acid (−)-**12** and alcohol (−)-**13** via the Keck protocol[20] [DCC, DMAP, camphorsulfonic acid (CSA), CH_2Cl_2, 77% yield] and desilylation of the resultant ester (−)-**14**[17] (3:1:1 AcOH/THF/H_2O) produced (−)-**15**[17] in 83% yield. The third fragment, TBS ether (+)-**16**,[21] was prepared from (3*S*)-3-hydroxybutyric acid and coupled with (−)-**15** in 96% yield (DCC, DMAP, CSA, CH_2Cl_2). Removal of the silyl and *tert*-butyl moieties in (−)-**17**[17] [5:1:5 trifluoroacetic acid (TFA)/CH_2Cl_2/thioanisole][22] provided seco acid (−)-**18**[17] (64%

Scheme 1



yield), which smoothly underwent Yamaguchi macrolactonization[23] (DMAP, 2,4,6-trichlorobenzoyl chloride, 91%). Finally, deprotection of (−)-**19**[17] (1:1 TFA/CH_2Cl_2) gave synthetic **1** in 90% yield, identical in all respects (400 MHz ^{1}H and 100 MHz ^{13}C NMR, IR, high-resolution FAB MS, optical rotation, melting point and mixed melting point, TLC and HPLC in four solvent systems) with a sample of the natural product.

The first total synthesis of (+)-macrosphelide A (**1**) has thus been achieved via a highly convergent, efficient strategy (11 steps, 10.6% overall yield). In conjunction with the conversion of **1** to **2**, the successful route also constitutes a formal construction of (+)-macrosphelide B, confirming the assigned structures of both congeners. Further refinements of the synthetic scheme and the preparation and biological evaluation of macrosphelide analogs will be reported in due course.

Acknowledgment. Financial support was provided by the Ministry of Education, Science and Culture (Japan), the Japan Keirin Association, a Kitasato University Research Grant for Young Researchers, and by the National Institutes of Health (Institute of General Medical Sciences) through grant GM-29028. The authors are also indebted to Dr. George T. Furst for assistance in interpreting the NMR data.

Supporting Information Available: Preparative procedures and characterization data for **1**−**6**, **8**−**19**; tables of X-ray data for **1** (18 pages). See any current masthead page for ordering and Internet access instructions.

JA971657W

(17) All synthetic compounds were purified by flash chromatography on silica gel. The structure assigned to each new compound is in accord with its IR, 400 or 270 MHz ^{1}H NMR, and 100 or 67.5 MHz ^{13}C NMR spectra, as well as appropriate parent ion identification by high-resolution mass spectrometry. In addition, compounds **8**−**19** gave satisfactory combustion analyses.

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