Multiplolides A and B, New Antifungal 10-Membered Lactones from *Xylaria multiplex*

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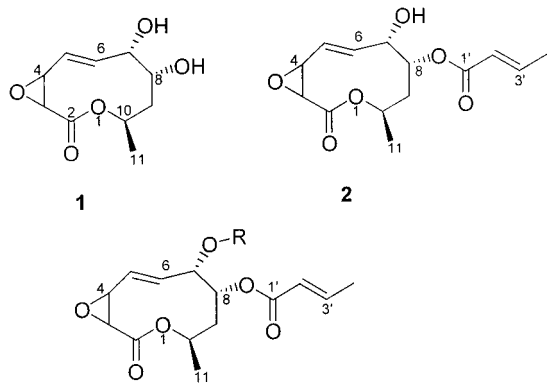
Two new 10-membered lactones, namely, multiplolides A (**1**) and B (**2**), were isolated from the broth extract of the fungus *Xylaria multiplex* BCC 1111. Chemical structures of **1** and **2** were elucidated on the basis of their spectral data. Multiplolides A (**1**) and B (**2**) exhibited antifungal activity against *Candida albicans* with IC_{50} values of 7 and 2 μg/mL, respectively. Both **1** and **2** were inactive in the screening systems toward the malarial parasite *Plasmodium falciparum* (at 20 μg/mL) and were not cytotoxic to BC-1 and KB cell lines (at 20 μg/mL).

As part of an ongoing research program on biologically active substances from Thai bioresources, we have evaluated several biological activities of extracts from plants and microorganisms. Fungi of the genus *Xylaria* are of particular interest, since crude extracts regularly show biological activity, particularly antimycobacterial, antimalarial, and antifungal. We report herein the isolation of two new antifungal 10-membered ring lactones, namely, multiplolides A (**1**) and B (**2**), from the broth extract of *Xylaria multiplex* BCC 1111.

The crude EtOAc extract of the culture broth of *X. multiplex* BCC 1111 was purified sequentially by Si gel and Sephadex LH-20 column chromatography, to yield multiplolides A (**1**) and B (**2**). The ESI-TOF mass spectrum of multiplolide A (**1**) gave an accurate mass of *m/z* 215.0923 [$(M + H)^+$, Δ +0.5 mmu], establishing the molecular formula of **1** as $C_{10}H_{14}O_5$. The IR spectrum of multiplolide A (**1**) exhibited an absorption peak at 1721 cm^{-1}, characteristic of an ester carbonyl. ^1H NMR ($CDCl_3$) of multiplolide A (**1**) showed a methyl doublet at δ 1.30, a nonequivalent methylene (at δ 1.21 and 2.21), five oxy protons (at δ 3.60, 3.75, 3.95, 4.50, and 5.25), and two olefinic protons (at δ 5.72 and 5.88). The $J_{H-5,H-6}$ value of 15.0 Hz revealed a *trans*-configuration of the olefinic protons in **1**. ^{13}C NMR ($CDCl_3$) of multiplolide A (**1**) showed 10 signals, attributable to one methyl, one methylene, seven methine, and one quaternary carbon, as determined by DEPT experiments. The $^1H-^1H$ COSY spectrum of multiplolide A (**1**) conclusively demonstrated the connectivity from H-3 to H-11. The epoxide moiety at carbons 3 and 4 in **1** was evident from the HMBC spectrum, in which the $^{13}C-^1H$ one-bond coupling constant ($^1J_{C-H}$) of 167 Hz (for C-3 and C-4) was observed. The HMBC spectrum of **1** also showed the correlation of both H-3 and H-10 to the carbonyl carbon (C-2), H-4 to C-6, H-5 to C-7, and H-9 to both C-7 and C-11. The NOESY spectrum of **1** revealed correlations of the methyl group to H-9_{ax}, and H-9_{ax} to both H-8 and H-7, suggesting that the methyl, H-9_{ax}, H-7, and H-8 were coplanar. Owing to the *trans*-configurated C_5-C_6 double bond, a *cis*-configuration of H-3 and H-4 of the oxirane moiety is a prerequisite for the formation of the 10-membered lactone ring in **1**. The $J_{H-3,H-4}$ of 4.5 Hz also suggested a *cis*-relationship of the epoxide protons in **1**. Despite this, the relative configuration of the epoxide moiety could not be assigned from available spectral data. Assignments of protons and carbons in multiplolide A (**1**) are shown in Table 1.



The ESI-TOF mass spectrum of **2** revealed the molecular formula of **2** as $C_{14}H_{18}O_6$ [observed *m/z* 283.1186 $(M + H)^+$, Δ +0.5 mmu]. The ^1H and ^{13}C NMR spectra ($CDCl_3$) of multiplolide B (**2**) were similar to those of multiplolide A (**1**), except that multiplolide B (**2**) showed an additional methyl (δ 1.80, d, J = 7.0 Hz), two *trans*-olefinic protons (δ 5.77, br d, J = 15.3 Hz; and 6.95, m), and four additional carbons (δ 165.2, 145.4, 122.2, and 17.8). The ^{13}C NMR spectral data of multiplolide B (**2**) showed 14 signals, which could be classified by the DEPT spectra as two methyl, one methylene, nine methine, and two quaternary carbons. The downfield shift of H-8 from δ 3.95 in **1** to 5.05 in **2** indicated that the C-8 hydroxyl in **1** was esterified. Analyses of the $^1H-^1H$ COSY, HMQC, and HMBC spectral data of multiplolide B (**2**) revealed that multiplolide B (**2**) was a crotonyl ester of multiplolide A (**1**). The HMBC spectrum of **2** clearly demonstrated the correlation of H-8 and H-3′ to carbonyl C-1′, confirming the presence of the crotonate

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Table 1. ^{1}H and ^{13}C NMR Spectral Data ($CDCl_3$) of Multiplolides A (**1**) and B (**2**)

	multiplolide A (**1**)		multiplolide B (**2**)	
C	δ_C, mult[a]	δ_H, mult, *J* in Hz	δ_C, mult[a]	δ_H, mult, *J* in Hz
2	167.2, s		166.9, s	
3	54.9, d	3.60, d, 4.5	54.6, d	3.55, d, 5.1
4	54.5, d	3.75, m	54.3, d	3.71, m
5	117.2, d	5.72, ddd, 15.4, 1.2, 1.2	118.6, d	5.74, dd, 15.0, 1.2
6	133.6, d	5,88, ddd, 15.4, 2.2, 0.9	132.7, d	5.83, br d, 15.1
7	72.2, d	4.50, m	69.8, d	4.53, br s
8	68.1, d	3.95, dd, 8.2, 2.7	70.5, d	5.05, dd, 8.3, 2.0
9	35.2, t	1.21, dd, 16.0, 3.7 (H-9_{eq})	32.2, t	1.14, dd, 16.4, 2.8 (H-9_{eq})
		2.21, ddd, 16.0, 8.3, 3.4 (H-9_{ax})		2.30, ddd, 16.4, 8.2, 3.5 (H-9_{ax})
10	68.2, d	5.25, m	67.9, d	5.17, m
11	17.6, q	1.30, d, 6.8	17.3, q	1.30, d, 6.8
1′			165.2, s	
2′			122.2, d	5.77, br d, 15.3
3′			145.4, d	6.95, m
4′			17.8, q	1.80, d, 7.0
7-O*H*				3.25, br s

[a] Multiplicity was determined by analyses of the DEPT spectra of **1** and **2**.

moiety at C-8 in **2**. The complete assignment of protons and carbons in multiplolide B (**2**) was established by analyses of the ^{1}H−^{1}H COSY, HMQC, and HMBC spectral data (Table 1).

The absolute configuration of multiplolide B (**2**) was addressed through the use of Mosher esters.[1] Both (*R*)- and (*S*)-methoxy trifluoromethylphenyl acetate (MTPA) esters (compounds **3** and **4**) of **2** were prepared and subjected to ^{1}H NMR analysis. The $\Delta\delta$ values [$\delta_{(-)}-\delta_{(+)}$] are shown in Figure 1, indicating that the absolute configuration of C-7 of **2** is represented as *S*. Therefore, the absolute configurations of both C-8 and C-10 are *R*. Multiplolide A (**1**) exhibited positive optical rotation similarly to that of multiplolide B (**2**), suggesting that they possess the same stereochemistry at C-7 (*S*), C-8 (*R*), and C-10 (*R*).

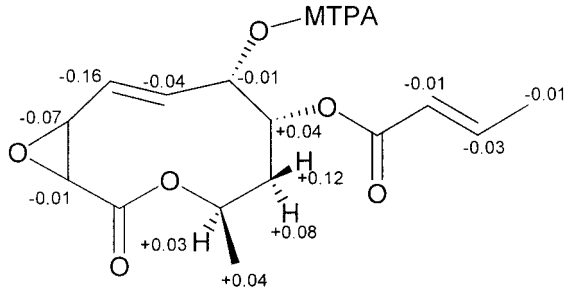

Figure 1. $\Delta\delta$ values [$\delta_{(-)}-\delta_{(+)}$] for the MTPA esters (**3** and **4**) of multiplolide B (**2**).

Multiplolides A (**1**) and B (**2**) exhibited antifungal activity against *Candida albicans* with the IC_{50} values of 7 and 2 μg/mL, respectively. Both **1** and **2** were inactive in the screening systems toward the malarial parasite *Plasmodium falciparum* (at 20 μg/mL) and were not cytotoxic to BC-1 and KB cell lines (at 20 μg/mL). Ten-membered lactones have been previously reported as metabolites in fungi, for example, diplodialides from *Diplodia pinea*,[2] decarestrictines from *Penicillium corylophilum*,[3] tuckolide from *Polyporus tuberaster*,[4] and pyrenolides from *Pyrenophora teres*.[5] Diplodialides and decarestrictines were previously found to be inhibitors of steroid biosynthesis, particularly cholesterol.[2,3] Dihydroisocoumarin (mellein) and diacid (2-hexylidene-3-methylsuccinic acid) were reported to be common metabolites in *X. multiplex*.[6] To our knowledge, the work described here is the first report on 10-membered ring lactones produced by the wood-decaying fungus *X. multiplex*.

Experimental Section

General Experimental Procedures. The ^{1}H, ^{13}C, DEPT, ^{1}H−^{1}H COSY, NOESY, HMQC, and HMBC experiments were carried out on a Bruker DRX 400 NMR spectrometer, operating at 400 MHz for proton and 100 MHz for carbon. The ESI-TOF mass spectra were obtained from a Micromass LCT mass spectrometer, and the lock mass calibration was applied for the determination of the accurate mass. The IR spectra and optical rotations were measured on a Perkin-Elmer 2000 spectrometer and Jasco DIP370 polarimeter, respectively. The UV spectra were recorded on a Cary 1E UV−vis spectrophotometer.

Fungal Material. The wood-decaying fungus *X. multiplex* BCC 1111 was collected from Ko Charng National Park, Than Mayom Waterfall, Trat Province, Thailand, and identified by Dr. Surang Thienhirun, the Royal Forest Department, Thailand. The fungus (registration no. BCC 1111) was deposited at the BIOTEC Culture Collection, Bangkok, Thailand. *X. multiplex* BCC 1111 was grown in a malt extract broth (MEB), containing (in 1 L of water) malt extract (6.0 g), maltose (1.8 g), dextrose (6.0 g), and yeast extract (1.2 g). The fungal culture was incubated at 25 °C for 24 days and then harvested for further study on chemical compositions.

Extraction and Isolation. The culture (6 L) of *X. multiplex* was filtered to separate cell and broth. The culture broth was extracted twice with an equal volume of EtOAc. EtOAc layers were combined and evaporated to dryness, yielding 0.87 g of a crude extract. The crude EtOAc extract was subsequently chromatographed on a Si gel column, eluted with gradient CH_2Cl_2 (100%) to CH_2Cl_2:MeOH (90:10). Fractions 1 and 2 were combined and further purified with a Sephadex LH-20 column (eluted with MeOH) to yield multiplolide B (**2**) (36 mg). The combined fractions 3−5, obtained from the Si gel column, were also chromatographed on a Sephadex LH-20 column (eluted with MeOH), furnishing multiplolide A (**1**) (22 mg).

Bioassays. The antifungal activity was assessed against *C. albicans*, employing the colorimetric method.[7] In our system, the IC_{50} value of the standard drug, amphotericin B, was 0.01 μg/mL. The antimalarial activity was evaluated against the parasite *Plasmodium falciparum* (K1, multidrug resistant strain), which was cultured continuously according to the method of Trager and Jensen.[8] Quantitative assessment of antimalarial activity in vitro was determined by means of the microculture radioisotope technique based upon the method described by Desjardins et al.[9] The inhibitory concentration (IC_{50}) represents the concentration that causes 50% reduction in parasite growth as indicated by the in vitro uptake of [^{3}H]-hypoxanthine by *P. falciparum*. An IC_{50} value of 1 ng/mL was observed for the standard compound, artemisinin, in the same test system. The cytoxicity of multiplolides A (**1**) and B (**2**) was determined, employing the colorimetric method as described

by Skehan and co-workers.[10] Ellipticine was used as the reference substance, exhibiting activity toward BC-1 and KB cell lines, both with an IC_{50} of 0.3 μg/mL.

Multiplolide A (1): colorless oil; $[\alpha]^{29}_D$ +6.7° (*c* 0.18, $CHCl_3$); UV (MeOH) λ_{max} end absorption; IR (neat) ν_{max} 3433, 1721, 1281, 1214, 1129, 1095, 1056, 980 cm^{-1}; ESI-TOF MS *m/z* 215.0923 $(M + H)^+$, calcd for $[C_{10}H_{14}O_5 + H]^+$ 215.0919; ^1H and ^{13}C NMR, see Table 1.

Multiplolide B (2): colorless oil; $[\alpha]^{29}_D$ +24.5° (*c* 0.40, $CHCl_3$); UV (MeOH) λ_{max} end absorption; IR (neat) ν_{max} 3499, 1720, 1656, 1443, 1364, 1308, 1280, 1211, 1180, 1053, 1008, 968 cm^{-1}; ESI-TOF MS *m/z* 283.1186 $(M + H)^+$, calcd for $[C_{14}H_{18}O_6 + H]^+$ 283.1181; ^1H and ^{13}C NMR, see Table 1.

Preparation of MTPA Esters. A reaction mixture consisting of multiplolide B (**2**) (ca 2 mg), pyridine (300 μL), and enantiomerically pure α-methoxy-α-trifluoromethyl phenylacetyl chloride (40 μL) was left standing at room temperature for 6 h. The mixture was dried under vacuum and dissolved in 5 mL of EtOAc, which was subsequently washed with diluted $NaHCO_3$ solution (6 × 5 mL) and finally with H_2O (2 × 5 mL). The EtOAc layer was dried, yielding MTPA ester of **2** (ca 2.2 mg). *S*-(−)-MTPA ester (**3**) of multiplolide B (**2**) was obtained as a colorless oil: ESI-TOF MS *m/z* 471.1612 $(M + H)^+$, calcd for $[C_{23}H_{25}O_7F_3 + H]^+$ 471.1630; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.57 (2H, m, aromatic protons of MTPA), 7.41 (3H, m, aromatic protons of MTPA), 6.94 (1H, m, H-3′), 6.09 (1H, br t, J = 1.1 Hz, H-7), 5.96 (1H, ddd, J = 17.0, 1.2 and 1.2 Hz, H-6), 5.74 (1H, dd, J = 15.8 and 1.6 Hz, H-2′), 5.35 (1H, dd, J = 17.0 and 1.2 Hz, H-5), 5.34 (1H, dd, J = 9.1 and 2.1 Hz, H-8), 5.30 (1H, m, H-10), 3.68 (1H, m, H-4), 3.64 (1H, d, J = 4.4 Hz, H-3), 3.61 (3H, s, −OCH_3 of MTPA), 2.33 (1H, ddd, J = 16.2, 8.3 and 3.6 Hz, H-9_{ax}), 1.88 (3H, dd, J = 7.0 and 1.2 Hz, H-4′), 1.40 (1H, dd, J = 16.3 and 3.4 Hz, H-9_{eq}), 1.40 (3H, d, J = 6.8 Hz, H-11). *R*-(+)-MTPA ester (**4**) of **2** was also obtained as a colorless oil: ESI-TOF MS *m/z* 471.1626 $(M + H)^+$, calcd for $[C_{23}H_{25}O_7F_3 + H]^+$ 471.1630; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.53 (2H, m, aromatic protons of MTPA), 7.43 (3H, m, aromatic protons of MTPA), 6.97 (1H, m, H-3′), 6.10 (1H, br t, J = 1.1 Hz, H-7), 6.00 (1H, ddd, J = 17.2, 1.2 and 1.2 Hz, H-6), 5.75 (1H, dd, J = 15.8 and 1.7 Hz, H-2′), 5.51 (1H, dd, J = 17.2 and 1.2 Hz, H-5), 5.30 (1H, dd, J = 8.0 and 2.2 Hz, H-8), 5.27 (1H, m, H-10), 3.75 (1H, m, H-4), 3.65 (1H, d, J = 4.8 Hz, H-3), 3.50 (3H, s, −OCH_3 of MTPA), 2.21 (1H, ddd, J = 16.3, 8.2 and 3.4 Hz, H-9_{ax}), 1.89 (3H, dd, J = 7.1 and 1.2 Hz, H-4′),1.37 (3H, d, J = 6.8 Hz, H-11), 1.32 (1H, dd, J = 16.3 and 3.3 Hz, H-9_{eq}).

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Supporting Information Available: The ^1H and ^{13}C NMR spectra ($CDCl_3$) of multiplolides A (**1**) and B (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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