DOI: 10.1002/cbdv.202000928

FULL PAPER

CHEMISTRY & BIODIVERSITY

β-Resorcylic Acid Derivatives, with Their Phytotoxic Activities, from the Endophytic Fungus *Lasiodiplodia theobromae* in the Mangrove Plant *Xylocarpus granatum*

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Nine new β-resorcylic acid derivatives, (15*S*)-*de*-*O*-methyllasiodiplodin (**1**), (13*S*,15*S*)-13-hydroxy-*de*-*O*-methyllasiodiplodin (**2**), (14*S*,15*S*)-14-hydroxy-*de*-*O*-methyllasiodiplodin (**3**), (13*R*,14*S*,15*S*)-13,14-dihydroxy-*de*-*O*-methyllasiodiplodin (**4**), ethyl (*S*)-2,4-dihydroxy-6-(8-hydroxynonyl)benzoate (**5**), ethyl 2,4-dihydroxy-6-(8-hydroxyheptyl) benzoate (**6**), ethyl 2,4-dihydroxy-6-(4-methoxycarbonylbutyl)benzoate (**7**), 3-(2-ethoxycarbonyl-3,5-dihydroxyphenyl)propionic acid (**8**), and isobutyl (*S*)-2,4-dihydroxy-6-(8-hydroxynonyl)benzoate (**9**), together with a known ethyl 2,4-dihydroxy-6-(8-oxononyl)benzoate (**10**) were obtained from *Lasiodiplodia theobromae* GC-22. The structures of these compounds were elucidated by extensive spectroscopic analyses. Compounds **1**, **3**, and **6** showed growth inhibitory effects against *Digitaria ciliaris*. Conversely, treatment with compounds **5**, **6**, **7**, **9**, and **10** stimulated elongation activity toward the root of *Lactuca sativa*. These data expand the repertoire of new β-resorcylic acid derivatives that may function as lead compounds in the synthesis of new agrochemical agents.

Keywords: β-resorcylic acid, endophyte, mangrove plant, *Lasiodiplodia theobromae*, phytotoxicity.

Introduction

During the last two decades, endophytic fungi from various plant habitats have attracted much attention in the field of natural products because of their sustainable biosynthesis of bioactive molecules with diverse structures.[1] Most plants in the natural ecosystem are symbiotic with endophytic fungi, including species with different symbiotic relationships and ecological functions. Endophytic fungi can have a significant impact on the host's adaptation to environmental conditions such as drought, salinity, heat, or cold. Moreover, endophytic fungi can significantly affect the growth and viability of a variety of competing organisms, which live in their natural habitats.[2] In these natural habitats, all organisms must adapt morphologically and physiologically. This biological diversity is reflected in the synthesis of structurally distinct compounds that represent a promising source of novel bioactive agents. We have previously reported that the fungal strain *Pseudocosmospora* sp. isolated from the Indonesian mangrove plant, *Acanthus ebracteatus* Vahl, produced cytotoxic compounds.[3] In our ongoing investigation to discover novel bioactive secondary metabolites from endophytes, we isolated and identified nine new polyketide-like compounds **1**–**9**, each containing a β-resorcylic acid moiety, along with one known compound **10** from the endophytic strain *L. theobromae*

Supporting information for this article is available on the WWW under https://doi.org/10.1002/cbdv.202000928

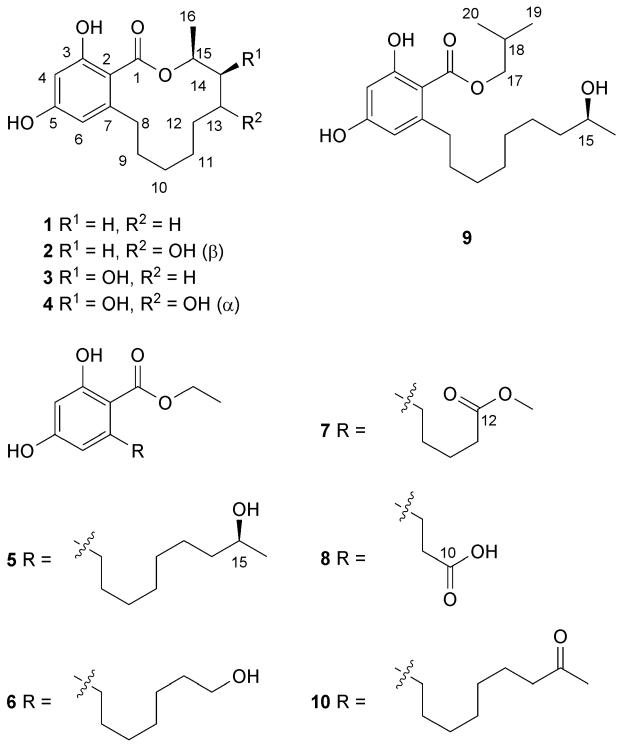

Figure 1. Chemical structures of compounds **1**–**10**.

GC-22 isolated from the mangrove plant *X. granatum*. *L. theobromae* is phytopathogenic fungus, belonging to the family Botryosphaeriaceae (*Figure 1*). It is distributed in tropical and subtropical regions.[4] The genus *Lasiodiplodia* is a valuable resource for structurally novel natural products with diverse bioactivities.[5] For example, *Lasiodiplodia* produce numerous bioactive secondary metabolites, including derivatives of lasiodiplodins,[6] substituted 2-dihydrofuranones, melleins, scytalones, and cyclo-(Trp-Ala).[7] Herein, we report the isolation, structural determination, and biological activities.

Results and Discussion

Compound **1** was isolated as an optically active white amorphous solid. Its molecular formula of $C_{16}H_{22}O_4$ was established by HR-ESI-TOF-MS analysis, which indicated six double bond equivalents. The IR spectrum of **1** showed bands for hydroxy groups (3358 cm^{-1}), a ketone functionality (1640 cm^{-1}), and a substituted aromatic ring (1464 and 837 cm^{-1}). These observations were consistent with the appearance of signals in the ^{13}C-NMR and DEPT spectral data (*Table 1*), which indicated the presence of one conjugated ketone carbonyl atom (δ(C) 171.8), and six aromatic carbons atoms (δ(C) 100.7, 104.4, 110.8, 148.7, 162.1, and 164.9), that accounted for five double bond equivalents. The planar of **1** was determined based on a detailed analysis of ^{1}H- (*Table 2*), ^{13}C-, and 2D-NMR (COSY, HSQC, HMBC) spectra. A literature search revealed that **1** was identical to (+)-(*R*)-*de*-*O*-methyllasiodiplodin, except for the specific rotation at $[\alpha]_D^{20} = -42.6$ ($c = 0.7$, MeOH) for **1** and $[\alpha]_D^{20} = +35.5$ ($c = 0.58$, MeOH) for (+)-(*R*)-*de*-*O*-methyllasiodiplodin.[8–11] In addition, the absolute configuration of **1** was elucidated by comparing its experimental CD spectrum to the ECD spectra calculated for isomers of **1** (*Figure 2*). Thus, the absolute configuration at C-15 of compound **1** was determined as (15*S*). Therefore, the structure of compound **1** was elucidated (*Figure 1*) and is referred to as *ent*-*de*-*O*-methyllasiodiplodin.

Compound **2** was obtained as a white amorphous powder and had a molecular formula of $C_{16}H_{22}O_5$ based on its HR-ESI-TOF-MS data. The ^{13}C-NMR, DEPT, and HMBC spectra of **2** confirmed the presence of 16 carbon atoms, including a carbonyl group, one methyl group, six methylene groups, four methine groups, and four quaternary carbons. Comparison of the NMR data (*Tables 1* and *3*) of **1** and **2** suggested that they shared a similar carbon skeleton. The difference in their carbon skeletons was the presence of an additional oxygenated methine in **2**, which was confirmed by the ^{1}H,^{1}H-COSY spin-coupling system H_2-C(8)/H_2-C(9)/H_2-

Table 1. ^{13}C-NMR Data for compounds **1**–**4** (150 MHz, CD_3OD, δ in ppm).

Position	1	2	3	4
	δ(C)	δ(C)	δ(C)	δ(C)
1	171.8	171.7	170.9	170.9
2	104.4	103.6	105.8	105.9
3	164.9	165.3	163.5	163.4
4	100.7	100.6	100.5	100.6
5	162.1	163.6	161.9	161.9
6	110.8	110.9	110.3	110.3
7	148.7	148.8	147.3	147.3
8	33.2	33.7	32.7	32.5
9	30.8[a]	34.3[a]	29.9[a]	29.5[a]
10	27.1[a]	31.6[a]	26.6[a]	29.0[a]
11	24.3[a]	27.7[a]	22.7[a]	25.9[a]
12	24.1[a]	18.1[a]	21.8[a]	19.1[a]
13	30.8[a]	69.3	30.0	70.2
14	21.0	40.4	69.1	72.7
15	74.6	72.0	75.7	74.3
16	19.1	20.3	11.8	13.1

[a] Interchangeable.

C(10)/H_2-C(11)/H_2-C(12)/H–C(13)/H_2-C(14)/H–C(15)/Me–C(16) and the key HMBCs from H–C(15) to C-1 (*Figure 3*). The NOE correlation from H–C(15) to H–C(13) suggested that these protons were on the same side (*Figure 4*). The absolute configuration of **2** was elucidated by comparing the calculated ECD data (at the B3LYP/6-311++G(d) level with the CPCM model in MeOH solution) and the experimental CD spectrum (*Figure 2*). The experimental CD curve of **2** matched well with the computer-simulated ECD curve of the (13*S*,15*S*) of **2**. In addition, **2** represents a new enantiomer derivative of (13*R*,15*R*)-13-hydroxy-*de*-*O*-methyllasiodiplodin.[12] Therefore, the structure of **2** was designated as (13*S*,15*S*)-13-hydroxy-*de*-*O*-methyllasiodiplodin (*Figure 1*).

The molecular formula of compound **3** was determined to be $C_{16}H_{22}O_5$ by HR-ESI-TOF-MS analysis. Compound **3** has the same molecular formula as compound **2**. The ^{13}C- and ^{1}H-NMR spectroscopic data of **3** (*Tables 1* and *3*) were very similar to those of **2**, with small differences in the chemical shifts for C-13 through C-15. Inspection of the ^{1}H- and ^{13}C-NMR data suggested that **3** is an isomer of **2**. Based on the HMBCs of H-16 to the oxygenated methine carbon at C-14 (δ(C)/δ(H) 69.1/4.03–4.08) (*Figure 3*), the hydroxy group was located at C-14. The agreement between the calculated ECD spectra of (14*S*,15*S*)-**3** with the experimental CD data of **3** suggested that the absolute configuration of the unsaturated lactone moiety is (14*S*,15*S*) (*Figure 2*). Additionally, the ^{13}C-NMR chemical shifts were also calculated to confirm the structure of **3**. The experimental and calculated ^{13}C-NMR chemical

Table 2. ^{13}C-NMR Data for compounds **5**–**9** (150 MHz, δ in ppm).

Position	**5**[a]	**6**[a]	**7**[b]	**8**[a]	**9**[a]
	δ(C)	δ(C)	δ(C)	δ(C)	δ(C)
1	171.4	171.4	171.5	171.1	171.6
2	104.1	104.1	105.1	103.9	104.4
3	165.0	164.7	165.6	165.0	164.5
4	100.5	100.5	101.7	101.0	100.5
5	162.3	162.3	160.4	162.2	162.2
6	110.6	110.6	110.9	110.7	110.4
7	147.9	147.9	148.0	145.7	147.6
8	36.5	36.5	36.6	31.6	36.1
9	32.0[c]	32.3[c]	31.3	35.8	31.8[c]
10	29.4[c]	29.3[c]	25.1	175.3	29.4[c]
11	29.5[c]	31.9[c]	34.1	61.1	29.4[c]
12	29.6[c]	32.3[c]	174.4	13.0	29.4[c]
13	25.6[c]	25.7[c]	61.5		25.1[c]
14	38.9	61.7	14.3		38.9
15	67.3	60.9			67.2
16	22.2	13.3			22.2
17	60.9				71.3
18	13.3				27.7
19					18.4
20					18.4
OCH_3			51.7		

[a] Recorded in CD_3OD. [b] Record in $CDCl_3$. [c] Interchangeable.

Table 3. ^{1}H-NMR Data of compounds **1**–**4** (600 MHz in CD_3OD, δ in ppm and J in Hz).

Position	**1**	**2**	**3**	**4**
	δ(H)	δ(H)	δ(H)	δ(H)
4	6.14 (s)	6.18 (d, J=1.8)	6.14 (d, J=1.8)	6.14 (d, J=2.4)
6	6.14 (s)	6.12 (d, J=1.8)	6.12 (d, J=1.8)	6.13 (d, J=2.4)
8	2.35–2.44 (m)	2.05–2.10 (m)	2.19 (td, J=10.0, 4.4)	2.23–2.35 (m)
	3.03–3.12 (m)	3.67–3.71 (m)	3.06 (td, J=10.0, 4.4)	2.92–3.02 (m)
9	1.28–1.35[a]	1.44–1.54[a]	1.35–1.44[a]	1.43–1.52[a]
	1.35–1.57[a]	1.55–1.66[a]	1.53–1.62[a]	1.58–1.69[a]
10	1.28–1.35[a]	1.44–1.54[a]	1.35–1.44[a]	1.32–1.39 (m)
		1.55–1.66[a]	1.53–1.62[a]	1.43–1.52[a]
11	1.35–1.57[a]	1.44–1.54[a]	1.35–1.44[a]	1.58–1.69[a]
			1.46–1.62[a]	
12	1.35–1.57[a]	1.44–1.54[a]	1.46–1.62[a]	1.43–1.52[a]
13	1.35–1.57[a]	3.96–4.00 (m)	1.67–1.77 (m)	3.77–3.87[a]
			1.79–1.86 (m)	
14	1.62–1.69 (m)	1.55–1.66[a]	4.03–4.08 (m)	3.77–3.87[a]
	1.73–1.82 (m)	2.21–2.27 (m)		
15	4.98–5.05 (m)	5.15–5.18 (m)	5.06–5.11 (m)	5.40–5.44 (m)
16	1.25 (d, J=6.0)	1.34 (d, J=6.0)	1.32 (d, J=6.6)	1.36 (d, J=6.7)

[a] Overlapped signals.

shifts of **3** (14*S**,15*S**) and **3** (14*R**,15*S**) showed good linear relationships with linear regression values of $R^2 = 0.9983$ and $R^2 = 0.9974$, respectively, as shown by the parity plots (*Figure S61* and *Table S1*). Therefore, the structure of **3** (*Figure 1*) is referred to as (14*S*,15*S*)-14-hydroxy-*de*-*O*-methyllasiodiplodin, which is an enantiomer of (14*R*,15*R*)-14-hydroxy-*de*-*O*-methyllasiodiplodin.[11,13,14]

Compound **4** has the molecular formula $C_{16}H_{22}O_6$, which was determined by HR-ESI-TOF-MS studies. Compound **4** has an additional oxygen atom compared with **2** and **3**. The ^{13}C- and ^{1}H-NMR spectral data (*Tables 1* and *3*) for **4** corresponded to those of **2** and **3**, except for the presence of one more oxymethine signal [δ(C)/δ(H) 70.2 d/3.77 – 3.87 (C-13)] in **4**. An HMBC experiment (*Figure 3*) was conducted on **4** to determine the connectivity of the hydroxy groups at C(13) and C(14). Next, the relative configuration of **4** was assigned based on the coupling constants and NOE experiments. The β-configurations of the C(15) methyl group, C(14) hydroxy group, and C(13) α-hydroxy group were inferred from NOE results of the acetonide derivative **4a** from H–C(16) to H–C(13) (*Figure 4*). Based upon the coupling constant between H–C(14) and H–C(15) ($J_{14,15} = 2.0$), these **4a** hydrogens were found to be co-facial. The determination of the absolute configuration at C(13) was examined with a modified version of Mosher's method.[15] Using this method, we detected chemical shift differences between the (−)-(*S*)- and (+)-(*R*)-MTPA esters (compounds **4b** and **4c**, respectively) of compound **4** (*Figure 5*). Consequently, the absolute configuration at C(13) in **4** was found to be (*R*). Therefore, the structure of **4** (*Figure 1*) was designated as (13*R*,14*S*,15*S*)-13,14-dihydroxy-*de*-*O*-methyllasiodiplodin.

Compound **5** was isolated as a white amorphous powder, with the molecular formula $C_{18}H_{28}O_5$ with 5 degrees of unsaturation as deduced from HR-ESI-TOF-MS data. The ^{1}H-NMR spectrum of **5** displayed two aromatic proton signals at δ(H) 6.12 ($J = 2.4$, H–C(4)) and 6.18 ($J = 2.4$, H–C(6)) accounting for a tetrasubstituted aromatic ring, one methyl proton signal at

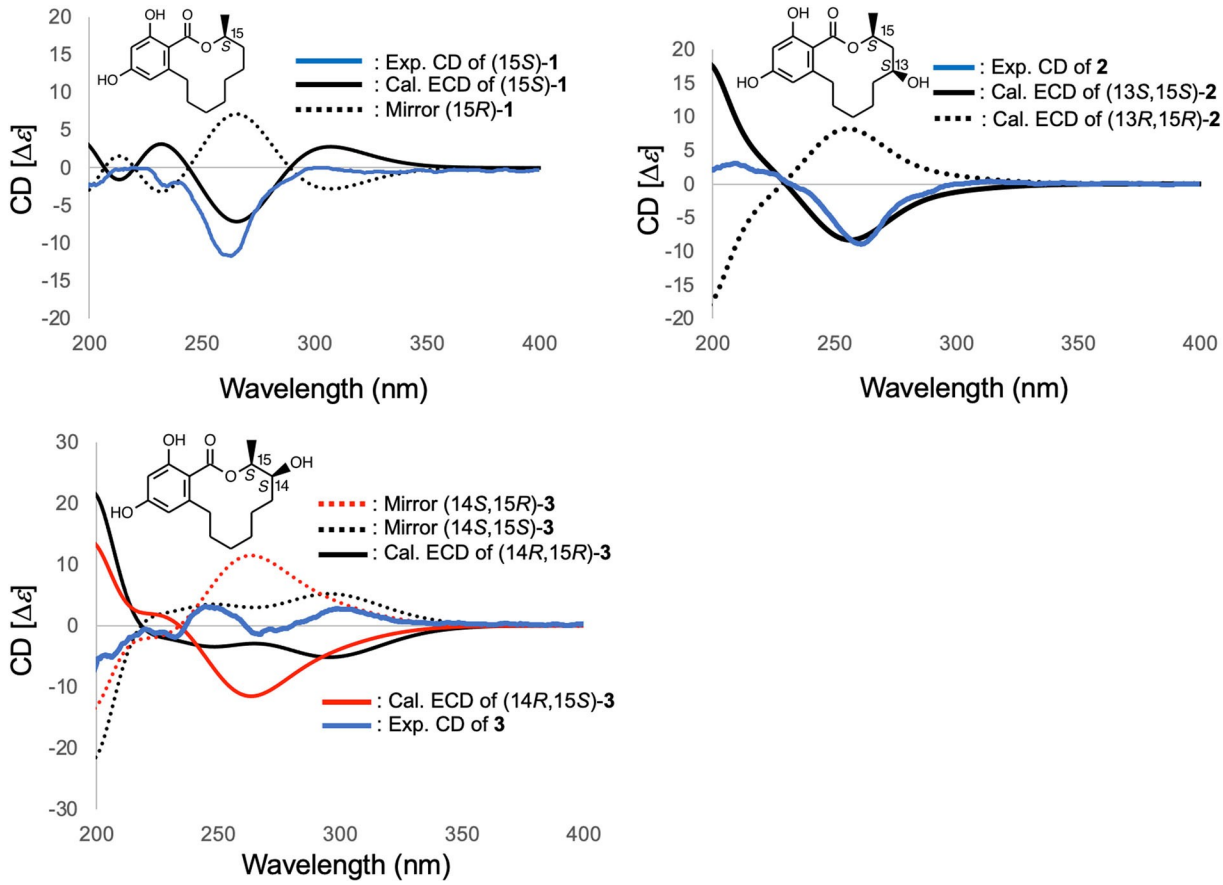

Figure 2. Calculated and experimental CD spectra of **1**, **2**, and **3**.

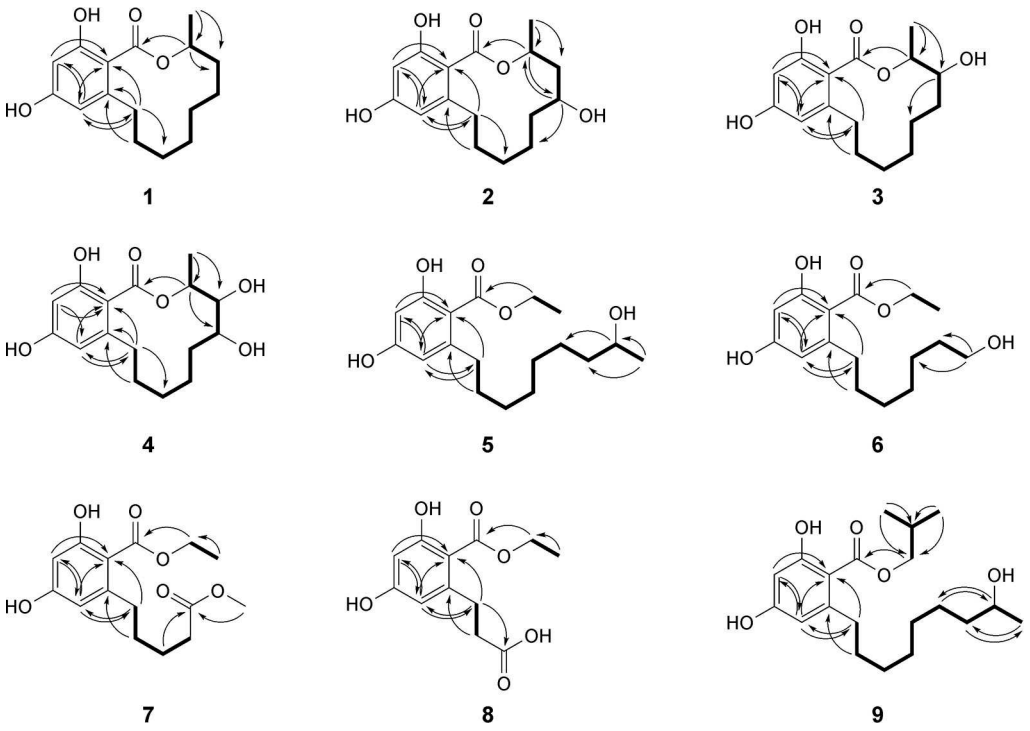

Figure 3. ^{1}H,^{1}H-COSY (bold lines) and selected HMBC (arrows) data of **1**–**9**.

δ(H) 1.10 (J=6.6, Me(16)), one oxygenated methine proton signal at δ(H) 3.64–3.70 (H–C(15)), as well as one oxygen-bearing ethyl group with proton signals at δ(H) 4.32 (J=7.2, H–C(17)) and 1.34 (J=7.2, H–C(18)) (*Table 4*). Analysis of the ^{13}C-NMR and DEPT spectra revealed that **5** contained two methyl groups (C-16 and C-18), one ester carbonyl (C(1)), eight methylene groups (C(8) to C(14)), and six aromatic carbons (C(2) to C(7)) (*Table 2*). A detailed chemical shift analysis of the aromatic carbons at δ(C) 165.0,

Table 4. ^{1}H-NMR Data of compounds **5**–**9** (600 MHz, δ in ppm and J in Hz).

Position	**5**[a]	**6**[a]	**7**[b]	**8**[a]	**9**[a]
	δ(H)	δ(H)	δ(H)	δ(H)	δ(H)
4	6.12 (d, J=2.4)	6.15 (d, J=1.8)	6.28 (d, J=1.8)	6.16 (d, J=1.8)	6.13 (d, J=1.8)
6	6.18 (d, J=2.4)	6.13 (d, J=1.8)	6.21 (d, J=1.8)	6.23 (br. s)	6.18 (d, J=1.8)
8	2.73–2.78 (m)	2.73–2.78 (m)	2.86 (t, J=7.5)	3.11 (t, J=8.1)	2.81 (t, J=7.8)
9	1.24–1.33[c]	1.27–1.34[c]	1.55–1.59 (m)	2.52 (t, J=7.8)	1.48–1.54[c]
10	1.24–1.33[c]	1.27–1.34[c]	1.64–1.70 (m)		1.26–1.38[c]
11	1.45–1.52[c]	1.45–1.52[c]	2.33 (t, J=7.2)	4.37 (q, J=7.5)	1.26–1.38[c]
12	1.45–1.52[c]	1.45–1.52[c]		1.37 (t, J=7.5)	1.26–1.38[c]
13	1.24–1.33[c]	1.27–1.34[c]	3.39 (q, J=7.2)		1.26–1.38[c]
14	1.31–1.43 (m)	3.51 (t, J=6.6)	1.40 (t, J=7.2)		1.48–1.54[c]
15	3.64–3.70 (m)	4.32 (q, J=7.0)			3.64–3.70 (m)
16	1.10 (d, J=6.6)	1.30 (t, J=7.0)			1.11 (d, J=6.0)
17	4.32 (q, J=7.2)				4.10 (d, J=7.2)
18	1.34 (t, J=7.2)				2.01–2.10 (m)
19					1.01 (d, J=6.6)
20					1.01 (d, J=6.6)
OH			11.82 (s)		
OCH_3			3.66 (s)		

[a] Recorded in CD_3OD. [b] Record in $CDCl_3$. [c] Overlapped signals.

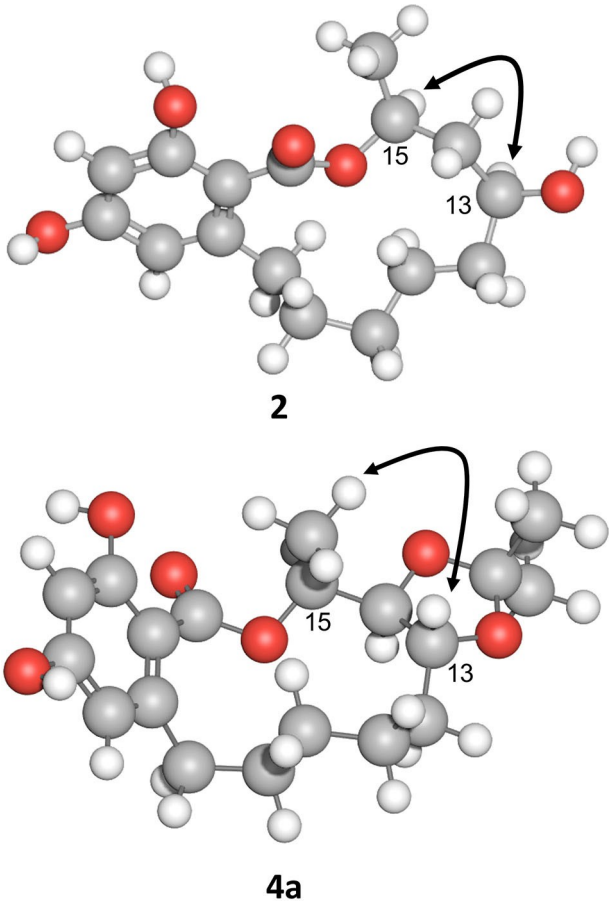

Figure 4. Key NOE correlations for compounds **2** and **4a**.

162.3, 147.9, 110.6, 104.1, and 100.5 together with an ester carbonyl at δ(C) 171.4 suggested that **5** was a β-resorcylic acid derivative. This structure was further confirmed by the HMBCs from H–C(4) to C(2), as well as C(6) and H–C(6) to C(2) and C(4) (*Figure 3*). The structure of **5** was finally elucidated by 2D-NMR, including COSY, HSQC, and HMBC. The absolute C(15) configuration of **5** was defined using Mosher's reagents to generate the diastereoisomeric (*S*)- and (*R*)-MTPA esters (**5a** and **5b**, respectively) (*Figure 5*). Thus, the absolute configuration of **5** was assigned as (15*S*). In addition, these data indicated that **5** is an enantiomer of ethyl (*R*)-2,4-dihydroxy-6-(8-hydroxynonyl)benzoate.[16] Therefore, the structure of **5** (*Figure 1*) is referred to as ethyl (*S*)-2,4-dihydroxy-6-(8-hydroxynonyl)benzoate.

Compound **6** was obtained as a white amorphous powder. Its molecular formula was assigned as $C_{16}H_{24}O_5$ based on HR-ESI-TOF-MS spectra. Sixteen signals from the ^{13}C-NMR and DEPT spectra revealed that **6** contained one methyl group, eight methylene groups, two methine groups, four quaternary carbons, and one ester carbonyl carbon. The ^{1}H-NMR spectra (*Table 4*) along with the HSQC spectra showed signals indicating the presence of one methyl group proton (δ(H) 1.30), eight methylene protons (δ(H) 1.27–1.34, 1.45–1.52, 2.73–2.78, 3.51), including an ethyl ester methylene proton (δ(H) 4.32), and two *meta* aromatic protons (δ(H) 6.13 and 6.15). A detailed chemical shift analysis of the aromatic carbons at δ(C) 164.7, 162.3, 147.9, 110.6, 104.1, and 100.5 together with an ester carbonyl at δ(C) 171.4 suggested that **6** was a β-resorcylic acid derivative, similar to **5**. Extensive analysis of 2D-NMR, including COSY, HSQC, and HMBC (*Figure 3*), revealed the structure of **6** (*Figure 1*), which we named ethyl 2,4-dihydroxy-6-(8-hydroxyheptyl)-benzoate.

Compound **7** was isolated as a white amorphous powder and its molecular formula was assigned as $C_{15}H_{20}O_6$ based on HR-ESI-TOF-MS data. Fifteen signals from the ^{13}C-NMR (*Table 2*) and DEPT spectra revealed that **7** contained two methyl groups, five methylene groups, two methine groups, four quaternary carbons, and two ester carbons. The ^{1}H-NMR spectra (*Table 4*) and the HSQC spectra revealed two methyl group protons [δ(H) 1.40 and 3.66 (s)], two aromatic protons (δ(H) 6.21 and 6.28), and five methylene group protons (δ(H) 1.55–1.59, 1.64–1.70, 2.33 and 2.86). Extensive analysis of 2D-NMR, including COSY, HSQC, and HMBC,

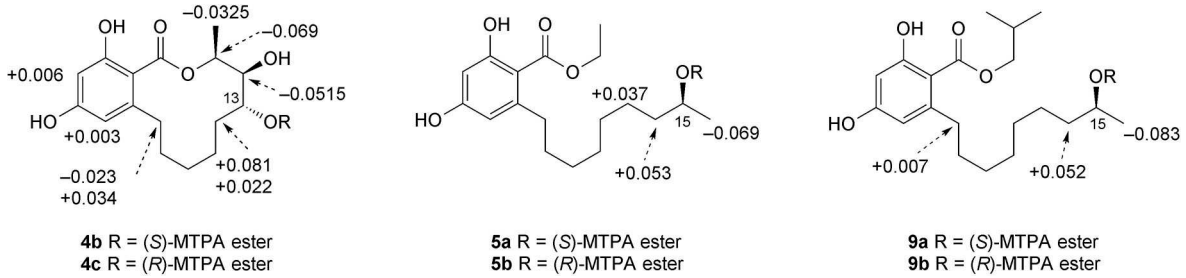

Figure 5. $\Delta\delta = \delta S - \delta R$ values in ppm obtained from the MTPA esters (**4b**, **4c**, **5a**, **5b**, **9a** and **9b**).

revealed the planar structure of **7** as described below (*Figure 3*). Two independent coupling fragments (H_2-C(8)/H_2-C(9)/H_2-C(10)/H_2-C(11) and H–C(13)/Me(14)) were present in the ^{1}H,^{1}H-COSY spectrum. In order to connect these structures, the HMBC spectrum was evaluated; specifically, the correlations of H–C(4) and H–C(6)/C-2, as well as H–C(13)/C(1) provided firm evidence of an ethyl β-resorcylic acid ester moiety. The linkage of the alkyl chain to C(7) of the ethyl benzoate was assigned by HMBCs from H–C(9)/C(7), H–C(8)/C(2) and C(6), as well as OCH_3/C(12). Based on the above evidence, the structure of **7** (*Figure 1*) was designated as ethyl 2,4-dihydroxy-6-(4-methoxycarbonylbutyl)-benzoate.

Compound **8** yielded a white amorphous powder. The HR-ESI-TOF-MS data of **8** revealed that its molecular formula was $C_{12}H_{14}O_6$. The IR spectrum of **8** showed absorptions at 3300 cm^{-1} (OH moiety). The ^{1}H- and ^{13}C-NMR assignments of **8** (*Tables 2* and *4*) were based on the COSY, HMQC, and HMBC spectra. When the ^{1}H-NMR spectrum of **8** was compared to that of **7**, we found that **8** lacked the methoxy protons as well as two methylene groups assigned to C(9) and C(10) of **7**. Moreover, **8** uniquely contained two coupled methylene groups, which indicated that the methyl pentanoate moiety was replaced by a propionic acid at C(7). The structure of **8** was further confirmed by HMBC experiments (*Figure 3*). Based on the above evidence, the structure of **8** (*Figure 1*) was designated as 3-(2-ethoxycarbonyl-3,5-dihydroxyphenyl)propionic acid.

Compound **9** was obtained as an amorphous solid with a molecular formula of $C_{20}H_{32}O_5$, as indicated by HR-ESI-TOF-MS data. The ^{1}H- and ^{13}C-NMR data of **9** were similar to those of **5**. The isobutyl moiety of **9** was readily assigned from ^{1}H,^{1}H-COSY, HMQC, and HMBCs (*Figure 3*), which suggested that the ethyl moiety present in **5** was replaced with the isobutyl moiety in **9**. The absolute configuration at C(15) in **9** was confirmed with a modified version of Mosher's method based on the differences between the ^{1}H-NMR chemical shifts of the (*R*)-MTPA ester and the (*S*)-MTPA ester derivatives (*Figure 5*). Based on these analyses, the structure of **9** (*Figure 1*) is referred to as isobutyl (*S*)-2,4-dihydroxy-6-(8-hydroxynonyl)benzoate.

In addition, compound **10** was identified by comparison of their spectroscopic data as ethyl 2,4-dihydroxy-6-(8-oxononyl)benzoate.[17] In 1971, the 12-membered lasiodiplodin compound and *de-O*-methyllasiodiplodin were isolated from a culture broth of the fungus *Botryodiplodia theobromae*.[10] These lasiodiplodin derivatives are often separated from various natural resources and have a variety of biological activities.[5] (*R*)-lasiodiplodin and (*R*)-*de-O*-methyllasiodiplodin are the most widely studied metabolites in this family. The absolute configuration of a methyl group at C(15) had been determined as (*R*) by means of chemical synthesis.[9,18,19,20] In several previous reports, the configuration of C(15) was deduced based on the comparison of spectral data and proposed biosynthetic pathways.[8,12,21] Recently, the absolute configurations of (11*R*,15*R*), (11*S*,15*R*), and (11*S*,15*S*)-11-hydroxylasiodiplodin, as well as (12*R*,14*S*,15*R*)-12,14-dihydroxy-*de-O*-methyllasiodiplodin were assigned via theoretical and experimental CD methods.[14,22] A recent report showed that (*R*)-lasiodiplodin was involved in the regulation of plant growth.[8] The β-resorcylic macrolactone, *trans*-resorcylide was previously isolated from culture filtrates of *Penicillium* sp.[23] In seedling tests, low concentrations (100 ppm) of *trans*-resorcylide exhibited phytotoxicity toward Chinese cabbage, lettuce, and rice.[23] In this study, all the compounds were subjected to the plant regulatory assay utilizing *L. sativa* and *D. ciliaris* (*Figures 6* and *7*). In this assay, the positive control 2,4-dichlorophenoxyacetic acid, significantly inhibited the growth of *L. sativa* and *D. ciliaris*. Compounds **1**, **3**, and **6** showed phytotoxic effects against *D. ciliaris* in a dose-dependent manner (*Figure 7*). In contrast, compound **4** had weak phytotoxic effects, which attested that the position of the mono-hydroxy group at C(14) had a significant impact on the phytotoxic activity. These results revealed that the presence of a 13-OH in the macrocyclic lactone ring of methyllasiodiplodin resulted in diminished phytotoxic activity. Among the tested compounds, only **5**, **7**, **9**, and **10** exhibited increased elongation activity toward lettuce root (*Figure 6*). Moreover, compounds **7**, **9**, and **10** displayed an enhanced elongation activity toward southern crabgrass root (*Figure 7*). These results indicated that the hydroxy or carbonyl group on the side chain attached to C(7) plays an important role in plant growth activity. On the other hand, low concentrations (25 μg/mL) of **6** did not inhibit root growth of *L. sativa* and *D. ciliaris*. In many plant species, it is known that growth-promoting compounds are produced during the processing of growth after germination. It may be postulated that **6** did not exhibit growth inhibitory activity during the early seedling development stage because of the secretion of growth-promoting exudates (e.g., self-defense substances) from plants.[24] In addition, compounds **1**, **3**, and **6** were also evaluated for phytotoxic activity against *D. ciliaris* leaves (*Figure 8*). Compounds **3** and **6** exhibited phytotoxic effects on the leaves, which appeared as visible lesions. In contrast, compound **1** did not display any phytotoxic effects on *D. ciliaris*

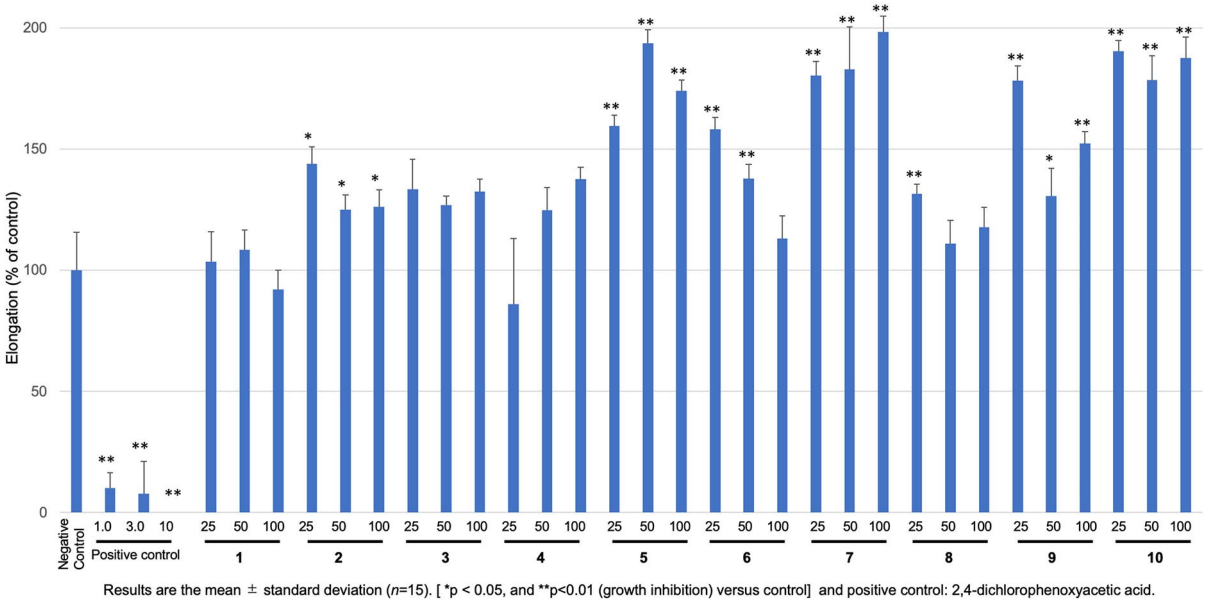

Figure 6. Effects of **1**–**10** on the root growth of *Lactuca sativa* (lettuce) (μg/mL).

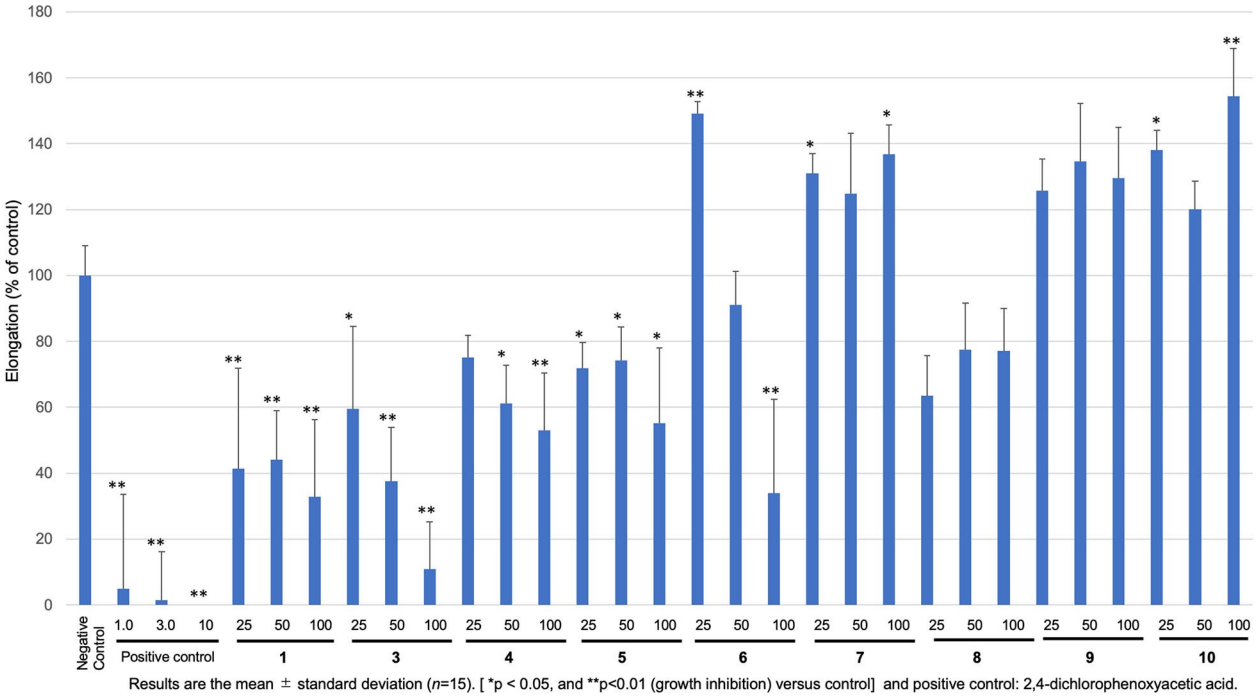

Figure 7. Effects of **1** and **3**–**10** on the root growth of *Digitaria ciliaris* (southern crabgrass) (μg/mL).

leaves. Although the inhibitory activity of **6** against root growth was lower than that of **3**, these results indicated that **6** could be effective lead compounds for the developments of herbicides. Compounds **1**–**10**, which have been isolated, identified, and partially characterized in this study, have unique structures, and possess a wide variety of activities. Therefore, it may be difficult to precisely define the structure–activity relationships or mode of actions of these *de-O*-methyllasiodiplodin derivatives in plants. However, our results with isolated compounds will provide advantageous information for future studies focused on developing plant protective compounds for agricultural applications.

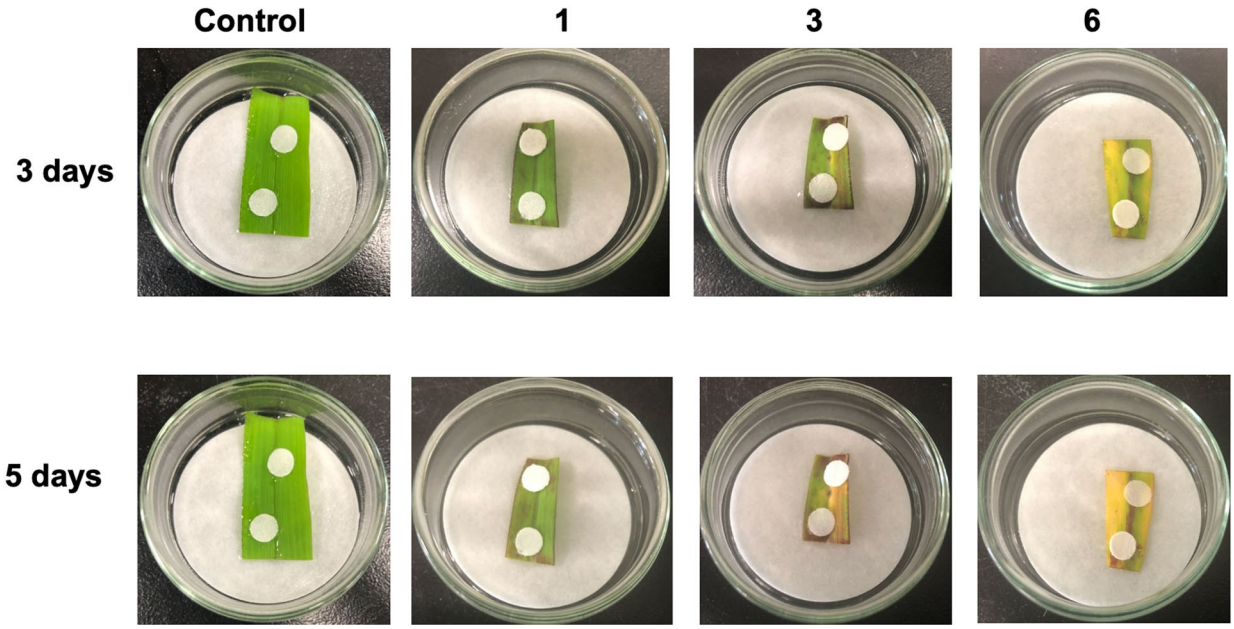

Figure 8. Phytotoxic effects on the leaves of *Digitaria ciliaris* by **1**, **3**, and **6** (3 and 5 days).

Conclusions

In summary, nine new β-resorcylic acid derivatives (compounds **1**–**9**), along with a known compound **10**, were obtained from the endophytic strain *L. theobromae* GC-22 isolated from the mangrove plant *X. granatum*. Compound **3** displayed significant phytotoxic activity against *D. ciliaris*. Moreover, compounds **5**, **7**, **9**, and **10** showed enhanced root elongation activity in *L. sativa*. Our results suggested that these β-resorcylic acid analogs may be utilized as lead compounds for further development of novel agrochemical agents.

Experimental Section

General

Optical rotations were measured using a Horiba SEPA-300 polarimeter (Horiba, Japan). IR and UV/VIS spectra were respectively recorded with Horiba FT710 (Horiba, Japan) and Shimadzu UV-1800 spectrometer (Shimadzu, Japan). Mass spectra were obtained with a Synapt G2 (Water Corporation, USA) and JEOL HX110 mass spectrometer (JEOL, Japan). NMR data were recorded on JEOL ECZ-600 spectrometer (JEOL, Japan) at 600 MHz for ^{1}H and 150 MHz for ^{13}C. Chemical shift are given on a δ (ppm) scale with TMS as an internal standard. ^{1}H, ^{13}C, DEPT, COSY, HMQC, and HMBC spectra were recorded using standard JEOL pulse sequences. Column chromatography were conducted on silica gel 60 (Kanto Chemical Co., Inc., Japan) and ODS (Fuji Silysia, Japan). Flash chromatography were conducted using Büchi Flash Chromatography C-601 (Büchi, Switzerland) and packed column Biotage® SNAP Ultra (Biotage, Sweden) 10 g (25 μm). HPLC separations were carried out using Shimadzu LC-20AT and UV/VIS detector SPD-20A (Shimadzu, Japan) on reversed-phase high performance liquid chromatography (RP-HPLC) packed column C-18 UG80 (Shiseido, Japan). Thin layer chromatography (TLC) were carried out on precoated silica gel 60 F_{254} plates (Merck, Germany), and spot were detected by spraying with 1% vanillin in H_2SO_4 followed by heating, or by UV irradiation.

Fungal Material and Fermentation

The fungal strain GC-22 was isolated from inner tissue of dead branch collected in the Santolo beach area (southern latitude 7°65′00′, east longitude 107°68′76′), Garut, West Java, Indonesia. This strain was identified as *Lasiodiplodia theobromae* using DNA analysis of the 18S rRNA regions. This fungus was deposited at our laboratory in the Faculty of Agriculture, Yamagata University. *L. theobromae* was cultivated on sterilized brown rice (120 g in five 1 L flasks) at 25 °C for four weeks.

Fermentation, Extraction, and Isolation

The fungal strain GC-22 was cultivated on sterile steamed unpolished rice (1,000 g) at 25 °C for eight weeks. The moldy unpolished rice was extracted with MeOH (1.5 L), and MeOH extract was concentrated. The resulting aqueous concentrated was partitioned into hexane layer (0.5 L), AcOEt layer (1.0 L) and aqueous layer (0.3 L). Purifications of eluates were monitored by the characteristic intense blue coloration with 10% vanillin in H_2SO_4 on TLC plates. The AcOEt layer (12.0 g) was chromatographed on a silica gel column with stepwise elution of hexane/AcOEt (100:0–0:100) and AcOEt/MeOH (50:50, 0:100), respectively, to afford fractions (1.1–1.12). Fractions 1.3 and 1.4 were combined and further re-chromatographed on a silica gel column using $CHCl_3$ and an increasing ratio of AcOEt (100:0–0:100) to afford fractions 2.1–2.12. Fractions 2.10 and 2.11 ($CHCl_3$/AcOEt, 10:90, 0:100, 100 mg) was further separated by ODS chromatography eluted with MeOH/H_2O (80:20) to give compounds **1** (50.0 mg) and **2** (3.0 mg). Fractions 1.4 and 1.5 were combined and further re-chromatographed on a silica gel column using $CHCl_3$ and an increasing ratio of AcOEt (100:0–0:100) to afford fractions 3.1–3.12. Fractions 3.3 and 3.4 ($CHCl_3$/AcOEt, 80:20, 70:30, 70 mg) were separated by ODS chromatography eluted with a stepwise gradient of H_2O/MeOH to give 13 fractions (4.1–4.13). Fraction 4.5 was purified by HPLC eluting with 80% MeOH/H_2O to afford compounds **3** (15 mg), **4** (11 mg), and **5** (5.0 mg). Fractions 1.6 and 1.7 were combined and further re-chromatographed on a silica gel column using $CHCl_3$ and an increasing ratio of AcOEt (100:0–0:100) to afford fractions 4.1–4.12. Fractions 4.3, 4.4 and 4.5 ($CHCl_3$/AcOEt, 80:20, 70:30, 60:40, 110 mg) were separated by ODS chromatography eluted with a stepwise gradient of H_2O/MeOH to give 13 fractions (5.1–5.13). Fraction 5.5 was purified by HPLC eluting with 80% MeOH/H_2O to afford compounds **7** (13 mg), **8** (17 mg), and **9** (11 mg). Fraction 1.11 (AcOEt/MeOH, 50:50, 100 mg) was subjected to silica gel column, eluting with a stepwise gradient of $CHCl_3$/AcOEt to yield 13 fractions (5.1–5.12). Fr. 5.8 (45 mg) was separated by ODS chromatography eluted with a stepwise gradient of H_2O/MeOH to give fractions 6.4 that was further purified by preparative HPLC eluting with 80% MeOH/H_2O to yield compounds **6** (5.0 mg) and **10** (7.0 mg).

(**15*S***)-***de-O*-Methyllasiodiplodin** (**1**). White amorphous powder. $[\alpha]_D^{20} = -42.6$ ($c = 0.7$, MeOH). UV (MeOH, λ_{max} (log ε):)): 217 (4.0), 264 (3.7), 302 (3.4). IR (KBr): 3358 (OH), 2939, 1640 (C=O), 1464, 1309, 1261, 1210, 1173, 1146, 1088, 1021, 837. ECD (MeOH, λ_{max} ($\Delta\varepsilon$)): 264 (−12.3) (*Figure S68*). ^{1}H- and ^{13}C-NMR: see *Tables 1* and *3*. HR-ESI-TOF-MS: 301.1412 ($[M+Na]^+$, $C_{16}H_{22}NaO_4^+$; calc. 301.1416).

(**13*S*,15*S***)-**13-Hydroxy-*de-O*-methyllasiodiplodin** (**2**). White amorphous powder. $[\alpha]_D^{20} = -17.7$ ($c = 0.7$, MeOH). UV (MeOH, λ_{max} (log ε)): 217 (3.5), 265 (3.5), 302 (3.2). IR (KBr): 3423 (OH), 2930, 1645 (C=O), 1456, 1310, 1257, 1170, 1092, 1024. ECD (MeOH, λ_{max} ($\Delta\varepsilon$)): 267 (−9.5). ^{1}H- and ^{13}C-NMR: see *Tables 1* and *3*. HR-ESI-TOF-MS: 317.1368 ($[M+Na]^+$, $C_{16}H_{22}NaO_5^+$; calc. 317.1365).

(**14*S*,15*S***)-**14-Hydroxy-*de-O*-methyllasiodiplodin** (**3**). White amorphous powder. $[\alpha]_D^{20} = -13.3$ ($c = 0.06$, MeOH). UV (MeOH, λ_{max} (log ε)): 218 (3.7), 264 (3.4), 302 (3.0). IR (KBr): 3311 (OH), 2939, 1645 (C=O), 1456, 1260, 1170, 1036. ECD (MeOH, λ_{max} ($\Delta\varepsilon$)): 250 (+3.6), 267 (−2.4), 307(2.7). ^{1}H- and ^{13}C-NMR: see *Tables 1* and *3*. HR-ESI-TOF-MS: 317.1363 ($[M+Na]^+$, $C_{16}H_{22}NaO_5^+$; calc. 317.1365).

(**13*R*,14*S*,15*S***)-**13,14-Dihydroxy-*de-O*-methyllasiodiplodin** (**4**). Yellow amorphous powder. $[\alpha]_D^{20} = -8.6$ ($c = 3.6$, MeOH). UV (MeOH, λ_{max} (log ε)): 217 (4.0), 265 (3.7), 303 (3.3). IR (KBr): 3339 (OH), 2939, 1642 (C=O), 1617, 1304, 1260, 1164, 1027. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *3*. HR-ESI-TOF-MS: 333.1311 ($[M+Na]^+$, $C_{16}H_{22}NaO_6^+$; calc. 333.1314).

Ethyl (*S*)-2,4-Dihydroxy-6-(8-hydroxynonyl)benzoate (**5**). White amorphous powder. $[\alpha]_D^{20} = +9.4$ ($c = 2.6$, MeOH). UV (MeOH, λ_{max} (log ε)): 219 (4.0), 265 (3.7), 302 (3.3). IR (KBr): 3432 (OH), 2920, 1652 (C=O), 1456, 1316, 1263, 1167, 1108, 1018. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *4*. HR-ESI-TOF-MS: 347.1834 ($[M+Na]^+$, $C_{18}H_{28}NaO_5^+$; calc. 347.1834).

Ethyl 2,4-Dihydroxy-6-(8-hydroxyheptyl)benzoate (**6**). White amorphous powder. UV (MeOH, λ_{max} (log ε)): 216 (3.9), 264 (3.6), 302 (3.2). IR (KBr): 3395 (OH), 2939, 1648 (C=O), 1459, 1313, 1263, 1170, 1105, 1021. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *4*. HR-ESI-TOF-MS: 319.1522 ($[M+Na]^+$, $C_{16}H_{24}NaO_5^+$, calc. 319.1522).

Ethyl 2,4-Dihydroxy-6-(4-methoxycarbonylbutyl)benzoate (**7**). White amorphous powder. UV (MeOH, λ_{max} (log ε): 217 (3.6), 264 (3.4), 300 (3.0). IR

(KBr): 3376 (OH), 2939, 1711, 1648 (C=O), 1446, 1316, 1266, 1164. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *4*. HR-ESI-TOF-MS: 319.1168 ($[M+Na]^+$, $C_{15}H_{20}NaO_6^+$; calc. 319.1158).

3-(2-Ethoxycarbonyl-3,5-dihydroxyphenyl)propionic acid (**8**). White amorphous powder. UV (MeOH, λ_{max} (log ε)): 217 (3.9), 264 (3.6), 301 (3.2). IR (KBr): 3300 (OH), 2967, 1735, 1652 (C=O), 1316, 1266, 1170, 1108, 1018, 838. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *4*. HR-ESI-TOF-MS: 277.0686 ($[M+Na]^+$, $C_{12}H_{14}NaO_6^+$; calc. 277.0688).

Isobutyl (*S*)-2,4-Dihydroxy-6-(8-hydroxynonyl)-benzoate (**9**). White amorphous powder. $[\alpha]_D^{20} = +6.0$ ($c = 0.3$, MeOH). UV (MeOH, λ_{max} (log ε)): 217 (3.8), 264 (3.5), 302 (3.1). IR (KBr) 3367 (OH), 2930, 1648 (C=O), 1620, 1465, 1316, 1263, 1204, 1170, 1015. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *4*. HR-ESI-TOF-MS: 375.2144, ($[M+Na]^+$, $C_{20}H_{32}NaO_5^+$; calc. 375.2148).

Formation of the Acetonide **4a** *from* **4**

1.5 mg of **4** was stirred overnight in 2,2-dimethoxylpropane (2 mL) and acetone (0.5 mL) with catalytic amounts of *p*-toluenesulfonic acid. The reaction mixture was poured into saturated $NaHCO_3$ and extracted with AcOEt. Evaporation of the organic layer yielded a residue which was purified by column chromatography on silica gel (hexane/AcOEt) to give acetonide **4a** (1.0 mg).

^{1}H-NMR (600 MHz, $CDCl_3$): δ(H) 6.25 (d, $J = 1.8$, H–C(4), 1H), 6.27 (m, $J = 1.8$, H–C(6), 1H), 5.67 (qd, $J = 7.0$, 2.0, H–C(15), 3H), 3.01–3.05 (m, H–C(8), 1H), 2.50–2.53 (m, H–C(8), 1H), 3.99 (dd, $J = 10.0$, 2.0, H–C(14), 1H), 3.87 (td, $J = 10.0$, 2.0, H–C(13), 1H), 1.43–1.55, 1.60–1.17, 1.90–1.92 (m, H–C(9), H–C(10), H–C(11), H–C(12)), 11.65 (s, 3-OH, 1H), 1.39 (CH_3 of acetonide moiety, 3H), 1.40 (s, CH_3 of acetonide moiety, 3H). ^{13}C-NMR (150 MHz, $CDCl_3$): δ(C) 170.9 (C(1)), 150.2 (C(2)), 160.4 (C(3)), 110.9 (C(4)), 165.6 (C(5)), 101.7 (C(6)), 148.8 (C(7)), 34.5(C(8)), 20.5 (C(9)) 27.2 (C(10)) 28.5 (C(11)), 29.3 (C(12)), 70.2 (C(13)), 72.7 (C(14)), 81.2 (C(15)), 14.4 (C(16)), 26.8, 27.0, and 107.3 (CH_3 and C of acetonide moiety). HR-ESI-TOF-MS: 373.1621 ($[M+Na]^+$, $C_{19}H_{26}NaO_6^+$; calc. 373.1627).

Preparation of MTPA Ester Derivatives (**4b** *and* **4c**) *from* **4**

To **4** (1.0 mg) in dry pyridine were added (−)-(*R*)-MTPACl (10 μL), the mixture was stirred at room temperature for 24 h. Purification by column chromatography have done on silica gel (hexane/AcOEt) to afford the (*S*)-MTPA ester (**4b**, 0.9 mg). Compound **4** (1.0 mg) was treated with (+)-(*S*)-MTPACl (10 μL) in the same procedure to afford the (*R*)-MTPA ester (**4c**, 0.9 mg).

4b. HR-ESI-TOF-MS: 5549.1740 ($[M+H]^+$, $C_{26}H_{29}F_3NaO_8^+$; calc. 549.1711). ^{1}H-NMR (600 MHz, $CDCl_3$): δ(H) 1.20–1.31 (overlapped signals, H–C(9), H–C(10), H–C(11)), 1.403 (d, $J = 7.0$, H–C(16), 3H), 1.875–1.9157 (m, H–C(12), 1H), 1.934–1.976 (m, H–C(12), 1H), 2.255–2.297 (m, H–C(8)), 3.117–3.119 (m, H–C(8)), 3.55 (OMe of MTPA, 3H), 4.158 (dd, $J = 7.0$, 3.0, H–C(14), 1H), 5.248–5.29 (m, H–C(15), 1H), 5.338 (t, $J = 7.0$, H–C(13), 1H), 6.217 (d, $J = 1.8$, H–C(4), 1H), 6.286 (d, $J = 1.8$, H–C(6), 1H), 7.42–7.45 (m, Ph of MTPA, 3H), 7.55–7.58 (m, Ph of MTPA, 2H), 11.60 (s, 3-OH, 1H).

4c. HR-ESI-TOF-MS: 549.1729 ($[M+H]^+$, $C_{26}H_{29}F_3NaO_8^+$; calc. 549.1711). ^{1}H-NMR (600 MHz, $CDCl_3$): δ(H) 1.435 (d, $J = 7.0$, H–C(16), 3H), 1.65–1.83 (overlapped signals, H–C(9), H–C(10), H–C(11)), 1.853–1.895 (m, H–C(12), 2H), 2.203–2.245 (m, H–C(8)), 3.12–3.162 (m, H–C(8)), 3.546 (3H, OMe of MTPA, 3H), 4.227 (dd, $J = 7.0$, 3.0, H–C(14), 1H), 5.30–5.342 (m, H–C(13) and H–C(15), 1H), 6.214 (d, $J = 1.8$, H–C(4), 1H), 6.280 (d, $J = 1.8$, H–C(6), 1H), 7.41–7.47 (m, Ph of MTPA, 3H), 7.50–7.57 (m, Ph of MTPA, 2H), 11.68 (s, 3-OH, 1H).

Preparation of MTPA Ester Derivatives (**5a** *and* **5b**) *from* **5**

Using the same procedure as that just described with **4**, **5** (1.0 mg and 1.0 mg) was treated with (−)-(*R*)-MTPACl (2.0 mg) and (*S*)-(+)-MTPACl (2.0 mg) to, respectively afford (−)-(*S*)- and (+)-(*R*)-MTPA esters **5a** (0.8 mg) and **5b** (0.8 mg).

5a. HR-ESI-TOF-MS: 563.2259 ($[M+H]^+$, $C_{28}H_{35}F_3NaO_7^+$; calc. 563.2232). ^{1}H-NMR (600 MHz, $CDCl_3$): δ(H) 1.258 (d, $J = 7.2$, H–C(16), 3H), 1.27–1.35 (overlapped signals, H–C(10), C(11), C(12), C(13)), 1.398 (3H, t, $J = 7.2$, H–C(18)), 1.529–1.571

(m, H–C(9)), 1.668–1.709 (m, H–C(14)), 2.79–2.832 (m, H–C(8)), 2.844–2.886 (m, H–C(8)), 3.55 (OMe of MTPA, 3H), 4.39 (q, $J=7.2$, H–C(17), 2H), 5.140–5.182 (m, H–C(15), 1H), 6.186 (d, $J=1.8$, H–C(4), 1H), 6.275 (d, $J=1.8$, H–C(6), 1H), 7.28–7.35 (m, Ph of MTPA, 3H), 7.39–7.41 (m, Ph of MTPA, 2H), 11.78 (s, 3-OH, 1H).

5b. HR-ESI-TOF-MS: 563.2253 ($[M+H]^+$, $C_{28}H_{35}F_3NaO_7^+$; 563.2232. ^{1}H-NMR (600 MHz, $CDCl_3$): δ(H) 1.21–1.30 (overlapped signals, H–C(9), C(10), C(11), C(12), C(13)), 1.327 (d, $J=7.2$, H–C(16), 3H), 1.40 (t, $J=7.2$, H–C(18), 3H), 1.601–1.670 (m, H–C(14)), 2.852–2.854 (m, H–C(8)), 2.782–2.824 (m, H–C(8)), 3.42 (s, OMe of MTPA, 3H), 4.39 (q, $J=7.2$, H–C(17), 2H), 5.167–5.169 (m, H–C(15), 1H), 6.286 (d, $J=1.8$, H–C(4), 1H), 6.195 (d, $J=1.8$, H–C(6), 1H), 7.42–7.43 (m, Ph of MTPA, 3H), 7.56–7.58 (m, Ph of MTPA, 2H), 11.80 (br. s, 3-OH, 1H).

Preparation of MTPA Ester Derivatives (**9a** *and* **9b**) *from* **9**

9a. HR-ESI-TOF-MS: 591.2574 ($[M+Na]^+$, $C_{30}H_{39}F_3NaO_7^+$; 591.2545). ^{1}H-NMR (600 MHz, $CDCl_3$): δ(H) 0.863 (dd, $J=6.9$, 2.1, H–C(19), C(20), 6H), 1.225 (d, $J=6.6$, H–C(16), 3H), 1.57–1.59 (overlapped signals, H–C(9), H–C(10), C(11), C(12), C(13)), 1.626–1.694 (m, H–C(14), 1H), 1.795–1.878 (m, H–C(14), 1H), 2.609 (t, $J=8.4$, H–C(8), 2H), 3.528 (OMe of MTPA, 3H), 3.793 (d, $J=7.2$, H–C(17), 2H), 5.084–5.136 (m, H–C(15), 1H), 6.913 (d, $J=1.8$, H–C(6), 1H), 6.945 (d, $J=2.4$, H–C(4), 1H), 7.31–7.39 (m, Ph of MTPA, 3H), 7.49–7.52 (m, Ph of MTPA, 2H), 11.56 (s, 3-OH, 1H).

9b. HR-ESI-TOF-MS: 591.2585 ($[M+Na]^+$, $C_{30}H_{39}F_3NaO_7^+$; calc. 591.2545). ^{1}H-NMR (600 MHz, $CDCl_3$): δ(H) 0.866 (d, $J=7.2$, H–C(19), C(20), 6H), 1.308 (d, $J=6.0$, H–C(16), 3H), 1.23–1.28 (overlapped signals, H–C(9), C(10), C(11), C(12), C(13)), 1.581–1.635 (m, H–C(14)), 1.795–1.878, (m, H–C(14)), 2.602 (t, $J=8.1$, H–C(8), 2H), 3.367 (OMe of MTPA, 3H), 3.510 (d, $J=6.6$, H–C(17), 2H), 5.10–5.140 (m, H–C(15), 1H), 6.917 (d, $J=1.2$, H–C(6), 1H), 6.941 (s, H–C(4), 1H), 7.36–7.38 (m, Ph of MTPA, 3H), 7.51–7.54 (m, Ph of MTPA, 2H), 11.56 (s, 3-OH, 1H).

Seedling Growth Assays

Two plants, *Digitaria ciliaris* and *Lactuca sativa*, were used as receiver plants for testing the phytotoxic activity of the isolated compounds. Seeds of *D. ciliaris* and *L. sativa* were surface sterilized with a flowing tap water before use. Isolated compounds were dissolved in methanol to give solutions at 10, 30, and 100 μg/mL and added to Petri dishes (4.0 cm diameter) lined with filter papers. After complete evaporation of methanol, distilled water (1 mL) was added to each Petri dish followed by addition of 15 seeds. All Petri dishes were stored in the dark at 25 °C. Seedlings were allowed to grow for 7 (for *L. sativa*) or 10 (for *D. ciliaris*) days before shoot and root length were measured. Three replicates were made for each treatment ($N=15$).

Phytotoxicity Assay

Fresh *Digitaria ciliaris* leaves were washed three times with sterilized water. The leaves were cut into 1.0× 3.0 cm rectangle with scissors and placed in a sterile glass dish. A 1 mg amount of test compound was dissolved in MeOH aqueous solution contains 90% (v/v) water to produce a 1.0 mg/0.1 mL test solution. Two filter piece discs (8 mm diameter) were placed on the leaves, and then, 10 μL of MeOH aqueous solution or test solution was added to each disc. The effect was recorded after 3 and 5 days of observation.

ECD Calculations

In order to clarify the absolute configurations of **1**, **2**, and **3**, computational methods were utilized. The DFT and TDDFT calculations were carried out in the gas phase with Gaussian 09 software.[25] The conformational analysis was initially performed using the GMMX program[26] with the MMFF94 force field. The selected conformer was optimized at B3LYP/6-31G(p) using DFT. The ECD calculations were conducted using the TDDFT method for 30 excited states at the B3LYP/6-311++G (d) level in the gas phase. The CD spectra were generated by the program SpecDis using a Gaussian band shape with 0.3 eV.

Calculated ^{13}C-NMR Data

Conformational analyses were performed by random searching with an energy cutoff of 3.5 kcal/ mol using the GMMX program software.[26] The MMFF94S force field was employed. The conformers were reoptimized in the gas phase at the DFT/B3LYP/6-31G(p) level

using the Gaussian 09 program. The ^{13}C-NMR shielding constants of **3** were calculated by the GIAO method at the mPW1PW91/6-311G(d) level of theory in the gas phase. The computational ^{13}C-NMR data were obtained by linear regression.

Acknowledgements

The authors thank Prof. Hiroyuki Konno and Prof. Takako Aboshi in Yamagata University for CD and MS experimental supports, respectively. This work was partly supported by MEXT KAKENHI Grant Number 19 K05709 (Y. S.) and the author (U. S.) was also supported by World Class Professor Grant Number T/83/D2.3/KK.04.05/2019, Indonesia).

Author Contribution Statement

Shiho Sato and Wataru Suehiro carried out the isolation and purification process, elucidated the chemical structure, and contributed to the biological activity assay. Desi Harneti, Rani Maharani, Unang Supratman, Fajar Fauzi Abdullah, and Supriatno Salam performed the experiments (sample collections), analyzed the data, and wrote the article. Ferry Ferdiansyah Sofian and Takuya Koseki analyzed the data (biological activity). Yoshihito Shiono elucidated the chemical structure, prepared the draft article, and conceived the project.

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Received November 15, 2020
Accepted January 15, 2021

