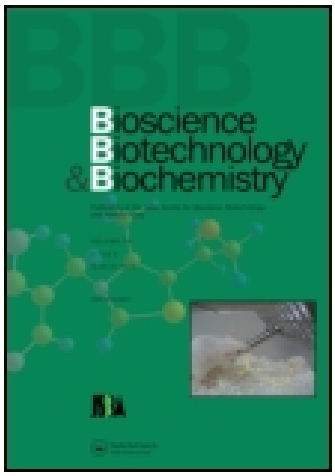

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Synthesis of 7-Methoxyapigeninidin and Its Fungicidal Activity against Gloeocercospora sorghi

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Note

Synthesis of 7-Methoxyapigeninidin and Its Fungicidal Activity against *Gloeocercospora sorghi*

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In the structure of sakuranetin, which was isolated as a phytoalexin from the rice plant, the methoxy group at C-7 has been shown to be important for its high activity. Apigeninidin was isolated as a phytoalexin from sorghum, but it had no methoxy group at C-7. We prepared 7-methoxyapigeninidin and compared its fungicidal activity with that of apigeninidin. The 7-methoxyapigeninidin showed higher activity against sorghum fungi than apigeninidin, suggesting that the methoxy group at C-7 was important for the high fungicidal activity.

Key words: phytoalexin; sakuranetin; naringenin; apigeninidin; 7-methoxyapigeninidin

From the rice plant, we have previously reported the isolation of the antifungal flavonoid sakuranetin (**1**; 5,4-dihydroxy-7-methoxyflavanone) as a phytoalexin.[1] Phytoalexins are antimicrobial secondary metabolites which accumulated in plants by microbial invasion. Sakuranetin **1** inhibits spore germination of *Pyricularia oryzae* at approximately 15 ppm.[1] In contrast, naringenin (**2**; 5,7,4′-trihydroxyflavanone) which is a precursor of sakuranetin **1**, shows much less fungicidal activity, scarcely inhibiting spore germination at up to 50 ppm.[1] The only chemical-structural difference between these two compounds is a hydroxy or methoxy group at the C-7 position. Therefore, we have speculated that methylation of this hydroxy group at C-7 may be important for high fungicidal activity.[1]

From the sorghum, apigeninidin (**3**; 5,7,4′-trihydroxyflavylium chloride) and luteolinidin were isolated as fungitoxic compounds and were considered to be sorghum phytoalexins.[2] Apigeninidin **3** and naringenin **2** have three hydroxy groups at the same positions, *i.e.*, the 5, 7, and 4′ positions, but no methoxy group at C-7 (Fig. 1). According to our previous speculation, the fungitoxic activity of apigeninidin **3** may be increased by methylation at C-7. Therefore, we synthesized apigeninidin **3** and 7-methoxyapigeninidin (**4**; 5,4′-dihydroxy-7-methoxyflavylium chloride) and compared its fungicidal activity each other.

Apigeninidin **3** was synthesized from naringenin **2** by the method of Sweeny *et al.*[3] The 7-methoxyapigeninidin **4** was synthesized from sakuranetin **1** according to the same method (Fig. 2).[3] Sakuranetin **1** was prepared from naringenin **2** by treating with ethereal diazomethane under anhydrous conditions in a 75% yield. The analytical data for synthetic sakuranetin **1** showed good accordance with that of natural sakuranetin.[1] After acetylating the hydroxy groups, sakuranetin **1** was converted to 7-methoxyapigeninidin **4** by $NaBH_4$ reduction and following chloranil dehydrogenation.[3] In the ^{1}H-NMR spectrum of 7-methoxyapigeninidin **4**, aromatic protons which are characteristic to

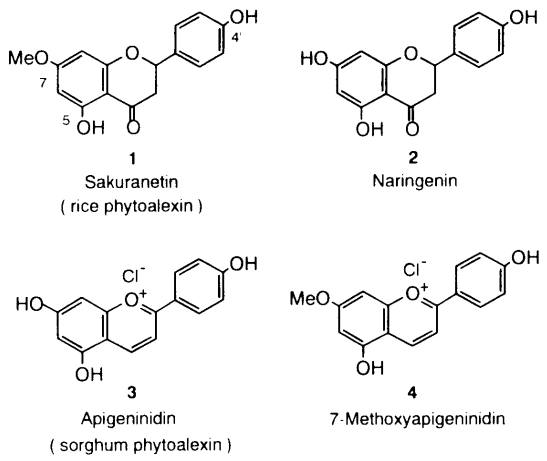

Fig. 1. Structure of the Compounds.

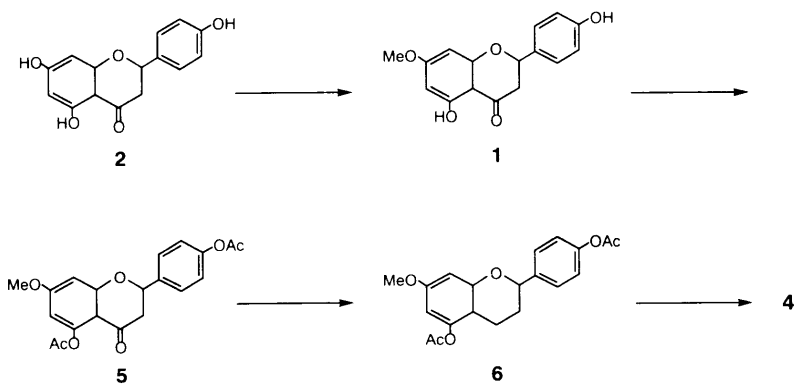

Fig. 2. Synthetic Scheme for 7-Methoxyapigeninidin **4**.

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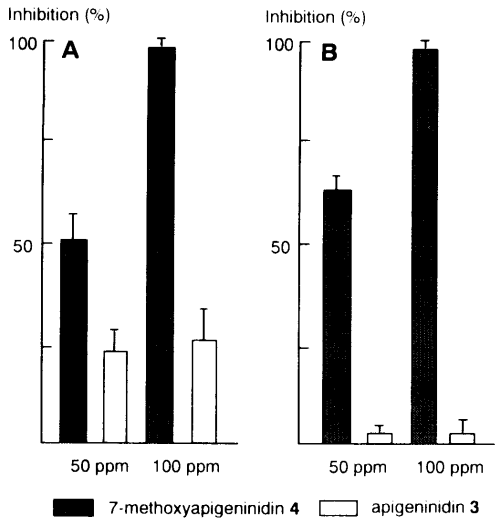

Fig. 3. Antifungal Activity of 7-Methoxyapigeninidin **4** and Apigeninidin **3**.

Bars indicate SE. **A**, against radial growth of *Gloeocercospora sorghi*; **B**, against spore germination of *Pyricularia oryzae*.

flavylium compounds and three singlet methyl protons were observed. These aromatic protons showed good accordance with those of apigeninidin **3** reported by Nilsson.[4] The ultraviolet absorption spectrum of 7-methoxyapigeninidin **4** was dependent on the pH of the solution. The maximum absorption of 7-methoxyapigeninidin **4** was observed at 475 nm in a solution at pH 2, and at 550 nm in a solution at pH 10, these values are good accordance with those of apigeninidin **3**.[5] These characteristic UV absorbance data also validated the structure of synthetic 7-methoxyapigeninidin **4**.

The I_{50} concentration for inhibiting the radial growth of *Gloeocercospora sorghi*, by 7-methoxyapigeninidin **4** was 50 ppm, while apigeninidin **3** showed only 25% inhibition at 100 ppm (Fig. 3). Although the radial growth of *Pyricularia oryzae* was not inhibited by either apigeninidin **3** or 7-methoxyapigeninidin **4** at 100 ppm (data not shown), its spore germination was inhibited by 7-methoxyapigeninidin **4** with an I_{50} concentration of 50 ppm. Apigeninidin **3** was almost inactive against the germination of *Pyricularia oryzae*, even at a high concentration (Fig. 3). This fairly high fungicidal activity of 7-methoxyapigeninidin **4** against both species is very surprising when compared to that of apigeninidin **3**.

These results suggest that the methoxy group at C-7 is very important for high activity in flavylium-type compound as in the case of flavone-type compound (sakuranetin **1** *vs.* naringenin **2**). We have recently succeeded in purifying the enzyme which catalyzes methylation of the hydroxy group at C-7 in naringenin **2** to produce sakuranetin **1** from rice leaves, and discussed the importance of this enzyme to the plant self-defence mechanism.[6] Although the existence of the enzyme which catalyzes the methylation of apigeninidin **3** has not yet been confirmed nor the presence of 7-methoxyapigeninidin **4** in sorghum, it would be very interesting to investigate if sorghum has the same methylation mechanism as that in the rice plant in future.

Experimental

Fungal inhibition assays. The fungus was cultivated on a potato dextrose agar medium at 25 °C for 1 week under dark. Experiments were initiated by placing plugs (1 cm in diameter), taken from the cultures of *Gloeocercospora sorghi*, mycelial side up on the medium (8.5 cm diameter), containing appropriate amounts of synthetic apigeninidin **3** and 7-methoxyapigeninidin **4**. Synthetic apigeninidin **3** and 7-methoxyapigeninidin **4** were added to the autoclaved medium when its temperature came down to below 60 °C. The stability of these synthetic compounds in this medium was confirmed by a TLC analysis with a solvent of ethyl acetate–formic acid–water–HCl (85 : 6 : 8 : 1, v/v/v/v, upper phase).[2] After 1 week, the radial growth of the cultures was measured, and the fungicidal activity was evaluated against an untreated control as a percentage. The spore germination inhibitory activity against *Pyricularia oryzae* was assayed as previously described.[1]

Methylation of naringenin **2**. A diazomethane solution (prepared from *p*-toluenesulfonyl-*N*-methyl-*N*-nitrosamide and a 50% KOH solution in ether) was added to 1.0 g of naringenin **2** in 400 ml of dry diethylether. After stirring at room temperature overnight, the solution was concentrated to give a crude product. Silica-gel column chromatography (EtOAc–*n*-hexane = 1 : 3) gave 790 mg of sakuranetin **1** in 75% yield. ^{1}H- and ^{13}C-NMR, and EI-MS data were in good accord with those of authentic sakuranetin.[1]

Acetylation of sakuranetin **1**. Five hundred mg of synthetic sakuranetin **1** was dissolved in 1 ml of pyridine and 1 ml of acetic anhydride and stirred at room temperature overnight. Removal of the excess solvents *in vacuo* gave a crude acylate, which was purified by silica-gel column chromatography (EtOAc–*n*-hexane = 1 : 3) gave 595 mg of acetylated sakuranetin **5** in a 92% yield. ^{1}H-NMR δ (500 MHz, $CDCl_3$): 2.28 (3H, s), 2.36 (3H, s), 2.70 (1H, dd, J = 16.0 Hz and 3.0 Hz), 2.96 (1H, dd, J = 16.0 and 13.0 Hz), 3.78 (3H, s), 5.42 (1H, dd, J = 13.0 and 3.0 Hz), 6.35 (1H, d, J = 2.8 Hz), 6.40 (1H, d, J = 2.8 Hz), 7.15 (2H, br. d, J = 8.8 Hz), 7.45 (2H, br. d, J = 8.8 Hz); MS *m/z*: 370 (M^+), 328 (100), 285, 167.

Reduction of acetylated sakuranetin **5**. To a solution of 300 mg of acetylated sakuranetin **5** in 15 ml of tetrahydrofuran and 15 ml of ethanol, 60 mg of sodium borohydride was added. After stirring for 30 minutes; an additional 60 mg of sodium borohydride was added, and stirring was continued for 2 h more at room temperature. The reaction solution was poured into 90 ml of cold 0.5% acetic acid in water and then extracted with chloroform (30 ml, three times). Drying (Na_2SO_4) and evaporating the solvent gave 350 mg of a product. Without purification, this product was treated with pyridine (1 ml) and acetic anhydride (1 ml) in the same manner as that previously described to give 250 mg of compound **6** in an 87% yield after silica-gel column chromatography (EtOAc–*n*-hexane = 1 : 3). ^{1}H-NMR δ (500 MHz, $CDCl_3$): 1.97 (1H, m), 2.15 (1H, m), 2.28 (6H, s), 2.60 (2H, m), 3.73 (3H, s), 4.98 (1H, br. d, J = 10.5 Hz), 6.28 (1H, d, J = 2.5 Hz), 6.38 (1H, d, J = 2.5 Hz), 7.10 (2H, d, J = 8.2 Hz), 7.42 (2H, d, J = 8.2 Hz); MS *m/z*: 356 (M^+, 100), 314, 371, 153.

Synthesis of 7-methoxyapigeninidin **4**. A mixture of the compound **6** (200 mg), 700 mg of chloranil, 24 ml of HOAc, 5 ml of water, and 1.5 ml of 6 N HCl was kept for 1 h with stirring. After cooling, the reaction mixture was diluted with 250 ml of 0.01 N HCl in MeOH and passed through a column of polyclar AT (previously washed 3 times with 3 N HCl). The column was eluted with a second 250 ml of the same solvent. The combined eluates were concentrated *in vacuo* at 30 °C to give 68 mg of 7-methoxyapigeninidin **4** as a red solid (40% yield). ^{1}H-NMR δ (500 MHz, DMSO-d_6): 4.01 (3H, s), 6.82 (1H, br. s), 7.13 (2H, d, J = 10.5 Hz), 7.39 (1H, d, J = 2.1 Hz), 8.32 (1H, d, J = 10.5 Hz), 8.43 (2H, d, J = 10.5 Hz), 9.15 (1H, d, J = 10.5 Hz). HR-FAB-MS (positive mode): found; 269.0839 ($[M-Cl]^+$, $C_{16}H_{13}O_4$); calculated, 269.0814.

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