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A POTENT ANTIFUNGAL BENZOQUINONE IN ETIOLATED SORGHUM SEEDLINGS AND ITS METABOLITES

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Key Word Index—*Sorghum bicolor*; Gramineae; sorghum; seedlings; antifungal substance; metabolisms; structures; benzoquinone.

Abstract—A potent antifungal substance, 2-hydroxy-5-methoxy-3-(8′*Z*,11′*Z*,14′-pentadecatrien)yl-1,4-benzoquinone (Q-1), together with its metabolite Q-2, was isolated from etiolated sorghum seedlings and identified. $[ED]_{50}$ of Q-1 was *ca* 6 $\mu g\ m^{-1}$ against the rice blast fungus, *Pyricularia oryzae*. Exposure of the etiolated seedlings to light resulted in a rapid decrease in the Q-1 level, and an increase in the level of another metabolite, Q-3. Structures of the Q-2 and Q-3 metabolites, which were both derived as a result of oxidative cleavage of the quinone chromophore in Q-1, were determined from spectroscopic data.

INTRODUCTION

Recently, Suzuki *et al.* [1] and Bouillant *et al.* [2] have independently identified five 5-alk(en)ylresorcinols including 5-(8′*Z*-heptadecen)ylresorcinol, as antifungal agents, in the extracts of whole etiolated rice seedlings and four 5-alk(en)ylresorcinols including 5-(12′*Z*-heptadecen)ylresorcinol from root exudates of etiolated rice seedlings, respectively. Accumulation of these rice resorcinols occurs specifically in etiolated plants [1, 3]. In addition, 5-alk(en)ylresorcinols are known to be active against a wide range of fungi [1, 4, 5]. That these resorcinols with potential antifungal properties are present at high levels suggests that they may help defense against microbes in the early seedling stage.

To verify the presence of specific compounds such as the rice alk(en)ylresorcinols, we examined antifungal substances in sorghum (*Sorghum bicolor* L.) seedlings. Sorghum, together with rice, is one of gramineous crop plants not containing a cyclic hydroxamate (widely distributed in Gramineae), and has been reported to be involved in the defense against insects and microbes [6]. In this report, the isolation and identification of a potent antifungal substance, Q-1, which accumulated specifically in etiolated sorghum seedlings and of its two metabolites Q-2 and Q-3 are described; their structures are shown in Fig. 1. Of these metabolites, Q-2 was produced under dark conditions and Q-3 on exposure of the etiolated seedlings to light.

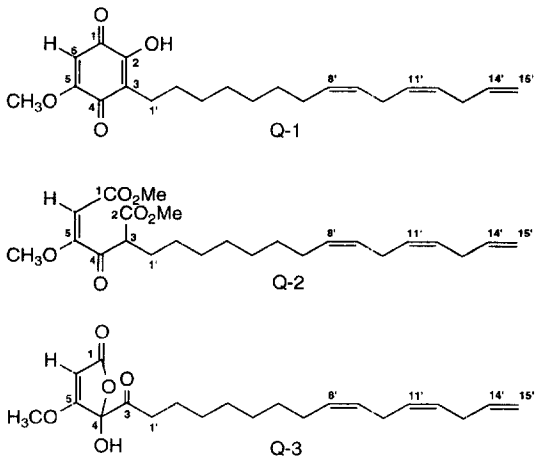

Fig. 1. Structures of the antifungal benzoquinone Q-1 and its metabolites Q-2 and Q-3.

RESULTS AND DISCUSSION

Eight-day-old etiolated or greened seedlings were homogenized with methanol. The methanol extract was partitioned between dichloromethane and water and then *n*-butanol and water to give dichloromethane and *n*-butanol soluble fractions. Table 1 lists the antifungal activities of the four fractions. These antifungal activities were evaluated by use of a spore germination test with the rice blast fungus, *Pyricularia oryzae* [1]. The four fractions completely inhibited spore germination of the rice blast fungus at a fresh weight equivalent concentration. When the $[ED]_{50}$ values of the antifungal activities for these four fractions were compared on a fresh weight concentration basis, there

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Table 1. Antifungal activity of fractions from etiolated and greened sorghum seedlings against *Pyricularia oryzae*

Fraction	Inhibition of spore germination $[ED]_{50}$	
	Etiolated*	Greened*
CH_2Cl_2 Fraction	0.015†	0.13†
n-BuOH Fraction	0.29	0.18

* Eight-day-old seedlings at 28 .
† g fresh weight concentration.

is a large difference between the dichloromethane fraction from the greened and that from the etiolated seedlings: the antifungal activity in the extract isolated from the etiolated seedlings was *ca* 9 times higher than that in the extract isolated from the greened seedlings (Table 1). This result suggests that the antifungal substances specific to etiolated seedlings exist in the dichloromethane fraction. Therefore, identification of the antifungal substances in the dichloromethane fraction from the etiolated seedlings was performed. The dichloromethane fraction of the extract isolated from 9-day-old etiolated seedlings (*ca* 740 g fr. wt) was separated using silica-gel column chromatography to give two active fractions, Fr-a and Fr-b, which exhibited *ca* 10 and 90% of the total antifungal activity, respectively. An active substance Q-1 (200 mg) from Fr-b, and a metabolite of Q-1, Q-2 (1 mg), together with Q-1 (1 mg) from Fr-a, were obtained through silica-gel column chromatography.

Q-1 was obtained as a pale yellow powder. Its spectroscopic data (see Experimental) were coincident with those of 2-hydroxy-5-methoxy-3-(8′*Z*,11′*Z*,14′-*n*-pentadecatrien)yl-1,4-benzoquinone which was identified in the root exudates of etiolated sorghum seedlings and was characterized as an autoxidation product of the hydroquinone form, the host-germination stimulant for *Striga asiatica* [7].

Q-2, a pale yellow oil, has the molecular formula $C_{24}H_{36}O_6$ as determined from high-resolution FAB mass spectrometry (*m/z* 420.2513, $[M]^+$) and from ^{13}C NMR spectral data. The ^1H NMR spectrum revealed signals for the homoconjugated triene group (7′-H to 15′-H) in Q-1 at δ 2.04 (2H, *q*, —CH$_2$—CH=CH—), 2.83 (4H, *t*, =CHCH$_2$CH=), 5.3–5.5 (4H, *m*, —CH=CH—), 5.81, 4.99 and 5.05 ppm (each 1H, *m*, —CH=CH$_2$), and signals for three methoxy groups at δ 3.69, 3.72 and 3.73 ppm. In addition, signals at δ 1.93 (2H, *m*), 3.81 (1H, *dd*) and 5.16 ppm (1H, *s*, olefinic H) were observed instead of signals at δ 2.44 (2H, *dd*), 1.45 (*m*), 5.84 (1H, *s*) and 7.27 (*s*) ppm which had been assigned to the 1′-, 2′-, 6-H and 2-OH in Q-1. The ^{13}C NMR spectrum revealed 24 carbon signals; in addition to signals for the triene group (C-7′ to C-15′) in Q-1, signals for three methoxy carbons at δ 51.7, 52.3 and 57.0 ppm, four quaternary carbons at δ 166.7, 169.4, 199.2 and 167.2 ppm, two methine carbons at δ 92.8 and 57.9 ppm and six methylene carbons at δ 29.7, 29.6, 29.3, 29.2, 27.7 and 27.3 ppm were observed. The FG-HMQC data correlated the three methoxy and olefinic proton signals with the carbon signals: δ_H 3.68/δ_C 51.7, δ_H 3.72/δ_C 52.3, δ_H 3.73/δ_C 57.0 and δ_H 5.16/δ_C 92.8 ppm [Fig. 2(a)]. The results of a differential NOE experiment and FG-HMBC correlation data showed the presence of the three partial structures illustrated in Fig. 2(b). In the NOE experiment, irradiation of the two methoxy proton signals at δ 3.68 and 3.73 ppm enhanced the olefinic proton signal at δ 5.16 ppm, indicating that both methoxy groups are located near the olefinic proton at δ 5.16 ppm. In the FG-HMBC spectrum, the three methoxy proton signals at δ 3.68, 3.72 and 3.73 ppm were long-range coupled with each of the carbon signals at δ 166.7, 169.4 and 167.2 ppm, and the olefinic proton signal at δ 5.16 ppm with the carbon signals at δ 167.2 and 199.2 ppm. The methine proton at δ 3.81 ppm which was coupled with the methylene proton signal at δ 1.93 ppm in the ^1H-^1H COSY spectrum was assigned to be adjacent to the remaining car-

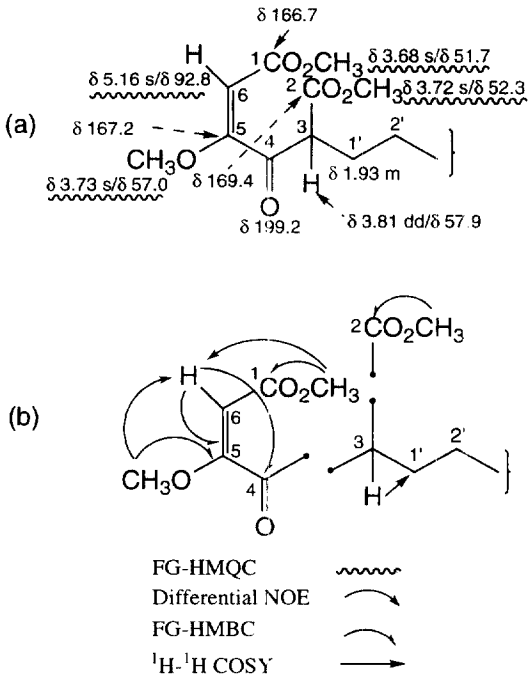

Fig. 2. Partial structures of Q-2.

bomethoxy group (δ_H 3.72/δ_C 52.3 and δ_C 169.4 ppm), the ketone group at C-4 and the methylene at C-1′. The remaining C-2′ to C-6′ structure of Q-2, though not determined solely from the NMR data, were deduced from an assumption that Q-2 is derived from Q-1.

Q-1 levels in etiolated and greened seedlings were determined, and the rate of accumulation in etiolated seedlings were monitored. The content of Q-1 in sorghum seedlings was estimated from UV absorption of the Q-1 fraction partially purified by silica gel TLC of the dichloromethane fraction. Comparison of the Q-1 level in etiolated with that in greened seedlings was performed using 9-day-old seedlings grown at 28°. The Q-1 level was *ca* 650 $\mu g\ g^{-1}$ fresh weight in the seedlings under dark conditions and 150 $\mu g\ g^{-1}$ fresh weight in the seedlings continuously exposed to light. This result shows that the accumulation of Q-1 in sorghum seedlings is specific to the etiolated seedlings. Figure 3 shows the time course of the Q-1 accumulation in etiolated seedlings. The Q-1 level increased linearly with time, reaching a level of *ca* 420 $\mu g\ g^{-1}$ fresh weight on day 9. This indicates that Q-1 was newly produced after germination. During this time-course experiment, the effect of light irradiation on the levels of Q-1 in the etiolated seedlings was also examined. Seven-day old etiolated seedlings were continuously exposed to light for 2 days at 30°. The level of Q-1 in these seedlings dropped markedly by 18% in one day, in a similar manner to the rice resorcinol levels. Since Q-1 still accumulated in the greened seedlings to some extent as mentioned above, the rapid decrease in the Q-1 level induced by exposure to light was most likely caused by an unusually high rate of degradation of Q-1 [3].

For confirmation of the high rate of degradation of Q-1, identification of Q-1 metabolites was attempted. Eight-day-old etiolated seedlings grown at 28° were continuously exposed to light for 2 days at 30°. The dichloromethane fraction from *ca* 700 g fresh weight of the seedlings was separated into three fractions, Fr-a to Fr-c, in a manner similar to that in the isolation Q-1 and Q-2 procedure. Purification of Fr-c by prep. TLC on silica gel gave Q-3 (17.7 mg).

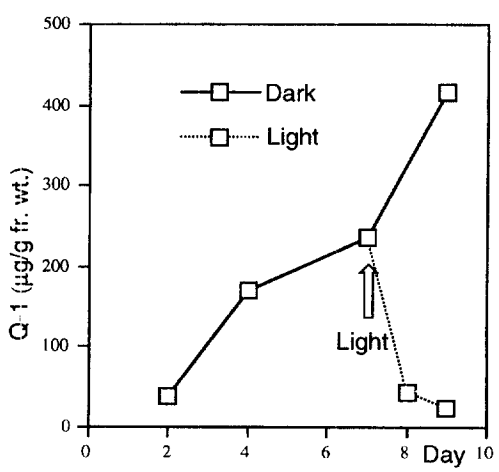

Fig. 3. Time course of Q-1 accumulation and effect of light exposure on Q-1 level in etiolated sorghum seedlings. Etiolated seedlings were grown for 7 days at 28° and 2 days at 30°. The 7-day-old etiolated seedlings were exposed to continuous light for 2 days at 30°.

The molecular formula of Q-3 was determined to be $C_{21}H_{30}O_5$ from high-resolution FAB mass spectrometry (*m/z* 361.2014, $[M-H]^-$) and ^{13}C NMR spectral data. The ^1H NMR spectrum revealed signals for the homoconjugated triene group (7′-H to 15′-H) of Q-1 and signals for a methoxy group at δ 3.94 ppm (*s*), methylenes at δ 2.34 and 2.71 (each 1H, *dd*) and 1.63 ppm (2H, *m*), an olefinic methine at δ 5.31 (*s*) and a hydroxy proton at δ 5.45 (*s*) ppm. The ^{13}C NMR spectrum revealed 21 carbon signals; in addition to signals for the triene group (C-7′ to C-15′), those for a methoxy carbon at δ 60.2 ppm, four quaternary carbons at δ 169.4, 98.9, 200.9 and 176.5 ppm, a methine carbon at δ 91.3 ppm and six methylene carbons at δ 34.5, 23.1, 28.8, 29.0, 29.1 and 29.5 ppm were observed. The ^1H-^{13}C COSY data correlated the five proton signals at δ 5.31, 3.94, 2.34 and 2.71 and 1.63 ppm in Q-3 with the carbon signals: δ_H 5.31/δ_C 91.3, δ_H 3.94/δ_C 60.2, δ_H 2.34 and 2.71/δ_C 34.5 and δ_H 1.63/δ_C 23.1 ppm [Fig. 4(a)]. The results of a differential NOE experiment and FG-HMBC correlation data [Fig. 4(b)] showed that the partial structure illustrated in Fig. 4 is present in Q-3. In the NOE experiment, irradiation of the methoxy proton signal at δ 3.94 ppm enhanced the olefinic proton signal at δ 5.31 ppm, indicating that the methoxy group is located near the olefinic proton. In the FG-HMBC spectrum, the methoxy proton signal at δ 3.94 ppm was long-range coupled with a carbon signal at δ 176.5 ppm.

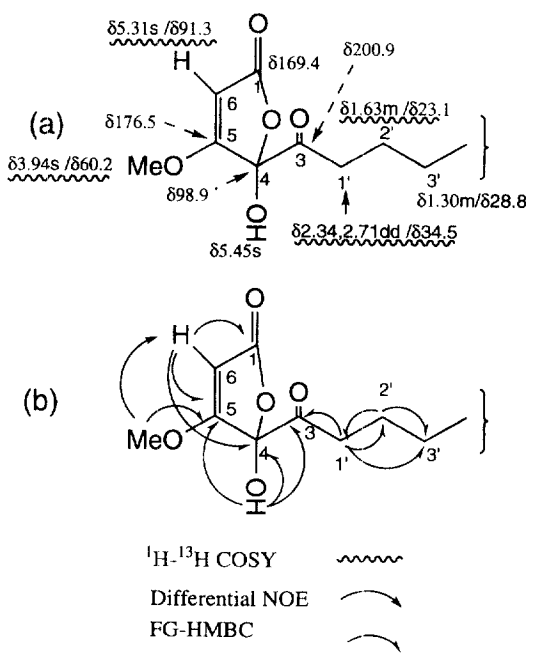

Fig. 4. Partial structure of Q-3.

an olefinic proton signal at δ 5.31 ppm, three carbon signals at δ 169.4, 176.5 and 98.9 ppm, and a hydroxy proton signal at δ 5.45 ppm, three carbon signals at δ 98.9, 200.9 and 176.5 ppm. Additional ^1H-^{13}C long-range couplings were also observed for the methylene proton signals at δ 2.34 and 2.71 (each *dd*) and 1.63 ppm (*m*); the former methylene proton signals were long-range coupled with the carbon signals at δ 200.9, 23.1 and 28.8 ppm and the latter multiplet proton signal was coupled with the carbon signals at δ 34.5 and 28.8 ppm. These couplings revealed the presence of the partial structure (b) illustrated in Fig. 4. The absorptions of Q-3 at ν 1750 cm^{-1} in the IR spectrum and λ_{max}^{EtOH} 224 nm (ε 8900) in the UV spectrum, being due to an α,β-unsaturated γ-lactone chromophore, are consistent with this partial structure (b) of Q-3 deduced from the NMR and mass spectral data. Q-3 is formed by connection of the partial structure (b) illustrated in Fig. 4, the triene group (C-7′ to C-15′) and the remaining three methylene carbons of C-4′ to C-6′ at δ 29.0, 29.1 and 29.5 ppm. Thus, the structure of Q-3 was determined to be that shown in Fig. 1.

The antifungal activities of Q-1 and Q-3 were investigated. Q-1 strongly inhibited spore germination of the fungus *Pyricularia oryzae* at an $[ED]_{50}$ of 6 $\mu g\ ml^{-1}$. Many quinone compounds exhibiting antimicrobial activities are known [8]. The $[ED]_{50}$ values of the antifungal activity for Q-3 and Q-1 methyl ether which was obtained by diazomethane treatment of Q-1 were *ca* 160 and 110 $\mu g\ ml^{-1}$, respectively. Loss of the quinone chromophore and blocking of the enol group in Q-1 resulted in a marked reduction of its inhibitory activity. These results demonstrate that the presence of both the quinone and the enol group in Q-1 molecule is essential for its potent biological activity.

As mentioned above, the etiolated seedlings, grown in the dark, produced mainly Q-1 but on exposure to light it rapidly declined and Q-3 predominated. Both products result from oxidative cleavage of the quinone chromophore. Cleavage of the bond between C-1 and C-2 of Q-1 results in the dicarboxylic acid form of Q-2, which appeared to be methylated with methanol during the extraction process. Cleavage of the bonds between C-1 and C-2 and C-2 and C-3 of Q-1 results in Q-3 accompanied by the liberation of the C-2 carbon of Q-1.

The role of Q-1 in defense against microbes remains unclear. As Chang *et al.* described, Q-1 and its hydroquinone are present in the root exudates [7]. The fact that appreciable amounts of Q-1 exhibiting the potent antifungal activity were present on the root surface indicates that Q-1 may be involved in the defense against microbes.

EXPERIMENTAL

Sorghum seedlings. Seeds (*Sorghum bicolor* L. cv. Super Sugar) were sterilized with 70% EtOH for 3 min, planted in pots filled with vermiculite and grown in a growth chamber (28–30°).

Comparison of antifungal activity of extracts isolated from etiolated and greened seedlings. Whole 8-day-old (*ca* 1 g fr. wt) seedlings were homogenized with MeOH (50 ml) and the filtrate was concd. The resulting residue was extracted with CH_2Cl_2 (10 ml × 3) and then *n*-BuOH (10 ml × 2). After concn, each fr. was subjected to the bioassay.

Isolation of Q-1 and Q-2. Whole 9-day-old etiolated sorghum seedlings (*ca* 740 g fr. wt) were homogenized with MeOH (5 l) and the homogenate was allowed to stand for a month. The filtrate was concd *in vacuo* and the resulting material was extracted with CH_2Cl_2. The CH_2Cl_2 fr. (2.03 g) was passed through a silica-gel column with EtOAc–C_6H_6 (1:49 and then 1:19) and then MeOH–$CHCl_3$ (1:19) as the eluents, giving 3 frs (Fr-a: 0.151 g, Fr-b: 1.482 g and Fr-c: 0.584 g). Fr-b was purified by column chromatography on silica gel with EtOAc–hexane (3:7 and then 2:3) as the eluent to give Q-1 (200 mg) as the active substance. CC of Fr-a with EtOAc–hexane (1:4) as the eluent gave Q-2 (1 mg) and Q-1 (1 mg).

Q-1, IR ν_{max}^{film} cm^{-1}: 3340, 2910, 2850, 1660, 1635, 1595, 1460, 1440, 1300, 1200, 910; UV $\lambda_{max}^{CH_2Cl_2}$ nm (ε): 411 (400), 288 (7300), 283 (7300); ^1H NMR (270 MHz, $CDCl_3$) δ ppm: 5.84 (1H, *s*, 6-H), 2.44 (2H, *dd*, *J* = 7.9, 6.9 Hz, 1′-H), 1.45 (2H, *m*, 2′-H), 1.30 (8H, *m*, 3′,4′,5′, 6′-H), 2.03 (2H, *dt*, *J* = 6.6, 6.3 Hz, 7′-H), 2.80 (4H, *m*, 10′, 13′-H), 5.3–5.5 (4H *m*, 8′, 9′, 11′, 12′-H), 5.82 (1H, *ddt*, *J* = 17.1, 1.6, 1.6 Hz, 14′-H), 4.98 (1H, *ddt*, *J* = 9.9, 1.6, 1.6 Hz, 15′-H), 5.05 (1H, *ddt*, *J* = 17.1, 1.6, 1.5 Hz, 15′-H), 3.86 (3H, *s*, 5-OMe), 7.27 (1H, *s*, 2-OH); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ ppm: 182.8 (*s*, C-1), 151.5 (*s*, C-2), 119.2 (*s*, C-3), 181.7 (*s*, C-4), 160.0 (*s*, C-5), 136.8 (*d*, C-6), 22.5 (*t*, C-1′), 27.9 (*t*, C-2′), 29.1, 29.2, 29.4, 29.5 (each *t*, C-3′, 4′, 5′, 6′), 27.1 (*t*, C-7′), 25.5 (*t*, C-10′), 31.4 (*t*, C-13′), 126.7, 127.5, 129.2, 130.3 (each *d*, C-8′, 9′, 11′, 12′), 136.8 (*d*, C-14′), 114.6 (*t*, C-15′), 56.7 (*q*, 5-OMe); EIMS (probe) 70 eV, *m/z* (rel. int.): 358 ($[M]^+$, 62), 168 (100), 167 (79).

Q-1 methyl ether: treatment of Q-1 with diazomethane in Et_2O at 0° for 30 min gave Q-1 methyl ether in a quantitative yield. ^1H NMR (270 MHz, $CDCl_3$) δ ppm: 5.72 (1H, *s*, 6-H), 2.43 (2H, *t*, *J* = 7.0 Hz, 1′-H), 1.30 (10H, *m*, 2′ 3′, 4′, 5′, 6′-H), 2.04 (2H, *dt*, *J* = 6.6, 6.3 Hz, 7′-H), 2.80 (4H, *m*, 10′, 13′-H), 5.3–5.5 (4H, *m*, 8′, 9′, 11′, 12′-H), 5.82 (1H, *ddt*, *J* = 17.1, 1.6, 1.6 Hz, 14′-H), 4.98 (1H, *ddt*, *J* = 9.9, 1.6, 1.6 Hz, 15′-H), 5.05 (1H, *ddt*, *J* = 17.1, 1.6, 1.5 Hz, 15′-H), 3.80 (3H, *s*, 5-OMe), 4.05 (3H, *s*, 2-OMe); high-resolution EIMS (probe) 70 eV, *m/z*: found, 372.2278 $[M]^+$, $C_{23}H_{32}O_{24}$; calcd, 372.2298.

Q-2, IR ν_{max}^{film} cm^{-1}: 3450, 2920, 2850, 1738, 1720, 1620, 1435, 1360, 1210; UV λ_{max}^{EtOH} nm (ε): 268 (sh, 2200), 233 (sh, 5000); ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 3.81 (1H, *dd*, *J* = 9.2, 5.8 Hz, 3-H), 5.16 (1H, *s*, H-6), 1.30 (10H, *m*, 2′, 3′, 4′, 5′, 6′-H), 2.04 (2H, *q*, *J* = 6.5 Hz, 7′-H), 5.3–5.5 (4H, *m*, 8′, 9′, 11′, 12′-H), 2.79 and 2.83 (each 2H, *t*, *J* = 5.8 Hz, 9′, 13′-H), 5.81 (1H, *m*, 14′-H), 4.99 and 5.05 (each 1H, *m*, 15′-H),

3.68 (3H, *s*, 1-OMe), 3.72 (3H, *s*, 2-OMe), 3.73 (3H, *s*, 5-OMe); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 166.7 (*s*, C-1), 169.4 (*s*, C-2), 57.9 (*d*, C-3), 199.2 (*s*, C-4), 167.2 (*s*, C-5), 92.8 (*d*, C-6), 29.7, 29.6, 29.3, 29.2, 27.7, 27.3 (each *t*, C-1′, 2′, 3′, 4′, 5′, 6′), 27.2 (*t*, C-7′), 25.6 (*t*, C-10′), 31.5 (*t*, C-13′), 126.8, 127.6, 129.3, 130.4 (each *d*, C-8′, 9′, 11′, 12′), 136.8 (*d*, C-14′), 114.7 (*t*, C-15′), 51.7 (*q*, 1-OMe), 52.3 (*q*, 2-OMe), 57.0 (*q*, 5-OMe); EIMS (probe) 70 eV, *m/z* (rel. int.): 420 ($[M]^+$, 21), 389 (39), 373 (29), 361 (30), 341 (14), 329 (54), 144 (100), 116 (76); high-resolution FABMS *m/z*: found, 420.2513 $[M]^-$, $C_{24}H_{36}O_6$; calcd, 420.2512.

Isolation of Q-3. Eight-day-old etiolated seedlings grown at 28° were continuously exposed to light for 2 days at 30°. Extraction of the harvested material (*ca* 700 g fr. wt) followed by partitioning and purification as described above gave the three frs Fr-a to Fr-b. Crude Q-1 (124 mg) from Fr-b and crude Q-3 (24 mg) from Fr-c were obtained. The crude Q-3 was then purified by prep. TLC on silica gel with MeOH–$CHCl_3$ (3:97) to give pure Q-3 (17.7 mg) as a colourless powder.

Q-3, IR ν_{max}^{film} cm^{-1}: 3400, 2920, 2850, 1750, 1735, 1640, 1450, 1320, 1200, 1020; UV λ_{max}^{EtOH} nm (ε): 268 (sh, 200), 224 (8900); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 5.31 (1H, *s*, 6′-H), 2.34 and 2.71 (each 1H, *dd*, J = 18.1, 7.3 Hz, 1′-H), 1.63 (2H, *m*, 2′-H), 1.30 (8H, *m*, 3′, 4′, 5′, 6′-H), 2.04 (2H, *m*, 7′-H), 5.3–5.5 (4H, *m*, 8′, 9′, 11′, 12′-H), 2.79 and 2.83 (each 2H, *t*, J = 5.9 Hz, 9′, 13′-H), 5.82 (1H, *ddt*, J = 17.1, 10.3, 6.3 Hz, 14′-H), 4.98 (1H, *ddt*, J = 10.3, 2.0, 2.0 Hz, 15′-H), 5.05 (1H, *ddt*, J = 17.1, 2.0, 2.0 Hz, 15′-H), 5.45 (1H, *s*, 3-OH), 3.94 (3H, *s*, 5-OMe); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 169.4 (*s*, C-1), 98.9 (*s*, C-3), 200.9 (*s*, C-4), 176.5 (*s*, C-5), 91.3 (*d*, C-6), 34.5 (*t*, C-1′), 23.1 (*t*, C-2′), 28.8 (*t*, C-3′), 29.0, 29.1 (each *t*, C-4′, 5′), 29.5 (*t*, C-6′), 27.1 (*t*, C-7′), 25.5 (*t*, C-10′), 31.5 (*t*, C-13′), 127.7, 130.2 (each *d*, C-8′, 9′), 126.8, 129.2 (each *d*, C-11′, 12′), 136.8 (*d*, C-14′), 114.7 (*t*, C-15′), 60.2 (*q*, 5-OMe); high-resolution FABMS *m/z*: found, 361.2014 $[M-H]^-$, $C_{21}H_{29}O_5$; calcd, 361.2015.

Quantification of Q-1. Whole seedlings (*ca* 1 g fr. wt) were homogenized in MeOH (50 ml) and the filtrate was concd. The resulting residue was extracted with CH_2Cl_2 (10 ml × 3). A sample equal to 0.25 g fr. wt was loaded on silica gel plate (2 cm wide) and the plate was developed with MeOH–CH_2Cl_2 (3:97). The purple zone was collected and eluted with MeOH–$CHCl_3$ (1:9, total 20 ml). The eluate was concd and analysed as a CH_2Cl_2 soln by UV absorption (291 nm).

Spore germination assay using Pyricularia oryzae. The spore germination test was conducted using the rice blast fungus, *Pyricularia oryzae*, according to a previously described procedure [1]. The activity of Q-2 was not determined.

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