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Synthesis and Biotransformation of Plausible Biosynthetic Intermediates of Salicylaldehyde-Type Phytotoxins of Rice Blast Fungus, *Magnaporthe grisea*

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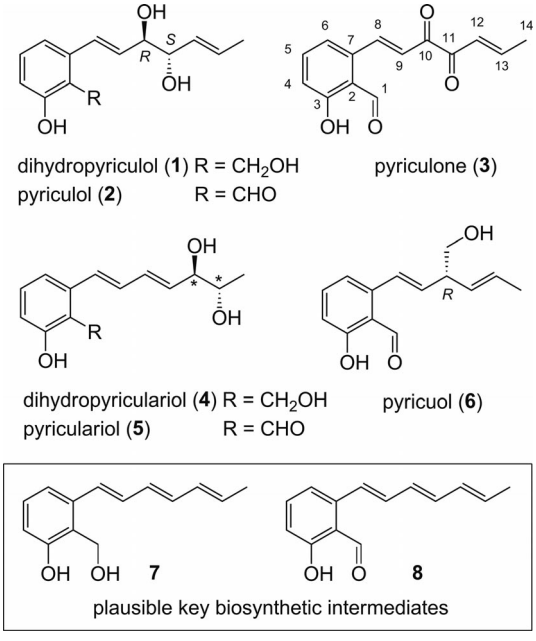
Rice blast fungus, *Magnaporthe grisea*, produces a series of salicylaldehyde-type phytotoxins such as pyriculol, pyricuol, and pyriculariol. Plausible biosynthetic intermediates of these phytotoxins were synthesized in deuterium labeled forms using the Stille coupling reaction as the key step, and subjected to biotransformation by the fungus. Heptatrienylsalicylaldehyde was converted into dihydropyriculol by the fungus by a shaking culture. A new biosynthetic pathway comprising both oxidation and reduction is proposed.

Introduction

Rice blast fungus *Magnaporthe grisea* (Hebert) Barr. (the perfect stage of *Pyricularia oryzae* Cavara), which causes rice blast disease, is the most serious pest of rice.[1] The fungus produces several main phytotoxic compounds according to the fermentation method, i.e. (12*R*,13*S*)-dihydropyriculol (**1**),[2] (12*R*,13*S*)-pyriculol (**2**),[3] and pyriculone (**3**)[4] by a shaking culture, racemic dihydropyriculariol (**4**)[6] and pyriculariol (**5**)[7] by an aeration and agitation culture, and tenuazonic acid by a stationary culture in a soy sauce–sucrose medium (Scheme 1). (*R*)-Pyricuol (**6**)[5] together with **2** have been produced by a shaking culture with a potato dextrose medium. These salicylaldehyde-type phytotoxins are common metabolites of blast fungi of various host plants.[8] To develop new methods to control the blast disease a better knowledge of its biosynthesis is necessary. Although a number of gene clusters of the fungus potentially involved in pathogenicity have been clarified, that encoding the biosynthesis of these toxins has not yet been specified.[9] Little is known about the biosynthesis of these phytotoxic compounds (Scheme 1), except that they are derived from seven units of acetyl CoA like other salicylaldehyde-type congeners.[10,11] Nukina et al. suggested that heptatrienylsalicyl alcohol (**7**) is an important biosynthetic intermediate of salicylaldehyde-type polyketide synthesis,[8,12] because the fungus could oxidize (*Z*)- and (*E*)-1-phenylpropenes to racemic *erythro*-diol and optically active *threo*-diol, respectively.[13] We also assumed that heptatrienylsalicylaldehyde (**8**) could be an intermediate, because Iwasaki et al. reported that aldehyde **2** was converted to alcohol **1** by the fungus.[14] We began the biosynthetic studies using possible labeled intermediates. In this paper, synthesis and biotransformation of deuterium labeled **7** and **8** are described.



Scheme 1. Salicylaldehyde-type phytotoxins from rice blast fungus, *Magnaporthe grisea*.

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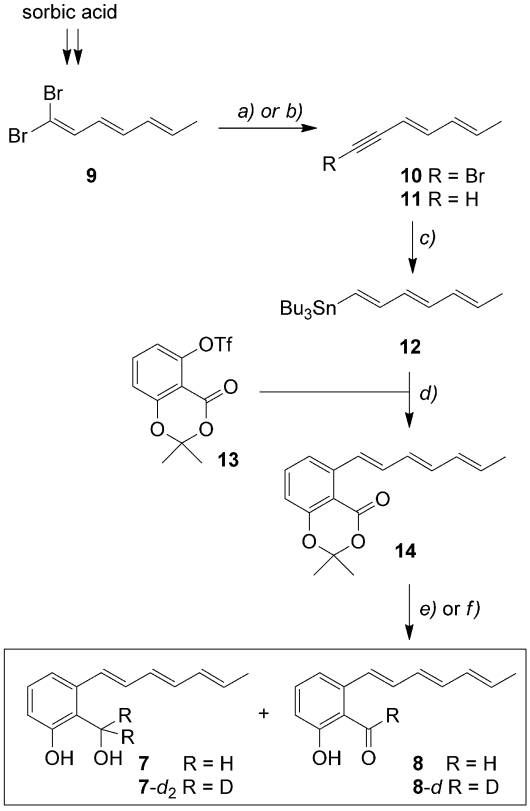
Results and Discussion

Synthesis of Substrates

Synthesis of the side chain fragment started from the known dibromide **9**[15] (Scheme 2), which was prepared from sorbic acid in three steps. Treatment of **9** with NaHMDS afforded the bromo-alkyne **10**, which was subjected to palladium-catalyzed hydrostannylation[16] to give (*E*)-stannane **12** accompanied by a small amount of regio- and stereoisomers. Hydrostannylation of the corresponding terminal alkyne **11** was not reproducible. The Stille coupling reaction with the known triflate **13**[17,18] successfully furnished unstable lactone **14**. The carbonyl moiety of **14** was reduced with $LiAlH_4$[19] at –78 °C to give **7** in 43% yield. In addition, half-reduced **8** was also obtained in 34% yield. For biotransformation the corresponding α-deuterio derivatives **7**-d_2 and **8**-*d* were prepared in a similar manner using $LiAlD_4$.



Scheme 2. Synthetic scheme of plausible biosynthetic intermediates (dba = dibenzylideneacetone). a) NaHMDS, THF –78 °C (80% for **10**). b) BuLi, THF, –78 °C. c) Bu_3SnH, PPh_3, Pd_2dba_3, THF. d) P(2-furyl)$_3$, Pd_2dba_3, LiCl, *N*,*N*-dimethylformamide (DMF, 35% from **10**). e) $LiAlH_4$, THF, –78 °C (43% for **7** and 34% for **8**). f) $LiAlD_4$, THF, –78 °C (36% for **7**-d_2 and 20% for **8**-*d*).

Biotransformation of 7-d_2 and 8-*d* by *Magnaporthe grisea*

Compounds **7**-d_2 and **8**-*d* were each subjected to biotransformation. A solution of each substrate in ethyl acetate (EtOAc) was introduced into the culture broth (soy sauce–sucrose medium) of *M. grisea* and the culture was incubated using a reciprocal shaker for 1 d at 24 °C. Each broth was filtered, the filtrate extracted into EtOAc, and the extract analyzed by liquid chromatography– mass spectrometry (LC–MS). A substantial amount of **1** was detected in each extract (A: +EtOAc, B: +**7**-d_2 in EtOAc, C: +**8**-*d* in EtOAc, and D: control). The LC–MS [ESI(+)] showed that extract B contained only unlabeled **1**, which is quite similar to that of extracts A and D, but almost equal amounts of unlabeled and labeled **1** and **1**-*d* were detected in extract C (Figure 1). The presence of EtOAc and **7**-d_2 did not affect the pattern of fermentation products.

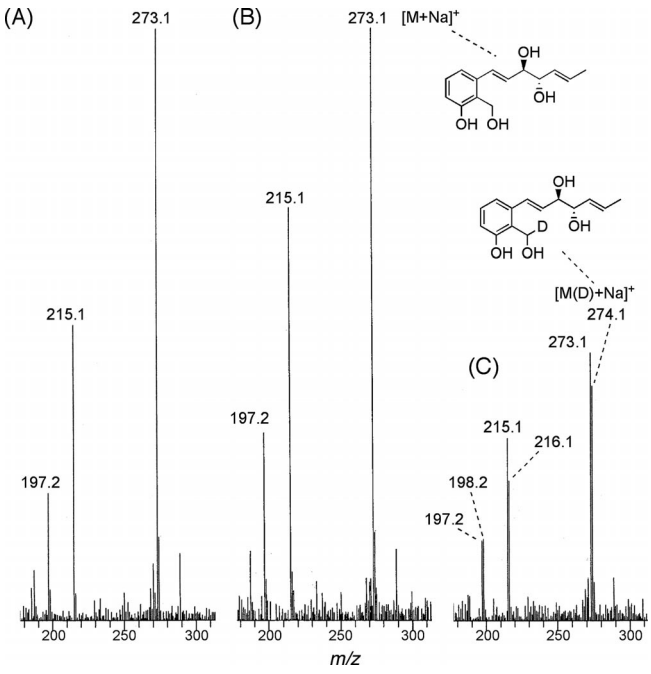
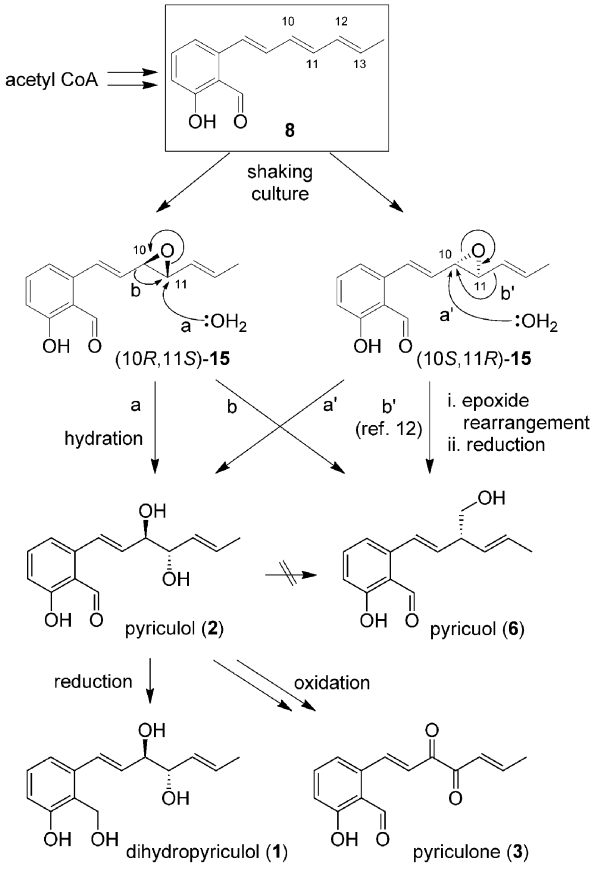


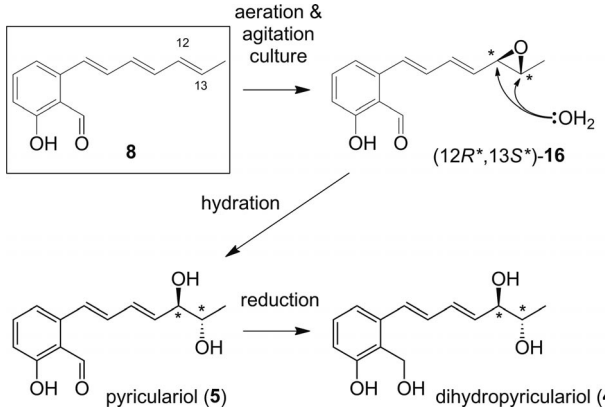
Figure 1. Regions of LC–MS [ESI(+)] of extracts A (+EtOAc), B (+**7**-d_2 in EtOAc), and C (+**8**-*d* in EtOAc).

The production of **1** and **1**-*d* was also confirmed by ^1H NMR analysis of extract C. A pair of broad singlets (δ_H = 5.01 ppm, 1 H for **1**; and δ_H = 4.99 ppm, 0.5 H for **1**-*d*) were observed. These results indicate that **8** is the most possible biosynthetic intermediate for the phytotoxins. Thus, the dihydro derivatives **1** and **4** are produced at the later stages by the reduction of **2** and **5**, respectively. Although there still remains the possibility that **7**-d_2 was too polar to permeate the cell membrane, it did not seem to inhibit the biosynthetic pathway. New biosynthetic pathways are proposed in Schemes 3 and 4. When the fungus is fermented by a shaking culture, epoxidation of the 10,12-double bond of **8** occurs to give (10*R*,11*S*)-**15** and/or (10*S*,11*R*)-**15** (Scheme 3). For (10*R*,11*S*)-**15**, after the formation of **2** by hydration of the epoxy ring at C-11 (route a), reduction and oxidation afford **1** and **3**, respectively. (10*S*,11*R*)-**15** can also furnish **2** by route a′ by hydration at C-10. Thus, the biosynthetic conversion could be performed by way of stereoselective epoxidation followed by regioselective hydration, and/or nonstereoselective epoxidation followed by *S*-carbon specific hydration. Our stereoselective synthesis of **6**[12]

showed that it is presumably formed not from **2** (through pinacol rearrangement) but from (10*R*,11*S*)- and/or (10*S*,11*R*)-**15** by epoxide rearrangement followed by reduction when the fungus is fermented in a potato dextrose medium (route b and/or b′).



Scheme 3. A newly proposed biosynthetic pathway for the salicylaldehyde-type phytotoxins (shaking culture).



Scheme 4. A newly proposed biosynthetic pathway for the salicylaldehyde-type phytotoxins (aeration and agitation culture).

On the other hand, racemic pyriculariol (**5**)[7c] is formed by an aeration and agitation culture (Scheme 4). If *trans*-epoxide **16** is produced in enantiomerically pure form, completely non-regioselective hydration is necessary to give racemic **5**. Thus, it is rather plausible that both epoxidation and hydration occur in a nonselective manner. Reduction of the formyl group leads to **4**. Both oxidation and reduction would occur during the later stage of the polyketide synthesis.

It is often the case with fungi that they produce different secondary metabolites under different fermentation conditions. The rice blast fungus also has the ability to produce various metabolites according to natural nutritional status. It would be interesting to know the relationships between the order of phytotoxicity (**3** > **6** > **2** > **5**) and the environmental conditions that produce them. It is somewhat curious that both oxidation and reduction occur during the later stage of the biosynthesis. However, oxidation from salicylalcohols to salicylaldehydes is restricted. Further synthetic and fermentative studies along with genetic analysis will clarify the whole biosynthetic pathway of these salicylaldehyde-type phytotoxins.

Conclusions

Plausible biosynthetic intermediates for the salicylaldehyde-type phytotoxins of the rice blast fungus were synthesized using the Stille coupling reaction as the key step. Deuterium-labeled heptatrienylsalicylaldehyde (**8**-*d*) was biotransformed into deuteriodihydropyriculol by the fungus, and a new biosynthetic pathway containing both oxidation and reduction is proposed.

Experimental Section

General: FTIR spectra were recorded as films with a Jasco 4100 spectrometer (ATR, Zn-Se). ^{1}H and ^{13}C NMR spectra were recorded with Varian Inova 600 (600 MHz for ^{1}H and 150 MHz for ^{13}C) and Varian Inova 500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometers with tetramethylsilane (δ = 0 ppm for ^{1}H) and $CDCl_3$ (δ = 77.0 ppm for ^{13}C) as internal standards. Mass spectra were recorded with a Jeol JMS–700 spectrometer. LC–MS data were recorded with a Thermo-LCQ Deca system. Merck silica gel 60 (70–230 mesh) was used for column chromatography.

(3*E*,5*E*)-1-Bromohepta-3,5-dien-1-yne (10): To a solution of **9** (4.12 g, 16.4 mmol) in dry THF (80 mL) was added NaHMDS (1.07 M in hexane, 25.0 mL, 26.8 mmol) at –78 °C, and the mixture was stirred at this temperature for 1 h. The reaction was quenched with sat. aq. NH_4Cl solution and extracted into pentane. The organic layer was washed with H_2O and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel. Elution with pentane/Et_2O (100:1) gave **10** (2.25 g, 13.2 mmol, 80%) as a yellow oil. R_f = 0.80 (SiO_2, hexane/EtOAc = 10:1). FTIR (ATR): $\tilde{\nu}$ = 3024, 2962, 2934, 2911, 2870, 2849, 2189 (C≡C), 2157 (C≡C) 1641, 1445, 1291, 980 (C–Br), 928 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.79 (d, *J* = 7.3 Hz, 3 H, 7-H), 5.45 (d, *J* = 15.7 Hz, 1 H, 3-H), 5.85 (dq, *J* = 15.1, 7.3 Hz, 1 H, 6-H), 6.08 (dd, *J* = 15.1, 10.7 Hz, 1 H, 5-H), 6.60 (dd, *J* = 15.7, 10.7 Hz, 1 H, 4-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 18.3 (C-7), 50.3, 79.6, 107.8, 130.6, 133.5, 143.6 ppm. EI-MS: *m*/*z* = 172 [M(^{81}Br)]$^{+\cdot}$, 170 [M(^{79}Br)]$^{+\cdot}$, 91 [M – Br]$^+$, 89, 65, 39. HRMS (EI): calcd. for $C_7H_7{}^{79}Br$ [M(^{79}Br)]$^{+\cdot}$ 169.9726; found 169.9732.

(1*E*,3*E*,5*E*)-Tributylhepta-1,3,5-trienylstannane (12): To a solution of **10** (80 mg, 0.47 mmol), PPh_3 (10 mg, 0.04 mmol), and Pd_2dba_3

(3.7 mg, 0.0040 mmol) in dry THF (5 mL) was added Bu_3SnH (314 mg, 1.08 mmol) dropwise and the mixture was stirred at 20 °C for 0.5 h. The mixture was filtered through a Celite pad, and the filtrate was concentrated in vacuo to give **12** as a dark brown oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.78–1.03 (m, 15 H, 1′, 4′-H), 1.25–1.40 (m, 6 H, 3′-H), 1.45–1.55 (m, 6 H, 2′-H), 1.78 (d, J = 6.7 Hz, 3 H, 7-H), 5.73 (dq, J = 14.9, 6.7 Hz, 1 H, 6-H), 6.03–6.13 (m, 3 H), 6.20 (d, J = 18.6 Hz, 1 H, 1-H), 6.56 (dd, J = 18.6, 9.3 Hz, 1 H, 2-H) ppm. Crude **12** was used in the next step without further purification.

(1′*E*,3′*E*,5′*E*)-6-(Hepta-1′,3′,5′-trienyl)-2,2-dimethyl-4*H*-1,3-benzodioxin-4-one (14): To a solution of Pd_2dba_3 (15 mg, 0.017 mmol), LiCl (101 mg, 2.38 mmol), P(2-furyl)$_3$ (9.9 mg, 0.043 mmol), and **13** (383 mg, 1.17 mmol) in dry DMF (5 mL) was added a solution of crude **12** (63 mg) in dry DMF (7.5 mL), and the mixture was stirred at 20 °C for 36 h. After addition of KF (1 g), the mixture was extracted with EtOAc. The organic layer was washed with H_2O and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel. Elution with hexane/EtOAc (100:1 to 25:1) gave **14** (44.8 mg, 0.166 mmol, 35% from **10**) as a colorless oil. FTIR (ATR): $\tilde{\nu}$ = 3071, 2997, 2938, 2853, 1727 (C=O), 1572, 1474, 1379, 1318, 1269, 1251, 1207, 1080, 1044, 998, 967, 925, 690 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.71 (s, 6 H, gem-Me_2), 1.81 (d, J = 6.9 Hz, 3 H, 7′-H), 5.81 (dq, J = 15.1, 6.8 Hz, 1 H), 6.17 (dd, J = 15.1, 7.8 Hz, 1 H), 6.33–6.42 (m, 2 H), 6.79–6.87 (m, 2 H), 7.33 (d, J = 7.8 Hz, 1 H), 7.42 (dd, J = 8.3, 7.8 Hz, 1 H), 7.69 (d, J = 15.6 Hz, 1 H, 1′-H) ppm. Lactone **14** was unstable and used in the next steps without further purification.

(1′*E*,3′*E*,5′*E*)-6-(Hepta-1′,3′,5′-trienyl)-2-hydroxybenzenemethanol-α,α-d_2 (7-d_2) and (1′*E*,3′*E*,5′*E*)-6-(Hepta-1′,3′,5′-trienyl)-2-hydroxybenzaldehyde-α-*d* (8-*d*): To a suspension of $LiAlD_4$ (38 mg, 0.91 mmol) in dry THF (6 mL) was added a solution of **14** (61 mg, 0.23 mmol) in dry THF (6 mL) at –78 °C and the mixture was stirred at this temperature for 3 h. A mixture of 0.1 M aq. HCl/MeOH was added, and the resulting mixture was diluted with Et_2O. The organic layer was washed with H_2O and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel. Elution with hexane/EtOAc (4:1) gave **7**-d_2 (18 mg, 0.082 mmol, 36%) and **8**-*d* (9.7 mg, 0.045 mmol, 20%). **7**-d_2: colorless crystals. FTIR (ATR): $\tilde{\nu}$ = 3200 (br., O–H), 3011, 2959, 2927, 2852, 1721, 1591, 1577, 1464, 1284, 1079, 1035, 997, 945, 791, 743 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.81 (d, J = 6.8 Hz, 3 H, 7′-H), 2.21 (br. s, 1 H, CD_2OH), 5.79 (dq, J = 14.7, 6.8 Hz, 1 H, 6′-H), 6.17 (dd, J = 14.7, 11.3 Hz, 1 H), 6.25 (dd, J = 15.1, 7.3 Hz, 1 H), 6.32 (dd, J = 15.1, 9.7 Hz, 1 H), 6.60–6.67 (m, 2 H), 6.77 (d, J = 8.3 Hz, 1 H), 7.03 (d, J = 7.8 Hz, 1 H), 7.14 (dd, J = 8.3, 7.8 Hz, 1 H, 4-H), 7.5 (s, 1 H, ArOH) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 18.4 (C-7′), 115.6, 118.1, 127.5, 129.0, 130.2, 131.0, 131.6, 132.7, 134.4, 156.9 (C-2) ppm. MS (EI): *m*/*z* = 218 $[M]^{+\cdot}$, 200 $[M - H_2O]^+$, 185 $[M - H_2O - CH_3]^+$, 167, 149, 146, 117, 68, 54, 31, 27. HRMS (EI): calcd. for $C_{14}H_{16}D_2O_2$ $[M]^{+\cdot}$ 218.1270; found 218.1270. **8**-*d*: colorless crystals. FTIR (ATR): $\tilde{\nu}$ = 3019, 2905 (D–CO), 1637 (C=O), 1598, 1456, 1339, 1311, 1242, 1210, 1177, 991, 784, 739, 714 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.83 (d, J = 6.8 Hz, 3 H, 7′-H), 5.86 (dq, J = 15.1, 6.8 Hz, 1 H, 6′-H), 6.17 (dd, J = 14.6, 7.8 Hz, 1 H), 6.31 (dd, J = 15.1, 10.7 Hz, 1 H), 6.70 (dd, J = 15.1, 10.7 Hz, 1 H), 6.84 (d, J = 8.3 Hz, 1 H), 6.93 (d, J = 13.2 Hz, 1 H, 1′-H), 6.99 (d, J = 7.8 Hz, 1 H), 7.44 (dd, J = 8.3, 7.8 Hz, 1 H), 11.95 (s, 1 H, OH) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 18.5 (C-7′), 116.5, 118.1, 124.7, 129.5, 131.4, 132.5, 136.2, 136.3, 137.0, 142.8, 163.0 (C-2), 200.5 (C-α) ppm. MS (EI): *m*/*z* = 215 $[M]^{+\cdot}$, 200, 197 $[M - H_2O]^+$, 186, 182 $[M - H_2O - CH_3]^+$, 149, 148, 91, 84, 77, 39, 18. HRMS (EI): calcd. for $C_{14}H_{13}DO_2$ $[M]^{+\cdot}$ 215.1052; found 215.1052.

(1′*E*,3′*E*,5′*E*)-6-(Hepta-1′,3′,5′-trienyl)-2-hydroxybenzenemethanol (7) and (1′*E*,3′*E*,5′*E*)-6-(Hepta-1′,3′,5′-trienyl)-2-hydroxybenzaldehyde (8): In the same manner as described for the deuterio derivatives, reduction of **12** with $LiAlH_4$ furnished alcohol **7** (43%) and aldehyde **8** (34%). **7**: FTIR (ATR): $\tilde{\nu}$ = 3382 (br., O–H), 3012, 2958, 2923, 2852, 1712, 1593, 1577, 1464, 1362, 1258, 1224, 1193, 1000, 981, 961, 912, 801, 769, 735 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.81 (d, J = 6.8 Hz, 3 H, 7′-H), 2.30 (br. s, 1 H, CH_2OH), 4.99 (s, 2 H, α-H), 5.79 (dq, J = 15.1, 6.8 Hz, 1 H, 6′-H), 6.16 (pseudo dd, J = 15.1, 10.3 Hz, 1 H, 5′-H), 6.25 (dd, J = 15.1, 6.8 Hz, 1 H, 3′-H), 6.32 (dd, J = 14.8, 9.8 Hz, 1 H, 4′-H), 6.60–6.67 (m, 2 H, 2′,1′-H), 6.76 (d, J = 7.8 Hz, 1 H, 3-H), 7.02 (d, J = 7.8 Hz, 1 H, 5-H), 7.14 (t, J = 7.8 Hz, 1 H, 4-H), 7.55 (br. s, 1 H, ArOH) ppm. ^{1}H NMR (600 MHz, CD_2Cl_2): δ = 1.80 (d, J = 6.7 Hz, 3 H, 7′-H), 2.30 (br. s, 1 H, CH_2OH), 5.00 (s, 2 H, α-H), 5.81 (dq, J = 14.7, 6.7 Hz, 1 H, 6′-H), 6.16 (ddd, J = 14.7, 10.2, 1.2 Hz, 1 H, 5′-H), 6.29 (dd, J = 15.0, 7.3 Hz, 1 H, 3′-H), 6.33 (dd, J = 15.0, 10.2 Hz, 1 H, 4′-H), 6.64–6.70 (m, 2 H, 1′,2′-H), 6.74 (d, J = 8.4 Hz, 1 H, 3-H), 7.04 (d, J = 7.8 Hz, 1 H, 5-H), 7.14 (t, J = 7.8 Hz, 1 H, 4-H), 7.60 (br. s, 1 H, ArOH) ppm. ^{13}C NMR (150 MHz, CD_2Cl_2): δ = 18.5 (C-7′), 60.4 (C-α), 115.8 (C-3), 118.1 (C-5), 122.1 (C-1), 127.9 (C-1′), 129.2 (C-4), 130.6 (C-3′), 131.4 (C-6′), 131.9 (C-5′), 132.8 (C-2′), 134.7 (C-4′), 137.1 (C-6), 157.1 (C-2) ppm. MS (EI): *m*/*z* = 216 $[M]^{+\cdot}$, 198 $[M - H_2O]^+$, 183 $[M - H_2O - CH_3]^+$, 165, 149, 144, 128, 115, 91, 81, 76, 54, 40. HRMS (EI): calcd. for $C_{14}H_{16}O_2$ $[M]^{+\cdot}$ 216.1145; found 216.1149. **8**: colorless crystals. FTIR (ATR): $\tilde{\nu}$ = 3015, 2955, 2926, 2854 (H–CO), 1733, 1645 (C=O), 1601, 1456, 1331, 1311, 1236, 997, 772 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.82 (d, J = 6.8 Hz, 3 H, 7′-H), 5.86 (dq, J = 14.6, 6.8 Hz, 1 H, 6′-H), 6.14–6.20 (m, 1 H), 6.30 (dd, J = 15.1, 10.7 Hz, 1 H), 6.40 (dd, J = 15.1, 10.7 Hz, 1 H), 6.70 (dd, J = 15.1, 10.7 Hz, 1 H), 6.84 (d, J = 8.3 Hz, 1 H), 6.995 (d, J = 8.8 Hz, 1 H), 7.00 (d, J = 15.1 Hz, 1 H, 1′-H), 7.44 (dd, J = 8.3, 7.8 Hz, 1 H, 4-H), 10.34 (s, 1 H, CHO), 11.93 (s, 1 H, OH) ppm.

Biotransformation: The fungus *M. grisea*, stored at 10 °C on a slant agar culture, was cultured in darkness at 24 °C on a reciprocal shaker, using 500 mL Sakaguchi flasks each containing 100 mL of soy sauce–sucrose medium. The medium contained 50 mL of commercial soy sauce and 50 g of sucrose in 1 liter of tap water and 0.01% silicone antifoaming agent. On the sixth day of the culture, when the growth of the fungus had almost reached its maximal stage, a solution of (A) EtOAc (100 μL), (B) **7**-d_2 (7.0 mg) in EtOAc (100 μL), (C) **8**-*d* (9.7 mg) in EtOAc (100 μL), or (D) nothing was introduced into each flask with a sterile microsyringe. Cultivation was continued for a further day under the same conditions. Each culture broth was filtered, and the filtrates were independently and repeatedly extracted into EtOAc. Each extract was washed with H_2O and brine, dried with Na_2SO_4, and concentrated in vacuo to give crude extracts (A) 13.6 mg, (B) 21.7 mg, (C) 16.9 mg, and (D) 19.8 mg. Each residue was diluted with MeOH and analyzed with LC–MS (L-column2 ODS, Φ = 4.6 × 150 mm, eluting solvent: a linear gradient of MeOH/H_2O from 95:5 to 100:0 in 15 min at a flow rate of 1.0 mL/min). Compound **1** eluted at 10.6 min, ESI-MS (+): *m*/*z* = 273.09 $[M + Na]^+$, and ESI-MS (–): *m*/*z* = 249.10 $[M - H]^-$ in each extract. Compound **1**-*d* was eluted at 10.4 min, ESI-MS (+): *m*/*z* = 274.08 $[M + Na]^+$, and ESI-MS (–): *m*/*z* = 250.09 $[M - H]^-$ in extract B. ^{1}H NMR (600 MHz, $CDCl_3$) of a mixture of **1** and **1**-*d* (1:1): δ = 1.75 (d, J = 6.0 Hz, 3 H, 14-H), 4.17–4.21 (m, 1 H, 11-H), 4.30–4.34 (m, 1 H, 10-H), 4.99 (br. s, 0.5 H, 1-H of **1**-*d*), 5.01 (s, 1 H, 1-H for **1**), 5.56 (dd, J = 15.0, 6.7 Hz, 1 H, 12-H), 5.83 (dq, J = 15.5, 7.0 Hz, 1 H, 13-H), 6.04 (dd, J = 15.3,

5.6 Hz, 1 H, 9-H), 6.82 (d, J = 7.3 Hz, 1 H), 6.85 (d, J = 15.0 Hz, 1 H, 8-H), 6.95 (d, J = 7.3 Hz, 1 H), 7.17 (t, J = 7.3 Hz, 1 H) ppm. The ^{1}H NMR spectrum was almost identical to those reported.[2]

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[1] a) *Rice Blast Disease*, CAB International, Wallingford, UK, **1994**; b) G. S. Khush, in: *Progress in Irrigated Rice Research*, International Rice Research Institute (IRRI), Manila, Philippines, **1989**, pp. 79–92; c) G. S. Khush, *Plant Mol. Biol.* **2005**, 79–92.
[2] a) S. Iwasaki, H. Muro, K. Sasaki, S. Nozoe, S. Okuda, *Tetrahedron Lett.* **1973**, *14*, 3537–3542; b) Y. Kono, S. Sekido, I. Yamaguchi, H. Kondo, Y. Suzuki, G. C. Neto, A. Sakurai, H. Yaegashi, *Agric. Biol. Chem.* **1991**, *55*, 2785–2791.
[3] S. Iwasaki, H. Muro, K. Sasaki, S. Nozoe, S. Okuda, *Tetrahedron Lett.* **1969**, *10*, 3977–3980.
[4] M. Nukina, T. Otuki, T. Kurebayashi, K. Hosokawa, *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu* **1996**, *38*, 391–396.
[5] J.-C. Kim, J.-Y. Min, H.-T. Kim, K.-Y. Cho, S.-H. Yu, *Biosci. Biotechnol. Biochem.* **1998**, *62*, 173–174.
[6] M. Nukina, *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu* **1987**, *29*, 705–712.
[7] a) M. Nukina, T. Sassa, M. Ikeda, T. Umezawa, H. Tasaki, *Agric. Biol. Chem.* **1981**, *45*, 2161–2162; b) the (12*R*,13*S*) absolute configuration of natural pyriculariol had been determined by our previous synthesis: A. Sasaki, K. Tanaka, Y. Sato, S. Kuwahara, H. Kiyota, *Tetrahedron Lett.* **2009**, *50*, 4637; c) however, our further synthetic and spectroscopic studies revealed that the natural pyriculariol is a racemate: H. Kiyota, A. Sasaki, K. Tanaka, Y. Nakamura, R. Ueda, Y. Suzuki, S. Kuwahara, M. Nukina, *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu* **2011**, *53*, 661–666.
[8] a) M. Nukina, T. Sassa, M. Ikeda, in: *Shokubutsu Byogai no Kagaku* (Eds.: A. Ichihara, T. Ueno), Gakkai Shuppan Center, Tokyo, **1997**, pp. 74–83 (in Japanese, CAS No. 130, 321619); b) M. Nukina, in: *Molecular Genetics of Host-Specific Toxins in Plant Disease* (Eds.: K. Kohmoto, O. C. Yoder), Kluwer Academic Publishers, The Netherlands, **1998**, pp. 165–166.
[9] a) J. R. Xu, X. Zhao, R. A. Dean, *Adv. Gen.* **2007**, *57*, 175–218; b) J. Collemare, A. Billard, H. U. Böhnert, M.-H. Lebrun, *Mycol. Res.* **2008**, *112*, 207–215.
[10] A. J. Birch, P. A. Massy-Westropp, C. J. Moye, *Aust. J. Chem.* **1955**, *8*, 539–544.
[11] J. Staunton, K. J. Weissman, *Nat. Prod. Rep.* **2001**, *18*, 380–416.
[12] K. Tanaka, Y. Nakamura, A. Sasaki, R. Ueda, Y. Suzuki, S. Kuwahara, H. Kiyota, *Tetrahedron* **2009**, *65*, 6115–6122.
[13] M. Nukina, T. Otsuki, N. Kuniyasu, *Biosci. Biotechnol. Biochem.* **1994**, *58*, 2293–2294.
[14] S. Iwasaki, H. Muro, S. Nozoe, S. Okuda, Z. Sato, *Tetrahedron Lett.* **1972**, *13*, 13–16.
[15] H. J. Bestmann, H. Frey, *Liebigs Ann. Chem.* **1980**, 2061–2071.
[16] H. X. Zhang, F. Guibé, G. Balavoine, *J. Org. Chem.* **1990**, *55*, 1857–1867.
[17] A. Fürstner, I. Konetzki, *Tetrahedron* **1996**, *52*, 15071–15078.
[18] X. Wang, J. A. Porco Jr., *J. Am. Chem. Soc.* **2003**, *125*, 6040–6041.
[19] N. Bajwa, M. P. Jennings, *J. Org. Chem.* **2006**, *71*, 3646–3649.

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