Pentaketides Relating to Aspinonene and Dihydroaspyrone from a Marine-Derived Fungus, *Aspergillus ostianus*

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Three new pentaketides, aspinotriols A (**1**) and B (**3**) and aspinonediol (**5**), were isolated together with two known compounds, aspinonene (**7**) and dihydroaspyrone (**9**), from the marine fungus *Aspergillus ostianus* strain 01F313, which was collected in Pohnpei and cultured with bromine-modified artificial seawater. The structures of the new compounds were determined by spectroscopic analyses including 1D and 2D NMR. Although **1** and **3** are diastereomers, they show nearly superimposable ^{1}H and ^{13}C NMR spectra. The absolute configurations of compounds **1**, **3**, **5**, and **9** were elucidated by the modified Mosher's method.

A growing number of marine fungi have been reported to produce novel and potentially useful bioactive secondary metabolites, and the vast majority of compounds reported from marine fungi are from strains that, based on morphological characteristics, are closely related or identical to terrestrial species.[1,2] We previously reported three antibacterial chlorine-containing components of *Aspergillus ostianus* strain 01F313 that had been isolated from an unidentified marine sponge collected at Pohnpei.[3] The chlorine must have originated from the cultivation medium composed of natural seawater. Expecting that bromine-containing compounds might be obtained when a medium composed of a bromide solution in place of seawater was used, we cultivated the same strain in a bromine-modified 1/2 PD medium. Although we were unable to isolate brominated compounds, we found that the metabolites were considerably different from those obtained from the strain cultured in the seawater medium and succeeded in isolating three new compounds, named aspinotriols A (**1**) and B (**3**) and aspinonediol (**5**), as well as two known compounds, aspinonene (**7**)[4,5,6] and dihydroaspyrone (**9**).[6] This paper elucidates the structures of the new compounds and the absolute configurations of all compounds.

Strain *A. ostianus* 01F313 was cultured in a 1/2 PD medium containing bromine-modified artificial seawater (see the Experimental Section). After the mycelial cake was removed by filtration, the filtrate was subjected to HP-20 extraction. The extract was separated by silica gel flash column chromatography (FCC) followed by reversed-phase (ODS) HPLC to give compounds **1**, **3**, **5**, **7**, and **9** (Figure 1).

Aspinotriol A (**1**), $[\alpha]^{25}_{D}$ −10.1 (*c* 0.23, MeOH), has a molecular formula of $C_9H_{16}O_3$ deduced from HRTOFMS [$(M + Na)^+$ *m/z* 195.1012, calc 195.0997]. The ^{1}H NMR (400 MHz, CD_3OD) spectrum (Table 1) showed the presence of two secondary methyls [δ 1.294 (d, J = 6.4 Hz), 1.289 (d, J = 6.4 Hz)], three olefinic methines [δ 6.17 (d, J = 15.9 Hz), 5.97 (dd, J = 15.9, 6.4 Hz), 5.60 (d, J = 8.6 Hz)], an oxymethylene [δ 4.330 (s)], and two oxymethines [δ 4.76 (dq, J = 8.6, 6.4 Hz), δ 4.325]. The two olefins must be conjugated considering the absorption maximum at 231 nm in the UV spectrum, and one of them is a disubstituted *E*-olefin showing $^3J_{HH}$ of 15.9 Hz. The *Z*-configuration of the other olefin was confirmed by the NOESY correlations of H-4/H-6 and H-7/H-9. The ^{13}C NMR (100 MHz, CD_3OD) spectrum (Table 1) confirmed the presence of two methyl carbons (δ 23.9, 23.7), three olefinic methine carbons (δ 138.8, 135.0, 131.7), a quaternary olefinic carbon (δ 137.8), an oxymethylene carbon (δ 57.6), and two oxymethine carbons (δ 69.4, 64.7). These data, together with the ^{1}H−^{1}H COSY, HSQC, and HMBC spectra (Table 1), readily revealed the planar structure of aspinotriol A (**1**). At this stage, the stereochemistry of the two asymmetric carbons was unknown.

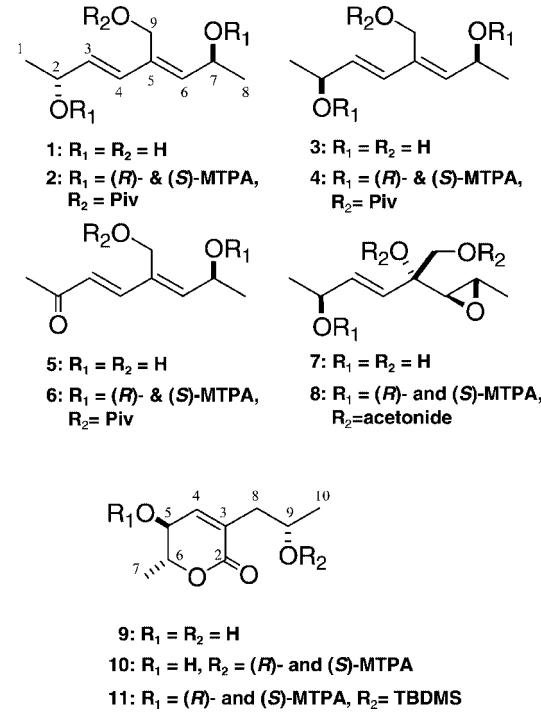

Figure 1. Structures of three new compounds, aspinotriols A (**1**) and B (**3**) and aspinonediol (**5**), together with the known compounds aspinonene (**7**) and dihydroaspyrone (**9**), and their derivatives. Piv: pivaloyl (2,2-dimethypropanoyl), acetonide: isopropylidene, TBDMS: *tert*-butyldimethylsilyl.

Aspinotriol B (**3**), $C_9H_{16}O_3$ [$(M + Na)^+$ *m/z* 195.1007, calc 195.0997], was obtained from the HPLC fraction, whose retention time (t_R = 19 min) was largely different from the fraction giving **1** (t_R = 13 min). We were puzzled to find that the ^{1}H (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD) spectra of **3** were practically identical to those of **1** (Table 2). Changing the NMR solvent to pyridine-d_5 gave the same results (Table 3). Analyses of the 1D and 2D NMR data of **3** (Table 2) led to the same planar structure as **1**, including the geometry of the diene group. Considering the chiroptical property of **3**, $[\alpha]^{25}_{D}$ +6.1 (*c* 0.21, MeOH), we

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Table 1. NMR Data (100/400 MHz, CD_3OD) of Aspinotriol A (**1**)

position	δ_C, mult.	δ_H (J in Hz)	COSY	HMBC	NOESY
1	23.9, CH_3	1.29, d (6.4)	2	2, 3	3
2	69.4, CH	4.33, m	1, 3, 4	1	
3	138.8, CH	5.97, dd (15.9, 6.4)	2, 4	1, 2, 4, 5	1, 2
4	131.7, CH	6.17, d (15.9)	2, 3, 6	2, 3, 5, 6, 9	2, 6
5	137.8, C				
6	135.0, CH	5.60, d (8.6)	4, 7, 9	4, 5, 8, 9	4, 8
7	64.7, CH	4.76, dq (8.6, 6.4)	6, 8	5, 8	9
8	23.7, CH_3	1.29, d (6.4)	7	6, 7	6
9	57.6, CH_2	4.33 (s)	6	4, 5, 6	3, 7

Table 2. NMR Data (100/400 MHz, CD_3OD) of Aspinotriol B (**3**)

position	δ_C, mult.	δ_H (J in Hz)	COSY	HMBC	NOESY
1	23.9, CH_3	1.29, d (6.4)	2	2, 3	3
2	69.4, CH	4.32, m	1, 3, 4	1, 3	
3	138.8, CH	5.97, dd (15.9, 6.4)	2, 4	1, 2, 4, 5, 6	1, 2
4	131.7, CH	6.17, d (15.9)	2, 3, 6	2, 3, 5, 6, 9	2, 6
5	137.8, C				
6	135.0, CH	5.60, d (8.6)	4, 7, 9	4, 5, 7, 8, 9	4, 8
7	64.7, CH	4.75, dq (8.6,6.2)	6, 8	5, 6, 8	9
8	23.7, CH_3	1.29, d (6.2)	7	6, 7	6
9	57.6, CH_2	4.33 (s)	6	4, 5, 6	3, 7

even wondered if **3** was the enantiomer of **1**, although this was of course impossible since the HPLC column (ODS) used for separation was achiral. We therefore undertook to elucidate the absolute configurations of the secondary alcohols of **1** and **3** (Figures 1 and 2) using the modified Mosher's method.[7,8]

The primary alcohol of **1** was protected with a pivaloyl (2,2-dimethylpropanoyl) moiety, and the resulting monopivaloyl ester was converted to di-(*R*)- and di-(*S*)-MTPA esters (**2**). The $\Delta\delta$ values [$\delta_{(S)\text{-MTPA}} - \delta_{(R)\text{-MTPA}}$] were calculated, revealing 2(*R*)- and 7(*S*)-configurations (Figure 2). Similar experiments performed on **3** through di-MTPA esters (**4**) gave 2(*S*)- and 7(*S*)-configurations of **3**. Thus, it was established that **1** and **3** were in an epimeric relationship at C-2. To our knowledge, it is very rare for such distinct diastereomers to show practically the same ^1H and ^{13}C NMR spectra.

Aspinonediol (**5**), $[\alpha]^{24}_D$ +2.4 (*c* 0.63, MeOH), had a molecular formula of $C_9H_{14}O_3$ deduced from HRTOFMS [$(M + Na)^+$ *m*/*z* 193.0869, calc 193.0841]. The ^1H NMR (CD_3OD) spectrum of **5** was similar to those of **1** and **3**, although one of the signals due to the oxymethines was missing, the signals of the disubstituted olefin appeared as lower field AB doublets at δ 7.24 (*J* 16.2 Hz) and 6.45 (*J* 16.2 Hz), and another olefin proton resonated at δ 6.09 (d, *J* 8.5 Hz). The ^{13}C NMR signal at δ 201.5 suggested the presence of a carbonyl carbon, and the UV maximum at 269 nm showed the existence of an $\alpha,\beta,\gamma,\delta$-unsaturated enone system (λ_{max}^{calc} 263 nm). Other NMR properties, including the 2D NMR data (Table 4), gave the planar structure **5** of aspinonediol. The *S*-configuration at C-7 was deduced by comparing the ^1H chemical shifts of (*R*)- and (*S*)-MTPA esters (**6**) (Figure 2). This finding suggested that **5** was the precursor of aspinotriols A (**1**) and B (**3**), whose 2-hydroxy groups are formed by enantiomerically nonspecific reduction of the carbonyl of **5**.

The spectroscopic data of **7**, $[\alpha]^{24}_D$ −7.3 (*c* 0.38, MeOH), were identical to those reported for aspinonene[4] except for the reported optical rotation value, $[\alpha]^{24}_D$ −78 (*c* 0.3, MeOH). The absolute stereochemistry of aspinonene has already been established by X-ray analysis. The marked difference in the specific rotation values made us suspect that **7** might be enantiomerically impure. To confirm the purity, the following experiments were performed. The primary and tertiary OH groups of **7** were protected by treatment with 2,2-dimethoxypropane, and the resulting acetonide[4] was converted to (*R*)- and (*S*)-MTPA esters (**8**). Each of the MTPA esters exhibited only a single set of ^1H NMR signals, implying that **7** was enantiomerically pure. The $\Delta\delta$ values obtained for **8** (Figure 2) indicated a 2(*S*)-configuration of **7**, which is consistent with the reported absolute configuration.

Compound **9** was identified as dihydroaspyrone by comparing its spectroscopic data with those reported in the literature.[6] The relative and absolute stereochemistry of **9** has been presumed on the basis of CD spectroscopy and biosynthetic reasoning.[6] This compound has two secondary hydroxy groups, one on the ring (C-5) and another on the side chain (C-9). The hydroxy group at C-5 appears more hindered than the one at C-9 because the former is neighboring a secondary methyl group. This supposition proved correct: when **9** was treated with 1.2 equiv of (*S*)- and (*R*)-MTPA chlorides, only the 9-OH was esterified, giving (*R*)- and (*S*)-MTPA (**10**), respectively, which enabled us to assign the *S*-configuration at C-9 (Figure 2). On the other hand, the more reactive hydroxy group at C-9 of **9** was first protected by a *tert*-butyldimethylsilyl (TBDMS) group, and the C-5 hydroxy group was esterified with (*R*)- and (*S*)-MTPA chlorides. The $\Delta\delta$ values obtained from the MTPA esters (**11**) (Figure 2) indicated an *S*-configuration for C-5. Since the *trans* relationship of H-5 and H-6 is obvious from the NOESY correlation between H-5 and Me-7, and because $J_{H\text{-}5,H\text{-}6}$ = 7.9 Hz observed for **9**, the absolute configuration of dihydroaspyrone was confirmed, as shown in structure **9**.

The biosynthesis of aspinonene (**7**) and its analogues has been exhaustively studied by Zeeck et al.[5,6] They experimentally confirmed that **7** is a pentaketide produced by a polyketide synthesis (PKS) via rearrangement of **I** to **II** (Figure 3) and decarboxylation followed by dehydration to afford 2(*S*),3(*S*),6(*S*),7(*S*)-diepoxide (**12a**). The 7(*S*)-configurations of **1**, **3**, **5**, and **7** and the 9(*S*)-configuration of **9** reinforce the proposed biosynthetic precursor **12a**.

We tested **1**, **3**, **5**, **7**, and **9** for inhibitory activity against MRSA (methicillin-resistant *Staphylococcus aureus*), as well as **1**, **7**, and **9** for cytotoxic activity against L-1210. No anti-MRSA activity was found at a concentration of 100 μg/disk for the tested samples. Compounds **7** and **10** showed toxicity against mouse lymphocytic leukemia cells at 25 ppm (27% and 25%, respectively), although **1** was inactive.

Experimental Section

General Experimental Procedures. Optical rotation values were determined on a JASCO P-1010 polarimeter. High-resolution MS spectra were obtained with a Waters LCT-Premier 2695 mass spectrometer. 1D and 2D NMR were recorded on Bruker ARX-400, JEOL-JNM-AL400, and JEOL-GSX-400 spectrometers. Chemical shifts (δ) are expressed in parts per million (ppm) with reference to the solvent signals. [^1H NMR: CD_3OD (δ 3.35), $CDCl_3$ (δ 7.25), C_5D_5N (δ 7.55 for H-3), acetone-d_6 (δ 2.05). ^{13}C NMR: CD_3OD (δ 49.0), $CDCl_3$ (δ 77.0), C_5D_5N (δ 135.5 for C-3).] Multiplicities of ^{13}C signals were determined by DEPT and HSQC spectra. Flash column chromatography (FCC) was carried out on silica gel (40–63 μm, Merck Co.). Thin-layer chromatography was carried out on silica gel 60 F_{254} plates (Merck Co.). Cultivation of the titled fungus was performed in a Sanyo grows cabinet NLR-350H. HPLC was done using a Tosoh CCPS. Recycle-HPLC was done using a Japan Analytical Industry Co., Ltd. LC-908.

Fungal Isolation and Identification. The fungus, designated as strain 01F313, was isolated from an unidentified marine sponge collected in Pohnpei in 2001. Isolation and identification of the strain are described in the literature.[3] The fungus is maintained on 1/10 YSA medium at the Marine Natural Products Laboratory, Faculty of Pharmaceutical Sciences, the University of Tokushima, Japan.

Cultivation of Fungus and Isolation of Metabolites. The fungus was cultured in 67 500-mL Erlenmeyer flasks each containing 150 mL of a 1/2 PD medium using 90% BrSW [NaBr (52.78 g), KBr (1.27 g), $CaBr_2$ (2.76 g), and $MgSO_4$ (6.60 g) dissolved in distilled H_2O (1 L); acidity of the medium was adjusted by 1 M HBr and 1 M NaOH to pH 8.3] for five weeks at 20 °C. The broth was filtered, and the filtrate was passed through a column packed with HP-20 (205 g). After washing with 1 L of distilled H_2O, MeOH (6 L) was passed through the column

Table 3. ^{1}H NMR Data (100/400 MHz, C_5D_5N) of Aspinotriol A (**1**) and Aspinotriol B (**3**)

	1			**3**		
position	δ_C, mult.	δ_H (J in Hz)	COSY	δ_C, mult.	δ_H (J in Hz)	COSY
1	24.4, CH_3	1.42, d (6.3)	2	24.4, CH_3	1.43, d (6.3)	2
2	68.1, CH	4.63, dq (6.3, 5.6)	1, 3, 4	68.2, CH	4.64, dq (6.3, 5.6)	1, 3, 4
3	135.5, CH	6.47, dd (15.9, 5.6)	2, 4	135.6, CH	6.49, dd (15.9, 5.6)	2, 4
4	130.9, CH	6.57, d (15.9)	2, 3, 6	130.9, CH	6.58, d (15.9)	2, 3, 6
5	137.7, C			137.8, C		
6	138.9, CH	5.97, d (8.2)	4, 7	138.9, CH	5.99, d (8.4)	4, 7
7	63.7, CH	5.19, dq (8.2, 6.3)	6, 8	63.8, CH	5.19, dq (8.4,6.3)	6, 8
8	24.7, CH_3	1.45, d (6.3)	7	24.8, CH_3	1.46, d (6.3)	7
9	57.5, CH_2	4.73/4.68, d (12.1)		57.6, CH_2	4.74/4.69, d (12.2)	

and the MeOH solution was concentrated. The brown extract (2.04 g) was separated by FCC (205 g of silica gel) eluted with a $CHCl_3$−MeOH gradient (1:0 to 0:1) to give 10 fractions. A part (209.3 mg) of fraction 8 (263.2 mg) was separated by HPLC using an ODS column [Capselpak C18 type SG120 A 5 μm; 10 mm × 250 mm; flow rate 2.0 mL/min; MeOH−H_2O (25:75) containing 0.1% CH_3COOH] into 16 fractions. Fraction 8-7 (107.5 mg) was identified as aspinonene (**7**). Fractions 8-11 (12.5 mg) and 8-15 (11.1 mg) were new compounds, aspinotriols A (**1**) and B (**3**), respectively. Fraction 7 (187.7 mg) of the FCC was separated by recycle-HPLC using the above-mentioned ODS column [Capselpak C18 type SG120 A 5 μm; 20 mm × 250 mm; flow rate 6.0 mL/min; MeOH−H_2O (30:70) containing 0.1% CH_3COOH] into 10 fractions. Fraction 7-7 (24.1 mg) was identified as dihydroaspyrone (**9**). Aspinonediol (**5**) was obtained as fraction 7-8 (7.5 mg).

Aspinotriol A (1): colorless oil; $[\alpha]^{25}_D$ −10.1 (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ε) 231 (4.22) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HRTOFMS *m/z* 195.1012 $[M + Na]^+$ (calc for $C_9H_{16}O_3Na$ 195.0997).

Di-(*R*)- and (*S*)-MTPA Esters (2). Pivaloyl chloride (2.6 μL; 3.6 equiv) was added to a solution of **1** (1.0 mg) in dry pyridine (0.1 mL), and the mixture was allowed to stand at room temperature for 3 h, when the TLC spot for the starting material spot had disappeared. Then, without isolation of the monopivaloyl ester, (*S*)-MTPA chloride (11 μL) was added to the solution and the mixture was kept at room temperature for 1 day. MeOH (0.1 mL) was added to destroy the excess acid chlorides, and after 30 min, trimethylsilyldiazaomethane (100 μL of 10% *n*-hexane solution) was added to methylate acids that might have formed by hydrolysis of the chlorides from moisture in the solvent. Pyridine was removed *in vacuo*, and the product was subjected to preparative TLC with *n*-hexane−EtOAc (9:1) as the developing solvent. The band at R_f 0.23 was scraped off to give di-(*R*)-MTPA ester (**2**) (1.0 mg; 25% yield). The location of the pivaloyl and MTPA groups was confirmed by HMBC analysis of **2**. Esterification of **1** with (*R*)-MTPA chloride was performed in the same manner, giving di-(*S*)-MTPA ester (**2**) (R_f 0.15) (1.3 mg: 33%). Di-(*R*)-MTPA ester (**2**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53–7.31 (10H, m, ArH), 6.15 (1H, d, J = 15.9 Hz, H-4), 6.01 (1H, dq, J = 9.0, 6.4 Hz, H-7), 5.87 (1H, dd, J = 15.9, 6.8 Hz, H-3), 5.62 (1H, dq, J = 6.8, 6.4 Hz, H-2), 5.50 (1H, d, J = 9.0 Hz, H-6), 5.03 (1H, d, J = 12.5 Hz, H-9a), 4.71 (1H, d, J = 12.5 Hz, H-9b), 3.54 (3H, brs, −OMe), 3.51 (3H, brs, −OMe), 1.43 (3H, d, J = 6.4 Hz, H-8), 1.37 (3H, d, J = 6.4 Hz, H-1), 1.14 (9H, s, −Piv); HRTOFMS *m/z* 711.2352 $[M + Na]^+$ (calc for $C_{34}H_{38}O_8F_6Na$ 711.2369). Di-(*S*)-MTPA ester (**2**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53–7.31 (10H, m, ArH), 6.04 (1H, d, J = 15.5 Hz, H-4), 6.01 (1H, dq, J = 9.4, 6.2 Hz, H-7), 5.80 (1H, dd, J = 15.5, 6.5 Hz, H-3), 5.64 (1H, dq, J = 6.5, 6.4 Hz, H-2), 5.54 (1H, d, J = 9.4 Hz, H-6), 4.97 (1H, d, J = 12.4 Hz, H-9a), 4.69 (1H, d, J = 12.4 Hz, H-9b), 3.55 (3H, brs, −OMe), 3.51 (3H, brs, −OMe), 1.43 (3H, d, J = 6.4 Hz, H-1), 1.35 (3H, d, J = 6.2 Hz, H-8), 1.12 (9H, s, -Piv); HRTOFMS *m/z* 711.2386 $[M + Na]^+$ (calc for $C_{34}H_{38}O_8F_6Na$ 711.2369).

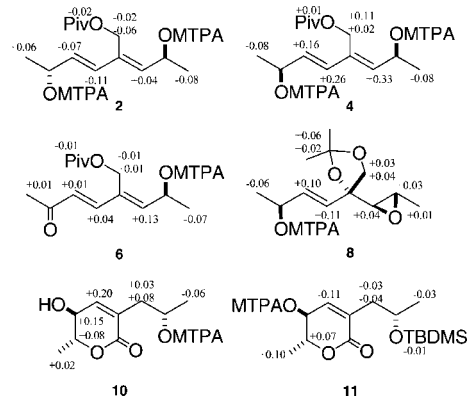

Figure 2. $\Delta\delta$ values obtained for the MTPA esters, **2**, **4**, **6**, **8**, **10**, and **11**. The ^{1}H NMR spectra were recorded for $CDCl_3$ solutions except for **4**, for which acetone-d_6 was used as a solvent.

Table 4. NMR Spectroscopic Data (100/400 MHz, CD_3OD) of Aspinonediol (**5**)

position	δ_C, mult.	δ_H (J in Hz)	COSY	HMBC	NOESY
1	27.3, CH_3	2.34, s		2, 3	
2	201.5, C				
3	128.4, CH	6.45, d (16.2)	4	2, 5	9
4	147.5, CH	7.24, d (16.2)	3	2, 6, 9	6
5	137.1, C				
6	148.2, CH	6.09, d (8.5)	7	4, 9	7
7	64.8, CH	4.81, dq (8.5, 6.4)	6, 8		6, 8, 9
8	23.6, CH_3	1.33, d (6.4)	7	6, 7	7
9	57.0, CH_2	4.38 (s)		4, 5, 6	3, 7

Di-(*R*)- and (*S*)-MTPA Esters (4). These esters were prepared as for **2** (see above). Pivaloyl chloride (1.3 μL; 3.5 equiv) was added to a solution of **3** (0.5 mg) in pyridine (50 μL), and after 2 h, (*S*)-MTPA chloride (4.3 μL; 3.5 equiv) was added. The mixture was allowed to stand at room temperature for 1 day, and *N*,*N*-dimethyl-1,3-propanediamine (3.5 μL) was added to convert the excess chloride to polar amides. After 10 min, the solvent was removed *in vacuo*, and the residue was separated by preparative TLC [*n*-hexane−EtOAc (9:1)], giving di-(*R*)-MTPA ester (**4**) (R_f 0.20) (0.8 mg; 40%). Esterification of **3** (0.5 mg) with (*R*)-MTPACl gave di-(*S*)-MTPA ester (**4**) (R_f 0.15) (0.5 mg; 25%). Acetone-d_6 was used as an NMR solvent to obtain well-separated signals. Di-(*R*)-MTPA ester (**4**): ^{1}H NMR (400 MHz, acetone-d_6) δ

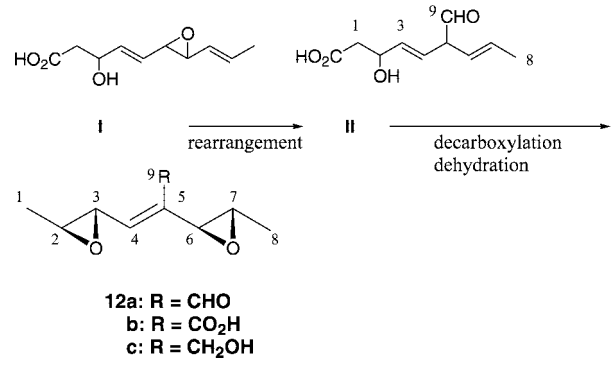

Figure 3. Biosynthetic pathway (Zeeck et al.) leading to **12a**−**c**, which are the precursors of **7** and aspyrone as well as **1**, **3**, **5**, and dihydroaspyrone (**9**). As for **I** and **II**, see text.

7.56–7.41 (10H, m, ArH), 6.16 (1H, d, J = 16.0 Hz, H-4), 6.09 (1H, dq, J = 9.5, 6.6 Hz, H-7), 5.91 (1H, dd, J = 16.0, 6.6 Hz, H-3), 5.69 (1H, dq, J = 6.6, 6.5 Hz, H-2), 5.54 (1H, d, J = 9.5 Hz, H-6), 5.04 (1H, d, J = 12.2 Hz, H-9a), 4.74 (1H, d, J = 12.2 Hz, H-9b), 3.59 (3H, brs, −OMe), 3.57 (3H, brs, −OMe), 1.47 (3H, d, J = 6.6 Hz, H-8), 1.47 (3H, d, J = 6.5 Hz, H-1), 1.13 (9H, s, Piv); HRTOFMS m/z 711.2424 $[M + Na]^+$ (calc for $C_{34}H_{38}O_8F_6Na$ 711.2369). Di-(*S*)-MTPA ester (**4**): ^{1}H NMR (400 MHz, acetone-d_6) δ 7.57–7.41 (10H, m, ArH), 6.42 (1H, d, J = 16.1 Hz, H-4), 6.14 (1H, dq, J = 9.8, 6.6 Hz, H-7), 6.07 (1H, dd, J = 16.1, 7.1 Hz, H-3), 5.87 (1H, d, J = 9.8 Hz, H-6), 5.70 (1H, dq, J = 7.1, 6.6 Hz, H-2), 5.06 (1H, d, J = 12.4 Hz, H-9a), 4.85 (1H, d, J = 12.4 Hz, H-9b), 3.54 (3H, brs, −OMe), 3.52 (3H, brs, −OMe), 1.39 (3H, d, J = 6.6 Hz, H-8), 1.39 (3H, d, J = 6.6 Hz, H-1), 1.14 (9H, s, Piv); HRTOFMS m/z 711.2318 $[M + Na]^+$ (calc for $C_{34}H_{38}O_8F_6Na$ 711.2369).

Aspinonediol (5): yellow oil; $[\alpha]^{24}_D$ +2.4 (*c* 0.63, MeOH); UV (MeOH) λ_{max} (log ε) 269 (4.43) nm; ^{1}H and ^{13}C NMR data, see Table 4; HRTOFMS m/z 193.0869 $[M + Na]^+$ (calc for $C_9H_{14}O_3Na$ 193.0841).

(*R*)- and (*S*)-MTPA Esters (6). These esters were prepared as for **2** (see above). Pivaloyl chloride (3.4 μL; 5.8 equiv) was added to a solution of **5** (0.8 mg) in pyridine (80 μL), and after 16 h, (*S*)-MTPA chloride (8.8 μL; 10 equiv) was added. The mixture was allowed to stand at room temperature for 10 h, and *N*,*N*-dimethyl-1,3-propanediamine (7.1 μL) was added to convert the excess chloride to polar amides. After 10 min, the solvent was removed *in vacuo*, and the residue was separated by preparative TLC [*n*-hexane−EtOAc (85:15)], giving (*R*)-MTPA ester (**6**) (R_f 0.17) (0.7 mg; 32%). Esterification of **5** (0.8 mg) with (*R*)-MTPACl gave (*S*)-MTPA ester (**6**) (R_f 0.17) (0.8 mg; 36%). (*R*)-MTPA ester (**6**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60–7.34 (5H, m, ArH), 6.99 (1H, d, J = 16.0 Hz, H-4), 6.36 (1H, d, J = 16.0 Hz, H-3), 6.04 (1H, dq, J = 9.3, 6.4 Hz, H-7), 5.87 (1H, d, J = 9.3 Hz, H-6), 5.08 (1H, d, J = 12.6 Hz, H-9a), 4.75 (1H, d, J = 12.6 Hz, H-9b), 3.53 (3H, brs, −OMe), 2.28 (3H, s, H-1), 1.47 (3H, d, J = 6.4 Hz, H-8), 1.17 (9H, s, −Piv); HRTOFMS m/z 493.1801 $[M + Na]^+$ (calc for $C_{24}H_{29}O_6F_3Na$ 493.1814). (*S*)-MTPA ester (**6**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60–7.34 (5H, m, ArH), 7.03 (1H, d, J = 16.2 Hz, H-4), 6.37 (1H, d, J = 16.2 Hz, H-3), 6.07 (1H, dq, J = 9.5, 6.1 Hz, H-7), 6.00 (1H, d, J = 9.5 Hz, H-6), 5.07 (1H, d, J = 12.5 Hz, H-9a), 4.76 (1H, d, J = 12.5 Hz, H-9b), 3.50 (3H, brs, −OMe), 2.29 (3H, s, H-1), 1.40 (3H, d, J = 6.1 Hz, H-8), 1.16 (9H, s, −Piv); HRTOFMS m/z 493.1835 $[M + Na]^+$ (calc for $C_{24}H_{29}O_6F_3Na$ 493.1814).

Aspinonene (7): colorless oil; $[\alpha]^{24}_D$ −7.3 (*c* 0.38, MeOH). All of the spectroscopic data except for optical rotation value were identical with those reported in the literature.[4]

(*R*)- and (*S*)-MTPA Esters (8). A solution of **7** (15 mg) and *p*-toluenesulfonic acid (1 mg) in 2,2-dimethoxypropane (2 mL) was stirred for 30 min at room temperature, and the solvent was removed by evaporation. The oily residue was separated by preparative TLC developing with $CHCl_3$−MeOH (14:1). The band at R_f 0.49 gave an acetonide (7.2 mg; 83%) that showed a ^{1}H NMR spectrum identical with that reported.[4] A solution of the acetonide (3.7 mg), (*R*)-MTPA acid (7.6 mg), 2-methyl-6-nitrobenzoic anhydride (13.4 mg), and 4-(dimethylamino)pyridine (8.7 mg) in CH_2Cl_2 (1 mL) was stirred at room temperature for 1 day.[9] The mixture was shaken with 10% aqueous citiric acid solution three times, washed with an aqueous $NaHCO_3$ solution and brine, and dried over Na_2SO_4. The crude reaction product (10.0 mg) was separated by preparative TLC developed with benzene−EtOAc (20:1). The band at R_f 0.38 gave (*R*)-MTPA ester (**8**; 4.1 mg) (57%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54–7.35 (5H, m, ArH), 5.83 (1H, dd, J = 15.6, 6.1 Hz, H-3), 5.75 (1H, d, J = 15.6 Hz, H-4), 5.63 (1H, dq, J = 6.6, 6.1 Hz, H-2), 3.92 (1H, d, J = 8.8 Hz, H-9a), 3.66 (1H, d, J = 8.8 Hz, H-9b), 3.00 (1H, dq, J = 5.1, 2.1 Hz, H-7), 2.62 (1H, d, J = 2.1 Hz, H-6), 1.43 (3H, d, J = 6.6 Hz, H-1), 1.38 (3H, s, J = 6.6 Hz, $-OC(CH_3)_2O-$), 1.30 (3H, s, $-OC(CH_3)_2O-$), 1.30 (3H, d, J = 5.1 Hz, H-8). (*S*)-MTPA ester (**8**) was obtained in the same manner in 57% yield. (*S*)-MTPA ester (**8**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54–7.35 (5H, m, ArH), 5.93 (1H, dd, J = 15.8, 6.5 Hz, H-3), 5.86 (1H, d, J = 15.8 Hz, H-4), 5.63 (1H, dq, J = 6.6, 6.5 Hz, H-2), 3.95 (1H, d, J = 8.9 Hz, H-9a), 3.70 (1H, d, J = 8.9 Hz, H-9b), 3.03 (1H, dq, J = 5.2, 2.1 Hz, H-7), 2.66 (1H, d, J = 2.1 Hz, H-6), 1.40 (3H, s, $-OC(CH_3)_2O-$), 1.37 (3H, d, J = 6.5 Hz, H-1), 1.36 (3H, s, $-OC(CH_3)_2O-$), 1.30 (3H, d, J = 5.2 Hz, H-8).

Dihydroaspyrone (9): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.62 (1H, brs, H-4), 4.38 (1H, dq, J = 7.9, 6.4 Hz, H-6), 4.20 (1H, dd, J = 7.9, 7.1 Hz, H-5), 4.01 (1H, m, H-9), 2.68 (1H, brd, J = 7.1 Hz, 5-OH), 2.47 (1H, dd, J = 14.3, 4.0 Hz, H-8a), 2.40 (1H, dd, J = 14.3, 8.0 Hz, H-8b), 2.28 (1H, brs, 9-OH), 1.45 (3H, d, J = 6.4 Hz, H-7), 1.22 (3H, d, J = 6.2 Hz, H-10); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.2 (C, C-2), 144.3 (CH, C-4), 129.1 (C, C-3), 79.4 (CH, C-6), 67.6 (CH, C-5), 66.9 (CH, C-9), 39.7 (CH_2, C-8), 23.4 (CH_3, C-10), 18.2 (CH_3, C-7). These NMR data agree well with those reported previously.[6]

(*R*)- and (*S*)-MTPA Esters (10). A solution of **9** (2.4 mg) and (*S*)-MTPA chloride (2.9 μL) in pyridine (0.1 mL) was kept at room temperature for 1 day. The solvent was removed *in vacuo*, and the residue was directly applied to preparative TLC developed with $CHCl_3$−MeOH (1:50) to give (*R*)-MTPA ester (**10**; R_f 0.23) (1.1 mg: 21%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56–7.36 (5H, m, ArH), 6.25 (1H, brs, H-4), 5.33 (1H, m, H-9), 4.15 (1H, dq, J = 8.8, 6.2 Hz, H-6), 3.86 (1H, brdd, J = 8.8, 7.0 Hz, H-5), 3.55 (3H, brs, OMe), 2.59 (1H, dd, J = 14.8, 3.9 Hz, H-9a), 2.48 (1H, dd, J = 14.8, 9.3 Hz, H-9b), 1.66 (1H, d, J = 7.0 Hz, 5-OH), 1.40 (6H, d, J = 6.2 Hz, H-7, H-10). Esterification of **9** (1.8 mg) with (*R*)-MTPA chloride (2.2 μL) was performed in the same manner, affording (*S*)-MTPA ester (**10**) (2.0 mg; 36%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51–7.37 (5H, m, ArH), 6.45 (1H, brs, H-4), 5.36 (1H, m, H-9), 4.23 (1H, dq, J = 8.8, 6.5 Hz, H-6), 4.01 (1H, brdd, J = 8.8, 7.3 Hz, H-5), 3.47 (3H, brs, −OMe), 2.62 (1H, dd, J = 14.0, 4.3 Hz, H-8a), 2.56 (1H, dd, J = 14.0, 7.8 Hz, H-8b), 1.82 (1H, d, J = 7.3 Hz, 5-OH), 1.42 (3H, d, J = 6.5 Hz, H-7), 1.34 (3H, d, J = 6.3 Hz, H-10).

(*R*)- and (*S*)-MTPA Esters (11). A solution of **9** (5.8 mg), TBDMS chloride (5.6 mg), and imidazole (6.4 mg) in DMF (0.5 mL) was stirred at 0 °C for 15 min and then at room temperature for 4 h. The mixture was diluted with diethyl ether and H_2O. The organic layer was separated. The aqueous layer was again extracted with diethyl ether three times, and the diethyl ether extract was combined with the first extract. Evaporation of the solvent gave the TBDMS ether (5.6 mg): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.55 (1H, brs, H-4), 4.26 (1H, m, H-6), 4.21 (1H, m, H-5), 4.06 (1H, m, H-9), 2.39 (1H, dd, J = 13.5, 4.4 Hz, H-8a), 2.31 (1H, dd, J = 13.5, 7.0 Hz, H-8b), 1.47 (3H, d, J = 6.4 Hz, H-7), 1.15 (3H, d, J = 6.4 Hz, H-10), 0.86 (9H, s, *t*-Bu), 0.03 (3H, s, SiMe), 0.02 (3H, s, SiMe). Equal sized portions of the TBDMS ether were esterified with (*S*)- and (*R*)-MTPA chlorides in pyridine to afford (*R*)-(1.6 mg: 33% yield) and (*S*)-MTPA (0.7 mg: 15% yield) esters (**11**), respectively. (*R*)-MTPA ester (**11**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54–7.37 (5H, m, ArH), 6.54 (1H, d, J = 2.8 Hz, H-4), 5.45 (1H, dd, J = 8.1, 2.8 Hz, H-5), 4.49 (1H, dq, J = 8.1, 6.6 Hz, H-6), 4.05 (1H, m, H-9), 3.53 (3H, brs, OMe), 2.46 (1H, dd, J = 13.3, 4.2 Hz, H-8a), 2.31 (1H, dd, J = 13.3, 7.8 Hz, H-8b), 1.32 (3H, d, J = 6.6 Hz, H-7), 1.14 (3H, d, J = 6.1 Hz, H-10), 0.86 (9H, s, *t*-Bu), 0.03 (3H, s, SiMe), 0.00 (3H, s, SiMe). (*S*)-MTPA ester (**11**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54–7.33 (5H, m, ArH), 6.43 (1H, d, J = 2.9 Hz, H-4), 5.45 (1H, dd, J = 7.8, 2.9 Hz, H-5), 4.56 (1H, dq, J = 7.8, 6.6 Hz, H-6), 4.02 (1H, m, H-9), 3.53 (3H, brs, OMe), 2.43 (1H, dd, J = 13.4, 4.2 Hz, H-8a), 2.27 (1H, dd, J = 13.4, 8.0 Hz, H-8b), 1.42 (3H, d, J = 6.6 Hz, H-7), 1.11 (3H, d, J = 6.0 Hz, H-10), 0.86 (9H, s, *t*-Bu), 0.02 (3H, s, SiMe), 0.01 (3H, s, SiMe).

Supporting Information Available: Copies of ^{1}H and ^{13}C 1D and 2D NMR spectra of compounds **1**, **3**, and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Bhadury, P.; Mohammad, B. T.; Phillip, C. *J. Ind. Microbiol. Biothechnol.* **2006**, *33*, 325–337.
(2) Jensen, P. R.; Fenical, W. In *Drugs From the Sea*; Fusetani, N., Ed.; Karger: Basel, 2000; pp 6–29.
(3) Namikoshi, M.; Negishi, R.; Nagai, H.; Dmitrenok, A.; Kobayashi, H. *J. Antibiot.* **2003**, *56*, 755–761.
(4) Fuchser, J.; Grabley, S.; Noltemeyer, M.; Phillips, R.; Tiericke, R.; Zeeck, A. *Liebigs Ann. Chem.* **1994**, 831–835.
(5) Fuchser, J.; Tiericke, R.; Zeeck, A. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1663–1666.
(6) Fuchser, J.; Zeeck, A. *Liebigs Ann./Recl.* **1997**, 87–95.
(7) Kusumi, T.; Ooi, T.; Ohkubo, Y.; Yabuuchi, T. *Bull. Chem. Soc. Jpn.* **2006**, *79*, 1–16.
(8) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
(9) Shiina, I.; Kubota, M.; Ibuka, R. *Tetrahedron Lett.* **2002**, *43*, 7535–7539.

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