Pergamon

PII: S0031-9422(98)00267-2

Phytochemistry, Vol. 49, No. 2, pp. 579–584, 1998

Printed in Great Britain
0031-9422/98/$-see front matter

THREE LASIODIPLODINS FROM *LASIODIPLODIA THEOBROMAE* IFO 31059

IN HONOUR OF PROFESSOR G. H. NEIL TOWERS 75TH BIRTHDAY

HIDEYUKI MATSUURA, KAORU NAKAMORI, E. A. OMER, CHIAKI HATAKEYAMA, TERUHIKO YOSHIHARA* and AKITAMI ICHIHARA

Department of Bioscience and Chemistry, Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan

(*Received* 21 *October* 1997; *received in revised form* 25 *February* 1998)

Key Word Index—*Lasiodiplodia theobromae*; potato micro-tuber inducing substances; lasiodiplodin.

Abstract—Three new fungal metabolites were isolated from the culture filtrate of the fungus, *Lasiodiplodia theobromae* IFO 31059 and identified as (5*R*) and (5*S*) 5-hydroxylasiodiplodins and 5-oxolasiodiplodin. The first two showed weak potato micro-tuber inducing activities, although only at very high dosage levels. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

In a previous paper [1], potato micro-tuber inducing substances, theobroxide, jasmonic acid and mellein from the culture filtrates of the fungus, *Lasiodiplodia theobromae* IFO 31059 were reported. Additional studies led us to isolate three hitherto unreported 12 member lactones from this fungus, and their structures were determined by means of ^{1}H-NMR, ^{13}C-NMR, DEPT, 2D-NMR and NOE experiments together with the advanced Mosher method and chemical conversions. Potato micro-tuber inducing activities were tested according to the method which utilises cultures of single-node segments of potato stems *in vitro* [2].

RESULTS AND DISCUSSION

The EtOAc extracts of the culture filtrates of *Lasiodiplodia theobromae* IFO 31059 were separated by CC on silica gel to afford 5-oxolasiodiplodin (**1**). Another culture filtrate of the same fungus was subjected to CC on charcoal, which following elution with EtOH gave (5*S*) 5-hydroxylasiodiplodin (**2**) and (5*R*) 5-hydroxylasiodiplodin (**3**).

Compound (**1**) was obtained as a white powder and gave a molecular formula of $C_{17}H_{22}O_5[M]^+$ by high resolution EI-mass spectrometry. Its IR spectrum showed bands due to hydroxyl (3390 cm^{-1}), carbonyl (1715 cm^{-1}), aromatic ring (1607 cm^{-1}) and ester (1260 cm^{-1}) groups. Its ^{1}H-NMR and ^{13}C-NMR spectra bore good resemblance to that of lasiodiplodin (**4**), which was isolated from *Lasiodiplodia theobromae* [3], except for the ^{1}H signals of H-4 (δ 2.88 and 2.64), H-6 (δ 2.47) and the ^{13}C resonance of C-5 (δ 210.2). Both the ^{13}C-NMR spectral and DEPT analyses revealed that **1** contained a ketone moiety (C-5, δ 210.2). Cross peaks between H-17/H-3/H-4 were also observed in the ^{1}H-^{1}H COSY spectrum. Irradiation of H-4 (δ 2.88 and 2.64) caused signal enhancements at C-3 (δ 69.1) and C-5 (δ 210.2) in an INAPT experiment, and irradiation of H-3 (δ 5.65) gave signal enhancements at C-1 (δ 168.0), C-4 (δ 49.1) and C-17 (δ 20.2). Accordingly, the structure of **1** is proposed as 5-oxolasiodiplodin (Fig. 1); Tables 1 and 2 give the complete ^{1}H-^{13}C NMR spectral analysis. Its structure was also confirmed by HMBC analysis, NOE experiments (Fig. 1) and by chemical conversion (Schemes 1 and 2). The absolute configuration at C-3 was determined as shown in Schemes 1 and 2. Lasiodiplodin (**4**), whose absolute configuration at C-3 was previously determined as (3*S*) [4], was converted to (2*S*) 9-(3′,5′-dimethoxy-2′-hydroxymethyl) phenyl-2-nonanol (**5**) (Scheme 1), whose $[\alpha]_D^{23}$ is $-5.6°$ ($CHCl_3$; c 2.85). Reduction of **1** gave the 5-hydroxylasiodiplodin intermediates which were converted into a compound apparently identical to **5**. Comparison of its ^{1}H-NMR and IR spectrum, and the EI-mass spectrometric fragmentation pattern of one substance gave good accordance to **5**, although the measured $[\alpha]_D^{25}$ was $-8.6°$ ($CHCl_3$; c 1.35 rather than $-5.6°$). Its absolute con-

* Author to whom correspondence should be addressed.

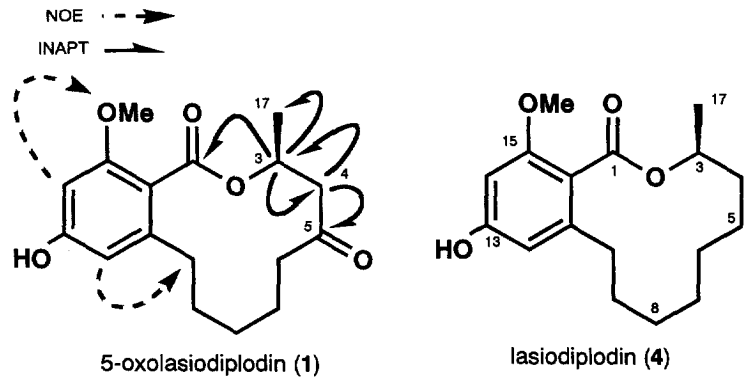
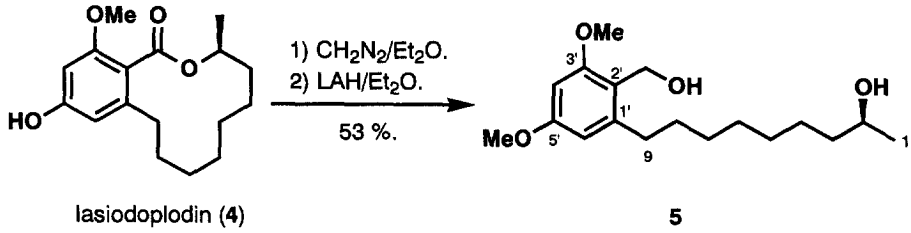


Fig. 1. The structures of **1** and **4** and INAPT and NOE experiments.

Table 1. The ^{1}H-NMR assignments for **1**, **2** and **3** (500 MHz, $CDCl_3$)

^{1}H (ppm)	1	2	3
H-3	5.65 (*ddq*, *J* = 10.3, 6.4, 2.8 Hz)	5.34 (*m*)	5.42 (*m*)
H-4a	2.88 (*dd*, *J* = 14.7, 10.4 Hz)	1.85 (*ddd*, *J* = 15.0, 6.1, 4.8 Hz)	1.81 (*ddd*, *J* = 14.8, 5.2, 1.3 Hz)
H-4b	2.64(*dd*, *J* = 14.7, 2.8 Hz)	2.15 (*ddd*, *J* = 15.0, 6.3, 3.5 Hz)	2.15 (*ddd*, *J* = 14.8, 10.1, 6.5 Hz)
H-5		4.07 (*m*)	3.86 (*m*)
H-6a	2.47 (*m*)	1.51 (*m*)	1.55 (*m*)
H-6b	2.47 (*m*)	1.67 (*m*)	1.65 (*m*)
H-7a	1.90 (*m*)	1.40 (*m*)[a]	1.42 (*m*)
H-7b	1.63 (*m*)	1.40 (*m*)[a]	1.42 (*m*)
H-8a	1.43 (*m*)	1.29 (*m*)[a]	1.42 (*m*)
H-8b	1.36 (*m*)	1.29 (*m*)[a]	1.42 (*m*)
H-9a	1.51 (*m*)	1.60 (*m*)	1.55 (*m*)
H-9b	1.51 (*m*)	1.60 (*m*)	1.65 (*m*)
H-10a	2.51 (*m*)	2.54 (*ddd*, *J* = 13.6, 6.0, 6.0 Hz)	2.44 (*ddd*, *J* = 12.0, 10.7, 5.5 Hz)
H-10b	2.30 (*m*)	2.65 (*ddd*, *J* = 13.6, 6.0, 6.0 Hz)	2.65 (*ddd*, *J* = 12.0, 10.7, 5.5 Hz)
H-12	6.19 (*d*, *J* = 2.1 Hz)	6.22 (*d*, *J* = 2.0 Hz)	6.22 (*d*, *J* = 2.1 Hz)
H-14	6.24 (*d*, *J* = 2.1 Hz)	6.25 (*d*, *J* = 2.0 Hz)	6.25 (*d*, *J* = 2.1 Hz)
H-17	1.43 (*d*, *J* = 6.4 Hz)	1.40 (*d*, *J* = 6.5 Hz)	1.40 (*d*, *J* = 6.4 Hz)
$PhOCH_3$	3.75 (*s*)	3.76 (*s*)	3.76 (*s*)

[a] exchangeable.



Scheme 1.

figuration at C-3 is proposed to be (3*S*) as for lasiodiplodin **4**.

Compounds **2** and **3** were obtained as colorless powders and both gave molecular formulae of $C_{17}H_{24}O_5[M]^+$ by high resolution FD- and EI-mass spectrometry. The IR spectra of **2** and **3** showed hydroxyl (3290 cm^{-1}), conjugated carbonyl (1680 cm^{-1} for **2** and 1670 cm^{-1} for **3**), aromatic ring (1590 cm^{-1} for **2** and 1570 cm^{-1} for **3**) and ester group (1250 cm^{-1}) absorptions. Additionally the ^{1}H- and ^{13}C-NMR spectra of **2** and **3** closely resembled that of lasiodiplodin (**4**), except for the signals of H-5 (δ 4.07 for **2** and δ 3.86 for **3**) and C-5 (δ 66.7 for **2** and δ 70.6 for **3**).

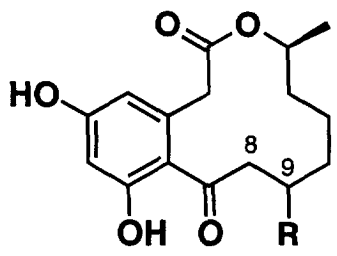
Therefore, the structures of **2** and **3** were considered to be 5-hydroxylasiodiplodins. The positions of

Table 2. The ^{13}C-NMR assignments for **1**, **2** and **3** (125.8 MHz, $CDCl_3$)

^{13}C (ppm)	**1**	**2**	**3**
C-1	168.0	168.2	168.7
C-3	69.1	69.5	70.5
C-4	49.1	40.4	43.5
C-5	210.2	66.7	70.6
C-6	42.0	36.7	36.2
C-7	21,6	22.0[a]	22.0[a]
C-8	27.2	24.9[a]	26.9[a]
C-9	29.0	30.0	29.7
C-10	32.6	30.0	31.1
C-11	142.0	142.9	143.0
C-12	108.7	108.3	108.3
C-13	157.9	158.1	158.3
C-14	97.0	96.9	96.9
C-15	158.2	157.4	157.7
C-16	115.9	117.0	116.3
C-17	20.2	19.9	21.4
$PhOCH_3$	55.7	55.8	55.8

[a] Exchangeable.

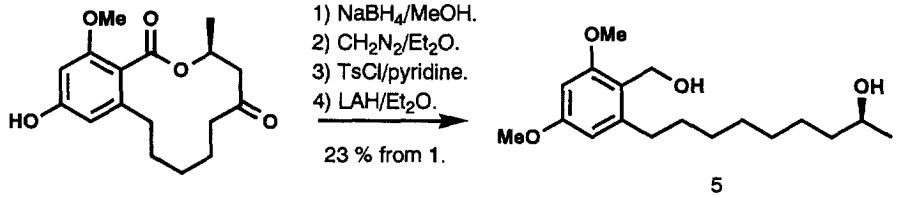
hydroxyl groups in **2** and **3** were determined to be at C-5 from the cross peaks (H-17/H-3/H-4/H-5, Fig. 2) in the ^1H-^1H COSY spectra. The complete NMR assignments are given in Tables 1–2 according to the results of ^1H-^{13}C COSY spectra. These structures were



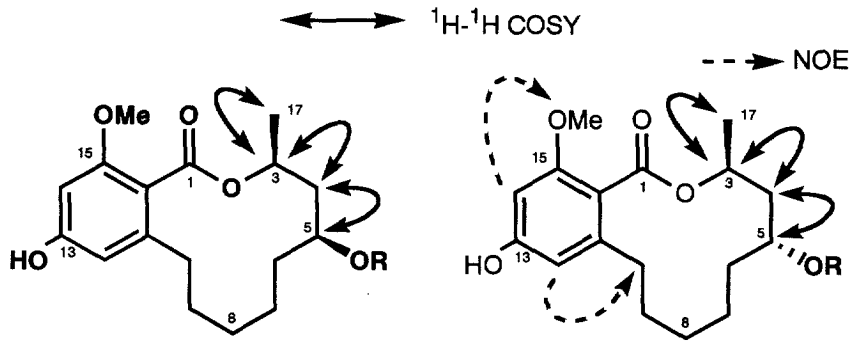
7 R=H; curvularin
8 R=H; 8,9-dehydro; dehydrocurvularin
9 R=OMe; 8-methoxycurvularin

Fig. 3. The structures of curvularin (**7**), dehydrocurvularin (**8**) and 8-methoxycurvularin (**9**).

also confirmed by HMBC spectral analysis, as well as by NOE experiments (Fig. 2), and chemical conversions. The ^1H-NMR spectra, EI-mass and IR spectra of the oxidized product **6** derived from **2** and **3** (Scheme 3) had good accordance with those of **1**, and the $[\alpha]_D^{25}$ showed $+5.4°$ ($CHCl_3$; c 0.45). Therefore the absolute configurations of C-3 of **2** and **3** are proposed to be (3*S*). In order to determine the absolute configurations of the hydroxyl moieties, **2** and **3** were converted to MTPA esters, **2a** and **3a** ((+)-MTPA ester), **2b** and **3b** ((-)-MTPA ester). The advanced Mosher method of determination of configuration [5]



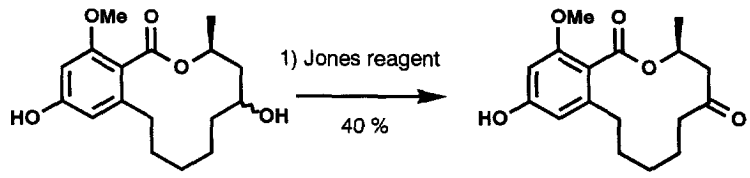
Scheme 2.



2 R=H; (5*S*) 5-hydroxylasiodiplodin
2a R=(+)-MTPA
2b R=(-)-MTPA

3 R=H; (5*R*) 5-hydroxylasiodiplodin
3a R=(+)-MTPA
3b R=(-)-MTPA

Fig. 2. The structures of (5*S*), (5*R*) 5-hydrolasiodiplodins (**2**), (**3**) and ^1H-^1H NMR and NOE experiments.



5-hydroxylasiodiplodins (**2** and **3**) 6

Scheme 3.

was applied to these esters. The assignments of **2a**, **2b**, **3a** and **3b** were achieved by ^{1}H-NMR and ^{1}H-^{1}H COSY spectral analyses. The differences between chemical shifts (^{1}H) **2a** and **2b** were analyzed (Table 3), and the absolute configuration of the hydroxyl group of **2** was determined to be (5*S*) based on the tendency of positive and negative values observed for their esters in Table 3. According to the same method, that of **3** is proposed as (5*R*).

Potato micro-tuber inducing activities of **1**, **2** and **3** were also evaluated according to the method using cultures of single-node segments of potato stem *in vitro* [2]. Under these conditions the activities of theobroxide and jasmonic acid are observed at the concentrations of 10^{-5} M and 10^{-6} M, respectively. The activities of **2** and **3**, on the other hand, were only noted at much higher concentrations (10^{-3} M), although **3** had a little higher activity. Compound **1** had no activity at the same concentrations.

Lasiodiplodin (**4**) was first isolated from *Lasiodiplodia theobromae* and synthesized by several groups [6–8], and lasiodiplodin analogs were isolated from other microorganisms. Among them, curvularin (**7**) dehydrocurvularin (**8**) and 8-methoxycurvularin (**9**) from *Penicillium A*-5-11 were reported to have unique activities [9]. That is, they have effects on the spindle formation of the embryos of urchin cells to give barrel-like spindles and terminate the first step of the cell division. Since (5*R*) and (5*S*) 5-hydroxylasiodiplodins have some structural similarities with curvularins, they might also have effects against the microtubules of the cells of potato stems to induce potato microtubers. The fact that jasmonic acid was reported to disrupt cortical microtubules in suspension cultures of tobacco BY-2 [10] and potato (cv May Queen) [11] cells is interesting; however, **2** and **3** only display very weak/moderate tuber-inducing properties.

Table 3. The $\Delta\delta$ values ($\delta(-)$-$\delta(+)$) for the esters of **2** and **3** in ppm (500 MHz)

	$\Delta\delta$ values ($\delta(-)$-$\delta(+)$)	
^{1}H (ppm)	**2**[a]	**3**[b]
H-3	0.03	−0.08
H-4a	0.08	−0.05
H-4b	0.05	−0.07
H-5	0.03	0.01
H-6	−0.43	0.07
H-7	−0.24	0.09
H-8	−0.05	0.09
H-9a	−0.42	−0.08
H-9b	−0.42	0.12
H-10a	−0.03	0.06
H-10b	0.03	−0.03
H-12	−0.01	−0.03
H-14	0.08	−0.07
H-17	0.00	0.08
Ph-OCH3		

[a] the $\Delta\delta$ values ($\delta(-)$-$\delta(+)$) for **2**. [b] the $\Delta\delta$ values ($\delta(-)$-$\delta(+)$) for **3**.

EXPERIMENTAL

General

Spectra were obtained with the following instruments: IR, Hitachi 285 spectrometer; optical rotations, JASCO DIP-4 polarimeter; NMR, Brüker AM-500 FT-NMR spectrometer and JEOL JNM-EX 270 FT-NMR system; FD- and EI-MS, JEOL JMS-O1SG-2 and JMS-DX-300 mass spectrometers, respectively.

Bioassay

The potato micro-tuber forming activity was examined according to the method using cultures of single-node segments of potato stem *in vitro* as previously described [2]. Briefly, single node segments of stem, prepared from etiolated potato shoots (*Solanum tuberosum* L. cv Irish Cobbler), were sterilized with 1% NaClO for 1 hr. Then three segments were planted horizontally in a 100 ml flask that contained 10 ml of basal medium (usually White's medium) supplemented with the compounds to be tested. Five replicates were prepared. The concn of sucrose in the medium was 2% by wt. 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.

Cultures and isolation of 5-*oxolasiodiplodin* (**1**)

The fungus was grown in 500 ml flasks in the stationary phase at 23°C in the dark for 35 days, with each culture containing 150 ml of 2% potato-sucrose medium. The culture filtrates (about 12 L, 80 flasks) were concentrated to nearly 1 L *in vacuo* and extracted with EtOAc. Evaporation of the EtOAc extracts *in vacuo* gave a dark brown oil (6 g). The oil was chromatographed on silica gel CC (C-200, Wako-gel, MeOH-$CHCl_3$, 1:100, 3:97, 20:80, v/v), and the eluents (MeOH-$CHCl_3$, 3:97, v/v) were further purified by HPLC (Inertsill PREP-ODS, 20 mm × 250 mm, GL Sciences Inc., MeOH-H_2O, 40:60, v/v, 6.0 ml min^{-1}, UV detector 260 nm) to afford **1** (45 mg). $[\alpha]_D^{25}$ +7° ($CHCl_3$; c 0.8); EI-MS *m/z* (rel. int.): 306 $[M]^+$ (100), 291 (7), 265 (8), 246 (16), 220 (24), 205 (15), 177 (91), 164 (44), 138 (31), 121 (4), 83 (13), 69 (11), 55 (8), 41 (7); EI-HR-MS *m/z*: 306.1469 $[M]^+$ (calcd for $C_{17}H_{22}O_5$: 306.1468); IR $\nu_{max}^{film}cm^{-1}$: 3390, 2936, 1715, 1607, 1260, 1163; ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Cultures and isolation of (5*S*) 5-*hydroxylasiodiplodin* (**2**) *and* (5*R*) 5-*hydroxylasiodiplodin* (**3**)

Growth conditions were as above except that the fungus was grown for 30 days. The culture filtrates were subjected to CC on charcoal and eluted with H_2O, EtOH and Me_2CO. The potato micro-tuber inducing substances were present in EtOH and Me_2CO eluents. The volatile components of the EtOH eluents were removed *in vacuo* to give a dark brown oil (12.9 g). A portion of the oil (5.8 g) was chromatographed twice on silica gel CC (C-200, Wako-gel, MeOH-$CHCl_3$-AcOH, 2:98:1, 5:95:1, 10:90:1, v/v/v) and (C-200, Wako-gel, Me_2CO-$CHCl_3$-AcOH, 5:95:1, v/v/v). The active fractions (27 mg) were further purified by prep. TLC (Merck, Me_2CO-$CHCl_3$, 3:7, Rf. value =0.6) and HPLC (Novapak C_{18}, 8 mm × 175 mm, Waters, MeOH-H_2O, 40:60, v/v, 0.5 ml min^{-1}, UV detector 210 nm) to afford (5*S*) 5-hydroxylasiodiplodin (**2**, 2.4 mg) and (5*R*) 5-hydroxylasiodiplodin (**3**, 2.2 mg). (5*S*) 5-hydroxylasiodiplodin (**2**). $[\alpha]_D^{23}$ +5.2° ($CHCl_3$; c 0.62); FD-MS *m/z* (rel. int.): 308 $[M]^+$ (100); EI-MS *m/z* (rel. int.): 308 $[M]^+$ (10), 290 $[M\text{-}H_2O]^+$ (8), 275 (6), 247 (14), 220 (20), 191 (19), 177 (100), 138 (80), 121 (18), 107 (15), 91 (11), 77 (26), 55 (22), 41 (10); FD-HR-MS *m/z*: 308.1617 $[M]^+$ (calcd for $C_{17}H_{24}O_5$: 308.1624); IR $\nu_{max}^{film}cm^{-1}$: 3290, 2900, 1680, 1590, 1250, 1080; ^1H NMR: Table 1; ^{13}C NMR: Table 2. (5*R*) 5-hydroxylasiodiplodin (**3**). $[\alpha]_D^{23}$ +8.8° ($CHCl_3$; c 0.70); FD-MS *m/z* (rel. int.): 308 $[M]^+$ (100); EI-MS *m/z* (rel. int.): 308 $[M]^+$ (3), 290 $[M\text{-}H_2O]^+$ (13), 275 (9), 247 (13), 220 (23), 191 (19), 177 (100), 164 (28), 138 (55), 121 (14), 106 (12), 91 (14), 77 (20), 55 (18), 41 (48); EI-HR-MS *m/z*: 308.1617 $[M]^+$ (calcd for $C_{17}H_{24}O_5$: 308.1624); IR $\nu_{max}^{film}cm^{-1}$: 3290, 2900, 1670, 1570, 1250, 1080; ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Preparation of **2a**, **2b**, **3a** *and* **3b**

Coupling reactions of **2** and **3** with (+)- and (-)-MTPA were achieved in toluene solution containing DMAP and DCC. The usual work-up followed by purification employing prep. TLC (Merck, Me_2CO-*n*-hexane, 4:6, v/v) gave **2a**, **2b**, **3a** and **3b**. (+)-MTPA ester of **2** (**2a**). EI-MS *m/z* (rel. int.): 740 $[M]^+$ (0.1), 507 (1), 489 (0.3), 189 (100), 105 (32), 77 (17); ^1H NMR (500 MHz, $CDCl_3$): δ 7.64 (2H, *m*), 7.53 (2H, *m*), 7.48 (3H, *m*), 7.40 (3H, *m*), 6.62 (1H, *d*, *J*=1.9 Hz), 6.54 (1H, *d*, *J*=1.9 Hz), 5.39 (1H, *m*), 5.28 (1H, *m*), 3.80 (3H, *s*), 3.68 (3H, *s*), 3.56 (3H, *s*), 2.70 (1H, *ddd*, *J*=14.0, 6.5, 6.5 Hz), 2.61 (1H, *ddd*, *J*=14.0, 6.5, 6.5 Hz), 2.27 (1H, *ddd*, *J*=15.0, 6.3, 3.4 Hz), 1.97 (1H, *ddd*, *J*=15.0, 5.5, 5.5 Hz), 1.81–1.73 (2H, *m*), 1.70–1.64 (2H, *m*), 1.53–1.47 (2H, *m*), 1.40–1.32 (2H, *m*), 1.25 (3H, *d*, *J*=6.5 Hz). (−)-MTPA ester of **2** (**2b**). EI-MS *m/z* (rel. int.): 740 $[M]^+$ (0.1), 507 (1), 489 (0.4), 189 (100), 105 (33), 77 (19); ^1H-NMR (500 MHz, $CDCl_3$): δ 7.62 (2H, *m*), 7.51 (2H, *m*), 7.47 (3H, *m*), 7.40 (3H, *m*), 6.61 (1H, *d*, *J*=1.7 Hz), 6.53 (1H, *d*, *J*=1.8 Hz), 5.42 (1H, *m*), 5.31 (1H, *m*), 3.80 (3H, *s*), 3.68 (3H, *s*), 3.53 (3H, *s*), 2.73 (1H, *m*), 2.58 (1H, *m*), 2.32 (1H, *m*), 2.05 (1H, *m*), 1.34 (2H, *m*), 1.33 (2H, *d*, *J*=6.4 Hz), 1.31 (2H, *m*), 1.25 (4H, *m*). (+)-MTPA ester of **3** (**3a**). EI-MS m/z (rel. int.): 740 $[M]^+$ (2), 507 (7), 189 (100), 105 (9); ^1H-NMR (500 MHz, $CDCl_3$); δ 7.63 (2H, *m*), 7.54 (2H, *m*), 7.47 (3H, *m*), 7.41 (3H, *m*), 6.58 (1H, *d*, *J*=2.0 Hz), 6.53 (1H, *d*, *J*=2.0 Hz), 5.42 (1H, *m*), 5.11 (1H, *m*), 3.80 (3H, *s*), 3.70 (3H, *s*), 3.58 (3H, *s*), 2.72 (1H, *m*), 2.58 (1H, *m*), 2.13 (1H, *m*), 2.02 (1H, *m*), 1.68 (2H, *m*), 1.38 (3H, *d*, *J*=6.5 Hz), 1.34 (4H, *m*). (−)-MTPA ester of **3** (**3b**). EI-MS *m/z* (rel. int.): 740 $[M]^+$ (1), 507 (5), 189 (100), 105 (17); ^1H NMR (500 MHz, $CDCl_3$): δ 7.63 (2H, *m*), 7.55 (2H, *m*), 7.47 (3H, *m*), 7.41 (3H, *m*), 6.59 (1H, *br s*), 6.50 (1H, *br s*), 5.36 (1H, *m*), 5.12 (1H, *m*), 3.79 (3H, *s*), 3.68 (3H, *s*), 3.57 (3H, *s*), 2.69 (1H, *m*), 2.64 (1H, *m*), 2.04 (1H, *m*), 1.97 (1H, *m*), 1.81–1.69 (3H, *m*), 1.55 (1H, *m*), 1.43 (4H, *m*), 1.31 (2H, *d*, *J*=6.4 Hz).

Preparation of (2*S*) 9-(3′, 5′-*dimethoxy*-2′-*hydroxymethyl*) *phenyl*-2-*nonanol* (**5**)

To a stirred solution of lasiodiplodin (**4**, 10 mg, 0.034 mmol) in MeOH (3 ml) was added a solution of CH_2N_2-Et_2O (3 ml), and the mixture was stirred at 0°C for 2 hour. The volatile components of the reaction mixture were removed under reduced pressure to give an oil. To a stirred suspension of LAH (69 mg) in Et_2O at 0°C was added a solution of the oil in Et_2O (5 ml), and the reaction was accomplished according to the usual manner to give an oil, which was purified by prep. TLC (Merck, MeOH-$CHCl_3$, 12:88, v/v) to afford (2*S*) 9-(3′,5′-dimethoxy-2′-hydroxymethyl) phenyl-2-nonanol (**5**, 5.7 mg, 0.018 mmol, 53%). $[\alpha]_D^{23}$ −5.6° ($CHCl_3$; c 2.85); EI-MS *m/z* (rel. int.): 310 $[M]^+$ (54), 292 (16), 277 (14), 182 (100), 167 (62), 152 (72); FD-HR-MS *m/z*: 310.2148 $[M]^+$ (calcd for $C_{18}H_{30}O_4$:

310.2145); IR $\nu_{max}^{film} cm^{-1}$: 3375, 2929, 1606; ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.33 (2H, *m*), 4.66 (2H, *s*), 3.84 (3H, *s*), 3.80 (3H, *s*), 2.65 (2H, *t*, J = 8.0, 7.9 Hz), 1.56–1.30 (18H, *m*), 1.18 (3H, *d*, J = 6.2 Hz).

Preparation of **5** *from* **1**

To a stirred solution of 5-oxolasiodiplodin (**1**, 12 mg, 0.04 mmol) in MeOH (3 ml) was added excess $NaBH_4$ at 0°C, and the mixture was stirred for 30 min. This reaction mixture was quenched with H_2O and extracted with EtOAc. The combined organic layers were washed with satd aq. $NaHCO_3$ and dried over Na_2SO_4. The volatile components of the organic layers were removed *in vacuo* to afford an oil, which was purified by HPLC (Inertsill PREP-ODS, 20 mm × 250 mm, GL Sciences Inc., MeOH-H_2O, 40:60, v/v, 6.0 ml min^{-1}, UV detector 210 nm) to give 5-hydroxyl-asiodiplodins. To a stirred solution of the 5-hydroxyl-asiodiplodins in MeOH (5 ml) at 0°C was added a solution of CH_2N_2-Et_2O (3 ml). The volatile components of the reaction mixture were removed *in vacuo* to give an oil. To a stirred solution of the oil in pyridine (2 ml) was added TsCl (88.1 mg), and the mixture was stirred for 14 hour at room temp. The reaction mixture was poured into Et_2O (25 ml) and washed with 0.1 N HCl (3 × 50 ml) and satd aq. NaCl (2 × 50 ml). The Et_2O layer was dried over Na_2SO_4 and concentrated up to give an oil. To a stirred suspension of excess LAH in Et_2O at 0°C was added a solution of the oil in Et_2O, and an usual work-up for this reaction was employed to afford an oil, which was purified as before to afford **5** (2.7 mg, 0.009 mmol, 33%). $[\alpha]_D^{25}$ −8.6° ($CHCl_3$; c 1.35); FD-MS *m/z*: 310 $[M]^+$ (100).

Preparation of **6**

To a stirred solution of **2** and **3** (6.4 mg, 0.02 mmol) in acetone at 0°C was added a solution of Jones reagent (5 ml), and the mixture was stirred for 30 min at 0°C. The reaction mixture was quenched with 2-propanol followed by H_2O and extracted with EtOAc (3 × 10 ml). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by prep. TLC (Merck, MeOH-$CHCl_3$, 5:95, v/v) to afford **6** (4.5 mg, 0.014 mmol, 70%). $[\alpha]_D^{25}$ +5.4° ($CHCl_3$; c 0.45); FD-MS *m/z* (rel. int.): 306 $[M]^+$ (100).

Acknowledgements—The authors thank Mr. K. Watanabe and Dr. E. Fukushi for FD-, EI-MS, HMBC and INAPT measurements.

REFERENCES

1. Nakamori, K., Matsuura, H., Yoshihara, T., Ichihara, A. and Koda, Y., *Phytochemistry*, 1994, **35**, 835.
2. Koda, Y. and Okazawa, Y., *Plant Cell Physiol*, 1988, **29**, 969.
3. Aldridge, D. C., Galt, S. and Turner, W.B., *J. Chem. Soc.* (*C*), 1971, 1623.
4. Lee, K.-H., Hayashi, N., Okano, M., Hall, I. H., Wu, R.-Y. and McPhail, A. T., *Phytochemistry*, 1982, **21**, 1119.
5. Ohtani, I., Kusumi, T., Kashman, Y. I. and Kakisawa, H., *J. Am. Chem. Soc*, 1991, **113**, 4092.
6. Gerlach von, H. and Thalmann, A., *Helv. Chim. Acta*, 1977, **60**, 2866.
7. Takahashi, T., Kasuga, K. and Tsuji, J., *Tetrahedron Lett*, 1978, **49**, 4917.
8. Danishefsky, S. and Etheredge, S. J., *J. Org. Chem*, 1979, **44**, 4716.
9. Kobayashi, A., Hino, T., Uneyama, K. and Kawazu, Y., *Symposium Papers, The Chemistry of Natural Products*, Hiroshima, 1985, p. 343.
10. Abe, M., Shibaoka, H., Yamane, H. and Takahashi, N., *Protoplasma*, 1990, **156**, 1.
11. Matsuki, T., Tazaki, H., Fujimori, T. and Hogetsu, T., *Biosci. Biotech. Biochem*, 1992, **56**, 1329.