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New Cytotoxic 14-Membered Macrolides from Marine-Derived Fungus *Aspergillus ostianus*

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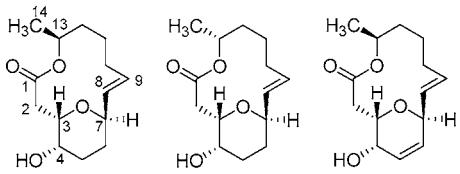
ABSTRACT



aspergillide A (**1**) aspergillide B (**5**) aspergillide C (**7**)

Three new 14-membered macrolides, named aspergillides A, B, and C (1, 5, and 7), were isolated from marine-derived fungus *Aspergillus ostianus* strain 01F313, cultured in a medium composed of bromine-modified artificial seawater. The structures of the new compounds were determined by analyses of 1D and 2D NMR spectra. Their absolute configurations were elucidated by the modified Mosher's method and chemical conversions. The new compounds showed cytotoxic activity against mouse lymphocytic leukemia cells (L1210).

Marine microorganisms are recognized as important sources of pharmacologically active metabolites.[1] In particular, a growing number of marine-derived fungi have been reported to produce novel bioactive secondary metabolites. Previously, we reported three antibacterial chlorine-containing components of *Aspergillus ostianus* strain 01F313 isolated from an unidentified marine sponge collected at Pohnpei, the Federated States of Micronesia.[2] The chlorine must have originated from cultivation medium composed of natural seawater. Expecting that bromine-containing compounds might be obtained when a medium in which bromide solution replaced seawater, we cultivated the same strain in a bromine-modified 1/2PD medium. Although we were unable to isolate the brominated compounds, we found that the metabolites were considerably different from those obtained from the strain cultured in seawater medium and succeeded in isolating three new macrolides: aspergillides A, B, and C (**1**, **5**, and **7**). This paper elucidates the absolute structures of the new compounds and describes their bioactivities.

Aspergillus ostianus strain 01F313 was cultured in a 1/2PD (potato-dextrose) medium containing bromine-modified artificial seawater (for the composition of the medium, see the experimental details). 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 FCC and HPLC to give compounds **1**, **5**, and **7**, aspergillides A, B, and C, respectively (Figure 1).

Aspergillide A (**1**), $[\alpha]_D^{27}$ −59.5 (*c* 0.45, $CHCl_3$), has a molecular formula of $C_{14}H_{22}O_4$, deduced from HRTOFMS [$(M+H)^+$ *m*/*z* 255.1633, calcd 255.1596; $(M + Na)^+$ *m*/*z* 277.1417, calcd 277.1416]. The IR absorptions at 3421 and 1729 cm^{-1} indicated the presence of a hydroxyl group and an ester group, respectively. Aspergillide A's ^1H NMR spectrum (400 MHz, $CDCl_3$) (Table 1) showed the presence of a disubstituted *E*-olefin [δ 5.79 (ddd, *J* 15.4, 8.6, 2.0 Hz),

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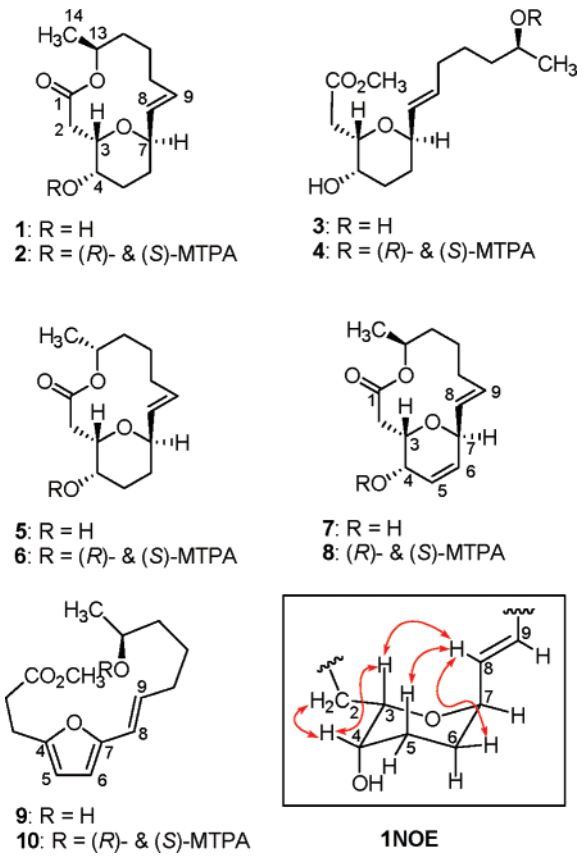

Figure 1. Structures of three new compounds, aspergillides A (**1**), B (**5**), and C (**7**), and their derivatives. NOEs essential for determining the relative stereochemistry of **1** are depicted in **1NOE**.

5.69 (ddd, *J* 15.4, 9.3, 3.2 Hz)], four oxymethines [δ 4.94 (ddq, *J* 6.6, 6.6, 2.2 Hz), 4.23 (m), 4.21 (m), 3.58 (m)], a methylene group adjacent to a carbonyl group [δ 2.63 (dd, *J* 15.4, 13.0 Hz), 2.39 (dd, *J* 15.4, 4.4 Hz)], and a secondary methyl group [δ 1.19 (d, *J* 6.6 Hz)]. The ^{13}C (100 MHz, $CDCl_3$) NMR spectrum (Table 1) confirmed the presence of an ester carbon (δ 170.1), two olefin carbons (δ 137.0, 132.1), four oxymethine carbons (δ 74.0, 71.5, 71.3, 66.7), and six methylene carbons (δ 40.5, 32.1, 31.0, 23.6, 21.9, 21.7). The signals in the ^{1}H NMR spectrum are well separated and the proton network from H_3-14 to H-2 of **1** was easily established by the COSY spectrum. Especially noteworthy is the fact that, in the HOHAHA experiment with a long mixing time (120 ms), a relayed cross-peak was observed between H_3-14 and H_2-2 signals, which suggests that aspergillide A has a linear acetogenin (a heptaketide = 14 carbons) framework.

The HMBC spectrum of **1** (Table 1) showed a cross-peak from H-13 (δ 4.94) to C-1 (δ 170.1) revealing the ester linkage between C-1 to C-13. Other HMBC correlation peaks between H-3 (δ 4.23)/C-7 (δ 71.3) and H-7 (δ 4.21)/C-3 (δ 74.0) indicate the presence of an ether linkage between C-3 and C-7 forming a tetrahydropyran ring. The residual oxygen must be due to a hydroxyl group. These findings allowed us to establish the planar structure of **1**.

Table 1. NMR Data ($CDCl_3$, 400/100 MHz) of Asperdillide A (**1**)[a]

	δ_C, mult	δ_H (*J* in Hz)	COSY correlation with	HMBC correlation to
1	170.1, C			
2	40.5, CH_2	2.63, dd (15.4, 13.0)	3	1, 3, 4
		2.39, dd (15.4, 4.4)	3	1, 3, 4
3	74.0, CH	4.23, m	2, 4	2, 4, 5, 7
4	66.7, CH	3.58, m	3, 5	6
5	21.7, CH_2	1.95, br.d (14.1)	4, 6	6, 7
		1.71, br.d (14.1)	4, 6	3, 4, 6, 7
6	21.9, CH_2	2.21, m	5, 7	4, 5, 7, 8
		1.38, m	5, 7	4, 5, 7, 8
7	71.3, CH	4.21, m	5, 6, 8	3, 8, 9
8	132.1, CH	5.79, ddd (15.4, 8.6, 2.0)	7, 9, 10	6, 7, 9, 10
9	137.0, CH	5.69, ddd (15.4, 9.3, 3.2)	8, 10	8, 7, 10, 11
10	31.0, CH_2	2.28, m	8, 9, 11	9, 11, 12
		2.10, m	9, 11	8, 9, 11, 12
11	23.6, CH_2	1.81, m	10, 12	10, 12, 13
		1.50, m	10, 12	9, 10, 12, 13
12	32.1, CH_2	1.88, m	11, 13	13
		1.49, m	11, 13	10, 11, 13
13	71.5, CH	4.94, ddq (6.6, 6.6, 2.2)	12, 14	1, 11, 12, 14
14	18.5, CH_3	1.19, d (6.6)	13	12, 13

[a] Carbon multiplicities were based on a DEPT experiment.

The relative stereochemistry of the tetrahydropyran ring was deduced mainly on the basis of the NOE correlations observed in the NOESY spectrum (**1NOE** of Figure 1): One of the olefin signals, H-8, shows the NOE cross-peaks to H-3, H-5β (δ 1.95), and H-6β (δ 1.38), which indicates that the tetrahydropyran ring has a chairlike conformation and that the olefin group (C_8=C_9) as well as H-3 and H-5β are in axial orientations. The NOE observed between H-3 and H-4 suggests the axial configuration of the 4-OH group. The small coupling constant ($J \approx 0$ Hz) between these two protons supports the axial/equatorial-like relation of the protons. The appearance of NOE between H-4 and one (δ 2.39) of H_2-2 reinforces the equatorial nature of C-2.

The most difficult part of elucidating the structure was assigning the configuration of H_3-14 relative to those of the tetrahydropyran substituents, since the secondary methyl is remote from the tetrahydropyran ring. We therefore intended to determine the absolute configurations of all the stereogenic centers of **1**.

Aspergillide A (**1**) was esterified with (*S*)- and (*R*)-MTPA chlorides to give the (*R*)- and (*S*)-MTPA esters (**2**), respectively. Application of the modified Mosher's method (**2** in Figure 2) determined the *S* configuration at C-4 of **1**.[3,4] Treatment of **1** with sodium methoxide in methanol afforded the methyl ester (**3**). Of the two secondary hydroxyl groups of the ring-cleaved product, 4-OH takes a sterically hindered axial position in the tetrahydropyran ring while 13-OH is

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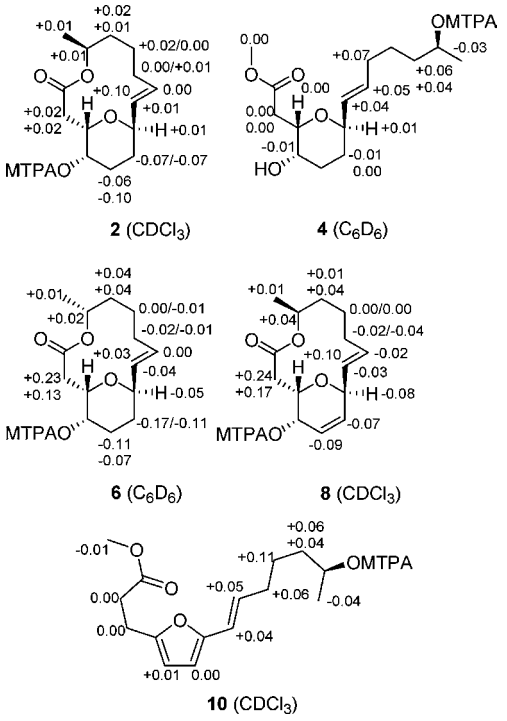

Figure 2. $\Delta\delta$ values [$\delta_{(S)-\mathrm{MTPA}} - \delta_{(R)-\mathrm{MTPA}}$] obtained for the MTPA esters **2**, **4**, **6**, **8**, and **10**. The solvents for the ^{1}H NMR spectra are shown in parentheses.

situated in a sterically less hindered methyl carbinol side-chain. Actually, treatment of **3** with (*S*)- (2.3 equiv) and (*R*)-MTPA (1 equiv) chlorides afforded mono-(*R*)- and mono-(*S*)-MTPA esters (**4**), respectively. The (*S*)-configuration at C-13 was deduced from the $\Delta\delta$ values depicted in **4** in Figure 2. Thus, the whole absolute stereochemistry of aspergillide A was established as shown in structure **1**.

Aspergillide B (**5**), $[\alpha]^{31}_{D}$ −97.2 (*c* 0.27, MeOH), has a molecular formula of $C_{14}H_{22}O_4$ deduced from HRTOFMS [$(M + Na)^+$ *m*/*z* 277.1439, calcd 277.1416]. The IR absorption at 3440 and 1727 cm^{-1} indicated the presence of a hydroxyl group and an ester group. Analyses of the ^{1}H and ^{13}C NMR as well as the 2D NMR spectra suggested that this compound was a diastereomer of aspergillide A (**1**). The *E*-configuration of C-8/C-9 olefin was confirmed by the *J* value (15.8 Hz) of the H-8/H-9 coupling, and the relative configuration of the tetrahydropyran ring was proved to be identical to that of **1** on the basis of the coupling constants determined by the decoupling difference spectrum (400 MHz, C_6D_6) and the NOEs (**5NOE** in Figure 3) observed for the protons on the tetrahydropyran ring. Furthermore, the (*S*)-configuration at C-4 was confirmed from the $\Delta\delta$ values (Figure 2) of the MTPA derivatives (**6**). These findings automatically established the (*R*)-configuration at C-13 of **5**.

The molecular formula of aspergillide C (**7**), $[\alpha]^{25}_{D}$ +66.2 (*c* 0.19, MeOH), ($C_{14}H_{20}O_4$; HRTOFMS [$(M + Na)^+$ *m*/*z* 275.1260, calcd 275.1259]), and the IR absorption at 3278 cm^{-1} (OH) and 1720 cm^{-1} (ester) suggested that it had a structure, in which one more olefin was incorporated in either **1** or **5**. Indeed, the ^{1}H NMR spectrum of **7** shows the *Z*-olefin

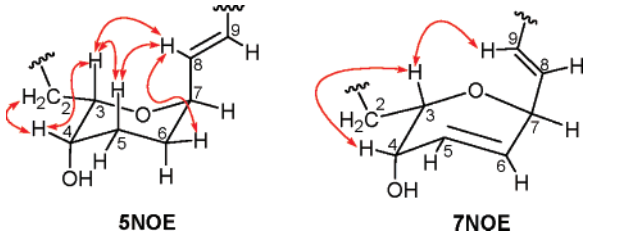

Figure 3. Essential NOEs for determinating the relative stereochemistry of **5** and **7**.

signals at δ 5.46 (br dd, *J* 10.4, 3.6 Hz) and 5.78 (dddd, *J* 10.4, 5.6, 2.4, 2.0 Hz) in addition to the *E*-olefin signals at δ 5.22 (br dd, *J* 15.6, 4.0 Hz)] and 5.98 (dddd, 15.6, 9.6, 6.0, 1.6) that have been observed for H-8/H-9 in the spectra of **1** and **5**. The location of the *Z*-olefin at C-5/C-6 was easily deduced from the COSY spectrum showing the correlations of H-5 (δ 5.78) and H-6 (δ 5.46) with H-4 (δ 3.27) and H-7 (δ 4.50), respectively. The planar structure and relative stereochemistry of the dihydropyran ring were deduced essentially in the same manner as described for **1** by the HMBC [H/C correlations: H-13/C-1; H-3/C-7 (Table 2)] and

Table 2. NMR Data (C_6D_6, 400/100 MHz) of Asperdillide C (**7**)[a]

	δ_C, mult	δ_H (*J* in Hz)	COSY	HMBC
1	170.0 C			
2	38.8, CH_2	2.91 (br dd, 14.0, 11.2)	2, 3	1, 3, 4
		2.28 (br dd, 14.0, 2.0)	2, 3	1, 3, 4
3	69.8, CH	4.08 (ddd, 11.2, 2.0, 1.6)	2, 4	1, 2, 4, 7
4	64.6, CH	3.27 (ddd, 11.2, 5.6, 1.6)	3, 5, 4-OH	5, 6
5	128.0, CH	5.78 (dddd, 10.4, 5.6, 2.4, 2.0)	4, 6	4, 7
6	131.8, CH	5.46 (br dd, 10.4, 3.6)	5, 7	4, 7
7	72.0, CH	4.50 (dddd, 4.0, 3.6, 2.0, 1.6)	5, 6, 8, 9	
8	126.2, CH	5.22 (br dd, 15.6, 4.0)	7, 9	7, 10
9	135.0, CH	5.98 (dddd, 15.6, 9.6, 6.0, 1.6)	8, 10	7, 10, 11
10	31.0, CH_2	2.00 (dddd, 12.8, 9.6, 6.0, 2.4)	9, 10, 11	8, 9, 11, 12
		1.65 (dddd, 12.8, 6.0, 2.4, 1.9)	9, 10, 11	8, 9, 11, 12
11	23.7, CH_2	1.46 (m)	10, 11 12	9, 10, 12, 13
		1.21 (m)	10, 11, 12	9, 10, 12, 13
12	32.1, CH_2	1.60 (m)	11, 12, 13	10, 11, 13, 14
		1.32 (m)	11, 12, 13	10, 11, 13, 14
13	69.5, CH	5.18 (m)	12, 14	1, 11
14	18.7, CH_3	1.03 (d, 6.8)	13	11, 12
4-OH		1.36	4	4

[a] Carbon multiplicities were based on a DEPT experiment.

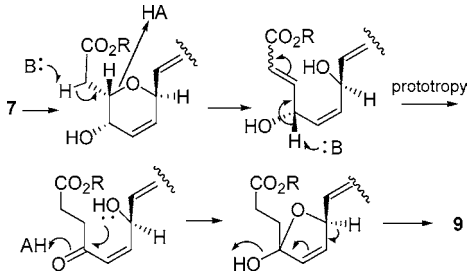

Figure 4. Probable reaction course to produce **9** from **7**.

NOESY [NOEs between H/H: H-9/H-3, H-3/H-4 (**7NOE** in Figure 3)] spectra together with the decoupling difference spectra (Table 2).

The absolute configuration at C-4 of **7** was determined to be *S* by the modified Mosher's method (**8** of Figure 2). In an attempt to obtain a ring-cleaved product, such as **3**, by treating **7** with sodium methoxide in methanol, an unexpected compound (**9**) was produced in 81% yield after acidification of the reaction mixture. The vinylfuran moiety of **9** was characterized by the NMR properties [furan protons at δ 5.90 (H-5, br.d, *J* 3.2 Hz) and 5.98 (H-6, d, *J* 3.2 Hz) and furan carbons at δ 159.6 (C-4), 107.7 (C-5), 111.9 (C-6), and 152.9 (C-7): olefin protons at δ 6.26 (H-9, dt, *J* 16.0, 6.8 Hz) and 6.15 (H-8, br.d, *J* 16.0 Hz) and olefin carbons at δ 126.9 (C-8) and 128.5 (C-9)]. A probable reaction course to produce **9** from **7** is shown in Figure 4. The (*S*)-configuration at C-13 of **9** was elucidated by the modified Mosher's method (**10** in Figure 2), establishing the absolute stereochemistry of aspergillide C (**7**).

Several 14-membered macrolides, such as cineromycins,[5] albocyclines,[6] and pikromycins,[7] have been reported. The present aspergillides A−C (**1**, **5**, and **7**) are the first examples of 14-membered macrolides that possess a tetrahydropyran ring.

Inhibitory activity of **1** and **7** against MRSA and the cytotoxicity of **1**, **5**, and **7** were examined. No anti-MRSA activity was found at a concentration of 100 μg/disk for **1** or **7**. Compounds **1**, **5**, and **7** showed toxicity against mouse lymphocytic leukemia cells (L1210) with LD_{50} values of 2.1, 71.0, and 2.0 μg/mL, respectively ($n = 3$).

Supporting Information Available: Experimental procedures and 1D and 2D NMR spectra of compounds **1**, **5**, **7**, and **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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