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Note

Novel Potato Micro-Tuber-Inducing Compound, (3*R*,6*S*)-6-Hydroxylasiodiplodin, from a Strain of *Lasiodiplodia theobromae*

Peng LI, Kosaku TAKAHASHI,† Hideyuki MATSUURA, and Teruhiko YOSHIHARA

Laboratory of Bio-Organic Chemistry, Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo, Hokkaido 060-8589, Japan

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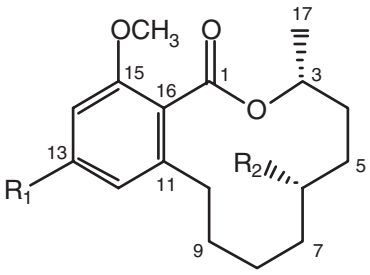
A novel potato micro-tuber-inducing compound was isolated from the culture broth of *Lasiodiplodia theobromae* Shimokita 2. The structure of the isolated compound was determined as (3*R*,6*S*)-6-hydroxylasiodiplodin by means of spectroscopic analyses, the modified Mosher method, and chemical conversion. The compound showed potato micro-tuber-inducing activity at a concentration of 10^{-4} M, using the culture of single-node segments of potato stems *in vitro*.

Key words: (3*R*,6*S*)-6-hydroxylasiodiplodin; potato micro-tuber-inducing activity; *Lasiodiplodia theobromae*

Lasiodiplodia theobromae (synonym *Botryodiplodia theobromae*) is a common pathogenic fungus found in the tropics and subtropics[1] on a great variety of host plants. This fungus causes numerous diseases, in particular causing rotting of fruits and root crops during storage. The culture filtrate of *L. theobromae* showed inhibitory activity for plant growth.[2] For several decades, this fungus has been the focus of considerable attention, and a variety of bioactive compounds for plants have been isolated from its culture filtrate. We have intensively studied in our laboratory some novel potato micro-tuber-inducing substances derived from *L. theobromae*.[3–5] However, bioassay results indicated that there were still active strains of *L. theobromae* previously uninvestigated which might yield biologically active compounds. During our screening for potato micro-tuber-inducing substances from strains of *L. theobromae*, a novel lasiodiplodin-related compound was found in the culture of the Shimokita 2 strain of *L. theobromae*. We describe here the isolation, structural elucidation, and biological activity of this compound.

Bioassay-guided fractionation was carried out during the isolation procedure for the micro-tuber-inducing compound, using a culture of single-node segments of potato stems. *L. theobromae* Shimokita 2 was isolated from tissue specimens collected from rotted mango branches on Miyako island in Japan. This fungus was statically incubated in one hundred 500-ml Erlenmeyer flasks containing 200 ml of a potato sucrose medium (3% sucrose) at 25 °C for 3 weeks. This culture broth (20-liter) was filtered through three layers of gauze and then concentrated to 1-liter. The filtrate was extracted with 1-liter of ethyl acetate, and the resulting extract was concentrated under reduced pressure to afford a brown residue (12 g). This residue was subjected to silica gel column chromatography, and successively eluted with $CHCl_3$, $CHCl_3$–MeOH (97:3), $CHCl_3$–MeOH (80:20) and MeOH. An active fraction (4 g) eluted with $CHCl_3$–MeOH (97:3) was subjected to silica gel column chromatography, using *n*-hexane–EtOAc (1:3). The fraction showing activity (154 mg) was further purified by crystallization to obtain compound **1** as colorless needles (75 mg).

The molecular formula was established as $C_{17}H_{24}O_5$ on the basis of HREI-MS data. This formula is identical to those of 5-hydroxylasiodiplodins.[4] The value for the specific rotation was +12.0° (*c* 0.1, MeOH, 25 °C). The IR spectrum of **1** revealed the presence of hydroxyl groups at 3375 cm^{-1}, a conjugated carbonyl group at 1677 cm^{-1}, and an aromatic ring at 1605 cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra of **1** were similar to those of 5-



1: R_1=OH, R_2=OH
2: R_1=OCH_3, R_2=O(-)-MTPA
3: R_1=OCH_3, R_2=O(+)-MTPA
4: R_1=OH, R_2=H

Fig. 1. Structures of (3*R*,6*S*)-6-Hydroxylasiodiplodin (**1**), **2**, **3** and **4**.

† To whom correspondence should be addressed. Tel: +81-11-706-3349; Fax: +81-11-706-2505; E-mail: kosaku@chem.agr.hokudai.ac.jp

hydroxylasiodiplodins.[4] The ^{1}H-NMR spectrum showed a pair of aromatic proton signals at δ_H 6.22 (1H, br.s) and δ_H 6.26 (1H, br.s). In addition, this spectrum contained a methoxy signal at δ_H 3.68 (3H, s) and a proton signal at δ_H 5.10 (1H, m) derived from a methine neighboring an ester group. The ^{13}C-NMR and DEPT spectra of **1** revealed 17 signals classified as two methyls, six methylenes, four methines, and five quaternary carbons, including one carbonyl carbon. Six degrees of unsaturation were inferred from the molecular formula. The HMBC data demonstrated correlations between $PhOCH_3$/C-15, OH-13/C-13, C14, and H-14/C-13, C-15, suggesting that a methoxy group was located *meta* to the phenolic hydroxyl group. Other key HMBC correlations were found between H-3/C-1, C-4, C-5, H-5a, H-5b/C-4, C-6, and OH (δ_H 4.31)/C-5, C-6, C-7; it was thus shown that the hydroxyl group at δ_H 4.31 was connected with C-6. Other HMBC correlations are shown in Table 1. Taking into consideration the degree of unsaturation of **1**, the planar structure of **1** was determined to be that of 6-hydroxylasiodiplodin.

The absolute configuration at C-6 of **1** was determined by a modified Mosher method.[6] In this process, compound **1** was first methylated with diazomethane, and converted to (−)-MTPA ester **2** and (+)-MTPA ester **3**. The assignments of **2** and **3** were achieved by ^{1}H-NMR and COSY spectral analyses, the proton chemical shift differences between **2** and **3** being listed in Table 2. The absolute configuration at C-6 of **1** was determined as (*S*) from tendency observed in the positive and negative values for the esters.

Table 1. NMR Spectral Data for **1** in DMSO-d_6

No.	δ_H[a]	δ_C[b]	HMBC
1		167.9	3
3	5.10 (1H, m)	71.3	4a, 4b, 5a, 5b, 17
4a	1.54 (1H, m)	28.4	3, 5a, 5b, 6, 17
b	1.95 (1H, m)		
5a	1.46 (1H, m)	29.5	3, 4a, 4b, 7a, 7b, 6-OH
b	1.60 (1H, m)		
6	3.58 (1H, m)	68.7	4b, 5a, 5b, 7a, 7b, 6-OH
7a	1.12 (1H, m)	32.3	5a, 5b, 9a, 9b, 6-OH
b	1.52 (1H, m)		
8a	1.11 (1H, m)	22.6	7a, 7b, 9a, 9b, 10a, 10b
b	1.26 (1H, m)		
9a	1.51 (1H, m)	29.9	7a, 8a, 10a, 10b
b	1.55 (1H, m)		
10a	2.36 (1H, m)	29.3	12
b	2.49 (1H, m)		
11		141.8	9a, 9b, 10a, 10b
12	6.22 (1H, br.s)	107.8	10a, 10b, 14, 13-OH
13		159.3	12, 14, 13-OH
14	6.26 (1H, br.s)	97.1	12, 13-OH
15		157.4	14, $PhOCH_3$
16		116.2	10a, 10b, 12, 14
17	1.21 (3H, d, J = 6.2 Hz)	18.8	4a
$PhOCH_3$	3.69 (3H, s)	55.8	
6-OH	4.31 (1H, d, J = 4.4 Hz)		
13-OH	9.66 (1H, s)		

[a], ^{1}H-NMR at 500 MHz referenced to DMSO-d_6 (δ2.49).
[b], ^{13}C-NMR at 125 MHz referenced to DMSO-d_6 (δ39.5).

Table 2. $\Delta\delta$ values [$\delta(-)$–$\delta(+)$] for the MTPA Esters of **2** and **3** in $CDCl_3$

No.	$\Delta\delta$values	No.	$\Delta\delta$values
H-3	0.04	H-8a	−0.10
H-4a	0.12	H-8b	−0.09
H-4b	0.09	H-9a	−0.06
H-5a	0.07	H-9b	−0.06
H-5b	0.11	H-10a	−0.03
H-7a	−0.04	H-10b	−0.04
H-7b	−0.03	H-17	0.04

To determine the absolute configuration at C-3, chemical conversion of **1** to lasiodiplodin (**4**) was attempted. Compound **1** was reacted with thionyl chloride to dehydrate the alcohol, and was then hydrogenated to afford compound **4**. The NMR and FD-mass spectrometric data for compound **4** were consistent with those of lasiodiplodin.[7,8] Moreover, the value for the specific rotation of **4** was +1.9° (*c* 0.1, $CHCl_3$, 25 °C), similar to the value for an authentic sample of lasiodiplodin of +2.0° under the same conditions. The absolute configuration of C-3 in **4** was therefore determined to be (*R*), so that the structure of **1** was that of (3*R*,6*S*)-6-hydroxylasiodiplodin.

A bioassay, using cultures of single-node segments of potato stems *in vitro*, indicated compound **1** to have activity at a concentration of 10^{-4} M. Under the same conditions, the activity of jasmonic acid was at a concentration of 10^{-5} M.

Experimental

General. IR spectra were measured with a Perkin-Elmer 2000 FT-IR spectrometer, and mass spectra were obtained with a Jeol JMS-SX102A mass spectrometer. Specific rotation values were measured with a Jasco DIP-370 digital polarimeter, and NMR spectra were recorded by a Brucker AMX-500 FT-NMR spectrometer.

Bioassay. The potato micro-tuber-inducing activity was evaluated by the method using cultures of single-node segments of the potato stem *in vitro* as previously described.[9]

(3*R*,6*S*)-6-hydroxylasiodiplodin (**1**): colorless needles; melting point: 235–236 °C; $[\alpha]_D^{25}$ +12.0° (*c* 0.1, MeOH); EI-MS *m/z* (rel.int): 308 $[M]^+$ (86), 290 $[M-H_2O]^+$ (21), 235 (50), 177 (100), 138 (89); EI-HR-MS *m/z*: 308.1630 $[M]^+$ (calcd. for $C_{17}H_{24}O_5$, 308.1624); IR ν_{max}^{film} cm^{-1}: 3375, 1677, 1605, 1199; ^{1}H- and ^{13}C-NMR data are shown in Table 1.

Preparation of 2 and 3. Compound **1** (20 mg) was methylated by diazomethane in MeOH (5 ml) at 0 °C for three hours, and after evaporating MeOH, the methylated derivative (11.3 mg) was obtained. To 5 mg of the resulting methylated derivative dissolved in pyridine were added DMAP (2 mg) and MTPA chloride (5 mg) [(−)-MTPA chloride for **2** and (+)-MTPA chrolide for

3]. After 48 hours, each reaction mixture was concentrated *in vacuo*. Each residue was subjected to HPLC analysis [Capcellpak C_{18} column, 4.6 mm × 250 mm; flow rate, 1.0 ml/min; UV detection, 280 nm; solvent, MeOH–H_2O (6:4)], 2.5 mg of (−)-MTPA ester **2**, and 3.0 mg of (+)-MTPA ester **3** being obtained. (−)-MTPA ester (**2**). FD-MS *m/z*: 538 $[M]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.49 (2H, m), 7.37 (3H, m), 6.29 (1H, s), 6.28 (1H, s), 5.29 (1H, m, H-3), 5.16 (1H, m, H-6), 3.78 (3H, s), 3.77 (3H, s), 3.51 (3H, s), 2.67 (1H, m, H-10b), 2.48 (1H, m, H-10a), 2.13 (1H, m, H-4b), 2.00 (1H, m, H-5b), 1.80 (1H, m, H-7b), 1.72 (1H, m, H-5a), 1.70 (1H, m, H-4a), 1.62 (2H, m, H_2-9, overlapped), 1.41 (1H, m, H-7a), 1.36 (1H, d, $J = 6.2$ Hz, H-17), 1.29 (1H, m, H-8b), 1.08 (1H, m, H-8a).

(+)-MTPA eater (**3**). FD-MS *m/z*: 538 $[M]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.47 (2H, m), 7.38 (3H, m), 6.29 (2H, s), 5.25 (1H, m, H-3), 5.15 (1H, m, H-6), 3.78 (3H, s), 3.77 (3H, s), 3.51 (3H, s) 2.71 (1H, m, H-10b), 2.51 (1H, m, H-10a), 2.04 (1H, m, H-4b), 1.89 (1H, m, H-5b), 1.83 (1H, m, H-7b), 1.68 (2H, m, H_2-9, overlapped), 1.65 (1H, m, H-5a), 1.58 (1H, m, H-4a), 1.45 (1H, m, H-7a), 1.38 (1H, m, H-8b), 1.32 (1H, d, $J = 6.2$ Hz, H-17), 1.18 (1H, m, H-8a).

*Preparation of **4** from **1***. To a stirred solution of compound **1** (30 mg) in pyridine (2 ml) was added thionyl chloride (0.2 ml) at 0 °C, and the mixture was stirred for 20 hours. This reaction mixture was poured into ice-cooled water (50 ml) and extracted with diethyl ether (50 ml). The organic layer was washed with a saturated NaCl aqueous solution (50 ml) and dried over Na_2SO_4. The organic layer was then concentrated under reduced pressure to afford an oil. The resulting oil was purified by PTLC [*n*-hexane–EtOAc (1:1)] to give a dehydrated mixture (9.5 mg). To a stirred solution of this mixture in MeOH (2 ml) at room temperature, Pd-C (2 mg) was added in the presence of hydrogen gas (H_2), and the mixture left for 3 hours. The reaction mixture was filtered through Celite and purified by PTLC [*n*-hexane–EtOAc (1:1)] to give **4** (2.5 mg) as a colorless oil. Compound **4**. $[\alpha]_D^{25}$ +1.9° (*c* 0.1, $CHCl_3$); FD-MS *m/z*: 292 $[M]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.25 (1H, d, $J = 2.2$ Hz), 6.22 (1H, d, $J = 2.2$ Hz), 5.30 (1H, m), 3.77 (3H, s), 2.63 (1H, m), 2.46 (1H, m), 1.34 (3H, d, $J = 6.4$ Hz), 1.25–1.93 (12H, m); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 167.4, 157.8, 157.8, 142.9, 118.9, 108.3, 96.8, 72.1, 55.9, 32.4, 30.4, 30.1, 26.5, 25.5, 24.2, 21.3, 19.5.

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