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Potato micro-tuber inducing hydroxylasiodiplodins from *Lasiodiplodia theobromae*

Qing Yang, Maki Asai, Hideyuki Matsuura, Teruhiko Yoshihara*

Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

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Abstract

Three hydroxylasiodiplodins were isolated from the mycelium extracts of *Lasiodiplodia theobromae* IFO 31059; and their structures were identified as (3*R*),(4*S*)-4-hydroxylasiodiplodin, (3*R*),(6*R*)-6-hydroxy-de-*O*-methyllasiodiplodin and (3*R*),(5*R*)-5-hydroxy-de-*O*-methyllasiodiplodin. All showed potato micro-tuber inducing activity. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Lasiodiplodia theobromae; Lasiodiplodin; Potato micro-tuber inducing substances

1. Introduction

The purpose of our research is to isolate potato micro-tuber inducing substances from microorganisms with the assistance of a bioassay using cultures of single-node segments of potato stems in vitro (Koda and Okazawa, 1998). *Lasiodiplodia theobromae* is a fungus (Aldridge et al., 1971) whose culture filtrate inhibits the growth of higher plants, and produces various organic metabolites, such as jasmonic acid, which has strong potato tuber inducing activity. Previous studies with *L. theobromae* IFO 31059 afforded some biologically active compounds, such as jasmonic acid, mellein, theobroxide (Nakamori et al., 1994) 5-oxolasiodiplodin (**1**) and 5-hydroxylasiodiplodins (**2a**) and (**2b**) (Matsuura et al., 1998) (Fig. 1). However, the bioassay results showed that there were still active fractions left uninvestigated which might yield biologically active compounds. As a result of successive studies, three previously unreported hydroxylasiodiplodins: (3*R*),(4*S*)-4-hydroxylasiodiplodin (**3**), (3*R*),(6*R*)-6-hydroxy-de-*O*-methyllasiodiplodin (**4**) and (3*R*),(5*R*)-5-hydroxy-de-*O*-methyllasiodiplodin (**5**) were obtained (Fig. 2). Their structures were determined by spectroscopic analysis and advanced Mosher method (Ohtani et al., 1991). We report herein the isolation, structural elucidation and potato micro-tuber inducing activity of these compounds.

2. Results and discussion

The ethyl acetate extract of the mycelium of *L. theobromae* IFO 31059 was separated by column chromatography. The fractions were subjected to a bioassay using single-node stem segments culture in vitro (Koda et al., 1988), with active fractions further purified by silica column chromatography, preparative TLC and HPLC to afford compounds **3**–**5**.

Compound **3** was obtained as a white powder and gave a molecular formula of $C_{17}H_{24}O_5$ $[M]^+$ by high resolution FD- and EI-mass spectrometry. The IR spectrum showed bands due to hydroxyl (3435 cm^{-1}), carbonyl (1735 cm^{-1}), aromatic ring (1578 cm^{-1}) and ester (1265 cm^{-1}) groups. The ^1H- and ^{13}C-NMR spectra of **3** resembled closely to those of **2**, the largest

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* Corresponding author. Tel.: +81-11-706-2505; fax: +81-11-706-2505.

E-mail address: yosihara@chem.agr.hokudai.ac.jp (T. Yoshihara).

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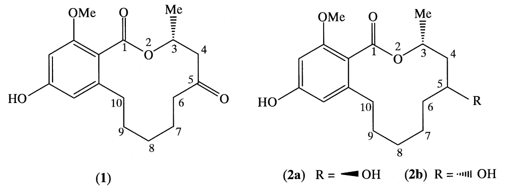


Fig. 1. Structures of 5-oxolasiodiplodin (**1**) and 5-hydroxylasiodiplodins (**2a**) and (**2b**).

difference being in the signals of H-3 (δ 4.73 for **3** and 5.34 for **2a**) and C-3 (δ 76.3 for **3** and 69.5 for **2a**). Compounds **3** and **2** appeared to be geometric isomers. Assignments of protons to the carbons were made in conjunction with HMBC and HMQC spectra (Tables 1 and 2). The ^1H–^1H COSY spectrum revealed cross peaks between H-17/H-3/H-4 (Fig. 2). The position of the hydroxyl group was determined to be at C-4 from the above analysis. The chemical structure of **3** was also confirmed by the NOE experiment (Figs. 3 and 4).

There were two asymmetric centers in **3** at C-3 and C-4, respectively. The absolute configuration at C-4 was determined by an advanced Mosher method (Ohtani et al., 1991), based on the analysis of differences in proton chemical shifts between the (*R*),(+)- and (*S*),(−)-MTPA esters of **3**. In this process, **3** was first methylated with iodomethane, and then converted to MTPA esters: (**3a**) [(*S*),(−)-MTPA ester] and (**3b**) [(*R*),(+)-MTPA ester]. The assignments of **3a** and **3b** were achieved by ^1H-NMR and ^1H–^1H COSY spectral analyses. The chemical shift differences between **3a** and **3b** (Table 3) indicated an *S* configuration at C-4. This was supported by the tendency of positive and negative values observed in the esters. The absolute configuration at C-3 was deduced from the relative configuration of C-3 with C-4 by the NOE experiment. It was determined as *R* from ^1H-NMR spin decoupling spectrum by irradiation of H-17 (δ 1.42) and H-4 (δ 3.62) showing the coupling constant to be 9.6 Hz between H-3 and H-4. Thus, the structure of **3** was proposed as (3*R*),(4*S*)-4-hydroxylasiodiplodin.

Table 1
^{13}C spectral data for compounds **3**–**5** (270 MHz, CD_3OD for **3** and **5**, CD_3COCD_3 for **4**)

Assignment	$\mathbf{3}^{a,b}$	$\mathbf{4}^{a,c}$	$\mathbf{5}^{a,b}$
1	171.9	173.1	173.1
3	76.3	76.8	73.4
4	76.2	32.5	41.9
5	31.9	37.5	70.7
6	23.9	66.0	35.7
7	27.2	33.2	19.5
8	28.6	23.9	29.1
9	32.6	31.8	33.0
10	32.1	34.4	35.0
11	145.1	150.0	150.1
12	110.8	111.7	112.2
13	162.3	163.6	163.9
14	99.3	101.8	101.9
15	160.9	166.9	166.6
16	118.8	105.1	105.5
17	19.8	21.9	21.7
$PhOCH_3$	57.7	–	–

[a] Assignments confirmed by two-dimensional experiments (COSY, HMQC and HMBC).

[b] CD_3OD signal at δ_C 49.0 as reference.

[c] CD_3COCD_3 signal at δ_C 206.5 as reference.

Compounds **4** and **5** were obtained as colorless powders. The infrared spectra indicated the presence of hydroxy (3425 cm^{-1}), conjugated carbonyl (1655 cm^{-1} for **4**, 1643 cm^{-1} for **5**), aromatic ring (1610 cm^{-1}) and ester (1251 cm^{-1}) moieties. The FD- and EI-mass spectra (MS) of **4** and **5** gave the same molecular formulae of $C_{16}H_{22}O_5$ $[M]^+$. The ^1H- and ^{13}C-NMR spectra of **4** and **5** bore good resemblance to that of 5-hydroxylasiodiplodins (**2**) except for the absence of a methoxyl signal in the NMR spectra, which suggested that compounds **4** and **5** were in the de-*O*-methyl lasiodiplodin group. The complete NMR spectral assignments of **4** and **5** are given in Tables 1 and 2 according to interpretation of the HMQC and HMBC spectra. The positions of the hydroxyl groups in **4** and **5** were determined to be at C-6 and C-5, respectively, based on analysis of their ^1H–^1H COSY spectra (Fig. 2). These interpretations were confirmed by NOE experiments (Fig. 3).

In order to determine the absolute configurations of the hydroxyl moieties, **4** and **5** were methylated with

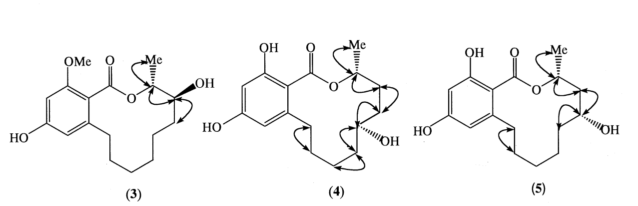


Fig. 2. Structures of (**3**)–(**5**) and their ^1H–^1H COSY experiments.

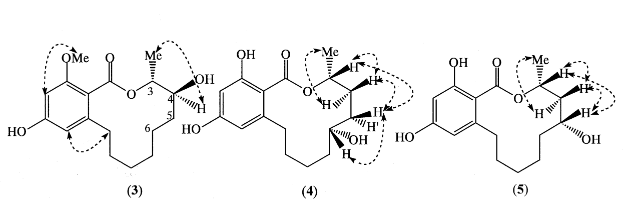


Fig. 3. NOE experiments of (**3**)–(**5**).

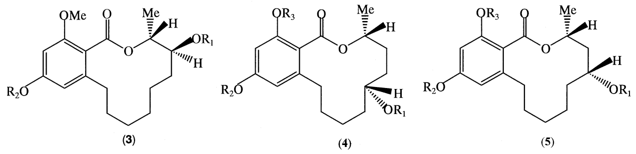
Table 2
^{1}H spectral data for compounds **3**–**5** (270 MHz, CD_3OD for **3** and **5**, CD_3COCD_3 for **4**)

Assignments	**3**^{a,b}	**4**^{a,c}	**5**^{a,b}
3	4.73 (*m*)	5.12 (*m*)	5.10 (*m*)
4	3.62 (*m*)	2.06 (*m*)	2.24 (*m*)
4′	–	1.67 (*m*)	1.62 (*m*)
5	1.82 (*m*)	1.70 (*m*)	4.00 (*m*)
5′	1.53 (*m*)	1.44 (*m*)	–
6	1.72 (*m*)	4.06 (*m*)	1.60 (*m*)
6′	1.72 (*m*)	–	1.30 (*m*)
7	1.34 (*m*)	1.85 (*m*)	1.50 (*m*)
7′	1.34 (*m*)	1.22 (*m*)	1.50 (*m*)
8	1.28 (*m*)	1.83 (*m*)	1.61 (*m*)
8′	1.28 (*m*)	1.60 (*m*)	1.42 (*m*)
9	1.62 (*m*)	1.63 (*m*)	1.50 (*m*)
9′	1.62 (*m*)	1.30 (*m*)	1.50 (*m*)
10	2.68 (*ddd*, $J = 13.6, 7.4, 3.7$ Hz)	3.70 (*m*)	3.68 (*m*)
10′	2.52 (*ddd*, $J = 13.5, 7.8, 3.7$ Hz)	2.20 (*m*)	2.05 (*m*)
12	6.23 (*d*, $J = 2.2$ Hz)	6.22 (*d*, $J = 2.6$ Hz)	6.08 (*d*, $J = 2.3$ Hz)
14	6.28 (*d*, $J = 2.2$ Hz)	6.28 (*d*, $J = 2.6$ Hz)	6.12 (*d*, $J = 2.3$ Hz)
17	1.42 (*d*, $J = 6.1$ Hz)	1.30 (*d*, $J = 6.6$ Hz)	1.28 (*d*, $J = 6.3$ Hz)
$PhOCH_3$	3.75 (*s*)	–	–

[a] Assignments confirmed by two-dimensional experiments (COSY, HMQC and HMBC).
[b] CD_3 signal at δ_H 4.78 as reference.
[c] CD_3COCD_3 signal at δ_H 206.5 as reference.

iodomethane, then converted to the corresponding MTPA esters: (**4a**) and (**5a**) [(*S*),(−)-MTPA esters], (**4b**) and (**5b**) [(*R*),(+)-MTPA esters]. The assignments of the MTPA esters were achieved by ^{1}H-NMR and ^{1}H–^{1}H COSY spectral analyses. The chemical shift differences between **4a** and **4b**, and **5a** and **5b** are shown in Table 3. According to the tendency of positive and negative values observed for the esters, both the absolute configurations of the hydroxyl groups of **4** and **5** were determined as *R*. The absolute configuration at chiral carbon 3 in both compounds **4** and **5** was deduced from the relative configuration of C-3 with another chiral carbon atom which was connected with a hydroxyl moiety. NOE and SPT experiments were applied to compounds **4** and **5**, respectively; the couplings between heterotopic protons on vicinal carbon atoms from C-3 up to another asymmetric center (C-6 for **4**, C-5 for **5**) were studied, and hence, the relative stereochemistry was defined.

In compound **4**, the SPT experiment was applied by irradiating H-17, H-5 and H-6, respectively, in order to measure the coupling constants between the vicinal protons. The *cis* protons H-3 and H-4′ and *trans* protons H-3 and H-4 were observed to have a value of 0 and 9 Hz, respectively. The values of about 4 Hz for H-4′ and H-5, 10 Hz for H-4 and H-5, 4 Hz for H-5



(**3**) (**4**) (**5**)

3 R_1=R_2=H
3a R_1=(-)-MTPA, R_2=CH_3
3b R_1=(+)-MTPA, R_2=CH_3

4 R_1=R_2=R_3=H
4a R_1=R_3=(-)-MTPA, R_2=CH_3
4b R_1=R_3=(+)-MTPA, R_2=CH_3

5 R_1=R_2=R_3=H
5a R_1=(-)-MTPA, R_2=R_3=CH_3
5b R_1=(+)-MTPA, R_2=R_3=CH_3

Fig. 4. MTPA derivatives of (**3**)–(**5**).

Table 3
^{1}H-NMR chemical shift differences between MTPA esters ($\Delta\delta = \delta(-) - \delta(+)$) of **3**–**5** (500 MHz, $CDCl_3$)

^{1}H (ppm)	**3**^{a}	**4**^{b}	**5**^{c}
H-3	0.05	−0.05	−0.04
H-4	0.03	−0.01	−0.05
H-4′	–	−0.06	−0.02
H-5	−0.04	−0.06	−0.03
H-5′	−0.01	−0.04	–
H-6	−0.02	0.03	0.11
H-6′	−0.02	–	0.03
H-7	−0.01	0.03	0.03
H-7′	−0.01	0.02	0.03
H-8	−0.02	0.03	0.10
H-8′	−0.02	0.02	0.03
H-9	−0.02	0.02	−0.02
H-9′	−0.02	0.02	0.03
H-10	−0.02	−0.14	−0.05
H-10′	−0.03	0.04	0.07
H-12	0.10	0.09	0.01
H-14	0	0	0.01
H-17	0.15	0	−0.06

[a] The $\Delta\delta$ values $(-\delta(-)-\delta(+))$ for **3**.
[b] The $\Delta\delta$ values $(\delta(-)-\delta(+))$ for **4**.
[c] The $\Delta\delta$ values $(\delta(-)-\delta(+))$ for **5**.

and H-6, and 9 Hz for H-5′ and H-6 suggested a *cis* correlation between H-4′ and H-5, as well as between H-5 and H-6. Based on the above analysis, the through-space relative correlation between H-3 and H-6 was proposed to be *cis*. Since the absolute configuration of C-6 was determined as *R*, therefore an *R* configuration was proposed for C-3. This conclusion was also confirmed by the NOE results of cross peaks H-17/H-4, H-3/H-5 and H-5/H-6 (Fig. 3). Thus, the structure of **4** was identified as (3*R*),(6*R*)-6-hydroxy-de-*O*-methyllasiodiplodin.

Compound **5** was studied in the same way as above. The coupling constant between H-3 and H-4 was determined to be 10 Hz, whereas the value of H-3 and H-4′ was found to be much smaller than 10 Hz. It was proposed that H-3 and H-4′ were vicinal *cis* protons, and H-3 and H-4 were vicinal *trans* protons. Furthermore, the correlation between H-4′ and H-5 was found to be *cis* with a value of about 6 Hz. Hence, it could be concluded that H-3 and H-5 were *cis* protons, and C-3 bore the same *R* configuration as C-5. The cross peaks H-3/H-4′/H-5 and H-17/H-4 observed in NOE (Fig. 3) also confirmed that H-3 and H-5, as well as H-17 and H-4, were aligned closely in space. Therefore, the structure of **5** was identified as (3*R*),(5*R*)-5-hydroxy-de-*O*-methyllasiodiplodin.

Our studies showed that the absolute stereochemistry of C-3 in the three hydroxylasiodiplodins was *R*. This conclusion was in accordance with the results of some successful synthesis of lasiodiplodin published in literature (Bracher and Schulte, 1996; Fürstner and Kindler, 1996; Fürstner et al., 1999; Jones and Huber, 1993; Jones et al., 1997) which confirmed an *R* configuration at C-3 in natural lasiodiplodin and de-*O*-methyl lasiodiplodin. Hence, the *S* configuration of C-3 in **1**, **2a** and **2b** earlier reported by us (Matsuura et al., 1998) has to be revised to *R*.

Using bioassay, compounds **3**–**5** were found to be active in potato micro-tuber induction at a concentration of 10^{-4} M, and the activity of **3** was stronger than that of the others. Under the same conditions, the activities of jasmonic acid and theobroxide, which are obtained from the same fungus, are observed at the concentration of 10^{-6} and 10^{-5} M, respectively. The negative control (using medium without any added compounds) showed no activity at all.

3. Experimental

3.1. General

Spectra were obtained with the following instruments: IR, Hitachi 285 spectrometer; optical rotations, JASCO DIP-4 polarimeter; NMR, Bruker AM-500FT-NMR spectrometer and JEOL JNM-EX 270 FT-NMR system; FD-and EI-MS, JEOL JMS01SG-2 and JMS-DX-300 mass spectrometers, respectively. Melting points were measured with a micro melting point apparatus, Yanaco.

3.2. Cultures and isolation

The fungus was cultured in a stationary flask containing 3% potato-sucrose medium 200 ml at 25°C in the dark for 35 days. The mycelium from 20 l culture filtrates was immersed in acetone for 3 months. The acetone filtrate was concentrated to nearly 1 l in vacuo and extracted with EtOAc. Evaporation of the EtOAc extract afforded a brown residue (11 g). The residue was subjected to chromatographic column (Si) and eluted with $CHCl_3$ (3 l), MeOH–$CHCl_3$ (3:97, v/v, 2 l), MeOH–$CHCl_3$ (20:80, v/v, 2 l), MeOH (2 l) successively. Every liter of eluate was collected as one aliquot. The potato micro-tuber inducing activity was observed in the two aliquots of 3% MeOH in $CHCl_3$.

3.3. Isolation of (3*R*),(4*S*)-4-hydroxylasiodiplodin (**3**)

The first aliquot of 3% MeOH in $CHCl_3$ eluate was concentrated under reduced pressure, the residue was separated using column chromatography (Si eluted with *n*-hexane–EtOAc, 50:50) and preparative TLC (silica gel 60 F_{254}, MeOH–$CHCl_3$, 8:92, R_f value = 0.40) successively. The fraction (10 mg) obtained from preparative TLC was purified further by HPLC (Wakosil-II 5C18 4.6 × 250 mm, MeOH–H_2O (containing 0.2% AcOH), 70:30, v/v, 0.5 ml/min, UV detector 210 nm, retention time: 11.4 min) to afford **3** (2 mg). $[\alpha]_D^{25}$ +2.0° (MeOH; *c* 1.0), mp 217–218°C: EI-MS *m*/*z* (rel. int.): 308 $[M]^+$ (41). 290 $[M - H_2O]^+$ (46), 264 (20), 236 (20), 235 (20), 220 (7), 191 (55), 177 (100), 138 (60), 121 (10), 107 (10), 91 (10), 77 (12), 57 (10), 44 (17); FD-MS *m*/*z*: 308 $[M]^+$ (100); FD-HR-MS *m*/*z*: 308.1611 $[M^+]$ (calcd. for $C_{17}H_{24}$ O_5: 308.1624): IR ν_{max}^{film} cm^{-1}: 3435. 2900, 1735, 1578, 1420, 1260, 1021; ^1H- and ^{13}C-NMR: Tables 1 and 2.

3.4. Isolation of (3*R*),(6*R*)-6-hydroxy-de-*O*-methyllasiodiplodin (**4**) and (3*R*),(5*R*)-5-hydroxy-de-*O*-methyllasiodiplodin (**5**)

After solvent evaporation in vacuo, the residue of the second aliquot of 3% MeOH in $CHCl_3$ eluate was applied to preparative TLC (MeOH–$CHCl_3$, 5:95, v/v, R_f value = 0.38) and silica column chromatography (*n*-hexane–EtOAc, 50:50), respectively to, finally afford two active fractions A (R_f value = 0.28) and B (R_f value = 0.40). Fraction A (15.7 mg) was purified by HPLC with a C_{18} column (Wakosil-II, 4.6 × 250 mm), eluted with a gradient mode of 70% methanol: 30% water containing 0.2% acetic acid to 100% methanol.

The eluate was monitored at 210 nm with a flow rate of 0.5 ml/min. Compound **4** (4 mg) was collected at retention time 18.2 min. $[\alpha]_D^{25}$ −5.36° (MeOH; *c* 0.4); mp 200–201°C ; EI-MS *m*/*z* (rel.int): 294$[M]^+$ (56), 276 $[M-H_2O]^+$ (9), 258 (15), 207 (42), 178 (40), 168 (49), 150 (100), 121 (21), 99 (10), 81 (17), 69 (24), 55 (21), 41 (20); FD-MS *m*/*z*: 294 $[M]^+$ (100); EI-HR-MS *m*/*z*: 294.1443 $[M]^+$ (calcd. for $C_{16}H_{22}O_5$: 294.1468); IR ν_{max}^{film} cm^{-1}: 3425, 1655, 1610, 1251; ^1H- and ^{13}C-NMR: Tables 1 and 2.

Fraction B 6.3 mg was purified by HPLC in the same way as that of fraction A to give compound **5** (2.1 mg, retention time: 17.1 min). $[\alpha]_D^{25}$ +19.6° (MeOH; *c* 0.5); mp 158–160°C ; EI-MS *m*/*z* (rel. int.): 294 $[M]^+$ (71), 276 $[M - H_2O]^+$ (0.1), 258 (36), 206 (43), 168 (100), 163 (71), 150 (60), 123 (15), 121 (13), 69 (16), 41 (11); FD-MS *m*/*z*: 294 $[M]^+$ (100); EI-HR-MS *m*/*z*: 294.1448 $[M]^+$ (calcd. for $C_{16}H_{22}O_5$: 294.1468); IR ν_{max}^{film} cm^{-1}: 3425, 1643, 1610, 1251; ^1H- and ^{13}C-NMR: Tables 1 and 2.

3.5. Preparation of (3a) and (3b)

A solution of compound **3** (1 mg, 3 μmol), anhydrous potassium carbonate (2.5 mg, 1.8 μmol) and iodomethane (three drops) in dry acetone (200 μl) was stirred at 50–60°C for 3 h. The reaction mixture was concentrated in vacuo, and the residue was extracted three times with *n*-hexane (3 ml × 3). After evaporating the *n*-hexane, the methyl derivative of **3** (0.9 mg, 2.8 μmol) was obtained. To a stirred solution of the resultant methyl derivative (0.45 mg, 1.4 μmol) in dry methylene dichloride (200 μl), DMAP (0.34 mg, 2.8 μmol), pyridine (one drop) and MTPACl (one drop) [(*R*),(−)-MTPACl for **3a** and (*S*),(+)-MTPACl for **3b**] were added. After 22 h, the reaction mix was concentrated in vacuo. The residue was subjected to preparative TLC on silica gel using *n*-hexane–EtOAc (5:1) to give the (*S*)-MTPA ester **3a** and (*R*)-MTPA ester **3b**, respectively.

Compound **3a**. FD-MS *m*/*z*: 538 $[M]^+$, ^1H-NMR (500 MHz, $CDCl_3$): δ 7.53 (2H, *m*), 7.41 (3H, *m*), 6.38 (2H, *s*), 5.18 (1H, *m*), 5.06 (1H, *m*), 3.90 (3H, *S*), 3.84 (3H, *S*), 3.54 (3H, *S*), 2.88 (1H, *m*), 2.72 (1H, *m*), 1.88 (1H, *m*), 1.62 (4H, *m*), 1.49 (1H, *m*), 1.36 (3H, *d*, *J* = 6.0 Hz), 1.27 (2H, *m*), 1.25 (2H, *m*).

Compound **3b**. FD-MS *m*/*z*: 538 $[M]^+$, ^1H-NMR (500 MHz, $CDCl_3$): δ 7.52 (2H, *m*), 7.40 (3H, *m*), 6.38 (1H, *s*), 6.28 (1H, *s*), 5.15 (1H, *m*), 5.01 (1H, *m*), 3.90 (3H, *s*), 3.80 (3H, *s*), 3.56 (3H, *s*), 2.90 (1H, *m*), 2.75 (1H, *m*), 1.92 (1H, *m*), 1.64 (4H, *m*), 1.50 (1H, *m*), 1.28 (2H, *m*), 1.27 (2H, *m*), 1.21 (3H, *d*, *J* = 6.0 Hz)

3.6. Preparation of (4a) and (4b)

Compound **4** (1 mg, 3.4 μmol) was methylated in the same manner as that used for compound **3**. However, it failed to methylate both hydroxy moieties on the benzene ring, even if excess iodomethane was used for prolonged time. Anyway, the monomethyl derivative of **4** was obtained (1 mg, 3.2 μmol) and its MTPA esters [(*R*),(−)-MTPACl for **4a** and (*S*),(+)-MTPACl for **4b**] were prepared as described above. The purification employing preparative TLC (*n*-hexane–EtOAc, 5:1) gave **4a** and **4b**, respectively.

Compound **4a**. FD-MS *m*/*z*: 740 $[M]^+$, ^1H-NMR (500 MHz, $CDCl_3$): δ 7.67 (2H, *m*), 7.51 (2H, *m*), 7.43 (3H, *m*), 7.39 (3H, *m*), 6.63 (1H, *d*, *J* = 2.3 Hz), 6.46 (1H, *d*, *J* = 2.3 Hz), 5.34 (1H, *m*), 5.17 (1H, *m*), 3.78 (3H, *s*), 3.64 (3H, *s*), 3.54 (3H, *s*), 3.25 (1H, *m*), 2.52 (1H, *m*), 2.15 (1H, *m*), 1.92 (2H, *m*), 1.66 (2H, *m*), 1.54 (2H, *m*), 1.27 (1H, *m*), 1.25 (2H, *m*), 1.24 (3H, *d*, *J* = 6.2 Hz).

Compound **4b**. FD-MS *m*/*z*: 740 $[M]^+$, ^1H-NMR (500 MHz, $CDCl_3$): δ 7.66 (2H, *m*), 7.50 (2H, *m*), 7.42 (3H, *m*), 7.39 (3H, *m*), 6.63 (1H, *d*, *J* = 2.3 Hz), 6.37 (1H, *d*, *J* = 2.3 Hz), 5.39 (1H, *m*), 5.14 (1H, *m*), 3.76 (3H, *s*), 3.71 (3H, *s*), 3.54 (3H, *s*), 3.39 (1H, *m*), 2.48 (1H, *m*), 2.16 (1H, *m*), 1.89 (2H, *m*), 1.72 (2H, *m*), 1.52 (2H, *m*), 1.31 (1H, *m*), 1.24 (3H, *d*, *J* = 6.2 Hz), 1.23 (2H, *m*).

3.7. Preparation of (5a) and (5b)

The methylation of compound **5** was achieved with both hydroxy moieties methylated by the same way as that used for **3**. This methyl derivative was used for the preparation of MTPA esters [(*R*),(−)-MTPACl for **5a** and (*S*),(+)-MTPACl for **5b**]. The usual work-up followed by purification using preparative TLC (*n*-hexane–EtOAc, 5:1) gave **5a** and **5b**, respectively.

Compound **5a**. FD-MS *m*/*z*: 538 $[M]^+$, ^1H-NMR (500 MHz, $CDCl_3$): δ 7.51 (2H, *m*), 7.38 (3H, *m*), 6.30 (2H, *s*), 5.38 (1H, *m*), 5.24 (1H, *m*), 3.79 (3H, *s*), 3.77 (3H, *s*), 3.53 (3H, *s*), 2.62 (2H, *m*), 2.25 (1H, *m*), 1.77 (2H, *m*), 1.64 (2H, *m*), 1.57 (5H, *m*), 1.27 (3H, *d*, *J* = 6.8 Hz).

Compound **5b**. FD-MS *m*/*z*: 538 $[M]^+$, ^1H-NMR (500 MHz, $CDCl_3$): δ 7.50 (2H, *m*), 7.39 (3H, *m*), 6.29 (2H, *s*), 5.41 (1H, *m*), 5.28 (1H, *m*), 3.79 (3H, *s*), 3.78 (3H, *s*), 3.52 (3H, *s*), 2.67 (1H, *m*), 2.55 (1H, *m*), 2.30 (1H, *m*), 1.66 (4H, *m*), 1.54 (5H, *m*), 1.36 (3H, *d*, *J* = 6.6 Hz).

3.8. Bioassay

Potato micro-tuber inducing activity was assayed using cultures of single-node segments of potato stems in vitro, as reported previously (Koda et al., 1988). In brief, single-node segments prepared from etiolated potato shoots (*Solanum tuberosum* L. cv Irish Cobber) were sterilized in a 1% solution of sodium hypochlorite for 1 h. Then three segments were planted in a 100 ml flask that contained 10 ml of medium (usually White's medium) supplement with the compound to be tested. The concentration of sucrose in the medium was 2% by weight. The medium was adjusted to pH 5.6 and solidified with 0.6% Bactoagar before being autoclaved. The cultures were maintained at 25°C in the dark for 3 weeks, and the rate of tuberization was calculated as the number of tuberized laterals divided by the total number of laterals that had emerged.

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