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Activity and Allelopathy of Soil of Flavone *O*-Glycosides from Rice

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Two flavone *O*-glycosides were isolated from allelopathic rice seedlings and have been identified as 5,4′-dihydroxy-3′,5′-dimethoxy-7-*O*-β-glucopyranosylflavone and 7,4′-dihydroxy-3′,5′-dimethoxy-5-*O*-β-glucopyranosylflavone. Considerable levels of these glycosides could be found in allelopathic rice tissues. They could not be detected in the soils growing these allelopathic rice seedlings. Only their aglycone, 5,7,4′-trihydroxy-3′,5′-dimethoxyflavone, could be found in the soil. Further experiments showed that two flavone *O*-glycosides were exuded from allelopathic rice roots to the rihzosphere and then transformed into their aglycone form, that is, 5,7,4′-trihydroxy-3′,5′-dimethoxyflavone, with a great diversity of biological activities on associated weeds and microbes by soil interactions once released. The glycosides degraded rapidly ($t_{1/2} < 2$ h), whereas their aglycone was more resistant toward degradation in paddy soils, in which the half-life ($t_{1/2}$) at low (25 μg/g) and high (200 μg/g) doses reached 19.86 $\pm$ 3.64 h ($r^2 = 0.97$) and 28.78 $\pm$ 3.72 h ($r^2 = 0.98$), respectively. Furthermore, the mobility of both glycosides and their aglycone in paddy soil was evaluated by soil TLC with bioassay. The mobility of the glycosides ($R_f = 0.418 \pm 0.069$, $n = 18$) is higher than that of the aglycone ($R_f = 0.361 \pm 0.048$, $n = 18$). The results suggested that two flavone *O*-glycosides are formed in rice biosynthesis and that storage of the allelochemicals and their aglycone 5,7,4′-trihydroxy-3′,5′-dimethoxyflavone is the agent of alleloapthic rice which interferes with weeds or microbes in paddy soil.

KEYWORDS: ***Oryza sativa*** **L.; allelopathy; flavone *O*-glycosides; bioactivity; soil TLC; soil dynamics**

INTRODUCTION

Higher plants biosynthesize a range of the flavones. The functions of flavones in higher plants as well as their roles in the interaction with other organisms offer several potential applications in ecology, agriculture, human nutrition, and pharmacology (*1*−*3*). Several flavones and their *O*- or *C*-glycosides have been found in rice (*Oryza sativa* L.), which present multiple biological and ecological problems (*4*−*6*). The antioxidative activity and therapeutic potential of the flavones contained in rice bran have been studied in recent years (*7*, *8*), and it has been found that the flavones play a role in species interactions with rice and other organisms, particularly in the rhizosphere (*9*). Several bioactivities have been described and evaluated for these compounds, including insect feedant, fungistatic, and phytotoxic (*5*, *10*). Rice flavone *C*- or *O*-glycosides were found to act as probing stimulants for planthoppers (*4*, *5*). Our previous studies showed that allelopathic rice seedlings could release sufficient quantities of 5,7,4′-trihydroxy-3′,5′-dimethoxyflavone into the environment to act as an allelochemical participating in the defense of rice against weeds and pathogens (*10*−*12*). Besides 5,7,4′-trihydroxy-3′,5′-dimethoxyflavone, however, it is still unclear whether there are other flavones or flavone glycosides involved in rice allelopathy. Accordingly, flavones in allelopathic rice may be a key type of allelochemical that interferes potently with other organisms in paddy ecosystems, but their interference mechanisms are largely unknown.

The flavone allelochemical of rice interferes with weeds or soil microbes whenever they are present in the soil in the vicinity of the target species in the paddy. These allelochemicals may be the result of interaction and modulation by soil microbes (*9*, *13*). The action of allelochemicals has tended to treat soil as a medium of support, and thus allelopathy methodology has been criticized due to neglect of soil (*14*, *15*). Rice allelopathy occurs if allelochemicals would be available to exert an allelopathic effect in soil under natural conditions (*10*, *11*). Unfortunately, there are no previous works about soil dynamics in rice allelochemicals. To elucidate allelopathic interference of rice with other organisms, detailed information about the transforma-

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tion, degradation, and mobility of rice allelochemicals in paddy soils is required. This study aimed to identify the allelochemicals of flavone glycosides from allelopathic rice and to assess the bioactivity of identified allelochemicals in soils. An attempt to clarify the allelochemicals and mechanism of allelopathic rice interference with associated weeds and soil microbes in paddy ecosystem is presented.

MATERIALS AND METHODS

Instruments. High-resolution mass spectrometer experiments were carried with an IonSpec Ultima FTMS and FABMS with a VG-ZAB-HS. IR spectra were recorded on a Bruker FT-IR infrared spectrophotometer. UV spectra were recorded on a Perkin-Elmer Lambda 5 UV−vis spectrophotometer. Optical rotation was measured with a Perkin-Elmer model-241 MC polarimeter. The NMR spectra were measured in deuterated dimethyl sulfoxide with Bruker AM-300 NMR and Bruker 600 NMR spectrometers (300 MHz for ^{1}H, 75 MHz for ^{13}C). All chemical shifts are reported as δ values relative to TMS.

Plant Materials and Paddy Soil. Four rice (*Oryza sativa* L.) varieties were selected for these studies. PI312777 and Huagan-1, putative allelopathic rice varieties, were screened from the USDA-ARS rice germplasm collection (*16*) and the Chinese rice germplasm collection (*12*), respectively. Lliaonong-11 and Huajingxian without the allelopathic trait are commercial cultivars in China.

Ripe seeds of two weeds (*Echinochloa crus-galli* and *Cyperus difformis*) and paddy soils were each collected from the Shenyang Experimental Station of Ecology, Chinese Academy of Sciences (northeastern China, N 41° 31′, E 123° 24′). The seeds of each weed were dried in sunlight and then stored in sealed glass jars, respectively. The soils were air-dried, mixed, and then sieved (2 mm mesh) to remove plant tissues. Soil was Albic luvisols (FAO classification) with a pH of 5.75 ± 0.28, organic matter content of 1.31 ± 0.22%, and the following fertility status: total N, 0.97 ± 0.12 g kg^{-1}; available N, 108.64 ± 19.12 mg kg^{-1}; total P, 0.41 ± 0.09 g kg^{-1}; available P, 29.76 ± 9.21 mg kg^{-1}; total K, 0.81 ± 0.17 g kg^{-1}; available K, 77.85 ± 12.43 mg kg^{-1}.

Isolation and Identification of Flavone Glycosides. The leaves of allelopathic PI312777 plants at the third leaf stage were washed, freeze-dried, and ground. The powders (3000 g) were extracted with hot EtOH for three times for 8 h each. The filtrate was concentrated in vacuo and gave a brown syrup. The syrup was partitioned with EtOAc and water. The water part was extracted with *n*-BuOH. The *n*-BuOH phase was concentrated in vacuo, and the residues (150 g) were subsequently subjected to silica gel CC (230−400 mesh, Merck) with EtOAc/EtOH (v/v, 1:9) mixture, providing several fractions. Fraction 2 was purified by silica gel CC with CH_2Cl_2/MeOH gradient elution to obtain two fractions. Removal of solvent from two fractions afforded pale yellow amorphous powders that were crystallized by EtOH/EtOAc (v/v, 5:1) to yield yellow prisms **1** (125 mg) and **2** (76 mg), respectively.

*5,4′-Dihydroxy-3′,5′-dimethoxy-7-O-β-glucopyranosylflavone (**1**):* mp 268−269 °C; $[R]_{D22}$ −68.5° (MeOH, *c* 1.0); HR-ESI-MS (positive), *m*/*z* 493.0867 ($[M + H]^+$, calcd for $C_{23}H_{24}O_{12}$; FAB-MS (positive), *m*/*z* 493 $[M + H]^+$ (*12*), 331 (*55*); IR, υ_{max} (KBr) 3410, 1705, 1651, 1600, 1500 cm^{-1}; UV, λ_{max} (EtOH) 312 (4.46), 287 (4.25) nm; ^{1}H NMR, δ 7.07 (1H, s, H-3), 6.46 (1H, d, J = 2.1 Hz, H-6), 6.93 (1H, d, J = 2.1 Hz, H-8), 7.36 (2H, s, H-2′ and H-6′), 3.88 (6H, s, −OCH_3), 5.05 (1H, d, J = 7.2 Hz, H-1″), 3.27 (1H, m, H-2″, overlap with D_2O), 3.30 (1H, m, H-3″), 3.16 (1H, m, H-4″), 3.44 (1H, m, H-5″), 3.73 (1H, dd, J = 4.8, 10.2 Hz, H-6″), 3.46 (1H, m, H-6″, overlap with H-5″), 12.96 (1H br s, 5-OH); ^{13}C NMR, δ 164.2 (s, C-2), 104.3 (d, C-3), 182.5 (s, C-4), 161.7 (s, C-5), 99.9 (d, C-6), 163.5 (s, C-7), 95.7 (d, C-8), 156.4 (s, C-9), 105.7 (s, C-10), 120.5 (s, C-1′), 104.9 (d, C-2′ and 6′), 148.7 (s, C-3′ and 5′), 139.8 (s, C-4′), 56.7 (q, 3′ and 5′ OCH_3), 100.5 (d, C-1″), 73.6 (d, C-2″), 76.9 (d, C-3″), 70.0 (d, C-4″), 77.7 (d, C-5″), 61.0 (t, C-6″).

*7,4′-Dihydroxy-3′,5′-dimethoxy-5-O-β-glucopyranosylflavone (**2**):* mp 272−274 °C; $[R]_{D22}$ −76.5° (MeOH, *c* 1.0); HR-ESI-MS (positive), *m*/*z* 493.09167 ($[M + H]^+$, calcd for $C_{23}H_{24}O_{12}$; FAB-MS (positive), *m*/*z* 493 $[M + H]^+$ (*15*), 331 (*61*); IR, υ_{max} (KBr) 3400, 1695, 1650, 1600, 1500 cm^{-1}; UV, λ_{max} (EtOH) 318 (4.46), 297 (4.25) nm; ^{1}H NMR, δ 6.49 (1H, s, H-3), 6.88 (1H, d, J = 3.0 Hz, H-6), 6.65 (1H, d, J = 3.0 Hz, H-8), 7.05 (2H, s, H-2′ and H-6′), 3.86 (6H, s, −OCH_3), 4.60 (1H, d, J = 7.2 Hz, H-1″), 3.50 (1H, dd, J = 5.6, 7.2 Hz, H-2″), 3.42 (1H, m, H-3), 3.36 (1H, m, H-4), 3.36 (1H, m, H-5″), 3.84 (1H, m, H-6″, overlap with −OCH_3), 3.60 (1H, dd, J = 5.6, 10.8, H-6″); ^{13}C NMR, δ 161.6 (s, C-2), 106.7 (d, C-3), 178.0 (s, C-4), 158.7 (s, C-5), 105.8 (d, C-6), 162.8 (s, C-7), 99.2 (d, C-8), 158.7 (s, C-9), 108.5 (s, C-10), 121.0 (s, C-1′), 103.8 (d, C-2′ and 6′), 148.1 (s, C-3′ and 5′), 139.5 (s, C-4′), 56.4 (q, 3′ and 5′ −OCH_3), 105.7 (d, C-1″), 73.6 (d, C-2″), 76.0 (d, C-3″), 70.2 (d, C-4″), 77.7 (d, C-5″), 61.9 (t,C-6″).

Bioassays. The inhibitory activity of two flavone *O*-glycosides on the growth of *E. crus-galli* and *C. difformis* was evaluated using a pot-culture method (*10*, *12*). At least 50 weed seeds were sown on each 6 × 9 cm pot containing 150 g of paddy soils. After emergence, the seedlings were thinned to 10 plants per pot, and then **1** and **2** at various concentrations tested (10−200 μg/g) were added to each of the treated pots, respectively. The control pots received water only. All pots were placed in a greenhouse with 20−30 °C night and daytime temperatures and 65−90% relatively humidity maintained. Pots were randomized once a week. The weeds were harvested after 4 weeks, and their shoots were clipped at the point of first root and dried for at least 48 h at 80 °C, and then dry weights were determined. Percentage of inhibition at different concentrations was obtained from the comparison of weed dry weights between the treated and control pots, respectively.

The effect of two flavone *O*-glycosides on soil microbial populations was determined with serial dilution and a pour plate method (*17*, *18*). Paddy soils (100 g) were placed in plastic pots (5 × 5 cm), and then **1** and **2** at the concentration of 50 or 200 μg/g were added to the treated pots, respectively. The control pots received sterile water only. All pots were placed in a dark environment growth chamber with 25 or 30 °C and 70% humidity for various time intervals of 0−25 days. Ten grams of soil from each pot was suspended in 10 mL of sterile water and 10-fold serially diluted. The CFU of bacteria including ammonifying bacteria were determined by spreading 100 mL of diluted samples on agar plates with beef extract−peptone medium and incubating at 25 ± 2 °C for 5 days. The CFU of actinomycetes were determined by probing colonies that developed with a dissecting needle: if the colony remained as a discrete, small mass, it was considered to be an actinomycete, whereas if the colony smeared and lost its periphery, it was considered to be a bacterium other than an actinomycete. These empirical observations were confirmed by microscopic examination of the colonies. The CFU of fungi were estimated on Rose Bengal (33 μg mL^{-1}) and streptomycin (30 μg mL^{-1}) agar on which 100 mL of 10-fold serially diluted soil samples was spread. The CFU of fungi were counted after incubation for 5−7 days at 25 ± 2 °C. The CFU of bacteria and fungi were determined on duplicate samples of soil from each pot and jar.

The same experiments described above were conducted three times for each determination under identical conditions.

Quantification Analysis of Flavone Glycosides. Fifty sterile and pregerminated seeds of four rice varieties tested were sown in plastic pots (5 × 5 cm) containing different media (75 mL Hoagland's solutions or 50 g paddy soils), respectively. All pots with three replicates for each of independent experiments were placed in a controlled environmental chamber (3 m^3) with a 12 h day length and approximately 350 μmol/m^2/s light intensity, 25−28 °C temperature, and 70% relative humidity. Rice tissues and media were collected at the third leaf stage, respectively.

Shoot and root samples (1 g) were each boiled in 10 mL of EtOH for 5 min and filtered. The Hoagland's solution (75 mL) was extracted with *n*-BuOH/EtOAc mixture (v/v, 1:1, 3 × 50 mL), and the soils (50 g) were extracted with 3 × 100 mL of 75% acetone/25% [water + EDTA (2%)] mixture and agitated for 24 h at 25 °C and then centrifuged at 2800*g* for 10 min. The filtrates of tissues and extracts of media were each concentrated in vacuo to give residues. The residues were respectively dissolved in 50% aqueous MeOH and loaded on reversed phase C_{18} Sep-Pak cartridge (Waters Co.), which was eluted with 50% aqueous MeOH and then MeOH. The MeOH fraction was concentrated with nitrogen gas to obtain 100 μL of concentrate for quantitative analysis.

Quantification analysis of glycosides **1** and **2** or their aglycone (5,7,4′-trihydroxy-3′,5′-dimethoxyflavone) in rice tissues and cultural media was carried out with an HPLC HP-1100 equipped with a Zorbax SB-C_{18} reversed column (Hypersil 150 mm × 4.6 mm, 5 μm) with a diode array detector. HPLC determination conditions were as follows: mobile phase, mixture of MeCN/1% aqueous AcOH (2:3, v/v), eluted at a flow rate of 0.80 mL/min at room temperature, and detected at 320 nm; injection volume of samples, 10 μL. **1**, **2**, and their aglycone in the tissues and media were each quantified by interpolating the peak area on the chromatograms of HPLC to a standard curve constructed by the peak area of authentic **1**, **2**, or their aglycone isolated from PI312777 plants described above or in our pervious literature (*10*, *11*). Triplicate injections of each concentration were made.

Soil Dynamics of Flavone Glycosides. Paddy soils (20 g) were each placed in 50 mL vials, and a mixture of glycoside **1** and **2** (w/w, 1:1) at different concentrations (25 or 200 μg/g) was added, respectively. The vials were placed in a dark environmental growth chamber (1 m^3) at 25 ± 1 °C and 70% humidity. The vials for incubation of the solution of glycosides were randomly taken from the environment chamber at various time intervals and then were each extracted with 75% acetone/25% [water + EDTA (2%)] mixture. The extracts were each concentrated in vacuo to give samples for their HPLC analysis as described above. The extraction was performed from the second hour and continued once every 3 h (a total of 98 h). Recoveries of the glycosides and their aglycone were 79.4 ± 5.1 and 83.4 ± 6.3%, respectively. Their half-lives ($t_{1/2}$) were each calculated according to the method of Macias et al. (*19*).

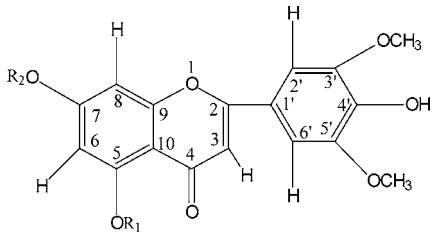
The mobility of the glycosides and their aglycone in paddy soils was evaluated using soil thin-layer chromatography (soil TLC) with bioassay. The paddy soils were air-dried and sieved to 125 μm. Then 30 g of soils was suspended in a dioxane/water (1:1, v/v) solvent to make a slurry, which was then spread as a 0.7 mm thick layer on a 20 × 20 cm glass plate. The plates were dried at room temperature and stored until used for chromatographic tests (*20*).

The glycosides and their aglycone were each sampled with a microsyringe at 1.0 cm from the bottom edge of the plates. After the spots had been deposited, the plates were allowed to develop in a closed glass chamber using distilled water as solvent. A sheet of filter paper dipping into the developing water fed water continuously to the substrate at the base of the plate, thus leading to a relatively uniform flow. During the development with solvent the whole device was held in a horizontal position. Solvent migration occurred at a distance 17.5 cm from the baseline. The surface of the soil TLC plate was uniformly sown with lettuce (*Lactuca sativa* L.) seeds and then cultured in the constant-humidity cabinet (25 °C). The germination of lettuce was observed after 2 days. The location with significant inhibition of lettuce germination was regarded as the migration distance of glycosides or their aglycone (R_1). The distances covered by the test chemicals on a soil TLC plate compared to that covered by water, that is, the R_f value, was calculated as $R_f = R_1/R_2$ (R_2 was the water migration distance). All manipulations as described above were conducted 18 times under identical conditions.

RESULTS AND DISCUSSION

On the basis of HR-ESI-MS data and various spectra, two compounds isolated from rice plants were subsequently identified as 5,4′-dihydroxy-3′,5′-dimethoxy-7-*O*-β-glucopyranosylflavone (**1**) and 7,4′-dihydroxy-3′,5′-dimethoxy-5-*O*-β-glucopyranosylflavone (**2**). Glycoside **1** has been found in rice and several plant species (*2*, *3*), but its isomer, **2**, has never been isolated from rice. Few studies have indicated that the active components of rice leaf and stem extract against the white-back planthopper (*Sogatella furcifera*) contained **2** through spectroscopic analyses (*5*). However, their aglycone, 5,7,4′-trihydroxy-3′,5′-dimethoxyflavone, has been extensively found in rice bran, husk, seedling, and cultural media (*6*−*8*, *10*−*12*).

Subsequent bioassays showed that glycosides **1** and **2** were highly active against *E. crus-galli* and *C. difformis*, but their weed-suppressive degree had no obvious difference (**Figure 1**).



Glycoside **1**, R_1=H, R_2=β-Glucopyranosyl;

Glycoside **2**, R_1=β-Glucopyranosyl, R_2=H;

Aglycone, R_1= R_2= H.

Soil bacteria and ammonifying bacteria populations were obviously influenced by the glycosides, particularly at the low concentrations tested (50 μg/g). Compared to the control soil, the significant increase in numbers of bacteria and ammonifying bacteria populations occurred after 3−5 days in soil to which the glycosides had been applied, and there were no significant differences after 7 days (**Figure 2**). Actinomycetes and fungi appeared to be less sensitive than bacteria to the exogenous application of glycosides in either high or low concentrations tested. A significant reduction in the population of actinomycetes occurred after 3 days in soil to which had been applied the glycosides at low concentrations (**Figure 2**). These results implied that flavone *O*-glycosides could exert the effect on associated weeds and soil bacteria if they would be continuously released from rice tissues to paddy soil in sufficient quantity.

Glycosides **1** and **2** could be found in four rice varieties tested, but their levels were highly varied with the allelopathic traits, the tissues, and cultural conditions. With the same tissues or cultural conditions, both allelopathic rice varieties had higher glycosides level than the non-allelopathic ones. Soil-cultured plants contained higher concentrations of the glycosides than hydroponic cultured plants. Under both soil and hydroponic cultural conditions tested, rice plants always contained glycosides **1** and **2** in almost equal quantities that had much higher concentrations in shoots than in roots (**Table 1**), indicating that

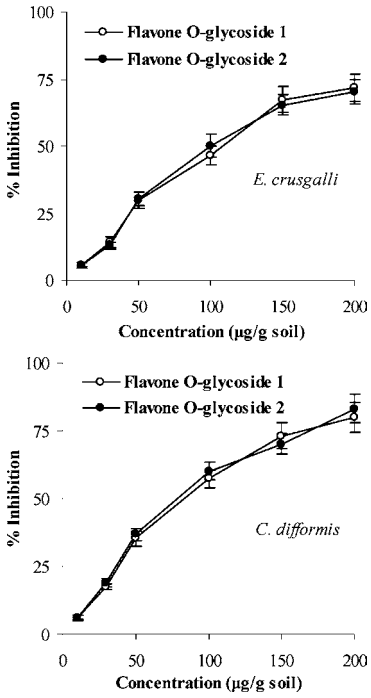


Figure 1. Inhibition of flavone *O*-glycosides on the growth of *E. crus-galli* and *C. difformis*.

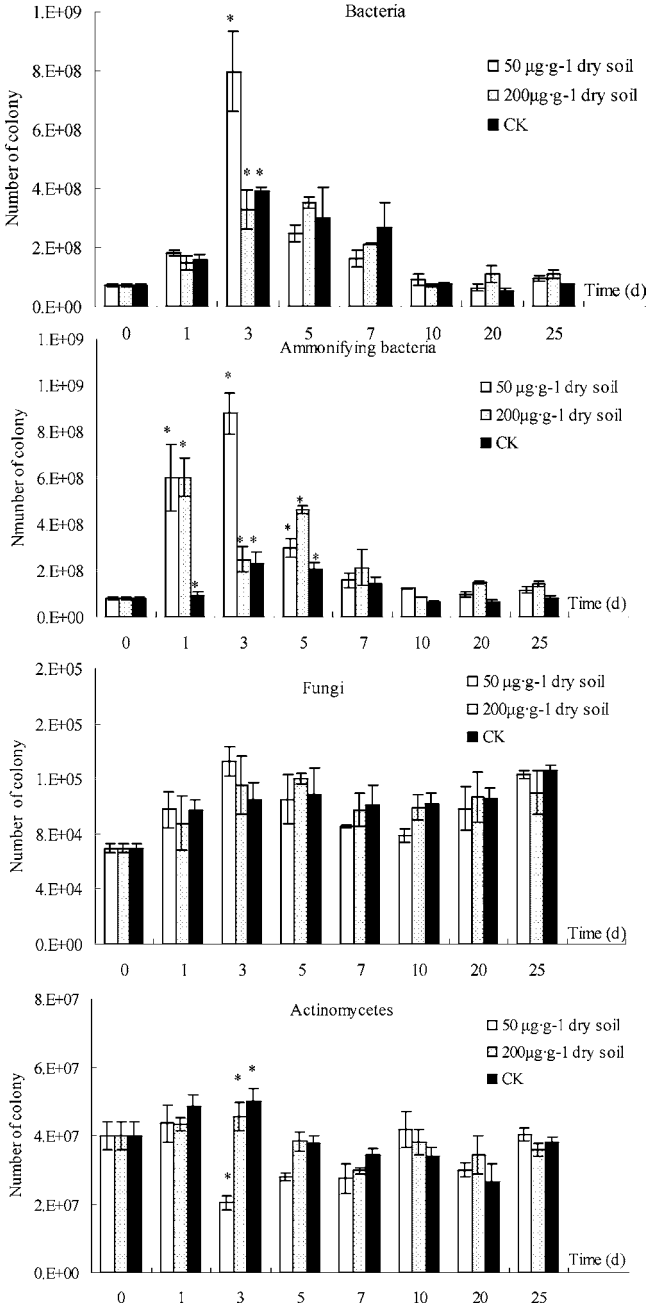


Figure 2. Effect of flavone *O*-glycosides on paddy soil microbial populations. There is no difference between the two flavone *O*-glycosides on soil microbial populations. Means ± SE from two flavone *O*-glycosides with the same concentrations for each determination are shown.

flavone *O*-glycosides were primarily synthesized in the shoots of rice seedlings. Furthermore, both glycosides and their aglycone could be simultaneously found in hydroponic solution, indicating that the glycosides were exuded from living rice into the environment. However, the glycosides could not be detected in the soils surrounding rice seedlings, and only their aglycone, 5,7,4′-trihydroxy-3′,5′-dimethoxyflavone, could be found in the soil (**Table 1**). In general, the glycosides occurring in plant tissues usually are almost immediately hydrolyzed by enzymes or microbes and release the aglycone once released into the environment (*15*). In this study, the effect of microbes or enzymes is much weaker under hydroponic culture than under soil culture, and thus the glycosides could be detected in hydroponic solution rather than in soil. Accordingly, it is

Table 1. Amounts of Flavone *O*-Glycosides in Rice Tissues and Cultural Media[a]

tissue or cultural medium	allelopathic variety		non-allelopathic variety	
	PI312777	Huagan-1	Liaonong-11	Huajingxian
Hydroponic Culture				
shoot (mg/g)				
glycoside **1**	0.31 ± 0.03a	0.37 ± 0.05a	0.17 ± 0.02b	0.16 ± 0.02b
glycoside **2**	0.26 ± 0.05a	0.30 ± 0.06a	0.14 ± 0.02b	0.18 ± 0.02b
root (mg/g)				
glycoside **1**	0.03 ± 0.01a	0.03 ± 0.01a	0.01 ± 0.00b	0.01 ± 0.00b
glycoside **2**	0.02 ± 0.01a	0.03 ± 0.01a	0.02 ± 0.00b	0.01 ± 0.00b
cultured solution (μg/g)				
glycoside **1**	1.38 ± 0.09a	1.43 ± 0.11a	0.70 ± 0.05b	0.61 ± 0.08b
glycoside **2**	1.40 ± 0.07a	1.45 ± 0.09a	0.05 ± 0.01b	0.59 ± 0.07b
aglycone[b]	0.11 ± 0.03a	0.09 ± 0.02a	tr[c]	tr
Soil Culture				
shoot (mg/g)				
glycoside **1**	0.82 ± 0.10a	1.01 ± 0.11b	0.35 ± 0.06c	0.42 ± 0.07c
glycoside **2**	0.80 ± 0.09a	1.05 ± 0.10b	0.38 ± 0.05c	0.41 ± 0.06c
root (mg/g)				
glycoside **1**	0.04 ± 0.01a	0.04 ± 0.01a	0.01 ± 0.00b	0.01 ± 0.00c
glycoside **2**	0.04 ± 0.01a	0.04 ± 0.01a	0.01 ± 0.00b	0.02 ± 0.01c
soil (μg/g)				
glycoside **1**	nd[d]	nd	nd	nd
glycoside **2**	nd	nd	nd	nd
aglycone[b]	1.66 ± 0.11a	1.70 ± 0.10a	tr	0.05 ± 0.01b

[a] Means ± SE from three independent experiments for each determination are shown. Data in a row followed by the same letter are not significantly different at $P = 0.05$, ANOVA with Duncan's multiple-range test. [b] 5,7,4′-Trihydroxy-3′,5′-dimethoxyflavone. [c] Trace. [d] Not detected.

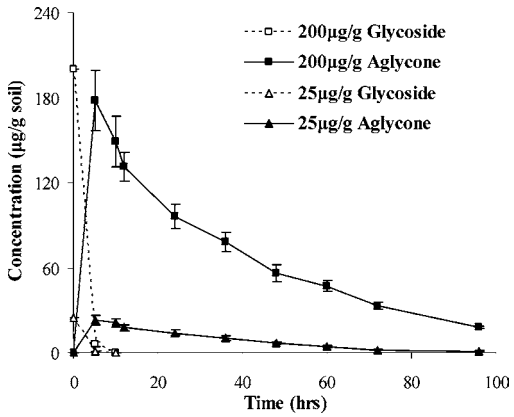


Figure 3. Transformation and degradation of flavone *O*-glycosides and their aglycone in paddy soil at different doses.

postulated that both glycosides were released from rice roots to the rihzosphere and then transformed into the aglycone 5,7,4′-trihydroxy-3′,5′-dimethoxyflavone by soil biotic and abiotic factors. To confirm this postulation, the flavone *O*-glycosides were added to soils, and then the amounts of glycosides and their aglycone were each analyzed at various time intervals. Initially, the soil contained the glycosides only. However, both glycosides and their aglycone were simultaneously found after 2 h. The glycoside amounts decreased rapidly and were not detected after 10 h whereas the aglycone amount increased greatly and reached a maximum at 5 h and then declined gradually with time (**Figure 3**). These results indicated the flavone *O*-glycosides degrade rapidly ($t_{1/2} < 2$ h), yielding their aglycone 5,7,4′-trihydroxy-3′,5′-dimethoxyflavone in paddy soils, whereas the aglycone was more resistant toward degradation in paddy soils, in which the half-life ($t_{1/2}$) at low (25 μg/g) and high (200 μg/g) doses reached 19.86 ± 3.64 h ($r^2 = 0.97$) and 28.78 ± 3.72 h ($r^2 = 0.98$), respectively.

The glycosides, given the short half-life, indicated that they could easily transform into the aglycone form by soil interactions once released. In addition, a weed-suppressive degree of the glycosides was in agreement with their aglycone in a previous paper (*10*). Therefore, bioactivities on weeds and microbes shown in **Figures 1** and **2** would be responsible for the action of the aglycone rather than glycosides. It must be pointed out that bioactive effects of the glycosides or their aglycone were observable only at levels greater than those detected in paddy soil. In this study, detection of the glycosides and their aglycone was conducted from the bulk soil under arbitrary conditions, rather than from the rihzosphere soil under intact conditions. Soil extractions dilute the compounds over large volumes. In the rhizosphere, the concentrations of the glycosides could be locally much higher. In this manner, they may have effects on rhizosphere bacteria, but it is not clear how they would affect weed growth.

The action of allelochemicals involved in allelopathic plant−plant interactions needs their presence in the soil in the vicinity of the target plant (*19*, *21*). Besides transformation or degradation processes taking place in the soil, migration or mobility of allelochemicals in the soil needs to be clarified (*20*, *22*). In this study, the mobility of flavone *O*-glycosides and their aglycone in paddy soil was evaluated by soil TLC with a combination of bioassay. As a result, no difference of the mobility was observed between glycosides **1** and **2**. However, the mobility of the glycosides ($R_f = 0.42 \pm 0.07$, $n = 18$) is higher than that of the aglycone ($R_f = 0.36 \pm 0.05$, $n = 18$). It appears from the results that the glycosides and their aglycone have good mobility and are not readily adsorbed in paddy soil, and thus they could easily be diffused from the rhizosphere to the bulk soil to affect weed germination and growth.

Higher plants roots exude an enormous range of potentially valuable small molecular weight compounds into the environment. These compounds play important roles in rhizosphere interactions with plants and other organisms (*9*, *13*). The allelochemicals exuded from roots to the soil have to be subjected to several processes including transformation, degradation, and mobility and biochemical interactions (*15*, *19*). This study showed that flavone *O*-glycosides biosynthesized in allelopathic rice could be exuded from root systems to the environment and could be transformed into their aglycone form with a great diversity of biological activities by soil interactions once released; thus, two flavone *O*-glycosides are formed in rice biosynthesis and storage of the allelochemicals, and their aglycone 5,7,4′-trihydroxy-3′,5′-dimethoxyflavone is the agent of alleloapthic rice which interferes with weeds or microbes in paddy soil. However, biotic and abiotic factors involved in soil dynamics of these alleochemicals remain obscure. Further clarification of these allelochemicals' diffusion through soil solution, final fate, and soil persistence as well affected factors such as soil type, mineral reactivity, and specific microbial activity is warranted.

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