Botcinins A, B, C, and D, Metabolites Produced by *Botrytis cinerea*, and Their Antifungal Activity against *Magnaporthe grisea*, a Pathogen of Rice Blast Disease

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Four new metabolites, botcinins A−D (**1**−**4**), were isolated from the culture filtrate of a strain of *Botrytis cinerea*. Their structures were determined by spectroscopic methods, mainly NMR techniques, molecular modeling, and the modified Mosher's method. They exhibited antifungal activities against *Magnaporthe grisea*, a pathogen of rice blast disease. Botcinins B and C have a MIC of 12.5 μM, and botcinins A and D are not active below 100 μM.

Rice (*Oryza sativa* L.) is one of the most important food crops and was cultivated on more than 153 million hectares worldwide in 2004 according to the International Rice Research Institute database. Rice blast disease caused by the fungus *Magnaporthe grisea* is a serious problem almost everywhere rice is grown. Enough rice to feed 60 million people is destroyed each year as a result of this disease.[1] Many scientists have been attempting to find new chemicals that are effective for protecting rice from this disease. In a search for fungal metabolites to inhibit melanin biosynthesis of *M. grisea*, which could be an ecologically sound method for preventing rice blast disease, our research group recently discovered a new fungal melanin inhibitor.[2] In a continuation of this approach, we found the presence of antifungal metabolites against *M. grisea* in a culture filtrate of *Botrytis cinerea* AEM 211 and succeeded in isolating four new metabolites designated botcinins A−D (**1**, **2**, **3**, and **4**). *B. cinerea* is a well-known pathogen of a number of commercial plants and produces many structurally diverse metabolites,[3−19] some of which are phytotoxic.[5−13,15,17−19] The structures of **1**−**4** were elucidated by spectroscopic methods, mainly 2D NMR techniques. The absolute configuration of **1** was determined by applying the modified Mosher's method. [20] We describe here the isolation and structure elucidation of **1**−**4** and the antifungal activity of these compounds against *M. grisea*.

Results and Discussion

Botrytis cinerea AEM 211 was cultured on a malt extract medium without shaking at 24 °C for 14 days in the dark. The metabolites in the culture filtrate were extracted with ethyl acetate and separated into neutral and acidic fractions. The neutral fraction was purified by chromatographic separations to give compounds **1**−**4** in respective yields of 32.8, 2.9, 1.7, and 0.6 mg/L.

Compound **1** was obtained as colorless needles. The molecular formula was established as $C_{22}H_{34}O_8$ (six unsaturations) on the basis of HRFABMS, ^{13}C NMR, and elementary analysis data. The NMR data (Table 1) for **1** revealed the presence of six methyls, three methylenes, seven sp^3 methines (five of which are oxygenated), two sp^2 methines, one oxygenated quaternary carbon, and three carbonyl carbons, indicating that **1** has one hydroxyl and two rings. The ^1H,^1H COSY data revealed three partial structures: −C(2)HCH_3−C(3)H−, −C(5)H−C(6)HCH_3−C(7)H−C(8)HCH_3−, and −C(2′)H=C(3′)H−C(4′)H−C(5′)-H_2−C(6′)H_2−C(7′)H_2−C(8′)H_3. Sharpening of the H-4′ resonance by disappearance of spin coupling with the hydroxyl proton after adding deuterium oxide to the NMR solution indicated a hydroxyl located at C-4′. Acetylation of **1** gave a monoacetate. The H-4′ resonance in the ^1H NMR spectrum of the acetyl derivative was observed 1.08 ppm downfield from the position in the spectrum of **1**, also supporting the C-4′ hydroxyl group. The $J_{H\text{-}2',H\text{-}3'}$ value of 15.6 Hz revealed that the geometry of the double bond between C-2′ and C-3′ was *E*. These partial structures were linked on the basis of HMBC data. A key correlation between H-5 and C-1 was obtained only with 3 Hz optimization. A selective HMBC experiment[21] using a Gaussian inversion pulse was performed to increase the selectivity, and a key long-range correlation between H-8 and C-4 was clearly observed. The relative stereochemistry was deduced on the basis of the NOE data, coupling constants, and molecular modeling. In the NOE experiments, irradiation of H-5 produced NOE enhancement of H-2, -3, and -7 resonances, irradiation of the 4-methyl protons caused NOE enhancement of H-6 and -8 resonances, and irradiation of H-6 produced NOE enhancement of H-8. No NOE was observed from the protons of the fatty acyl portion to those of the ring portion. The NOE of H-2 and H-3 caused by irradiation of H-5 and the coupling constant between H-2 and H-3 (J = 9.7 Hz) indicated a

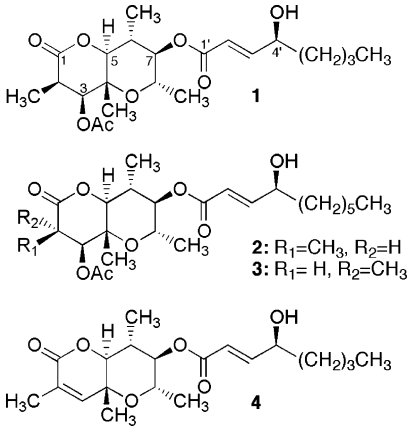

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Table 1. ^{1}H and ^{13}C NMR Data of **1**, **2**, **3**, and **4** in $CDCl_3$[a]

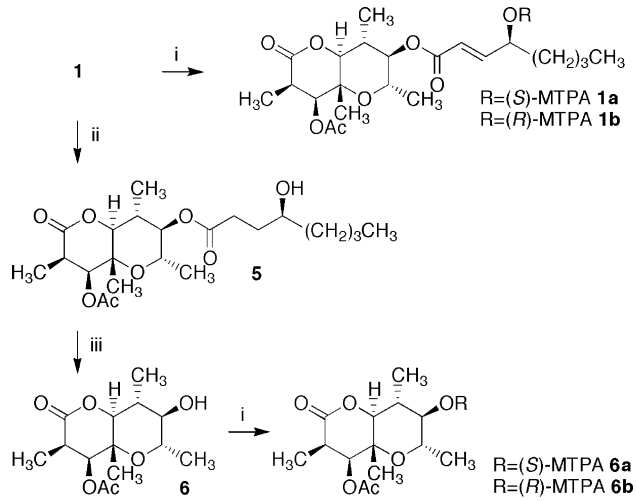
	1		**2**		**3**		**4**	
position	δ_C	δ_H (mult., J in Hz)	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	173.2		173.2		172.4		165.4	
2	37.3	3.16 (dq, 9.7, 7.1)	37.2	3.16 (dq, 9.7, 7.1)	42.0	2.61 (dq, 7.8, 7.6)	126.5	
2-CH_3	10.2	1.12 (d, 7.1)	10.2	1.11 (d, 7.1)	16.5	1.43 (d, 7.6)	16.8	1.91 (q, 1.6)
3	74.3	5.40 (d, 9.7)	74.3	5.40 (d, 9.7)	77.9	4.96 (d, 7.8)	148.3	6.65 (d, 1.6)
4	75.2		75.2		73.1		71.5	
4-CH_3	11.9	1.26 (s)	11.9	1.25 (s)	10.1	1.33 (s)	15.6	1.36 (s)
5	78.5	3.80 (d, 11.0)	78.5	3.80 (d, 10.8)	79.4	3.71 (d, 11.2)	82.2	3.87 (d, 11.7)
6	35.4	2.19 (ddq, 11.0, 10.1, 6.4)	35.4	2.18 (ddq, 10.8, 10.1, 6.2)	35.3	2.08−2.18 (m)	35.1	2.16 (ddq, 11.7, 10.1, 6.2)
6-CH_3	13.7	1.06 (d, 6.4)	13.6	1.05 (d, 6.2)	13.5	1.06 (d, 6.2)	13.0	1.07 (d, 6.2)
7	76.1	4.53 (dd, 10.1, 9.9)	76.0	4.52 (dd, 10.1, 9.7)	76.3	4.51 (dd, 10.1, 9.4)	76.9	4.52 (dd, 10.1, 9.9)
8	68.4	3.68 (dq, 9.9, 6.2)	68.4	3.67 (dq, 9.7, 6.0)	68.0	3.67 (dq, 9.4, 6.0)	68.7	3.77 (dq, 9.9, 6.2)
8-CH_3	18.2	1.09 (d, 6.2)	18.1	1.08 (d, 6.0)	18.1	1.07 (d, 6.0)	18.3	1.13 (d, 6.2)
1′	165.7		165.7		165.6		165.7	
2′	119.0	6.06 (dd, 15.6, 1.4)	119.0	6.05 (dd, 15.6, 1.4)	119.0	6.06 (d, 15.6, 1.4)	119.0	6.07 (dd, 15.6, 1.6)
3′	151.8	7.00 (dd, 15.6, 4.6)	151.8	7.00 (dd, 15.6, 4.6)	151.8	7.01 (dd, 15.6, 4.4)	151.8	7.02 (dd, 15.6, 4.6)
4′	71.1	4.34 (m)	71.0	4.32 (m)	71.1	4.33 (m)	71.1	4.34 (m)
5′	36.3	1.54−1.65 (m)	36.6	1.53−1.65 (m)	36.6	1.53−1.64 (m)	36.3	1.54−1.66 (m)
6′	27.4	1.32−1.45 (m)	25.2	1.25−1.50 (m)	25.2	1.26−1.37 (m)	27.3	1.25−1.46 (m)
7′	22.5	1.32−1.45 (m)	29.0	1.25−1.50 (m)	29.1	1.26−1.37 (m)	22.5	1.25−1.46 (m)
8′	13.9	0.91 (t, 6.9)	31.6	1.25−1.50 (m)	31.7	1.26−1.37 (m)	13.9	0.91 (t, 7.3)
9′			22.5	1.25−1.50 (m)	22.5	1.26−1.37 (m)		
10′			14.0	0.87 (t, 7.1)	14.0	0.88 (t, 6.5)		
CH_3CO	20.6	2.12 (s)	20.5	2.11 (s)	20.9	2.11 (s)		
	170.1		170.1		170.0			

[a] The assignments were based on ^{1}H,^{1}H COSY, HMQC, and HMBC data

boat form of the lactone ring, while the NOE of H-6 caused by irradiation of H-8 and the coupling constants between H-6 and H-7 (J = 10.1 Hz) and between H-7 and H-8 (J = 9.9 Hz) indicated the conformation of the ether ring to be a chair. The most favored conformation of each stereoisomer was searched with MM2 calculations using a computer. This process afforded two stereoisomers that differed from each other only in the configuration of C-4′, and their most preferable conformers were in good agreement with the NOE data and the coupling constants described above. In these conformers, the lactone ring and the ether ring have a boat and a chair form, respectively; H-2, H-3, H-5, H-7, 6-methyl, and 8-methyl were on the β configuration and H-6, H-8, and 4-methyl were on the α configuration of these rings. Conclusively, the relative configuration was determined to be 2*R**, 3*S**, 4*S**, 5*S**, 6*R**, 7*R**, 8*S**, although the stereochemistry of C-4′ has not yet been determined. The absolute stereochemistry of **1** was elucidated by a modification of Mosher's method[20] as follows. First, to determine the absolute configuration of C-4′ in the acyl portion, compound **1** was treated with (*R*)-MTPACl and (*S*)-MTPACl in the presence of 4-(dimethylamino)-pyridine and triethylamine to afford the (*S*)-MTPA ester **1a** and (*R*)-MTPA ester **1b**, respectively (Scheme 1). The chemical shift differential values, $\Delta\delta$ (δ_{S-R}), showed positive values for H-2′ and H-3′, while negative values were observed for H-5′, H-6′, H-7′, and H-8′, indicating that the configuration of C-4′ was *S*. Subsequently, to apply the modified Mosher's method to the ring portion, compound **1** was converted into the alcohol **6** via **5**. Since the ester linkage between the ring and the fatty acyl portions in **1** was resistant to both alkaline and acid hydrolysis, it was first hydrogenated with a catalytic amount of PtO_2 and H_2 gas followed by acid hydrolysis to remove the acyl portion. The alcohol **6** was converted into (*S*)-MTPA ester **6a** and (*R*)-MTPA ester **6b** (Scheme 1). The $\Delta\delta$ (δ_{S-R}) indicated the *R* configuration at C-7. Thus, the absolute stereochemistry of **1** was established as 2*R*, 3*S*, 4*S*, 5*S*, 6*R*, 7*R*, 8*S*, 4′*S*.

Botcinin B (**2**) was obtained as a colorless oil. The HRFABMS and NMR data for **2** afforded a molecular formula of $C_{24}H_{38}O_8$. The ^{1}H NMR and ^{13}C NMR spectra of **2** were very similar to those of **1**. The ^{13}C NMR data including DEPT data showed that **2** possesses two more

Scheme 1. Conversion of **1**[a]



[a] Conditions: (i) (*R*)- or (*S*)-MTPACl in CH_2Cl_2, DMAP, Et_3N, 0 °C, 0.5 h; (ii) H_2, PtO_2, EtOAc, rt, 1 h; (iii) 12 M HCl, 0 °C, 1 h.

methylenes than **1**. The fragment ions assignable to the fatty acyl portion were detected at *m/z* 85 and 169 in the EIMS spectrum of **1**, while they were observed at *m/z* 57 and 141 in the EIMS spectrum of **2**. Thus, **2** differs from **1** by the length of the acyl portion. The key NOE correlations were identical to those of **1**, indicating that **1** and **2** possess the same relative stereochemistry. The absolute configuration of C-4′ in the acyl portion was determined as *S* by the modified Mosher's method. In the biosyntheses of **1** and **2**, the ring portion is presumably first constructed, and then 4-hydroxyl-2-octenoic and 4-hydroxyl-2-decenoic acids attach to the ring to produce **1** and **2**, respectively. The optical rotations for these compounds, $[\alpha]^{25}_D$ −33 and $[\alpha]^{25}_D$ −35 for **1** and **2**, respectively, are almost the same, indicating that the chiral centers of **1** and **2** are probably the same.

Botcinin C (**3**) was obtained as colorless needles. The molecular formula, $C_{24}H_{38}O_8$, was determined by HR-FABMS and NMR, and it was the same as that of **2**. The ^{1}H NMR spectrum of **3** was similar to that of **2** except for some differences in chemical shifts and the shape of the resonances: The H-2 and H-3 resonances in the ^{1}H NMR

spectrum of **3** were observed 0.55 and 0.44 ppm upfield, respectively, from their positions in the spectrum of **2**, and $^2J_{H\text{-}2,H\text{-}3}$ in **3** was 7.8 Hz while it was 9.7 Hz in **2** (Table 1). The 2D NMR data revealed that **2** and **3** have the same planar structure. In the NOE experiments, irradiation of H-2 caused enhancement in the resonance of 4-methyl protons, whereas no effect was observed on H-5, suggesting that **3** is a 2-epimer of **2**. Molecular modeling of the 2-epimer of **2** showed that it also has a lactone ring in the boat form and an ether ring in the chair form and that the 2-H and 4-methyl protons are close enough to have NOEs. On the basis of these data, the absolute stereochemistry of **3** was suggested to be 2*S*, 3*S*, 4*S*, 5*S,* 6*R*, 7*R*, 8*S*, 4′*S*.

Botcinin D (**4**) was obtained as a colorless oil. The molecular formula of $C_{20}H_{30}O_6$ was determined by HREIMS and NMR. In the ^{13}C NMR spectrum of **4**, resonances due to two sp^2 methines (δ_C 126.5 and 148.3) appeared in place of resonances due to the acetoxyl at C-3 and to the two sp^3 methines at C-2 and at C-3 in the ^{13}C NMR spectrum of **1**. Therefore, compound **4** has another double bond between the quaternary carbon (C-2) and the methine carbon (C-3). When **1** was treated with aqueous KOH in an attempt to remove the fatty acyl portion, deacetylation and double-bond formation occurred. The resulting product was identical in all respects to **4**. Therefore, the absolute stereochemistry of **4** was determined to be 4*R*, 5*S,* 6*R*, 7*R*, 8*S*, 4′*S*.

Botcinin A (**1**) has a unique bicyclic unit, hexahydropyrano[3,2-*b*]pyran-2(3*H*)-one, and a hydroxy fatty acyl portion, 4-hydroxyoct-2-enoyl. Botcinin B (**2**) has the same bicyclic unit and a different acyl portion, 4-hydroxydec-2-enoyl. Botcinin C (**3**) is a C-2 epimer of **2**, and botcinin D (**4**) is a 3-deacetyl-2,3-didehydro derivative of **1**. To our knowledge, the bicyclic units of these botcinins represent a new group of natural products. Methyl epimers, such as **3**, at sites α to carbonyl group are not very common in fungal metabolites, but several examples were reported in *B. cinerea*.[14,17] The formation of these bicyclic units and their biosynthetic relationships require further investigation.

Compounds **1**−**6** were tested for antifungal activity against *Magnaporthe grisea*. Compounds **2** and **4** exhibited the most potent activity (MIC 12.5 μM) and were at least 8-fold more active than **1**, **3**, and **5** (MIC 100 μM). Compound **1** was less active than **2** and as active as **5**, indicating that the length of the acyl portion is important for the activity and that the C2′ double bond is not. Compound **2** was more active than **3**, suggesting that a change of the stereochemistry of only one carbon atom in the molecule influences the activity. Compound **4**, which has a double bond between C-2 and C-3, was one of the most active compounds despite the fact that the acyl portion of **4** is shorter than that of **2**. Compound **6** was inactive even at a concentration of 800 μM, indicating that the acyl portion plays an important role, and the combination of the acyl and the ring portions may be essential for the antifungal activity.

Experimental Section

General Experimental Procedures. Melting points are uncorrected. Optical rotations were measured with a Horiba SEPA-200 polarimeter. UV spectra were recorded with a Hitachi U-2001 spectrophotometer. NMR spectra were measured with a JEOL JNM-ECP 500 and/or a JEOL JNM-A 600 NMR spectrometer. Chemical shifts were referenced to $CDCl_3$ (δ_H 7.26, δ_C 77.0). Mass spectra were obtained with a JEOL AX505HA spectrometer (direct probe). *p*-Nitrobenzyl alcohol was the matrix used for FABMS. In EIMS the ionization voltage was 70 eV. In CIMS, the reaction gas was isobutane. HPLC was carried out with a Cosmosil 5C_{18}-AR column (Nacalai Tesque, 10 × 250 mm), with a flow rate of 0.8 mL/min and detection at 220 nm. Merck Kieselgel 60 F_{254} was used for TLC.

Molecular Modeling. Molecular modeling was performed on a HP workstation xw 4100 with MacroModel version 8.5 (Schrödinger, Inc., New York).

Fungal Material. *Botrytis cinerea* AEM 211 was isolated from a diseased strawberry in Tottori Prefecture by the Tottori Horticultural Experiment Station, gifted, and maintained on potato dextrose agar in our laboratory.

Fermentation and Extraction. The fungus was grown without shaking at 24 °C for 14 days in the dark in 500 mL conical flasks (50) containing liquid medium (200 mL/flask) composed of glucose (30 g/L), peptone (3 g/L), the extract from 50 g/L of malt, and H_2O. Metabolites were extracted from the culture filtrate with EtOAc (3 × 10 L) after adjusting the pH to 2.0 with 6 M HCl. The EtOAc solution was washed with 1 M $NaHCO_3$ (2 × 0.5 volume), dried over Na_2SO_4, and concentrated to dryness to give a residue (5.2 g).

Isolation. The residue was subjected to silica gel column chromatography (Daiso gel IR-60, 31 × 200 mm), with 1200 mL (240 mL × 5) each of 10, 20, 30, and 40% acetone in *n*-hexane as eluent. The fourth and fifth fractions (334 and 323 mg) eluted with 20% acetone in *n*-hexane were combined and subjected to silica gel column chromatography (Daiso gel IR-60, 22 × 184 mm), with 350 mL (5 mL × 20) each of 30% EtOAc in *n*-hexane as the eluent to give **1** (305 mg). The third fraction (186 mg), eluted with 20% acetone in *n*-hexane, was purified by Sephadex LH-20 column chromatography (20 × 900 mm, MeOH). Five milliliter portions of the eluent were collected. Fractions 49−51 were combined and further purified by HPLC (85% MeOH in 1% AcOH) to give **1** (23.2 mg, t_R 19 min). The second fraction (227 mg) eluted with 20% acetone in *n*-hexane was subjected to ODS flash column chromatography (Cosmosil 75C_{18}-PREP, 22 × 53 mm), with 100 mL (5 mL × 20) each of 60, 70, and 80% MeOH as the eluent. Fractions 9−14 of 70% MeOH were combined and purified by HPLC (75% MeOH in 1% AcOH) to give **2** (29.1 mg, t_R 75 min) and **3** (17.4 mg, t_R 80 min). Fractions 4−7 of 70% MeOH were combined to give **4** (6.3 mg).

Botcinin A (1): colorless needles; mp 109−111 °C; $[\alpha]^{25}_D$ −33 (*c* 0.50, EtOH); UV λ_{max} (log ϵ) 211 (4.08) nm; IR (KBr) ν_{max} 3508, 1750, 1726 cm^{-1}; NMR data, see Table 1. The selective HMBC experiment was performed using a 3.4 ms Gaussian-shaped pulse at 3.5 ppm. EIMS *m/z* 426 $[M]^+$ (1), 269 (20), 268 (94), 209 (26), 141 (84), 140 (32), 125 (36), 124 (48), 123 (56), 111 (26), 109 (100), 97 (95), 96 (29), 95 (29), 85 (63), 84 (33), 69 (53), 57 (71), 55 (33); FABMS *m/z* 427 $[M + H]^+$; HRFABMS *m/z* 427.2342 (calcd for $C_{22}H_{35}O_8$, 427.2332); *anal*. C 61.66%, H 8.06%, calcd for $C_{22}H_{34}O_8$, C 62.00%, H 8.03%.

Acetylation of Botcinin A (1). Compound **1** (6.7 mg, 0.016 mmol) was acetylated overnight with 1.0 mL of acetic anhydride and 0.5 mL of pyridine. The product was purified by HPLC (70% MeOH, 1.0 mL/min), giving acetylbotcinin A (5.0 mg): ^1H NMR ($CDCl_3$, 500 MHz) δ 0.90 (3H, t, *J* = 6.9 Hz, H-8′), 1.06 (3H, d, *J* = 6.4 Hz, 6-C*H*$_3$), 1.09 (3H, d, *J* = 6.0, 8-C*H*$_3$), 1.12 (3H, d, *J* = 6.9 Hz, 2-C*H*$_3$), 1.26 (3H, s, 4-C*H*$_3$), 1.28−1.71 (10H, m, H-3′-7′), 2.11 (3H, s, C*H*$_3$CO), 2.13 (3H, s, C*H*$_3$CO), 2.19 (1H, ddq, *J* = 11.0, 10.1, 6.0 Hz, H-6), 3.16 (1H, dq, *J* = 9.6, 7.4 Hz, H-2), 3.67 (1H, dq, *J* = 9.6, 6.0 Hz, H-8), 3.80 (1H, d, *J* = 11.0, 9.6 Hz, H-5), 4.53 (1H, dd, *J* = 10.6, 9.6 Hz, H-7), 5.41 (1H, d, *J* = 9.7 Hz, H-3), 5.42 (1H, m, H-4′), 5.94 (1H, dd, *J* = 15.6, 1.4 Hz, H-2′), 6.90 (1H, dd, *J* = 5.1, 15.6 Hz, H-2′); EIMS *m/z* 468 $[M]^+$ (7), 269 (23), 268 (98), 171 (16), 141 (100), 140 (28), 125 (18), 124 (41), 123 (43), 109 (62), 97 (82), 96 (18), 95 (29), 85 (15), 81 (16), 69 (35), 55 (12).

Botcinin B (2): colorless oil; $[\alpha]^{25}_D$ −35 (*c* 0.50, EtOH); UV λ_{max} (log ϵ) 210 (4.19) nm; IR (KBr) ν_{max} 3504, 1744 cm^{-1}; NMR data, see Table 1; EIMS *m/z* 454 $[M]^+$ (3), 269 (19), 268 (100), 171 (11), 169 (17), 140 (15), 125 (12), 124 (20), 123 (21), 109 (36), 97 (50), 85 (11), 69 (21); FABMS *m/z* 455 $[M + H]^+$; HRFABMS *m/z* 455.2620 (calcd for $C_{24}H_{39}O_8$, 455.2645).

Botcinin C (3): colorless needles; mp 95−97 °C; $[\alpha]^{25}_D$ −28 (*c* 0.26, EtOH); UV λ_{max} (log ϵ) 211 (4.11) nm; IR (KBr) ν_{max} 3526, 1750, 1726 cm^{-1}; NMR data, see Table 1; EIMS *m/z* 268 (100), 151 (30), 125 (23), 124 (30), 123 (33), 111 (22), 109 (46), 97 (81), 81 (22), 69 (42), 55 (21); FABMS *m/z* 455 $[M + H]^+$; HRFABMS *m/z* 455.2674 (calcd for $C_{24}H_{39}O_8$, 455.2645).

Botcinin D (4): colorless oil; $[\alpha]^{25}_D$ −18 (*c* 0.38, EtOH); UV λ_{max} (log ϵ) 211 (4.35) nm; IR (KBr) ν_{max} 3505, 1726 cm^{-1}; NMR data, see Table 1; EIMS *m/z* 366 $[M]^+$ (21), 208 (60), 165 (36), 141 (48), 125 (40), 124 (42), 123 (44), 97 (100); HREIMS *m/z* 366.2041 (calcd for $C_{20}H_{30}O_6$, 366.2042).

Alkaline Treatment of Botcinin A (1). Aqueous KOH (1 M, 14.8 μL) was added to a solution of **1** (3.4 mg, 0.0074 mmol) in DMF at 0 °C. After 10 min, the reaction mixture was diluted with ice water and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by silica gel column chromatography to give **4** (2.5 mg, 0.0068 mmol).

Hydrogenation of Botcinin A (1). A catalytic amount of PtO_2 in EtOAc (2 mL) was stirred 30 min under a hydrogen atmosphere at room temperature, and then **1** (12.7 mg, 0.030 mmol) in EtOAc (0.5 mL) was added. After stirring at room temperature for 1 h, the reaction mixture was filtered and evaporated in vacuo. The residue was purified by silica gel column chromatography to give **5** (12.4 mg, 0.029 mmol): colorless oil; $[\alpha]^{25}_D$ −44 (*c* 0.35, EtOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.90 (3H, t, J = 6.9 Hz, H-8′), 1.05 (3H, d, J = 6.0 Hz, 6-CH_3 or 8-CH_3), 1.07 (3H, d, J = 6.0, 6-CH_3 or 8-CH_3), 1.11 (3H, d, J = 7.4 Hz, 2-CH_3), 1.24 (3H, s, 4-CH_3), 1.24−1.88 (10H, m, H-3′-7′), 2.12 (3H, s, CH_3CO), 2.15 (1H, ddq, J = 11.0, 10.1, 6.0 Hz, H-6), 2.49 (2H, ddd, J = 7.3, 7.3, 3.7 Hz, H-2′), 3.15 (1H, dq, J = 9.7, 7.4 Hz, H-2), 3.58−3.66 (1H, m, H-5), 3.64 (1H, dq, J = 9.6, 6.0 Hz, H-8), 4.47 (1H, dd, J = 10.1, 9.6 Hz, H-7), 5.40 (1H, d, J = 9.7 Hz, H-3); CIMS 429 $[M + H]^+$.

Acid Hydrolysis of Compound 5. Aqueous HCl (12 M, 30 μL) was added to **5** (13.5 mg, 0.032 mmol) at 0 °C. After 1 h, the reaction mixture was diluted with ice water and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by silica gel column chromatography to give **6** (6.2 mg, 0.022 mmol): colorless needles; $[\alpha]^{25}_D$ −64 (*c* 0.64, EtOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.10 (3H, d, J = 7.1 Hz, 2-CH_3), 1.20 (3H, d, J = 6.4 Hz, 6-CH_3 or 8-CH_3), 1.22 (3H, d, J = 6.4 Hz, 6-CH_3 or 8-CH_3), 1.22 (3H, s, 4-CH_3), 1.97 (1H, ddq, J = 11.0, 10.1, 6.4 Hz, H-6), 2.12 (3H, s, CH_3CO), 2.91 (1H, ddd, J = 10.1, 9.7, 5.8, H-7), 3.13 (1H, dq, J = 9.7, 6.7 Hz, H-2), 3.50 (1H, dq, J = 9.7, 6.4 Hz, H-8), 3.71 (1H, d, J = 11.0 Hz, H-5), 5.37 (1H, d, J = 9.7 Hz, H-3); EIMS *m/z* 286 $[M]^+$ (15), 227 (44), 158 (70), 142 (51), 129 (28), 125 (36), 115 (25), 111 (23) 97 (37), 85 (100), 69 (41), 57 (24); CIMS *m/z* 287 $[M + H]^+$.

Preparation of MTPA Esters of 1 (1a and 1b). To a solution of **1** (4.2 mg, 0.010 mmol) in dry CH_2Cl_2 (1.5 mL) were added 4-(dimethylamino)pyridine (DMAP, 3.4 mg, 0.028 mmol), triethylamine (Et_3N, 7 μL, 0.050 mmol), and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPACl, 8 μL, 0.030 mmol) at 0 °C. After being stirred at room temperature for 0.5 h, the reaction mixture was diluted with EtOAc and the solution was washed with aqueous $NaHCO_3$ and brine. The solution was dried over Na_2SO_4, filtrated, and concentrated under reduced pressure. The residue was purified by TLC (acetone−*n*-hexane, 3:7) to give (*S*)-MTPA ester **1a** (5.8 mg, 0.0090 mmol, R_f 0.48). By the same procedure, (*R*)-MTPA ester **1b** (3.7 mg, 0.0058 mmol, R_f 0.45) was prepared with (*S*)-(+)-MTPACl from **1** (2.5 mg, 0.0059 mmol). (*S*)-MTPA ester **1a**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.84 (3H, t, J = 7.1 Hz, H-8′), 1.05 (3H, d, J = 6.4 Hz, 6-CH_3), 1.08 (3H, d, J = 6.0 Hz, 8-CH_3), 1.13 (3H, d, J = 7.1 Hz, 2-CH_3), 1.26 (3H, s, 4-CH_3), 1.14−1.33 (4H, m, H-6′, 7′), 1.72 (2H, m, H-5′), 2.13 (3H, s, CH_3CO), 2.18 (1H, ddq, J = 11.0, 10.4, 6.4 Hz, H-6), 3.16 (1H, dq, J = 9.7, 7.1 Hz, H-2), 3.54 (3H, s, CH_3O), 3.67 (1H, dq, J = 9.7, 6.0 Hz, H-8), 3.80 (1H, d, J = 11.0 Hz, H-5), 4.52 (1H, dd, J = 10.4, 9.7 Hz, H-7), 5.41 (1H, d, J = 9.7 Hz, H-3), 5.64 (ddt, J = 7.4, 1.2, 5.8 Hz, H-4′), 5.97 (1H, dd, J = 15.8, 1.4 Hz, H-2′), 6.91 (1H, dd, J = 15.8, 6.0 Hz, H-3′), 7.38−7.52 (5H, m, Ph); FABMS *m/z* 643 $[M + H]^+$. (*R*)-MTPA ester **1b**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.89 (3H, t, J = 7.1 Hz, H-8′), 1.04 (3H, d, J = 6.4 Hz, 6-CH_3), 1.06 (3H, d, J = 6.0, 8-CH_3), 1.13 (3H, d, J = 7.1 Hz, 2-CH_3), 1.26 (3H, s, 4-CH_3), 1.33 (4H, m, H-6′, 7′), 1.75 (2H, m, H-5′), 2.13 (3H, s, CH_3CO), 2.16 (1H, ddq, J = 11.0, 10.4, 6.4 Hz, H-6), 3.16 (1H, dq, J = 9.7, 7.1 Hz, H-2), 3.57 (3H, d, J = 0.9 Hz, CH_3O), 3.65 (1H, dq, J = 9.7, 6.0 Hz, H-8), 3.79 (1H, d, J = 11.0 Hz, H-5), 4.49 (1H, dd, J = 10.4, 9.7 Hz, H-7), 5.41 (1H, d, J = 9.7 Hz, H-3), 5.63 (ddt, J = 6.9, 1.4, 5.8 Hz, H-4′), 5.80 (1H, dd, J = 15.8, 1.4 Hz, H-2′), 6.84 (1H, dd, J = 15.8, 6.0 Hz, H-3′), 7.38−7.52 (5H, m, Ph); FABMS *m/z* 643 $[M + H]^+$.

Preparation of MTPA Esters of 6 (6a and 6b). Compound **6** (4.2 mg, 0.015 mmol) in dry CH_2Cl_2 was reacted with (*R*)-(−)-MTPACl in the presence of DMAP and Et_3N. The reaction product was purified by TLC (acetone−*n*-hexane, 3:7) to give (*S*)-MTPA ester **6a** (1.0 mg, 0.0020 mmol, R_f 0.64). Similarly, (*R*)-MTPA ester **6b** (1.2 mg, 0.0024 mmol, R_f 0.50) was prepared with (*S*)-(+)-MTPACl from **6** (2.3 mg, 0.0080 mmol). (*S*)-MTPA ester **6a**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.97 (3H, d, J = 6.0 Hz, 6-CH_3), 1.07 (3H, d, J = 6.2 Hz, 8-CH_3), 1.12 (3H, d, J = 7.1 Hz, 2-CH_3), 1.22 (3H, s, 4-CH_3), 2.12 (3H, s, CH_3CO), 2.21 (1H, ddq, J = 11.0, 10.5, 6.0 Hz, H-6), 3.15 (1H, dq, J = 9.7, 7.1 Hz, H-2), 3.56 (3H, d, J = 1.2 Hz, CH_3O), 3.64 (1H, dq, J = 10.1, 6.2 Hz, H-8), 3.79 (1H, d, J = 11.0 Hz, H-5), 4.62 (1H, dd, J = 10.5, 10.1 Hz, H-7), 5.40 (1H, d, J = 9.7 Hz, H-3), 7.39−7.58 (5H, m, Ph); CIMS *m/z* 503 $[M + H]^+$. (*R*)-MTPA ester **6b**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.97 (3H, d, J = 6.2 Hz, 6-CH_3), 1.09 (3H, d, J = 7.1, 8-CH_3), 1.12 (3H, d, J = 7.1 Hz, 2-CH_3), 1.23 (3H, s, 4-CH_3), 2.12 (3H, s, CH_3-CO), 2.18 (1H, ddq, J = 11.0, 10.6, 6.2 Hz, H-6), 3.15 (1H, dq, J = 9.7, 7.1 Hz, H-2), 3.53 (3H, d, J = 0.9 Hz, CH_3O), 3.68 (1H, dq, J = 9.7, 6.2 Hz, H-8), 3.77 (1H, d, J = 10.6 Hz, H-5), 4.63 (1H, dd, J = 10.6, 9.7 Hz, H-7), 5.40 (1H, d, J = 9.7 Hz, H-3), 7.42−7.58 (5H, m, Ph); CIMS *m/z* 503 $[M + H]^+$.

MTPA Esters of 2 (2a and 2b). Compound **2** (4.4 mg, 0.0097 mmol) in dry CH_2Cl_2 was reacted with (*R*)-(−)-MTPACl in the presence of DMAP and Et_3N. The reaction product was purified by TLC (acetone−*n*-hexane, 3:7) to give (*S*)-MTPA ester **2a** (3.1 mg, 0.0046 mmol, R_f 0.47). Similarly, (*R*)-MTPA ester **2b** (3.8 mg, 0.0057 mmol, R_f 0.45) was prepared with (*S*)-(+)-MTPACl from **2** (3.4 mg, 0.0075 mmol). (*S*)-MTPA ester **2a**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.86 (3H, t, J = 6.6 Hz, H-10′), 1.05 (3H, d, J = 6.4 Hz, 6-CH_3), 1.08 (3H, d, J = 6.0, 8-CH_3), 1.13 (3H, d, J = 6.9 Hz, 2-CH_3), 1.26 (3H, s, 4-CH_3), 1.17−1.34 (8H, m, H-6′, 7′, 8′, 9′), 1.71 (2H, m, H-5′), 2.13 (3H, s, CH_3CO), 2.18 (1H, ddq, J = 11.0, 10.4, 6.4 Hz, H-6), 3.15 (1H, dq, J = 9.7, 6.9 Hz, H-2), 3.54 (3H, s, CH_3O), 3.65 (1H, dq, J = 9.7, 6.0 Hz, H-8), 3.80 (1H, d, J = 11.0 Hz, H-5), 4.52 (1H, dd, J = 10.4, 9.7 Hz, H-7), 5.41 (1H, d, J = 9.7 Hz, H-3), 5.64 (ddt, J = 7.4, 1.2, 5.8 Hz, H-4′), 5.97 (1H, dd, J = 15.8, 1.4 Hz, H-2′), 6.91 (1H, dd, J = 15.8, 6.0 Hz, H-3′), 7.38−7.52 (5H, m, Ph); FABMS *m/z* 671 $[M + H]^+$. (*R*)-MTPA ester **2b**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.88 (3H, t, J = 6.9 Hz, H-10′), 1.04 (3H, d, J = 6.0 Hz, 6-CH_3), 1.06 (3H, d, J = 6.5, 8-CH_3), 1.13 (3H, d, J = 7.4 Hz, 2-CH_3), 1.26 (3H, s, 4-CH_3), 1.26−1.40 (8H, m, H-6′, 7′, 8′, 9′), 1.75 (2H, m, H-5′), 2.13 (3H, s, CH_3CO), 2.16 (1H, ddq, J = 11.0, 10.4, 6.0 Hz, H-6), 3.16 (1H, dq, J = 9.7, 7.4 Hz, H-2), 3.57 (3H, s, CH_3O), 3.65 (1H, dq, J = 9.7, 6.0 Hz, H-8), 3.79 (1H, d, J = 11.0 Hz, H-5), 4.49 (1H, dd, J = 10.4, 9.7 Hz, H-7), 5.41 (1H, d, J = 9.7 Hz, H-3), 5.62 (ddt, J = 7.4, 1.2, 5.8 Hz, H-4′), 5.81 (1H, dd, J = 15.8, 1.4 Hz, H-2′), 6.84 (1H, dd, J = 15.8, 6.0 Hz, H-3′), 7.38−7.52 (5H, m, Ph); FABMS *m/z* 671 $[M + H]^+$.

Antifungal Assay. MeOH solutions of the samples were placed in wells of a 96-microwell plate. As a control, only MeOH was also placed in the wells of the same plate. After air-drying, 100 μL of malt extract medium composed of glucose (30 g/L), peptone (3 g/L), and the extract from 50 g/L of malt was added to the samples. Then, an agar plug (1 mm^2) cut from the edge of a 1-week-old culture of *Magnaporthe grisea* (*Pyricularia oryzae* IFO 30733), grown at 28 °C in a Petri dish (90 × 20 mm) containing 20 mL of potato medium composed of sucrose (30 g/L), agar (20 g/L), and the extract from 200 g/L of potato, was inoculated onto the medium. After 3 days at 28

°C, the mycelium growth was observed and the minimum concentration to inhibit the growth of mycelia was checked. This antifungal assay was carried out three times.

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