Zygosporin D and Two New Cytochalasins Produced by the Fungus *Metarrhizium anisopliae*

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Zygosporin D (**3**) and two new cytochalasins (**4** and **5**) were isolated from the culture filtrate of the fungus *Metarrhizium anisopliae* and characterized on the basis of their spectral data and chemical conversions. The new cytochalasins, **4** and **5**, were determined to be deacetylcytochalasin C and (6*R*,16*S*,18*R*,21*R*)-18,21-dihydroxy-16,18-dimethyl-10-phenyl[11]cytochalasa-13(*E*),19(*E*)-diene-1,7,17-trione, respectively. Of these three cytochalasins, only zygosporin D is an effective inhibitor of shoot elongation of rice seedlings.

During our screening-based search for natural plant growth retardants among the metabolites produced by soil fungi,[1] we found that the fungus, *Metarrhizium anisopliae* (Metschn.) Sorok., produced growth retardant(s) to rice seedlings. *M. anisopliae*, one of the most important insect pathogens, has a high degree of specialization and occurs almost solely in two families of the Coleoptera, the Elateridae and Curculionidae.[2] Production of cytochalasins C (**1**) and D (**2**)[3] and of insecticidal cyclodepsipeptides, the destruxins,[4] by *M. anisopliae* has been reported. The culture filtrate from our fungus contained three cytochalasins, one of which showed plant growth-retardant activity and was identified as zygosporin D (**3**). The second, cytochalasin **4**, is the first isolation as a natural product, but it has been reported as a chemical conversion product of cytochalasin C (**1**). The third, **5**, was determined to be a new type of cytochalasin. These last two cytochalasins, **4** and **5**, had no retardant activity.

Cytochalasins **3**−**5** were isolated from the culture filtrate of the fungus in respective yields of 1, 11, and 0.4 mg/L. On the basis of the HREIMS and ^{13}C NMR data, they have the same molecular formula, $C_{28}H_{35}NO_5$. The NMR data for **3** suggested that it was zygosporin D, the deacetyl derivative of cytochalasin D (**2**). Spectral data reported in the original paper,[5] however, were not sufficient to identify it unambiguously as zygosporin D; therefore, chemical conversion was attempted. Compound **3** was acetylated by the usual method, affording **6**, which was identical in all respects to the compound obtained by acetylation of cytochalasin D (**2**).

The structure of cytochalasin **4** was clarified by comparing its NMR data with the data for **3** and by the chemical conversion. Two resonances (δ 114.0 and 148.1) due to the exocyclic double bond that are present in the ^{13}C NMR spectrum of **3** were missing; instead, the carbon signals (δ 126.2 and 132.6) due to a tetrasubstituted double bond were present in **4**. Furthermore, in the ^{1}H NMR spectrum of **4**, the signal (δ 1.54) due to the C-11 methyl was a singlet, not a doublet. These findings suggested that **4** is an isomer of **3**, a deacetyl derivative of cytochalasin C (**1**). Chemical conversion was performed to confirm this. Compound **4** was acetylated, giving **7**, which was identical in all respects to the compound obtained by the acetylation of cytochalasin C (**1**). Deacetylcytochalasin C is reported to have been obtained only by mild alkaline hydrolysis of cytochalasin C (**1**).[3] This is the first report of deacetylcytochalasin C as a natural product.

The structure of **5** was elucidated by comparing its NMR data with those of **4** and by chemical conversion. The molecular formula of **5** is the same as that of **3** and **4**. These compounds, therefore, have the same unsaturation. There were two resonances in the ^{13}C NMR spectrum of **5** assignable to ketonic carbonyl carbons (δ 210.4 and 214.2), whereas in that of **4** there was only one such resonance (δ 210.0). Four resonances assignable to olefinic carbons (δ 126.9, 127.2, 133.3, and 136.8) were present in the ^{13}C NMR spectrum of **5**, whereas **4** had six such resonances (δ 126.2, 126.4, 131.0, 131.7, 132.6, and 138.1). Instead of the two additional olefinic carbon signals present in the ^{13}C NMR spectrum of **4**, in that of **5** there were two signals due to sp^3 methine carbons (δ 35.6 and 45.8). The ^{1}H NMR spectrum of **5** had four methyl signals (one singlet and three doublets); in that of **4** there were also four methyl signals (three singlets and one doublet). The ^{1}H−^{1}H COSY spectrum of **4** indicated a coupling system in which the proton that resonated at δ 2.40 (H-8) was coupled with two protons at δ 3.78 (H-7) and 5.89 (H-13). In the ^{1}H−^{1}H COSY spectrum of **5** the proton that resonated at δ 3.79 (H-8) appeared to be coupled only with the proton at δ 5.66 (H-13). These NMR data indicate that **5** has a structure in which the double bond at C-5 in **4** is hydrogenated to a single bond and the hydroxy group at C-7 in **4** is oxidized to a ketone group. HMBC data support this conclusion.

Chemical conversion of cytochalasin D (**2**) and **5** confirmed this and established the stereochemistry of **5**. Catalytic hydrogenation of **2** afforded two products, **8** and **9**, which were diastereomers due to the configuration of C-6. NOE experiments were conducted to establish the stereochemistry of C-6. In those for **8**, irradiation of H-11 caused NOE enhancement of the signals due to H-3, H-5, and H-6, and irradiation of H-12 caused NOE enhancement of the signals due to H-5, H-6, and H-8. In those for **9**, irradiation of H-11 caused NOE enhancement of the signals due to H-3 and H-5, and irradiation of H-12 caused NOE enhancement of the signals due to H-3, H-6, and H-7. These results indicate that the absolute stereochemistry of C-6 in **8** is *R* and in **9** is *S*. Compound **8** was oxidized by pyridinium chlorochromate (PCC) yielding **10** which was identical in all respects to the compound obtained by the acetylation of **5**. The structure of **5**, therefore, was estab-

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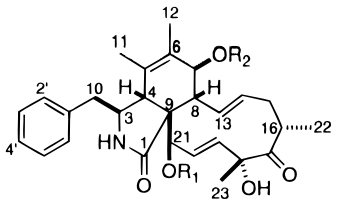
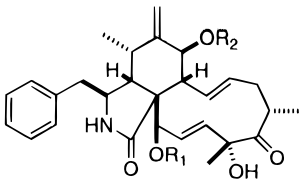
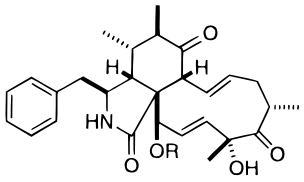
lished as (6*R*,16*S*,18*R*,21*R*)-18,21-dihydroxy-16,18-dimethyl-10-phenyl[11]cytochalasa-13(*E*),19(*E*)-diene-1,7,17-trione. To date only the protophomin from *Phoma exigua* has been reported as a 7-keto cytochalasin, in which the ketone function is in the cyclohexene ring.[6,7] The cytochalasin reported here is a new type, in which the 7-ketone function is in the cyclohexane ring.



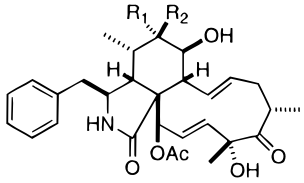
1: R_1 = Ac, R_2 = H (Cytochalasin C)
4: $R_1 = R_2$ = H
7: $R_1 = R_2$ = Ac



2: R_1 = Ac, R_2 = H (Cytochalasin D)
3: $R_1 = R_2$ = H (Zygosporin D)
6: $R_1 = R_2$ = Ac



5: R = H
10: R = Ac



8: R_1 = H, R_2 = CH_3
9: R_1 = CH_3, R_2 = H

In a microdrop bioassay of rice seedlings (*Oryza sative* L. cv. Yamahikari), the administration of 1 nmol/plant of **3** (zygosporin D) reduced the second leaf sheath length of treated plants to 41% of that of the water control. It is noteworthy that **3** had this effect without doing damage to the plant cells. The same amount of **4** (deacetylcytochalasin C) and **5** produced, respectively, 83 and 94% growth in terms of the second leaf sheath length of treated plants compared to the control.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a JASCO FT−IR-5300 spectrometer. Optical rotation was measured with a Horiba SEPA-200 high sensitive polarimeter. EIMS were obtained with a JEOL AX505HA spectrometer (direct probe, 70 eV). NMR spectra were measured with a JEOL Lambda 400 or a JEOL GX 270 NMR spectrometer. Chemical shifts were referenced against $CDCl_3$ (δ_H 7.26, δ_C 77.20) and DMSO-d_6 (δ_C 39.60). Preparative HPLC was performed on a Cosmosil 5C_{18}-AR column (Nacalai Tesque, 10 × 250 mm), monitoring at 220 nm.

Fungal Material. A fungus (A-173) isolated from a soil sample collected in Tottori Prefecture, Japan, was identified as *Metarrhizium anisopliae* (Metchn.) Sorok. *1883*, based on its morphological features.[2] It has been maintained on potato−dextrose agar.

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 its pH to 2.0 with HCl. The EtOAc extracts were dried over Na_2SO_4, concentrated, washed with 1M $NaHCO_3$ (2 × 0.5 volume), then dried over Na_2SO_4, and concentrated to dryness.

Purification. The residue (0.58 g) was purified by Si gel column chromatography (Daisogel IR-60, 11 × 135 mm). Elution was done with 650 mL (130 mL × 5) each of 10, 20, and 40% acetone in *n*-hexane. The first fraction, eluted with 40% acetone in *n*-hexane (195 mg), was recrystallized from EtOAc, yielding colorless needles of **4** (102 mg). The cytochalasins in the mother liquor were purified by Sephadex LH-20 column chromatography (20 × 400 mm, MeOH). The column was developed with MeOH, and 5-mL portions of the eluate were collected. Fractions 18−20 were combined and evaporated to dryness. The cytochalasins in the residue (35 mg) were purified by HPLC (70% MeOH, 1.0 mL/min), giving **3** as a white powder (12.3 mg, t_R 18.7 min), colorless needles of **4** (11.0 mg, t_R 21.8 min), and **5** as a white powder (4.3 mg, t_R 23.8 min).

Zygosporin D (3): $[\alpha]^{25}_D$ +20.6° (*c* 0.25, EtOH) and −15.5° (*c* 0.58, dioxane); IR (film) ν_{max} 3374 (OH, NH), 1700 (ketone CO), 1682 (amide CO) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.32 (2H, m, H-3′, -5′), 7.26 (1H, m, H-4′), 7.12 (2H, m, H-2′, -6′), 6.21 (1H, dd, J = 16.2, 2.4 Hz, H-20), 5.65 (1H, dd, J = 15.9, 10.2 Hz, H-13), 5.53 (1H, s, NH), 5.41 (1H, dd, J = 16.2, 2.6 Hz, H-19), 5.31 (1H, s, H-12), 5.27 (1H, ddd, J = 15.9, 10.8, 5.4 Hz, H-14), 5.11 (1H, s, H-12), 4.05 (1H, dd, J = 2.6, 2.4 Hz, H-21), 3.79 (1H, d, J = 10.7 Hz, H-7), 3.29 (1H, ddd, J = 8.3, 4.3, 3.8 Hz, H-3), 2.9−2.8 (1H, m, H-5), 2.88 (1H, dd, J = 13.6, 3.5 Hz, H-10), 2.83 (1H, dd, J = 10.7, 10.2 Hz, H-8), 2.72 (1H, ddq, J = 11.0, 1.6, 6.5 Hz, H-16), 2.57 (1H, m, H-4), 2.55 (1H, m, H-10), 2.48 (1H, ddd, J = 13.0, 11.0, 10.8 Hz, H-15), 2.00 (1H, ddd, J = 13.0, 5.4, 1.6 Hz, H-15), 1.55 (3H, s, H-23), 1.19 (3H, d, J = 6.8 Hz, H-22), 1.11 (3H, d, J = 6.8 Hz, H-11); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 210.2 (s, C-17), 175.0 (s, C-1), 148.1 (s, C-6), 137.3 (s, C-1′), 137.1 (d, C-20), 133.7 (d, C-19), 130.9 (d, C-14), 129.2 (d, C-2′, -6′), 128.9 (d, C-3′, -5′), 127.1 (d, C-4′), 127.0 (d, C-13), 114.0 (t, C-12), 77.7 (s, C-18), 76.4 (d, C-21), 69.7 (d, C-7), 54.2 (s, C-9), 53.5 (d, C-3), 50.0 (d, C-4), 45.6 (d, C-8), 45.3 (t, C-10), 42.3 (d, C-16), 37.7 (t, C-15), 32.9 (d, C-5), 24.2 (q, C-23), 19.4 (q, C-22), 13.9 (q, C-11); EIMS *m*/*z* 465 $[M]^+$ (5), 437 (15), 419 (13), 365 (36), 338 (100), 254 (58), 120 (18), 91 (53); HREIMS *m*/*z* 465.2500 (calcd for $C_{28}H_{35}NO_5$, 465.2515).

Acetylcytochalasin D (6). Compound **3** (3.2 mg) was treated overnight with 0.5 mL of acetic anhydride and 0.5 mL of pyridine. The products were purified by HPLC (85% MeOH, 1.0 mL/min), giving **6** (2.6 mg, t_R 16.1 min) as a white powder.

Compound 6: $[\alpha]^{25}_D$ +30.2° (*c* 1.00, EtOH); IR (film) ν_{max} 3451 (OH, NH), 1736 (acetyl CO), 1699 (ketone CO and amide CO), 1229 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.4−7.1 (5H, m, H-2′−6′), 6.09 (1H, dd, J = 15.6, 2.7 Hz, H-20), 5.66 (1H, dd, J = 2.7, 2.4 Hz, H-21), 5.60 (1H, s, NH), 5.57 (1H, dd, J = 15.9, 9.8 Hz, H-13), 5.24 (1H, s, H-12), 5.19 (1H, d, J = 10.2 Hz, H-7), 5.18 (1H, m, H-14), 5.11 (1H, dd, J = 15.6, 2.4 Hz, H-19), 5.03 (1H, s, H-12), 3.23 (1H, m, H-3), 3.07 (1H, dd, J = 10.2, 9.8 Hz, H-8), 2.83−2.66 (4H, m, H-5, -10, -16), 2.44 (1H, ddd, J = 13.2, 11.3, 10.2 Hz, H-15), 2.28 (3H, s, 21-OAc), 2.15 (1H, m, H-4), 1.93 (1H, m, H-15), 1.92 (3H, s, 7-OAc), 1.49 (3H, s, H-23), 1.17 (3H, d, J = 6.8 Hz, H-22), 0.91 (3H, d, J = 6.6 Hz, H-11); EIMS *m*/*z* 549 $[M]^+$ (14), 521 (76), 458 (67), 401 (59), 358 (61), 310 (66), 254 (100), 120 (40), 91 (93).

Cytochalasin D (**2**) (5 mg, Sigma, from *Zygosporium mansonii*) was subjected to the procedure used for **3**, giving **6** (4.6 mg).

Deacetylcytochalasin C (4): $[\alpha]^{25}_D$ +27.6° (*c* 0.25, EtOH); IR (film) ν_{max} 3434 (OH, NH), 1692 (ketone CO and amide CO), 1644 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 7.37−7.11 (5H,

m, H-2′−6′), 6.15 (1H, dd, J = 15.4, 2.4 Hz, H-20), 5.89 (1H, dd, J = 16.2, 10.3 Hz, H-13), 5.64 (1H, s, NH), 5.46 (1H, dd, J = 15.4, 2.4 Hz, H-19), 5.26 (1H, ddd, J = 16.2, 10.3, 5.4 Hz, H-14), 4.51 (1H, m, H-21), 3.78 (1H, br d, J = 9.7 Hz, H-7), 3.35 (1H, m, H-3), 3.00 (1H, br s, H-4), 2.96 (1H, dd, J = 13.5, 9.6 Hz, H-10), 2.81 (1H, dd, J = 13.5, 5.7 Hz, H-10), 2.72 (1H, m, H-16), 2.52 (1H, ddd, J = 12.8, 11.0, 10.3 Hz, H-15), 2.40 (1H, dd, J = 10.3, 9.7 Hz, H-8), 2.00 (1H, br dd, J = 12.8, 5.4 Hz, H-15), 1.69 (3H, s, H-12), 1.55 (3H, s, H-23), 1.54 (3H, s, H-11), 1.20 (3H, d, J = 7.0 Hz, H-22); ^{13}C NMR (DMSO-d_6, 67.8 MHz) δ 210.0 (s, C-17), 176.0 (s, C-1), 138.1 (d, C-20), 138.1 (s, C-1′), 132.6 (s, C-6), 131.7 (d, C-19), 131.0 (d, C-14), 129.6 (d, C-2′, -6′), 128.4 (d, C-3′, -5′), 126.5 (d, C-4′), 126.4 (d, C-13), 126.2 (s, C-5), 77.6 (s, C-18), 73.2 (d, C-21), 68.2 (d, C-7), 59.6 (d, C-3), 54.1 (s, C-9), 48.5 (d, C-4), 48.0 (d, C-8), 44.5 (t, C-10), 41.5 (d, C-16), 38.1 (t, C-15), 24.9 (q, C-23), 19.5 (q, C-22), 16.6 (q, C-12), 14.3 (q, C-11); EIMS m/z 465 $[M]^+$ (12), 437 (18), 419 (20), 365 (60), 338 (100), 320 (87), 256 (70), 120 (52), 91 (45); HREIMS m/z 465.2522 (calcd for $C_{28}H_{35}NO_5$, 465.2515).

Acetylcytochalasin C (7). Compound **4** (2.0 mg) was acetylated by the procedure used for **3**. The products were purified by HPLC (85% MeOH, 1.0 mL/min) giving **7** (1.8 mg, t_R 17.8 min) as colorless needles.

Compound 7: $[\alpha]^{25}_D$ +56.0° (c 0.25, EtOH); IR (film) ν_{max} 3362 (OH, NH), 1742 (acetyl CO), 1698 (ketone CO and amide CO), 1231, 1028 cm^{-1}; ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.4−7.1 (5H, m, H-2′−6′), 6.00 (1H, dd, J = 15.1, 2.2 Hz, H-20), 5.92 (1H, dd, J = 2.2, 2.2 Hz, H-21), 5.76 (1H, dd, J = 15.7, 10.8 Hz, H-13), 5.60 (1H, s, NH), 5.30 (1H, br d, J = 7.6 Hz, H-7), 5.16 (1H, m, H-14), 5.12 (1H, dd, J = 15.1, 2.2 Hz, H-19), 4.66 (1H, s, 18-OH), 3.33 (1H, t, J = 7.6 Hz, H-3), 2.97 (2H, d, J = 7.6 Hz, H-10), 2.78−2.38 (4H, m, H-4, 8, 15, 16), 2.28 (3H, s, 21-OAc), 1.94 (3H, s, 7-OAc), 1.49 (3H, s, H-23), 1.49 (3H, br s, H-11 or -12), 1.44 (3H, br s, H-11 or -12), 1.17 (3H, d, J = 7.6 Hz, H-22); EIMS m/z 549 $[M]^+$ (11), 521 (60), 489 (78), 458 (84), 429 (42), 401 (54), 358 (55), 338 (62), 320 (100), 310 (80), 240 (64), 228 (63), 120 (100), 91 (76).

Cytochalasin C (**1**) (5 mg, Sigma, from *Metarrhizium anisopliae*) was subjected to the procedure used for **3**, giving **7** (3.3 mg).

Compound 5: $[\alpha]^{25}_D$ −29.5° (c 0.2, EtOH); IR (film) ν_{max} 3387 (OH, NH), 1705 (ketone CO), 1695 (ketone and amide CO), 1032 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.32 (2H, m, H-3′, -5′), 7.26 (1H, m, H-4′), 7.13 (2H, m, H-2′, -6′), 6.20 (1H, dd, J = 15.9, 2.4 Hz, H-20) 5.69 (1H, br s, NH), 5.66 (1H, ddd, J = 15.6, 9.5, 1.5 Hz, H-13), 5.40 (1H, dd, J = 15.9, 2.2 Hz, H-19), 5.05 (1H, ddd, J = 15.6, 10.8, 4.6 Hz, H-14), 4.08 (1H, br s, H-21), 3.79 (1H, d, J = 9.5 Hz, H-8), 3.58 (1H, ddd, J = 9.0, 4.2, 3.4 Hz, H-3), 2.99 (1H, dd, J = 13.4, 3.4 Hz, H-10), 2.70 (1H, ddq, J = 11.2, 1.5, 6.8 Hz, H-16), 2.61 (1H, dd, J = 4.9, 4.2 Hz, H-4), 2.57 (1H, dd, J = 13.4, 9.0 Hz, H-10), 2.48 (1H, ddd, J = 13.0, 11.2, 10.8 Hz, H-15), 2.32 (1H, m, H-5), 2.03−1.95 (2H, m, H-6, -15), 1.52 (3H, s, H-23), 1.17 (3H, d, J = 6.8 Hz, H-22), 1.14 (3H, d, J = 7.1 Hz, H-12), 1.08 (3H, d, J = 7.1 Hz, H-11); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 214.2 (s, C-7), 210.4 (s, C-17), 173.8 (s, C-1), 136.8 (d, C-20), 136.7 (s, C-1′), 133.3 (d, C-14), 129.3 (d, C-2′, -6′), 129.1 (d, C-3′, -5′), 127.3 (d, C-4′), 127.2 (d, C-19), 126.9 (d, C-13), 77.6 (s, C-18), 76.5 (d, C-21), 55.6 (s, C-9), 53.2 (d, C-3), 51.0 (d, C-4), 50.4 (d, C-8), 45.8 (d, C-6), 45.8 (t, C-10), 42.3 (d, C-16), 38.1 (t, C-15), 35.6 (d, C-5), 24.2 (q, C-23), 19.3 (q, C-22), 15.9 (q, C-11 or -12), 15.8 (q, C-11 or -12); long-range correlations in the HMBC spectrum (optimized for $J_{C,H}$ of 7 Hz) C-1 (H-8), C-3 (NH, H-10), C-4 (H-11), C-5 (H-11, H-12), C-6 (H-11, H-12), C-7 (H-6, H-8, H-12), C-8 (H-21), C-9 (NH, H-8, H-21), C-10 (H-2′, H-6′), C-11 (H-6), C-12 (H-6), C-13 (H-8, H-15), C-14 (H-8, H-15), C-15 (H-13, H-22), C-16 (H-22), C-17 (H-15, H-22, H-23), C-18 (H-19, H-20, H-23), C-19 (H-20, H-21, H-23), C-20 (H-19), C-21 (H-19, H-20), C-23 (H-19), C-1′ (H-10), C-2′, -6′ (H-10); EIMS m/z 465 $[M]^+$ (12), 447 (14), 437 (37), 419 (20), 374 (37), 365 (60), 338 (100), 254(33), 190 (18), 120 (20), 91 (48); HREIMS m/z 465.2500 (calcd for $C_{28}H_{35}NO_5$, 465.2515).

Compounds 8 and 9. Cytochalasin D (**2**) (10 mg, Sigma, from *Zygosporium mansonii*) in EtOAc (3 mL) containing PtO_2 (2 mg) was stirred under H_2 at room temperature for 30 min. The reaction mixture was filtered and evaporated in vacuo. The products were separated by HPLC (65% MeOH, 0.8 mL/min) giving compounds **8** (4.0 mg, t_R 49.9 min) and **9** (3.7 mg, t_R 48.3 min).

Compound 8: $[\alpha]^{25}_D$ +13.0° (c 0.20, EtOH); IR (film) ν_{max} 3362 (OH, NH), 1744 (acetyl CO), 1692 (ketone CO and amide CO), 1028 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36−7.12 (5H, m, H-2′−6′), 6.13 (1H, dd, J = 15.9, 2.7 Hz, H-20), 5.65 (1H, dd, J = 15.7, 10.3 Hz, H-13), 5.62 (1H, dd, J = 2.7, 2.2 Hz, H-21), 5.52 (1H, s, NH), 5.27 (1H, ddd, J = 15.7, 11.1, 5.4 Hz, H-14), 5.13 (1H, dd, J = 15.9, 2.2 Hz, H-19), 4.64 (1H, s, OH), 3.55−3.45 (2H, m, H-3, 7), 2.91 (1H, dd, J = 13.5, 4.1 Hz, H-10), 2.73 (1H, m, H-16), 2.65 (1H, dd, J = 13.5, 9.5 Hz, H-10), 2.63 (1H, dd, J = 10.3, 9.7 Hz, H-8), 2.49 (1H, ddd, J = 12.4, 11.8, 11.1 Hz, H-15), 2.24 (3H, s, 21-OAc), 2.06 (1H, dd, J = 4.3, 3.8 Hz, H-4), 2.00 (1H, br dd, J = 12.4, 5.4 Hz, H-15), 1.77−1.63 (2H, m, H-5, 6), 1.50 (3H, s, H-23), 1.19 (3H, d, J = 7.0 Hz, H-22), 0.93 (3H, d, J = 6.2 Hz, H-12), 0.88 (3H, d, J = 6.2 Hz, H-11); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 210.3, 174.4, 169.6, 137.5, 133.9, 132.7, 131.1, 129.03, 128.96, 127.4, 127.1, 77.7, 77.2, 68.5, 53.8, 53.7, 50.9, 46.1, 45.4, 42.4, 37.7, 34.7, 33.3, 24.2, 20.8, 19.4, 16.1, 12.6; EIMS m/z 509 $[M]^+$ (4), 481 (39), 449 (16), 422 (25), 421 (44), 379 (20), 378 (43), 360 (24), 340 (67), 330 (38), 323 (33), 322 (30), 312 (29), 256 (30), 254 (29), 120 (37), 91 (100).

Compound 9: $[\alpha]^{25}_D$ −22.0° (c 0.10, EtOH); IR (film) ν_{max} 3387 (OH, NH), 1738 (acetyl CO), 1684 (ketone CO and amide CO), 1018 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.34−7.09 (5H, m, H-2′−6′), 6.13 (1H, dd, J = 15.9, 2.7 Hz, H-20), 5.67 (1H, dd, J = 15.6, 9.8 Hz, H-13), 5.60 (1H, dd, J = 2.7, 2.4 Hz, H-21), 5.56 (1H, s, NH), 5.30 (1H, ddd, J = 15.6, 10.7, 5.1 Hz, H-14), 5.12 (1H, dd, J = 15.9, 2.4 Hz, H-19), 4.64 (1H, s, OH), 3.61 (1H, m, H-3), 3.07 (1H, dd, J = 11.2, 5.6 Hz, H-7), 2.82 (1H, dd, J = 13.4, 4.9 Hz, H-10), 2.76−2.66 (2H, m, H-8, -16), 2.61 (1H, dd, J = 13.4, 9.3 Hz, H-10), 2.50 (1H, ddd, J = 12.7, 11.2, 11.0 Hz, H-15), 2.22 (3H, s, 21-OAc), 2.16 (1H, m, H-5), 2.04−1.96 (2H, m, H-4, -15), 1.84 (1H, s, OH), 1.72 (1H, m, H-6), 1.50 (3H, s, H-23), 1.18 (3H, d, J = 6.8 Hz, H-22), 1.01 (3H, d, J = 7.6 Hz, H-12), 0.87 (3H, d, J = 7.3 Hz, H-11); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 210.3, 174.5, 169.6, 137.3, 134.8, 132.8, 130.2, 129.03, 128.97, 127.4, 127.1, 77.6, 77.4, 74.0, 54.4, 53.9, 50.5, 47.0, 46.2, 42.4, 39.6, 37.8, 30.2, 24.2, 20.8, 19.4, 16.1, 15.1; EIMS m/z 509 $[M]^+$ (5), 481 (39), 449 (16), 422 (29), 421 (44), 406 (22), 390 (21), 378 (42), 360 (21), 341 (32), 340 (96), 330 (42), 322 (25), 312 (24), 272 (21), 256 (24), 254 (25), 120 (35), 91 (100).

Compound 10. Compound **8** (3.3 mg) in CH_2Cl_2 (2 mL) was added dropwise to the solution of PCC (8.7 mg) in CH_2Cl_2 (0.21 mL), and the whole was stirred for 1 h. Diethyl ether (2 mL) and anhydrous Mg_2SO_4 (5 g) then were added to the reaction mixture, which was stirred for 10 min. After filtering the mixture, the filtrate was concentrated in vacuo and purified by HPLC (65% MeOH, 0.8 mL/min), giving compound **10** (2.1 mg, t_R 51.0 min).

Compound 10: $[\alpha]^{25}_D$ −43.9° (c 0.5, EtOH); IR (film) ν_{max} 3431 (OH, NH), 1741 (acetyl CO), 1711 (ketone CO), 1700 (amide CO, sh), 1225 cm^{-1}; ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.36−7.11 (5H, m, H-2′−6′), 6.13 (1H, dd, J = 15.6, 2.7 Hz, H-20), 5.72 (1H, dd, J = 15.4, 9.7 Hz, H-13), 5.68 (1H, dd, J = 2.7, 2.4 Hz, H-21), 5.52 (1H, s, NH), 5.15 (1H, dd, J = 15.6, 2.4 Hz, H-19), 5.11 (1H, ddd, J = 15.4, 11.0, 4.8 Hz, H-14), 4.62 (1H, br s, OH), 3.71 (1H, d, J = 9.7 Hz, H-8), 3.53 (1H, m, H-3), 2.92 (1H, dd, J = 13.4, 4.1 Hz, H-10), 2.78−2.61 (3H, m, H-4, -10, -16), 2.53 (1H, ddd, J = 12.7, 11.2, 11.0 Hz, H-15), 2.30 (3H, s, 21-OAc), 2.10−1.95 (3H, m, H-5, -6, -15), 1.78 (1H, s, OH), 1.49 (3H, s, H-23), 1.19 (3H, d, J = 6.8 Hz, H-22), 1.11 (3H, d, J = 7.0 Hz, H-12), 0.97 (3H, d, J = 6.5 Hz, H-11); EIMS m/z 507 $[M]^+$ (3), 447 (21), 419 (25), 404 (39), 376 (42), 338 (100), 328 (20), 282 (22), 254 (26), 190 (43), 120 (20), 91 (44).

Compound **5** (1.4 mg) was acetylated by the procedure used for **3**, giving **10** (1.5 mg).

Microdrop Bioassay. Seeds of rice (*Oryza sativa* L. cv. Yamahikari) were soaked in H_2O for 48 h at 28° under fluorescent light. Sets of six germinated seeds were planted on 1% agar medium in 30-mL beakers and incubated for 48 h

under continuous light. A 1-μL sample containing 50% aqueous acetone was placed as a drop between the shoot and first leaf of a seedling. After incubation for 72 h under continuous light, the length of the second leaf sheath of the seedling was measured.

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