Pergamon

Phytochemistry, Vol. 35, No. 4, pp. 835–839, 1994

0031-9422/94 $6.00 + 0.00

POTATO MICRO-TUBER INDUCING SUBSTANCES FROM *LASIODIPLODIA THEOBROMAE*

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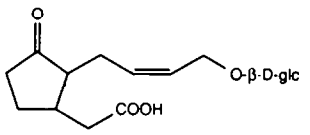
(*Received in revised form* 30 *June* 1993)

Key Word Index—*Lasiodiplodia theobromae*; potato micro-tuber inducing activity; mellein; jasmonic acid; theobroxide.

Abstract—Three potato-tuber inducing substances were isolated from *Lasiodiplodia theobromae* IFO 31059, and their structures identified as mellein, jasmonic acid and a previously unrecorded cyclohexene named theobroxide.

INTRODUCTION

The potential practical applications of potato (*Solanum tuberosum* L.) micro-tubers include: (1) reduction of time required for the improvement of plant breeding, (2) facilitating the judgement of potato varieties such as early or late ripening ones, (3) reduction of transportation costs, (4) expanding of productivity by the use of germless seedlings, (5) the succession of micro-tubers to the genetic superiority of mother tubers, (6) the study of tuber-inducing mechanisms of plants in general. In a recent paper, we described tuberonic acid glucoside (**1**) as the potato tuber-inducing substance of potato (*Solanum tuberosum* L.) [1] and its methyl ester, jasmonic acid, and polyacetylenic compounds as those of Jerusalem artichoke (*Helianthus tuberosus*) [2, 3], using a culture of single-node segments of potato stem *in vitro* [4]. We applied this method in our search for micro-tuber inducing substances from microorganisms. We now present the chemical structures of potato micro-tuber inducing substances, **2**–**4**, from *Lasiodiplodia theobromae*.



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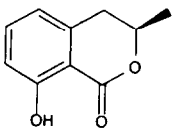

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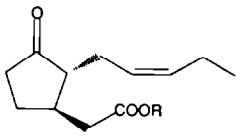

3 R=H
5 R=Me

RESULTS AND DISCUSSION

The culture filtrate of *Lasiodiplodia theobromae* IFO 31059 was subjected to column chromatography on charcoal and eluted with water, ethanol and acetone. The ethanol and acetone eluates showed potato micro-tuber inducing activity and compounds were further purified by silica gel and HPLC to afford **2**–**4**.

Compound **2** was obtained from acetone eluents as needles, and analysed for $C_{10}H_{10}O_3$ $[M]^+$ by high resolution EI-mass spectrometry. The IR spectrum showed hydroxy group (3600–3000 cm^{-1}) and hydrogen-bonded lactone carbonyl group (1610 cm^{-1}) absorptions. The ^1H NMR spectrum showed the presence of three aromatic protons (δ7.39, 1H, *dd*, J = 8.4, 7.4 Hz; δ6.87, 1H, *d*, J = 8.4 Hz; δ6.69, 1H, *d*, J = 7.4 Hz), a hydrogen-bonded phenolic group (δ11.03, 1H, *s*), and $ArCH_2CH(Me)O$–CO– (δ4.73, 1H, *m*; δ2.92, 2H, *d*, J = 6.4 Hz; δ1.53, 3H, *d*, J = 6.4 Hz). These signals had good accordance with those of mellein, which had already been isolated from the fungus, *Lasiodiplodia theobromae* [5]. The absolute configuration of mellein was proposed as (*R*) on the basis of the observation of a negative extremum at 257 nm in its CD [7] and this proposal was confirmed by the chiral synthesis of (*R*)-mellein [8].

Compound **3** was isolated from the ethanol eluents as a pale yellow oil. The ^1H NMR spectrum of **3** showed the presence of olefinic protons (δ5.45, 1H, *dt*, J = 10.7, 7.2 Hz; δ5.26, 1H, *dt*, J = 10.7, 4.7 Hz), carbonylmethylene proton (δ2.78, 1H, *br d*, J = 12.2 Hz), methyl protons (δ0.95, 3H, *d*, J = 7.5 Hz), and δ1.91 (1H, *m*), δ1.55 (1H,

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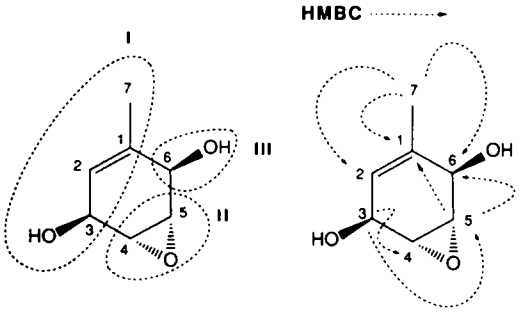
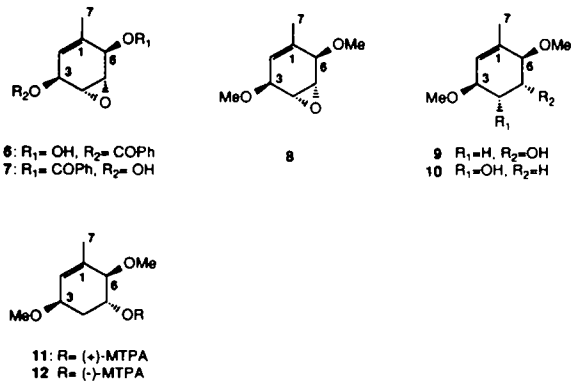


Fig. 1. The structure of theobroxide (**4**), partial structures and HMBC experiment for tuberonone.



m). Based on these signals, **3** was proposed to be jasmonic acid, which had already been isolated from the fungus, *Lasiodiplodia theobromae* [5] and this proposal was confirmed by the comparison of the EI-mass spectrum of **3** with that of authentic jasmonic acid. Its methyl ester derivative (**5**) exhibited $[\alpha]_D^{25} - 73.6°$ (MeOH; *c* 0.25), [lit. [9], $-90.2°$ (MeOH; *c* 0.32)] and the chemical structure of **3** was determined as (−)-jasmonic acid.

Compound **4** ($C_7H_{10}O_3$) was obtained from ethanol eluents as needles and analysed for $C_7H_8O_2$ [$M - H_2O$]$^+$ by high resolution EI-mass spectrometry. The IR spectrum showed hydroxy group (3500–3000 cm^{-1}) and epoxy group (1280 cm^{-1}) absorptions. ^{13}C NMR and DEPT experiments revealed that **4** contained seven carbons: one methyl, five methine, and one quaternary carbons, and the HMQC spectrum established the connection between carbons and directly attached protons (Table 1). Two signals (δ 4.04, 3.97) assigned to hydroxyl groups were eliminated by the addition of deuterium oxide. The partial structure I was revealed by spin decoupling experiments. However, the signals in the ^{1}H NMR spectrum were broad, so the structural analysis using spin decoupling experiments was not suitable. Therefore, in order to determine the connection of partial structures (I, II and III), the HMBC spectrum was measured. The cross-peaks of H-7/C-1, C-2, C-6, H-3/C-4, C-5, H-5/C-1, C-6 confirmed the skeleton of **4**, and other cross-peaks agreed with this result.

In order to determine the absolute configuration, **4** was converted to two monobenzoyl derivatives (**6** and **7**). The position of the benzoyl ester was established by ^{1}H NMR and ^{1}H–^{1}H COSY spectra. The circular dichroism spectrum [10] of **6** showed a positive Cotton effect of $\Delta\varepsilon_{249}$ (+), indicating the 3*S*-configuration in **6**, and that of **7** showed a negative one of $\Delta\varepsilon_{249}$ (−), indicating the 6*R*-configuration in **7**.

Table 1. ^{1}H (500 MHz, $CDCl_3$) and ^{13}C NMR (125.8 MHz, $CDCl_3$) spectral data for compound **4**

H		C	(ppm)
2	5.50 (1H, *br d*, *J* = 4.6 Hz)	1	135.0
3	4.44 (1H, *br s*)	2	121.4
4	3.29 (1H, *br s*)	3	62.9
5	3.35 (1H, *br s*)	4	51.8
6	4.23 (1H, *br d*, *J* = 6.3 Hz)	5	53.0
7	1.82 (3H, *s*)	6	66.1
		7	21.2
OH	4.04 (1H, *br d*, *J* = 8.2 Hz)		
OH	3.97 (1H, *br s*)		

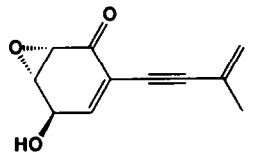
In order to determine the absolute configuration of the 4,5-epoxy moiety, **4** was converted to the dimethyl derivative **8**, and the cleavage of the 4,5-epoxide of **8** was carried out by lithium aluminium hydride in order to produce alcohols **9** and **10**. The structure and assignments of the protons of **9** and **10** were established by ^{1}H NMR, ^{1}H–^{1}H COSY spectra, and the advanced Mosher method [11] was applied to **9**. Esterification of **9** with (+) or (−)-MTPA by *N*,*N*′-dicyclohexylcarbodiimide and 4-dimethylaminopyridine in dichloromethane yielded **11** and **12** respectively. The assignments of **11** and **12** were achieved by ^{1}H NMR and ^{1}H–^{1}H COSY spectra, and the difference of the chemical shifts (^{1}H) between **11** and **12** was analysed. The tendency of positive values was observed in $-{}^3C\underline{H}(OMe)-$ and $-{}^4CH_2-$, and negative values in $-{}^6C\underline{H}(OMe)-$, $-{}^7Me$, and $^2CH=$, indicating the (5*R*)-configuration of **9** and consequently (4*R*,5*S*) in **4**. Based on these experiments, the absolute configuration of **4** was determined as (3*S*,4*R*,5*S*,6*R*)-3,6-dihydroxy-4,5-epoxy-1-methylcyclohex-1-ene.

(−)-Mellein (**2**) was isolated as a metabolite of *Aspergillus melleus* [12], *Aspergillus ochraceus* [13], and *Lasiodiplodia theobromae* [5], as a hair pencil component of the male oriental fruit moth, *Grapholitha molesta* [14], and as an inhibitor of cellulose synthesis of *Acetobactor xylinum* [15]. In the course of our study, **2** was isolated as a potato micro-tuber inducing substance (activity 1×10^{-4} M, weak activity), but its activity depended on the developmental stage of potato tubers used for bioassay. The possibility exists that mellein (**2**) may work upon cell walls by inhibition of cellulose synthesis, but this remains to be proven.

Jasmonic acid (**3**) is widely distributed in the plant kingdom and microorganisms and has a wide variety of physiological activities. Jasmonic acid (**3**) always shows activity at the concentration of 1×10^{-6} M. It has been reported to disrupt cortical microtubules in suspension cultures of tobacco BY-2 [16] and potato (cv May Queen) cells [17].

Table 2. ^{1}H NMR (270 MHz, $CDCl_3$) spectral data for **11** and **12**, and $\Delta\delta$ values [δ (−): **12**−δ (+): **11**] (270 MHz)

H	12	11	$\Delta\delta$ values [$\delta(-)-\delta(+)$]
2	5.68	5.71	−8.1
3	3.78	3.73	13.5
4a	2.14	2.08	16.2
4b	1.96	1.90	16.2
5	5.49	5.49	0.0
6	3.53	3.64	−24.3
7	1.75	1.79	−10.8



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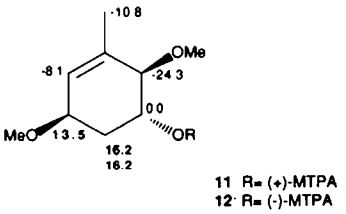

11 R= (+)-MTPA
12 R= (-)-MTPA

This is the first time **4** has been isolated from a natural source and showed potato micro-tuber inducing activity at a concentration of 5×10^{-6} M. Harveynone (**13**), related to **4**, was isolated as a mitotic arrester of sea urchin eggs [18] and efficiently inhibited their spindle formation and arrested first cell division. Compound **4** might possess a similar activity potential in the inhibition of plant microtubules formation.

According to some reports on the mechanism of tuber inducement, at the first stage of tuberization of potato, the radial expansion of pith cells at the stolon tip occurs, followed by cell division [19]. It is, therefore, feasible that the radial cell expansion of pith cells was induced by the disruption of cortical microtubules contained therein. From this hypothesis, **3** and **4** might play an important role concerning micro-tuber formation in potatoes.

EXPERIMENTAL

Bioassay. The tuber-forming activity was tested using cultures of single-node segments of potato stem *in vitro* as previously described [4].

Cultures and isolation. The fungus was grown in 500 ml flasks containing 150 ml of 2% potato–sucrose medium stationarily at 23° in the dark for 30 days. The culture filtrates were subjected to charcoal chromatography and eluted by H_2O, EtOH and Me_2CO. The potato micro-tuber inducing activity was observed in EtOH and Me_2CO eluents.

Isolation of mellein (**2**). The Me_2CO eluents (290 mg) of 30 flasks were subjected to silica gel CC (C-200; Wako-gel, $CHCl_3$–MeOH, 1:0, 19:1) to give **2** (44 mg). $[\alpha]_D^{22}$ −94° ($CHCl_3$; *c* 0.48); mp 47–48°; EI-MS *m/z* (rel. int.): 178 $[M]^+$ (92), 160 (49), 149 (24), 134 (100), 106 (27); EI-HR-MS *m/z*: 178.0610 $[M]^+$ (calcd for $C_{10}H_{10}O_3$: 178.0630); IR ν_{max}^{film} cm^{-1}: 3600–3000, 1610, 1590; ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.03 (1H, *s*), 7.39 (1H, *dd*, *J* = 8.4, 7.4 Hz), 6.87 (1H, *d*, *J* = 8.4 Hz), 6.69 (1H, *d*, *J* = 7.4 Hz), 4.73 (1H, *m*), 2.92 (2H, *d*, *J* = 6.4 Hz), 1.53 (3H, *d*, *J* = 6.4 Hz).

Isolation of (−)-jasmonic acid (**3**) *and theobroxide* (**4**). EtOH eluents were concd under red. pres. and the residue subjected to silica gel chromatography (C-200; Wako-gel, MeOH–$CHCl_3$–HOAc, 2:98:1, 5:95:1, 10:90:1, 1:1:0) to give active fractions A–D. Fraction A (7.1 g) of 63 flasks was chromatographed on silica gel (C-200, Wako-gel, *n*-hexane–EtOAc–HOAc, 90:10:1) to give **3** (44 mg). $[\alpha]_D^{24}$ −88.2° ($CHCl_3$; *c* 0.40); EI-MS *m/z* (rel. int.): 210 $[M]^+$ (20), 192 (4), 151 (31), 142 (27), 133 (13), 121 (10), 109 (21), 95 (20), 83 (100), 67 (25), 55 (32), 40 (66); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.45 (1H, *dt*, *J* = 10.7, 7.2 Hz), 5.26 (1H, *dt*, *J* = 10.7, 4.7 Hz), 2.78 (1H, *br d*, *J* = 12.2 Hz), 2.45–2.25 (4H, *m*), 2.35 (2H, *dd*, *J* = 7.5, 7.5 Hz), 2.19–2.10 (1H, *m*), 2.06 (2H, *dq*, *J* = 7.3, 7.3 Hz), 1.91 (1H, *m*), 1.55 (1H), 0.95 (3H, *d*, *J* = 7.5 Hz).

The active fraction C (7.1 g) of 77 flasks was chromatographed twice on silica gel CC (C-200; Wako-gel, EtOAc–C_6H_6–HOAc, 40:60:1, 70:30:1, 1:0:0), (C-200, Wako-gel, MeOH–$CHCl_3$–HOAc, 5:95:1, 10:90:1, 1:0:0), and further purified by HPLC (μBondapac C_{18}, 7.8 × 300 mm, Waters, MeCN–H_2O–HOAc, 400:600:1, 1.13 ml min^{-1}, UV detector 210 nm) to give **4** (28 mg). $[\alpha]_D^{24}$ −6.12° (EtOH; *c* 0.20); mp 94–96°; FD-MS *m/z* (rel. int.): 142.0 $[M]^+$ (100); EI-MS *m/z* (rel. int.): 142 $[M]^+$ (1), 124 $[M-H_2O]^+$ (11), 113 (10), 95 (62), 84 (30), 73 (78), 69 (62), 53 (33), 43 (77), 41 (100), 39 (66); EI-HR-MS *m/z*: 124.0517 $[M-H_2O]^+$ (calcd for $C_7H_8O_2$: 124.0524); IR ν_{max}^{film} cm^{-1}: 3500–3000 [-OH], 2830, 1410, 1280, 990, 790; ^{1}H, ^{13}C NMR: Table 1.

Preparation of compounds **5**–**12**. *Methyl (−)-jasmonate* (**5**). To the stirred soln of jasmonic acid (**3**) in Et_2O at room temp. was added excess CH_2N_2 in Et_2O. After 1 hr, the volatile components were removed under red. pres., and the residue was purified by prep. TLC (Merck, *n*-hexane–Et_2O, 1:1) to give **5**. $[\alpha]_D^{25}$ −73.6° (MeOH; *c* 0.25); EI-MS *m/z* (rel. int.): 224 $[M]^+$ (23), 156 (20), 151 (43), 109 (22), 95 (28), 83 (100), 76 (13), 55 (23), 41 (53); IR ν_{max}^{film} cm^{-1}: 2930, 1740, 1430, 1190, 990; ^{1}H NMR (270 MHz, $CDCl_3$): δ 5.44 (1H, *m*), 5.27 (1H, *m*), 3.70 (3H, *m*), 2.69 (1H, *m*), 2.50–2.05 (11H, *m*), 1.90 (1H, *m*), 1.50 (1H, *m*), 0.95 (3H, *t*, *J* = 7.3 Hz).

3-Benzoyl derivative (**6**) *of* **4** *and 6-benzoyl derivative* (**7**) *of* **4**. To the stirred mixture of **4** (50 mg), benzoic anhydride (61.3 mg) and dimethylaminopyridine (3.2 mg) was added triethylamine under Ar atmosphere with ice bath. The reaction mixture was stirred for 2 hr at 0°, room temp. The reaction mixture was extracted by Et_2O, and the combined organic layers were washed with 1 M aq. HOAc, satd aq. $NaHCO_3$ and NaCl and dried

over Na_2SO_4. The volatile components were removed under red. pres., and the residue purified by silica gel CC (C-200, Wako-gel, *n*-hexane–EtOAc, 2:3) and HPLC (μPolasil, 7.8 × 300 mm, Waters, *n*-hexane-EtOAc, 2:3, 1.0 ml min^{-1}, UV detector 245 nm) to give **6**, **7** and the dibenzoyl derivative of **4**. Compound **6**: EI-MS *m/z* (rel. int.): 185 $[M]^+$ (16), 167 (6), 149 (26), 125 (8), 111 (13), 100 (42), 83 (31), 71 (84), 57 (91), 43 (100), 41 (76); IR ν_{max}^{film} cm^{-1}: 3380, 2880, 1680, 1410, 1280, 1230, 1070, 990, 920, 790, 680; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.04 (2H, *d*, *J* = 7.6 Hz), 7.59 (1H, *dd*, *J* = 7.4, 7.4 Hz), 7.45 (2H, *dd*, *J* = 7.6, 7.6 Hz), 5.52 (2H, *br s*), 4.29 (1H, *d*, *J* = 10.2 Hz), 3.51 (1H, *br s*), 3.43 (1H, *br s*), 2.45 (1H, *d*, *J* = 10.4 Hz), 1.89 (3H, *s*); CD λ_{max}^{EtOH} nm ($\Delta\varepsilon$): 249 (+0.460), 261 (0), 274 (−0.073); UV λ_{max}^{EtOH} nm (ε): 273 (579), 281 (458). Compound **7**: EI-MS *m/z* (rel. int.): 149 (7), 125 (87), 111 (11), 97 (18), 85 (29), 71 (84), 57 (78), 43 (100), 41 (78); IR ν_{max}^{film} cm^{-1}: 3390, 2880, 1690, 1410, 1220, 1070, 930, 790, 680; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.06 (2H, *d*, *J* = 7.4 Hz), 7.60 (1H, *dd*, *J* = 7.4, 7.4 Hz), 7.47 (2H, *dd*, *J* = 7.7, 7.7 Hz), 5.74 (1H, *br d*, *J* = 4.9 Hz), 5.45 (1H, *br s*), 4.48 (1H, *m*), 3.45 (1H, *br s*), 3.44 (1H, *br d*, *J* = 1.1 Hz), 2.39 (1H, *d*, *J* = 9.9 Hz), 1.78 (3H, *s*); CD λ_{max}^{EtOH} nm ($\Delta\varepsilon$): 249 (−0.607); UV λ_{max}^{EtOH} nm (ε): 274 (374), 282 (301).

3,6-Dimethoxy-4,5-epoxy-1-methylcyclohex-1-ene (**8**). Powdered KOH (63 mg) was placed in a 30 ml one-neck round-bottomed flask equipped with glass stopper. The KOH powder was dissolved at room temp. in DMSO (0.56 ml) over 5 min, and to this soln was added **4** (20 mg) followed immediately by MeI (0.35 ml). The reaction was stopped by addition of H_2O (5 ml). The organic layers were extracted with Et_2O, and washed with H_2O and then dried over Na_2SO_4. The volatile components were removed under red. pres. and the residue purified by prep. TLC (Merck, EtOAc–C_6H_6, 2:3) to give **8** (22 mg). EI-MS *m/z* (rel. int.): 170 (8), 152 (53), 138 (81), 123 (74), 110 (66), 95 (78), 67 (67), 55 (39), 41 (100); IR ν_{max}^{film} cm^{-1}: 2920, 1440, 1090, 960, 830; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.58 (1H, *br d*, *J* = 3.7 Hz), 4.08 (1H, *br dd*, *J* = 2.4, 1.1 Hz), 4.03 (1H, *br s*), 3.45 (3H, *s*), 3.40 (3H, *s*), 3.36 (1H, *br d*, *J* = 1.4 Hz), 3.32 (1H, *br s*), 1.79 (3H, *br s*).

3,6-Dimethoxy-5-hydroxy-1-methylcyclohex-1-ene (**9**) *and 3,6-dimethoxy-4-hydroxy-1-methylcyclohex-1-ene* (**10**). LAH (16.4 mg) was placed in a 30 ml three-neck round-bottomed flask equipped with stirrer bar, septum cap, glass stopper, and Ar balloon. LAH was suspended in dry THF (5 ml), and to this soln was added **8** (105 mg) dropwise. The reaction mixture was stirred at *ca* 50° for 24 hr. The excess LAH was destroyed by addition of EtOAc and H_2O, and the ppt. dissolved by 6 M HCl and subjected to extraction with Et_2O, the combined organic layers were washed with saturated aq. $NaHCO_3$ and NaCl and then dried over Na_2SO_4. The volatile components were removed under red. pres. and the residue was purified by prep. TLC (Merck, MeOH–$CHCl_3$, 1:9) and HPLC (Inertsil ODS 5 μm, 4.6 × 250 mm, GL Sciences Inc., MeOH–H_2O, 3:7, 1.13 ml min^{-1}, UV detector 210 nm) to give **9** (6 mg) and **10** (4 mg). Compound **9**: EI-MS *m/z* (rel. int.): 154 (0.1), 140 (19), 128 (65), 122 (100), 107 (74), 99 (25), 91 (51), 79 (81), 65 (26), 53 (35), 39 (65); IR ν_{max}^{film} cm^{-1}: 3380, 2920, 1460, 1110; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.65 (1H, *m*), 4.08 (1H, *ddd*, *J* = 3.5 Hz), 3.79 (1H, *m*), 3.53 (1H, *br d*, *J* = 6.9 Hz), 3.49 (3H, *s*), 3.35 (3H, *s*), 2.12 (1H, *m*), 2.11 (1H, *ddd*, *J* = 13.4, 4.0, 0.9 Hz), 1.80 (3H, *br d*, *J* = 1.2 Hz), 1.73 (1H, *ddd*, *J* = 13.4, 10.6, 4.5 Hz). Compound **10**: EI-MS *m/z* (rel. int.): 172 (0.1), 154 (1), 140 (8), 128 (89), 107 (100), 98 (20), 91 (52), 77 (57), 40 (50); IR ν_{max}^{film} cm^{-1}; 3320, 2870, 1260, 1080; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.54 (1H, *br d*, *J* = 1.4 Hz), 3.87 (1H, *m*), 3.58 (1H, *m*), 3.42 (3H, *s*), 3.40 (3H, *s*), 2.31 (1H, *br s*), 2.24 (1H, *ddd*, *J* = 13.7, 3.8, 2.5 Hz), 1.79 (3H, *br t*, *J* = 1.5 Hz), 1.59 (1H, *m*).

(+)-MTPA ester (**11**) *of* **9**. 3,6-Dimethoxy-5-hydroxy-1-methylcyclohex-1-ene (**9**) (3 mg), (+)-MTPA (25 mg), 4-dimethylaminopyridine (19 mg), *N,N'*-dicyclohexylcarbodiimide (30 mg) were dissolved in dry CH_2Cl_2 under Ar atmosphere at room temp., and the mixture was stirred for 24 hr. The volatile components were removed, and the residue was purified by prep. TLC (Merck, EtOAc–C_6H_6, 1:4) to give **11** (3 mg). EI-MS *m/z* (rel. int.): 389 (0.1), 357 (0.1), 331 (0.5), 203 (0.7), 189 (7), 175 (5), 154 (8), 122 (100), 105 (21), 91 (37), 77 (25), 45 (31): IR ν_{max}^{film} cm^{-1}; 2930, 1750, 1440, 1360, 1240, 1800, 1100, 1020; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.53 (2H, *m*), 7.42 (3H, *m*), 5.71 (1H, *m*), 5.49 (1H, *ddd*, *J* = 8.6, 5.6, 3.0 Hz), 3.73 (1H, *m*), 3.64 (1H, *br d*, *J* = 5.6 Hz), 3.57 (3H, *s*), 3.42 (3H, *s*), 3.34 (3H, *s*), 2.08 (1H, *ddd*, *J* = 13.2, 6.2, 3.3 Hz), 1.90 (1H, *ddd*, *J* = 13.2, 8.3, 5.0 Hz), 1.79 (3H, *br d*, *J* = 0.7 Hz).

(−)-MTPA ester (**12**) *of* **9**. (−)-MTPA ester (**12**) (3 mg) from 3,6-dimethoxy-5-hydroxy-1-methylcyclohex-1-ene (**9**) (3 mg) was prepd by the same manner as that used for (+)-MTPA ester (**11**). EI-MS *m/z* (rel. int.): 389 (0.1), 357 (0.1), 331 (0.3), 203 (0.6), 175 (6), 154 (13), 122 (100), 105 (21), 91 (35), 77 (24), 45 (28); IR ν_{max}^{film} cm^{-1}; 2920, 1760, 1450, 1260, 1180, 1100, 1020; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.54 (2H, *m*), 7.42 (3H, *m*), 5.68 (1H, *m*), 5.49 (1H, *ddd*, *J* = 8.6, 5.6, 3.3 Hz), 3.78 (1H, *m*), 3.53 (4H, *m*), 3.35 (3H, *s*), 3.34 (3H, *s*), 2.14 (1H, *ddd*, *J* = 13.5, 5.9, 3.6 Hz), 1.96 (1H, *ddd*, *J* = 13.5, 8.4, 4.6 Hz), 1.75 (3H, *br s*).

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

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