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Lactones from a brown alga endophytic fungus (No. ZZF36) from the South China Sea and their antimicrobial activities

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Abstract—Two new metabolites named 6-oxo-de-*O*-methyllasiodiplodin (**1**) and (*E*)-9-etheno-lasiodiplodin (**2**), with three known compounds lasiodiplodin (**3**), de-*O*-methyllasiodiplodin (**4**), and 5-hydroxy-de-*O*-methyllasiodiplodin (**5**), were isolated from the mycelium extracts of a brown alga endophytic fungus (No. ZZF36) obtained from the South China Sea. Their structures were elucidated using spectroscopic methods, mainly 1D and 2D NMR. Additionally, the structure of compound **1** was confirmed by single crystal X-ray diffraction analysis. The antimicrobial activities of lasiodiplodins, and the 13-acetyl and 12,14-dibromo derivatives of lasiodiplodin were tested for the first time and the results were compared to each other.

As part of an ongoing research on biologically active products from marine endophytic fungi from the South China Sea,[1] we studied the chemical constituents of the unidentified endophytic fungus No. ZZF36, which was isolated from a brown alga (*Sargassum* sp.) collected from Zhanjiang sea area, China. Herein we reported the isolation[2] of two new 12-membered ring lactones, namely 6-oxo-de-*O*-methyllasiodiplodin (**1**) and (*E*)-9-etheno-lasiodiplodin (**2**), together with three known compounds, lasiodiplodin (**3**), de-*O*-methyllasiodiplodin (**4**), and 5-hydroxy-de-*O*-methyllasiodiplodin (**5**) (Fig. 1), from the mycelium extracts of the fungus No. ZZF36. Their antimicrobial activity had been tested and compared. Lasiodiplodin and its relatives are known to be very efficient inhibitors of prostaglandin biosynthesis, and exhibit significant anti-leukemic and potato micro-tuber inducing activities.[3] It is the first time to report the antimicrobial test of lasiodiplodins.

Compound **1** was obtained as colorless crystals, and the molecular formula $C_{16}H_{20}O_5$ was determined by HR-EI-MS. In the ^{1}H NMR of **1**, a methyl doublet at δ 1.40, a methine at δ 5.33, and two benzene protons at δ 6.19 and 6.26 were observed. ^{13}C NMR of **1** showed sixteen signals, attributable to one methyl, six methylene, three methine, and six quaternary carbons, including an ester carbonyl carbon (δ 171.61) and a ketone carbonyl carbon (δ 211.05), as determined by DEPT experiments. The ^{1}H–^{1}H COSY (Fig. 2) revealed contiguous sequence of coupled signals from H-17 to H-5 and H-7 to H-9. The HMBC data assembled the overall structure of **1** (Fig. 2 and Table 1), especially the multiple correlations from H-4, H-5, H-7, and H-9 to C-6 established the partial structure from C-3 to C-10. The correlations from H-10 to C-11 and C-12, and the correlation between the chelated hydroxyl groups and C-16 defined that the connection points of aliphatic ring and benzene ring were C-11 and C-16. Compound **1** was therefore identified as 6-oxo-de-*O*-methyllasiodiplodin.

The structure of compound **1** (Fig. 3) was confirmed by X-ray diffraction analysis[4] also. Crystals of **1** belong to the orthorhombic system, space group $P2_12_12_1$; a = 6.0733 (13) Å, b = 15.263 (3) Å, c = 16.229 (4), $\alpha = \beta = \gamma = 90°$; volume = 1504.3 (6) Å^3, Z = 4, D_{calcd} = 1.291 mg/m^3, m = 0.095 mm^{-1}, $F(000)$ = 624. The final value of R was 0.0370, $wR2$ = 0.0912 [$I > 2\sigma(I)$], GooF = 1.013.

Compound **2** was obtained as a white powder, the molecular formula was assigned as $C_{17}H_{22}O_4$ based on

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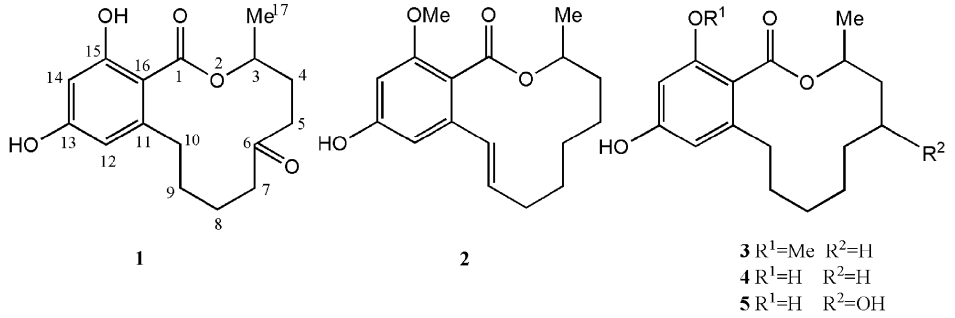

1 2

3 R^1=Me R^2=H
4 R^1=H R^2=H
5 R^1=H R^2=OH

Figure 1. Structures of compounds **1**–**5**.

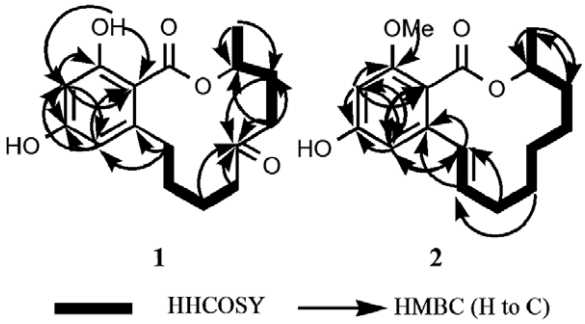

Figure 2. Important HHCOSY and HMBC correlations of **1** and **2**.

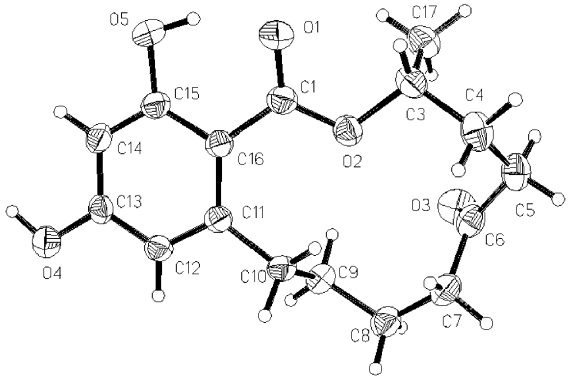

Figure 3. Molecular structure of **1**.

Table 1. NMR spectral data of 6-oxo-de-*O*-methyllasiodiplodin (**1**) and (*E*)-9-etheno-lasiodiplodin (**2**) (in $CDCl_3$)[a]

Position	6-Oxo-de-*O*-methyllasiodiplodin (**1**)[b]		(*E*)-9-etheno-lasiodiplodin (**2**)[c]	
	δC	δH	δC	δH
1	171.61 (C)		168.02 (C)	
3	73.02 (CH)	5.33 (1H, m)	72.37 (CH)	5.17 (1H, m)
4	31.23 (CH_2)	2.23 (2H, m)	33.54 (CH_2)	1.86 (1H, m)
				1.48 (1H, m)
5	40.06 (CH_2)	2.72 (1H, m)	27.29[d] (CH_2)	1.52[d] (1H, m)
		2.44 (1H, m)		1.42[d] (1H, m)
6	211.05 (C)		24.81[d] (CH_2)	1.60[d] (1H, m)
				1.58[d] (1H, m)
7	38.15 (CH_2)	2.76 (1H, m)	21.75 (CH_2)	1.37 (1H, m)
		2.56 (1H, m)		1.35 (1H, m)
8	22.13 (CH_2)	1.84 (1H, m)	31.80 (CH_2)	2.25 (2H, m)
		1.71 (1H, m)		
9	30.95 (CH_2)	1.60 (1H, m)	134.79 (CH)	5.83 (1H, dt, J = 15.5, 7.5 Hz)
		1.48 (1H, m)		
10	34.24 (CH_2)	3.09 (1H, m)	128.99 (CH)	6.39 (1H, d, J = 15.5 Hz)
		2.49 (1H, m)		
11	148.76 (C)		139.40 (C)	
12	110.45 (CH)	6.19 (1H, d, J = 2.4 Hz)	105.67 (CH)	6.33 (1H, s)
13	160.28 (C)		157.96 (C)	
14	101.61 (CH)	6.26 (1H, d, J = 2.4 Hz)	97.62 (CH)	6.33 (1H, s)
15	165.89 (C)		157.38 (C)	
16	105.30 (C)		115.99 (C)	
17	19.11 (CH_3)	1.40 (3H, d, J = 6.4 Hz)	20.11 (CH_3)	1.33 (3H, d, J = 6.2 Hz)
OMe			55.94	3.81 (3H, s)
13-OH				5.21 (1H, s)
15-OH		11.94 (1H, s)		

[a] TMS was used as internal standard, δ in ppm.
[b] Data of 1 at 400/100 MHz.
[c] Data for **2** at 500/125 MHz.
[d] May be interchanged.

Table 2. Tests of MIC (μg/mL) of compounds **1**, **3**–**7** against six bacterial and fungal strains[a]

Strains	Compounds							
	1	**3**	**4**	**5**	**6**	**7**	Amp[b]	Nys[b]
Staphylococcus aureus ATCC27154	>100	25	6.25	100	>100	>100	>100	NT[b]
Bacillus subtilis ATCC 6633	>100	50	12.5	>100	>100	6.25	100	NT
Escherichia coli ATCC 25922	>100	>100	>100	>100	>100	>100	6.25	NT
Salmonella enteritidis ATCC 13076	>100	>100	12.5	>100	>100	>100	50	NT
Candida albicans ATCC 10231	>100	>100	100	>100	>100	>100	NT	1.56
Fusarium oxysporum f.sp.*cubense*	>100	100	50	>100	>100	12.5	NT	3.125

[a] Results are expressed as the minimum inhibitory concentration (MIC).
[b] Ampicillin (Amp), Nystatin (Nys): positive control; NT, not tested.

HR-EI-MS. Seventeen signals in the ^{13}C NMR were classified by the DEPT spectra, including two methyl, five methylene, five methine, and five quaternary carbons. The ^{1}H NMR revealed a methyl doublet (δ 1.33), a methyl (δ 3.81), two benzene protons (δ 6.33), and three methine singles (δ 5.17, 5.83, 6.39). In the ^{1}H–^{1}H COSY (Fig. 2), the correlation from H-3 to H-10 was showed. To analyze the HMBC spectrum of **2** (Fig. 2), we assembled the overall structure of **2**. The geometrical configuration of the double bond was proved to be *E* by the coupling constants of $J_{9,10}$ = 15.5 Hz. So the structure of compound **2** was confirmed as (*E*)-9-etheno-lasiodiplodin.

Furthermore, three known compounds **3**, **4**, and **5** were identified by comparison of their spectroscopic data with those of literature.[3,5]

Except the minor component **2**, compounds were tested against six aerobic reference strains for their inhibitory activity, using a modified version of the 2-fold serial dilutions method as Fromtling et al. described.[6] Compound **4** exhibited the inhibiting activities to the other five microorganisms except for *Escherichia coli*, especially to *Staphylococcus aureus* with MIC = 6.25 μg/mL. **3** inhibited the in vitro growth of *S. aureus*, *Bacillus subtilis*, and *Fusarium oxysporum* when MIC = 25, 50 and 100 μg/mL, respectively. But **5** showed effect against *S. aureus* at 100 μg/mL only and inactive to all the others. In all the antimicrobial tests, compound **1** exhibited no activities (Table 2).

The analysis of the in vitro antibiotic data of lasiodiplodins is probably helpful for the study on the structure–activity relationship, though the data are far from enough. At C-15, compound **4** bears a hydroxy, and **3** bears a methoxy group. But **4** showed stronger antibacterial and antifungal activities than **3**. When the hydroxyl group of **3** was acetylated, its product **6**[7] was inactive to all the strains (Table 2). It implied that the hydroxyls in C-13 and C-15 probably have effect in antibiotic activities. Compound **5** bears one more hydroxyl group at C-5 (on the lactone ring) than **4**, but it exhibited worse antibiotic activity than **4**. In addition, compound **1** with a ketone carbonyl group showed no activity to all these six strains. An interesting result was that 12,14-dibromo lasiodiplodin **7**[8] showed stronger activity against *F. oxysporum* and *B. subtilis* (MIC = 12.5 and 6.25 μg/mL, respectively) than its parent compound **3** (MIC = 100 and 50 μg/mL, respectively). However, the bromination of **3** led to a decrease of the activity against *S. aureus* (from MCI = 25 μg/mL to none).

Acknowledgments

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2. The fungus was cultured stationary in a 500 mL Erlenmeyer flask containing 200 mL of liquid medium (glucose 10 g/L, peptone 2 g/L, yeast extract 1 g/L, and NaCl 3 g/L) at 25 °C for 30 days. The mycelium from 100 L culture filtrates was immersed in MeOH for one month. Evaporation of the MeOH extract gave brown oil (20 g). The oil was applied to a silica gel column, eluted with a gradient of petroleum ether (100%, 2 L), petroleum ether/EtOAc (90:10, v/v, 4 L) (fraction 1–4), and petroleum ether/EtOAc (70:30, v/v, 4 L) (fractions 5–8), successively. Every liter of eluate was collected as one fraction. The 4th fraction was concentrated under reduced pressure, the residue was separated by column chromatography (silica gel, petroleum ether/EtOAc, 90:10) and preparative TLC (silica gel F_{254}, petroleum ether/EtOAc, 90:10) to offer **4** (50 mg). Fraction 5 deposited crystals of **3** (2.8 g) when set aside overnight. Fraction 6 eluted with 20% EtOAc in petroleum ether eluate yielded **1** (4.6 mg) and further purified by preparative TLC (silica gel F_{254}, $CHCl_3$) offering **2** (2.5 mg). Compound **5** (30 mg) was obtained from fraction 8 eluted by elution with petroleum ether/EtOAc (80:20). Compound **1**: mp 143–145 °C; $[\alpha]_D^{20}$ +38.89° ($CHCl_3$, *c* 0.02); IR (KBr) ν_{max} 3304, 2924, 2853, 1685, 1613, 1580, 1491, 1461, 1334, 1259, 1216, 1191, 1167, 1132, 1102, 1024, 970 cm^{-1}; HR-EI-MS *m/z* 292.1300 $[M]^+$ (calcd for $C_{16}H_{20}O_5$, 292.1305). Compound **2**: mp 158–159 °C; $[\alpha]_D^{20}$ −23.81° ($CHCl_3$, *c* 0.02); IR

(KBr) ν_{max} 3353, 2923, 2854, 1698, 1645, 1621, 1586, 1463, 1431, 1408, 1384, 1311, 1263, 1200, 1170, 1129, 1095, 1017 cm^{-1}; HR-EI-MS *m/z* 290.1503 $[M]^+$ (calcd for $C_{17}H_{22}O_4$, 290.1513).

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4. All single-crystal data were collected using the hemisphere technique on a Bruker SMART 1000 CCD system diffractometer with graphite-monochromated Mo Kα radiation $\lambda = 0.71073$ Å at 293(2) K. The structure was refined by direct methods using SHELXTL Ver. 6.12. All non-hydrogen atoms were refined with anisotropic displacement parameters, and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters. CCDC (Deposit No: 294120) contains the supplementary crystallographic data. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: +44-(0)1223-336033; E-mail: deposit@ccdc.cam.ac.uk.
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7. A solution of **3** (20 mg) in acetic anhydride (3 mL) with pyridine (three drops) was stirred at room temperature overnight. The reaction mixture was separated by preparative TLC (silica gel F_{254}, petroleum ether/EtOAc, 50:50) to offer **6** (22.9 mg). Compound **6**: $C_{19}H_{26}O_5$; ^1H NMR (300 MHz, $CDCl_3$) δ 6.53 (1 H, d, $J = 1.8$ Hz), 6.47 (1H, d, $J = 1.8$ Hz), 5.27 (1H, m), 3.76 (3H, s), 2.69 (1H, m), 2.51 (1H, m), 2.26 (3H, s), 1.92 (1H, m), 1.64 (4H, m), 1.40 (4H, m), 1.30 (3H, d, $J = 6.6$ Hz), 1.20 (3H, m); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 169.21 (C), 167.97 (C), 157.28 (C), 152.03 (C), 142.71 (C), 122.82 (C), 114.77 (CH), 102.92 (CH), 72.66 (CH), 56.33 (CH_3), 32.67 (CH_2), 30.73 (CH_2), 30.30 (CH_2), 26.82 (CH_2), 25.75 (CH_2), 24.54 (CH_2), 21.54 (CH_2), 21.52 (CH_3), 19.75 (CH_3).
8. To a stirred solution of the compound **3** (20 mg) in 5 mL CCl_4 was added 2 equiv of NBS. After reflux for 24 h, the reaction mixture was separated by preparative TLC (silica gel F_{254}, $CHCl_3$) to offer **7** (9.8 mg). Compound **7**: $C_{17}H_{22}O_4Br_2$; ^1H NMR (300 MHz, $CDCl_3$) δ 5.28 (1H, m), 3.88 (3H, s), 2.82 (2H, m), 1.95 (1H, m), 1.71 (5H, m), 1.38 (3H, d, $J = 6.6$ Hz), 1.45 ~ 1.28 (6H, m); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 166.77 (C), 154.31 (C), 151.29 (C), 140.12 (C), 124.83 (C), 108.40 (C), 102.90 (C), 73.82 (CH), 62.43 (CH_3), 33.23 (CH_2), 31.31 (CH_2), 27.13 (CH_2), 27.01 (CH_2), 26.17 (CH_2), 24.73 (CH_2), 21.77 (CH_2), 19.95 (CH_3).