JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Flavonoids from the Grains of C1/R-S Transgenic Rice, the Transgenic *Oryza sativa* spp. *japonica*, and Their Radical Scavenging Activities

Jin-Gyeong Cho,[†] Na-Young Song,[†] Tae-Gyu Nam,[‡] Sabina Shrestha,[†] Hee-Jung Park,[†] Ha-Na Lyu,[†] Dae-Ok Kim,[‡] Gihwan Lee,[§] Young-Min Woo,[#] Tae-Sook Jeong,[⊥] and Nam-In Baek*[,†]

[†]Graduate School of Biotechnology, Institute of Life Sciences and Resources, Kyung Hee University, Yongin 446-701, Republic of Korea

[‡]Department of Food Science and Technology and Institute of Life Sciences and Resources, Kyung Hee University, Yongin 446-701, Republic of Korea

[§]Department of Functional Crop, National Institute of Crop Sciences, Rural Development Administration, Milyang 627-803, Republic of Korea

[#]Laboratory of Plant Functional Genomics, Division of Molecular and Life Sciences, Pohang University of Science and Technology, Pohang 790-784, Republic of Korea

[⊥]National Research Laboratory of Lipid Metabolism and Atherosclerosis, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-806, Republic of Korea

ABSTRACT: The transgenic rice cultivar of *Oryza sativa* spp. *japonica* cv. Hwa-Young, C1/R-S transgenic rice (C1/R-S rice), is a flavonoid-rich cultivar of rice. The grains of C1/R-S rice were extracted with aqueous MeOH, and the concentrated extract was partitioned with EtOAc, *n*-BuOH, and H_2O, successively. Repeated silica gel, octadecyl silica gel (ODS), and Sephadex LH-20 column chromatographies for the EtOAc and *n*-BuOH fractions afforded four new flavonoids (compounds **2**, **3**, **7**, and **8**) along with four known flavonoids: (+)-3′-*O*-methyltaxifolin (**1**), brassicin (**4**), isorhamnetin-4′-*O*-*β*-D-glucosyranoside (**5**), and 3′-*O*-methyltaxifolin-5-*O*-*β*-D-glucopyranoside (**6**). The new flavonoids were identified as 3′-*O*-methyltaxifolin-7-*O*-*β*-D-glucopyranoside (**2**), 3′-*O*-methyltaxifolin-4′-*O*-*β*-D-glucopyranoside (**3**), isorhamnetin-7-*O*-*β*-D-cellobioside (brassicin-4″-*O*-*β*-D-glucopyranoside) (**7**), and brassicin-4′-*O*-*β*-D-glucosyranoside (**8**) from the result of spectroscopic data including nuclear magnetic resonance spectrometry (NMR), mass spectrometry (MS), and infrared spectroscopy (IR). Also, quantitative analysis of major flavonoids (compounds **2**, **3**, and **8**) in C1/R-S rice, *O. sativa* spp. *japonica* cv. Hwa-Young (HY), and a hybrid of two cultivar (C1/R-S rice/HY) extracts was performed using HPLC experiment. The isolated flavonoids were evaluated for their radical-scavenging effect on DPPH and ABTS radicals.

KEYWORDS: *ABTS, DPPH, flavonoid, HPLC, Oryza sativa, transgenic rice*

INTRODUCTION

Flavonoids are ubiquitous in nature and are synthesized from phenylpropanoid derivatives by condensation with malonyl-CoA in the cytosol of plant cells.[1] There are a variety of structural forms including chalcone, flavanone, dihyroflavonol, anthocyanin, flavone, flavonol, isoflavonoid, and catechin. They are found in fruits, vegetables, nuts, seeds, and stems and represent a common constituent of the human diet. Flavanols are found in red grapes and red wine, flavanones are present in citrus foods, flavones are rich in green leafy spices, isoflavones are found in soy foods, anthocyanidins are found in berries, and flavonols are found in almost all human foods.[2] Flavonoids have a wide range of beneficial health effects. They have been reported to exhibit antiviral,[3] antiallergic,[4] antiplatelet,[4] anti-inflammatory,[5,6] antitumor,[5] and antioxidant activities.[5,7] The most well-known beneficial effects of flavonoids such as quercetin (flavonol),[8,9] apigenin (flavone),[10] and naringenin (flavanone)[11] are their antioxidant activities.

C1/R-S transgenic rice (C1/R-S rice) is a new cultivar rice from *Oryza sativa* spp. *japonica* cv. Hwa-Young (HY).[12] This cultivar has a high flavonoid content in the seeds, and in particular in rice endosperm tissue, not just in the seed coat or aleurone layer, because an endosperm-specific promoter was used to regulate the expression of maize *C1* and *R-S*. The endosperm is the nutritive tissue surrounding the embryo of a seed and makes up the majority of the rice grain that we eat. The total flavonoid contents in the C1/R-S rice seeds were 14 times higher than in the nontransgenic rice cultivar Hwa-Young.[13] Furthermore, many flavonoids have been identified through liquid chromatography−mass spectrometry−mass spectrometry (LC-MS/MS) of C1/R-S rice as compared with HY.[12] Therefore, this study was initiated to search for the principal flavonoids that manifest antioxidant activity.

The grains of C1/R-S rice were extracted in aqueous MeOH and solvent-fractionated using EtOAc, *n*-BuOH, and H_2O, successively. Repeated column chromatographies of the EtOAc and *n*-BuOH fractions afforded eight flavonoids, and the chemical structures were determined through spectroscopic data analyses. Among them, four were revealed to be new

Received: July 18, 2013
Revised: September 25, 2013
Accepted: September 26, 2013
Published: September 27, 2013

dx.doi.org/10.1021/jf403072c | *J. Agric. Food Chem.* 2013, 61, 10354−10359

compounds. The isolated compounds and C1/R-S rice were evaluated for scavenging activities of DPPH and ABTS radicals. Also, an HPLC experiment was carried out for quantitative analysis of major flavonoids.

MATERIALS AND METHODS

Sample Materials. C1/R-S rice grains were provided by Pohang University, Pohang, Korea, in May 2008. A voucher specimen (KHU090512) was deposited in the Laboratory of Natural Products Chemistry, Kyung Hee University, Yongin, Korea.

Instruments and Chemicals. For instrumental and chemicals, see previous papers.[14−16]

Extraction of C1/R-S Rice Grains and Isolation of Flavonoids. The dried powder of C1/R-S rice grains of white rice (1.6 kg) was extracted with 80% MeOH (5 L × 4) at room temperature for 12 h. The extract were filtered through filter paper and concentrated in a rotary vacuum evaporator to yield a brownish residue (52 g). The residue was suspended in 500 mL of water and successively partitioned with EtOAc (500 mL × 3) and *n*-BuOH (400 mL × 2), yielding concentrated extracts of EtOAc (C1RSRE, 5 g), *n*-BuOH (C1RSRB, 9 g), and H_2O (38 g), sequentially.

The EtOAc fraction (C1RSRE, 5 g) was fractionated with flash silica gel (SiO_2) column chromatography (Biotage SNAP Cartridge 100 g, Biotage, Uppsala, Sweden) eluting with *n*-hexane/EtOAc (8:1, 3 L → 5:1, 2 L) and $CHCl_3$/MeOH (15:1 → 7:1, each 1.5 L) to yield 14 fractions (C1RSRE-1 to C1RSRE-14). Flavonoid-rich fractions C1RSRE-10, -13, and -14, which were confirmed through TLC experiment, were selected for further isolation of flavonoids. C1RSRE-10 [751 mg, elution volume/total volume (V_e/V_t) 0.67−0.72] was subjected to an ODS column chromatography (3.5 cm × 4.5 cm) and eluted with acetone/H_2O (2:1, 2.0 L → 4:1, 1.0 L) to obtain 17 fractions (C1RSRE-10-1 to C1RSRE-10-17). C1RSRE-10-1 (118 mg, V_e/V_t 0.00−0.03) was applied to the octadecyl SiO_2 (ODS) column chromarography (3.0 × 4.0 cm) and eluted with MeOH/H_2O (5:7, 1.0 L), yielding 20 fractions (C1RSRE-10-1-1 to C1RSRE-10-1-20) and ultimately gave compound **1** [C1RSRE-10-1-4, 28.4 mg, V_e/V_t 0.11−0.16, TLC (RP-18 F_{254s}) R_f 0.60, MeOH/H_2O = 2:1]. C1RSRE-13 (368 mg, V_e/V_t 0.83−0.93) was separated by the ODS column chromatography (3.5 cm × 4.5 cm) and eluted with MeOH/H_2O (2:5, 2.0 L → 2:3, 0.5 L), yielding 15 fractions (C1RSRE-13-1 to C1RSRE-13-15) and ultimately gave compound **2** [C1RSRE-13-2, 37.5 mg, V_e/V_t 0.02−0.11, TLC (RP-18 F_{254s}) R_f 0.60, MeOH/H_2O = 1:1] and compound **3** [C1RSRE-13-7, 32 mg, V_e/V_t 0.27−0.48, TLC (RP-18 F_{254s}) R_f 0.55, MeOH/H_2O = 1:1]. C1RSRE-13-15 (100 mg, V_e/V_t 0.93−1.00) was separated by the ODS column chromatography (3.0 cm × 3.0 cm) and eluted with acetone/H_2O (2:3, 1.0 L), yielding six fractions (C1RSRE-13-15-1 to C1RSRE-13-15-6) and isolated compound **4** [C1RSRE-13-15-2, 12 mg, V_e/V_t 0.16−0.37, TLC (RP-18 F_{254s}) R_f 0.59, acetone/H_2O = 3:2]. The C1RSRE-14 fraction (218 mg, V_e/V_t 0.93−1.00) was subjected to the ODS column chromatography (3.0 cm × 4.0 cm) and eluted with acetone/H_2O (1:2, 1.2 L), yielding 10 fractions (C1RSRE-14-1 to C1RSRE-14-10) and ultimately gave compound **5** [C1RSRE-14-6, 5 mg, V_e/V_t 0.49−0.54, TLC (RP-18 F_{254s}) R_f 0.31, acetone/H_2O = 2:3].

The concentrated *n*-BuOH fraction (C1RSRB, 9 g) was subjected to the SiO_2 column chromatography (8.0 cm × 13.0 cm) and eluted with a gradient of $CHCl_3$/MeOH/H_2O (8:3:1, 16 L → 5:3:1, 6 L), resulting in 10 fractions (C1RSRB-1 to C1RSRB-10). Among the obtained fractions from the *n*-BuOH fraction, flavonoid-rich fractions C1RSRB-4, -7, and -8 were used for further isolation of flavonoids. The C1RSRB-4 fraction (1.26 g, V_e/V_t 0.13−0.20) was subjected to the ODS column chromatography (4.5 cm × 4.5 cm) and eluted with MeOH/H_2O (2:5, 1L → 1:1, 0.5 L) to produce eight fractions (C1RSRB-4-1 to C1RSRB-4-8). Fraction C1RSRB-4-1 (204 mg, V_e/V_t 0.00−0.14) was subjected to the ODS column chromatography (3.3 cm × 3.0 cm) and eluted with MeOH/H_2O (1:4, 0.8 L → 1:3, 0.6 L), yielding 13 fractions (C1RSRB-4-1-1 to C1RSRB-4-1-13) and ultimately gave compound **6** [C1RSRB-4-1-7, 7.9 mg, V_e/V_t 0.59−0.65, TLC (RP-18 F_{254s}) R_f 0.56, MeOH/H_2O = 1:1]. Fraction C1RSRB-7 (1.7 g, V_e/V_t 0.42−0.67) was subjected to the ODS column chromatography (4.5 cm × 5.0 cm) and eluted with acetone/H_2O (2:5, 1 L → 1:1, 0.5 L), yielding 11 fractions (C1RSRB-7-1 to C1RSRB-7-11) and ultimately gave compound **7** [C1RSRB-7-5, 10 mg, V_e/V_t 0.21−0.27, TLC (RP-18 F_{254s}) R_f 0.55, MeOH/H_2O = 2:1]. Fraction C1RSRB-8 (1.4 g, V_e/V_t 0.67−0.78) was subjected to the flash ODS column chromatography (Biotage SNAP Cartridge KP-C_{18}-HS 30 g, Biotage) and eluted with acetone/H_2O (1:5, 0.9 L → 2:3, 0.5 L), yielding 14 fractions (C1RSRB-8-1 to C1RSRB-8-14). Fraction C1RSRB-8-5 (80 mg, V_e/V_t 0.32−0.39) was applied to the Sephadex LH-20 coulmn chromatography (3.0 cm × 40.0 cm, acetone 33%, 0.8 L → 100%, 0.5 L), yielding seven fractions (C1RSRB-8-5-1 to C1RSRB-8-5-7) and ultimately gave compound **8** [C1RSRB-8-5-3, 7.5 mg, V_e/V_t 0.50−0.54, TLC (RP-18 F_{254s}) R_f 0.67, acetone/H_2O = 2:3].

*3′-O-Methyltaxifolin (**1**):* yellow amorphous powder (MeOH); mp 200−205 °C; $[\alpha]_D^{25}$ +10.5° (*c* 3.6, MeOH); EI/MS *m/z* 318 $[M]^+$, 289, 261, 166, 165, 164, 153, 137, 124, 95, 77, 69, 55; IR (CaF_2, ν, cm^{-1}) 3420, 1007, 849, 816; ^1H and ^{13}C NMR data were consistent with other literature.[17,18]

*3′-O-Methyltaxifolin-7-O-β-D-glucopyranoside (**2**):* yellow amorphous powder (MeOH); $[\alpha]_D^{26}$ −41.7° (*c* 0.8, MeOH); negative HRFAB/MS *m/z* 479.1189 $[M - H]^-$ (calcd for $C_{22}H_{23}O_{12}$, 479.1190); IR (CaF_2, ν, cm^{-1}) 3391, 2924, 1645, 1578, 1519, 1455, 1278, 1171, 1075, 1027; ^1H NMR (400 MHz, CD_3OD, δ_H) 7.08 (1H, br s, H-2′), 6.93 (1H, br d, *J* = 8.0 Hz, H-6′), 6.80 (1H, d, *J* = 8.0 Hz, H-5′), 6.17 (1H, d, *J* = 2.0 Hz, H-6), 6.15 (1H, d, *J* = 2.0 Hz, H-8), 4.96 (1H, d, *J* = 12.0 Hz, H-2), 4.94 (1H, d, *J* = 7.6 Hz, H-1″), 4.58 (1H, d, *J* = 12.0 Hz, H-3), 3.84 (3H, s, 3′-OCH_3), 3.82 (1H, dd, *J* = 12.0, 1.6 Hz, H-6″a), 3.65 (1H, dd, *J* = 12.0, 5.2 Hz, H-6″b), 3.44−3.28 (4H, H-2″, -3″, -4″, -5″); ^{13}C NMR (100 MHz, CD_3OD, δ_C) 199.1 (C-4), 166.9 (C-7), 164.4 (C-5), 163.9 (C-9), 148.6 (C-3′), 148.1 (C-4′), 129.4 (C-1′), 122.1 (C-6′), 115.8 (C-5′), 112.2 (C-2′), 103.3 (C-10), 101.1 (C-1″), 98.2 (C-8), 96.9 (C-6), 85.2 (C-2), 78.1 (C-3″), 77.6 (C-5″), 74.5 (C-2″), 73.6 (C-3), 70.9 (C-4″), 62.1 (C-6″), 56.4 (3′-OCH_3).

*3′-O-Methyltaxifolin-4′-O-β-D-glucopyranoside (**3**):* light yellow powder ($CHCl_3$/MeOH); $[\alpha]_D^{27}$ −35.7° (*c* 0.39, $CHCl_3$/MeOH); negative HRFAB/MS *m/z* 479.1189 $[M - H]^-$ (calcd for $C_{22}H_{23}O_{12}$, 479.1190); IR (CaF_2, ν, cm^{-1}) 3390, 1644, 1595, 1514, 1464, 1265, 1166, 1074; ^1H NMR (400 MHz, C_5D_5N, δ_H) 7.63 (1H, d, *J* = 8.4 Hz, H-5′), 7.46 (1H, br s, H-2′), 7.34 (1H, br d, *J* = 8.4 Hz, H-6′), 6.51 (1H, br s, H-6), 6.39 (1H, br s, H-8), 5.71 (1H, d, *J* = 6.8 Hz, H-1″), 5.43 (1H, d, *J* = 11.6 Hz, H-2), 5.01 (1H, d, *J* = 11.6 Hz, H-3), 4.51 (1H, br d, *J* = 11.6 Hz, H-6″a), 4.44−4.06 (5H, H-2″, -3″, -4″, -5″, -6″b), 3.66 (3H, s, 3′-OCH_3); ^{13}C NMR (100 MHz, C_5D_5N, δ_C) 198.3 (C-4), 168.6 (C-7), 164.9 (C-5), 163.6 (C-9), 149.9 (C-3′), 148.3 (C-4′), 131.9 (C-1′), 121.6 (C-6′), 115.6 (C-5′), 112.6 (C-2′), 101.9 (C-1″), 101.6 (C-10), 97.5 (C-6), 96.2 (C-8), 84.6 (C-2), 78.8 (C-3″), 78.5 (C-5″), 74.8 (C-2″), 73.1 (C-3), 71.1 (C-4″), 62.2 (C-6″), 55.9 (3′-OCH_3).

*Brassicin (**4**):* yellow crystals (MeOH); mp 214−216 °C; $[\alpha]_D^{26.7}$ −39.3° (*c* 0.2, MeOH); EI/MS *m/z* 478 $[M]^+$; IR (CaF_2, ν, cm^{-1}) 3367, 1649, 1595, 1514, 1498, 1285, 1206, 1170, 1073, 1027; ^1H and ^{13}C NMR data were consistent with other literature.[19]

*Isorhamnetin-4′-O-β-D-glucosyranoside (**5**):* yellow powder (MeOH); $[\alpha]_D^{26.8}$ −31.9° (*c* 0.06, MeOH); positive FAB/MS *m/z* 479 $[M + H]^+$; IR (CaF_2, ν, cm^{-1}) 3450, 1654, 1560; ^1H and ^{13}C NMR data were consistent with other literature.[20,21]

*3′-O-Methyltaxifolin-5-O-β-D-glucopyranoside (**6**):* yellow amorphous powder (MeOH); $[\alpha]_D^{26.9}$ −31.1° (*c* 0.34, MeOH); negative FAB/MS *m/z* 479 $[M - H]^-$; IR (CaF_2, ν, cm^{-1}) 3367, 2924, 1654, 1609, 1519, 1450, 1372, 1278, 1073, 1029; ^1H and ^{13}C NMR data were consistent with other literature.[22]

*Brassicin-4″-O-β-D-glucopyranoside (**7**):* yellow amorphous powder (MeOH); $[\alpha]_D^{26.7}$ −87.8° (*c* 0.54, $CHCl_3$/MeOH); negative HRFAB/MS *m/z* 639.1566 $[M - H]^-$ (calcd for $C_{28}H_{31}O_{17}$, 639.1561); IR (CaF_2, ν, cm^{-1}) 3334, 1704, 1649, 1616, 1596, 1500, 1356, 1318, 1285, 1207, 1166, 1071, 1027; ^1H NMR (400 MHz, CD_3OD, δ_H) 7.81 (1H, br s, H-2′), 7.68 (1H, br d, *J* = 8.0 Hz, H-6′), 6.88 (1H, d, *J* = 8.0 Hz, H-5′), 6.69 (1H, br s, H-6), 6.41 (1H, br s, H-

8), 5.08 (1H, d, J = 7.6 Hz, H-1″), 4.45 (1H, d, J = 7.6 Hz, H-1‴), 3.89 (3H, s, 3′-OCH_3), 3.98−3.24 (12H, H-2″, -3″, -4″, -5″, -6″, -2‴, -3‴, -4‴, -5‴, -6‴); ^{13}C NMR (100 MHz, CD_3OD, δ_C) 177.5 (C-4), 164.3 (C-5), 162.1 (C-7), 157.6 (C-9), 150.0 (C-4′), 148.5 (C-2), 148.4 (C-3′), 136.0 (C-3), 123.9 (C-1′), 122.9 (C-6′), 116.3 (C-5′), 112.6 (C-2′), 106.2 (C-10), 104.6 (C-1‴), 101.3 (C-1″), 100.1 (C-6), 95.8 (C-8), 80.2 (C-4″), 78.1 (C-5‴), 77.9 (C-3‴), 76.8 (C-5″), 76.2 (C-3″), 74.9 (C-2‴), 74.5 (C-2″), 71.4 (C-4‴), 62.5 (C-6‴), 61.6 (C-6″), 56.7 (3′-OCH_3).

Brassicin-4′-O-β-D-glucopyranoside (**8**): yellow amorphous powder (MeOH); $[\alpha]_D^{26.5}$ −69.7° (c 0.09, MeOH); negative HRFAB/MS m/z 639.1566 $[M - H]^-$ (calcd for $C_{28}H_{31}O_{17}$, 639.1561); IR (CaF_2, ν, cm^{-1}) 3327, 1647, 1595, 1502, 1356, 1069; ^{1}H NMR (400 MHz, C_5D_5N, δ_H) 8.52 (1H, br s, H-2′), 8.35 (1H, br d, J = 8.8 Hz, H-6′), 7.96 (1H, d, J = 8.8 Hz, H-5′), 7.42 (1H, d, J = 2.0 Hz, H-8), 7.12 (1H, d, J = 2.0 Hz, H-6), 6.10 (1H, d, J = 7.2 Hz, H-1″), 6.08 (1H, d, J = 7.2 Hz, H-1‴), 4.86−4.47 (12H, H-2″, -3″, -4″, -5″, -6″, -2‴, -3‴, -4‴, -5‴, -6‴), 4.17 (3H, s, 3′-OCH_3); ^{13}C NMR (100 MHz, C_5D_5N, δ_C) 177.8 (C-4), 163.7 (C-7), 162.1 (C-9), 156.6 (C-5), 150.1 (C-3′), 149.2 (C-4′), 147.1 (C-2), 135.3 (C-3), 125.8 (C-1′), 121.9 (C-6′), 115.5 (C-5′), 112.6 (C-2′), 106.0 (C-10), 101.8 (C-1″), 101.7 (C-1‴), 99.7 (C-6), 95.1 (C-8), 79.3, 79.1 (C-5″, -5‴), 78.6 (C-3″, -3‴), 74.8 (C-2″, -2‴), 71.2 (C-4″, -4‴), 62.3 (C-6″, -6‴), 56.0 (3′-OCH_3).

Quantitative Analysis of Flavonoids Using HPLC. Flavonoids were extracted from 500 mg of powder in 3.5 mL of 70% methanol. This process involved 2 min of vigorous vortexing, 30 min of sonication, and incubation for 3 h at room temperature. After the mixture had been centrifuged for 20 min at 180 rpm, 0.9 mL of supernatant was taken. After partitioning with 0.9 mL of *n*-hexane to remove lipids, the supernatant was taken filtered through a 0.45 μm syringe filter. High-performance liquid chromatography (HPLC) (Agilent 6410B, RRLC system, Agilent Technologies, Palo Alto, CA, USA) analysis was carried out on a C18 column (Agilent, Eclipse Plus C18, 1.8 μm, 100 × 2.1 mm) with gradient elution with solvents A (0.1% formic acid) and B (MeOH/acetonitrile = 1:1). The concentration of B was 5−30% at 0−30 min, 30−100% at 30−40 min, 100−100% at 40−54 min, and 100−2% at 54−55 min. The flow rate was 0.2 mL/min, the injection volume was 2 μL, and a photodiode array detector was used at 280 nm.

Free Radical Scavenging Activity. 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was determined by using the method developed by van den Berg et al. with slight modification.[23] The 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity was determined using the modified method of Brand-Williams et al.[24]

RESULTS AND DISCUSSION

Chemical Structure Elucidation. The MeOH extracts of C1/R-S rice were fractionated using EtOAc, *n*-BuOH, and H_2O. Because the TLC experiment confirmed the aqueous fraction was mainly composed of sugars such as glucose and sucrose, the organic phases were used for further isolation of active metabolites. From the EtOAc and *n*-BuOH fractions, four new flavonoids (**2**, **3**, **7**, and **8**) and four known flavonoids (**1**, **4**, **5**, and **6**) were isolated through repeated SiO_2, ODS, and Sephadex LH-20 column chromatographies (Figure 1). The known compounds, dihydroflavonols **1** and **6** and flavonols **4** and **5**, were identified as (+)-3′-*O*-methyltaxifolin, 3′-*O*-methyltaxifolin-5-*O*-β-D-glucopyranoside, brassicin, and isorhamnetin-4′-*O*-β-D-glucosyranoside, respectively, on the basis of the spectroscopic data, namely, NMR, MS, IR, and the specific rotation value and confirmed by comparison of the data with those reported in the literature.[17−22]

Compound **2** was obtained as a yellow amorphous powder and showed absorbance bands due to a hydroxyl group (3391 cm^{-1}) and an aromatic double bond (1645, 1455 cm^{-1}) in the IR spectrum. The molecular formula was determined to be $C_{22}H_{24}O_{12}$ from the pseudomolecular ion peak $[M - H]^-$ at

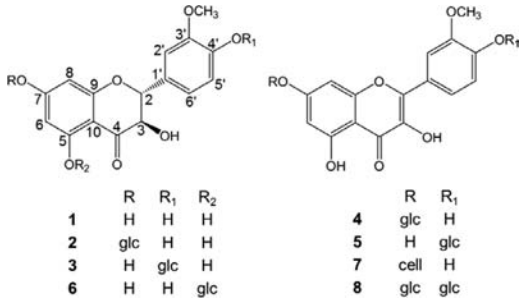

Figure 1. Structures of flavonoids. glc, β-D-glucopyranosyl; cell, β-D-cellobiosyl.

m/z 479.1189 in the high-resolution negative FABMS (calcd for $C_{22}H_{23}O_{12}$, 479.1190). In the ^{1}H NMR spectrum, three olefin methine proton signals observed at δ_H 7.08 (1H, br s), 6.93 (1H, br d, J = 8.0 Hz), and 6.80 (1H, d, J = 8.0 Hz) were of a typical 1,2,4-trisubstituted benzene ring, and two olefin methine proton signals observed at δ_H 6.17 (1H, d, J = 2.0 Hz) and 6.15 (1H, d, J = 2.0 Hz) were due to the meta coupling protons in the A ring. Two oxygenated methine proton signals at δ_H 4.96 and 4.58 (each 1H, d, J = 12.0 Hz) ascribable to H-2 and H-3 of a dihydroflavonol were also observed, and the coupling constant indicated that two protons were in a trans configuration. An anomer proton signal (δ_H 4.94, 1H, d, J = 7.6 Hz), four oxygenated methine proton signals (δ_H 3.44−3.28), one oxygenated methylene signal (δ_H 3.82, 1H, dd, J = 12.0, 1.6 Hz, H-6″a; 3.65, 1H, dd, J = 12.0, 5.2 Hz, H-6″b) due to a sugar moiety, and one methoxy proton signal (δ_H 3.84, 3H, s, 3′-OCH_3) were also observed. The proton NMR signals led to the conclusion that compound **2** was a 5,7,3′,4′-tetrahydroxy dihydroflavonol with one methoxy and one hexose. In the ^{13}C NMR spectrum, 22 carbon signals, including one methoxy (δ_C 56.4) and one hexose signal, were observed. The multiplicity of each carbon was determined using a distortionless enhancement by a polarization transfer (DEPT) experiment. One ketone carbon signal at δ_C 199.1 (C-4); five oxygenated olefin quaternary carbon signals at δ_C 166.9 (C-7), 164.4 (C-5), 163.9 (C-9), 148.6 (C-3′), and 148.1 (C-4′); two olefin quaternary carbon signals at δ_C 129.4 (C-1′) and 103.3 (C-10); five olefin methine carbon signals at δ_C 122.1 (C-6′), 115.8 (C-5′), 112.2 (C-2′), 98.2 (C-8), and 96.9 (C-6); and two oxygenated methine carbon signals at δ_C 85.2 (C-2) and 73.6 (C-3) were observed. The sugar signals were identified as β-D-glucopyranose from the chemical shifts of the carbon signals of the sugar moiety: a hemiacetal carbon signal at δ_C 101.1 (C-1″); four oxygenated methine carbon signals at δ_C 78.1 (C-3″), 77.6 (C-5″), 74.5 (C-2″), and 70.9 (C-4″); and an oxygenated methylene carbon signal at δ_C 62.1 (C-6″) were observed. The coupling constant of the anomer proton signal (7.6 Hz) confirmed the β linkage of the anomer hydroxyl group. The methoxy proton signal at δ_H 3.84 was correlated with the oxygenated olefin quaternary carbon signal at δ_C 148.6 (C-3′) in the HMBC spectrum, confirming the position of the methoxy group. The connection between the glucopyranosyl unit and the C-7 hydroxy of the aglycon was verified by the cross-peak observed between the anomer proton signal at δ_H 4.94 (H-1″) and the oxygenated olefin quaternary carbon signal at δ_C 166.9 (C-7) in the HMBC spectrum. The optical rotation was determined as $[\alpha]_D^{25}$ +10.5° (c 3.6, MeOH). Therefore, compound **2** was 3′-*O*-methyltaxifolin-7-*O*-β-D-glucopyranoside, a new dihydroflavonol glycoside.

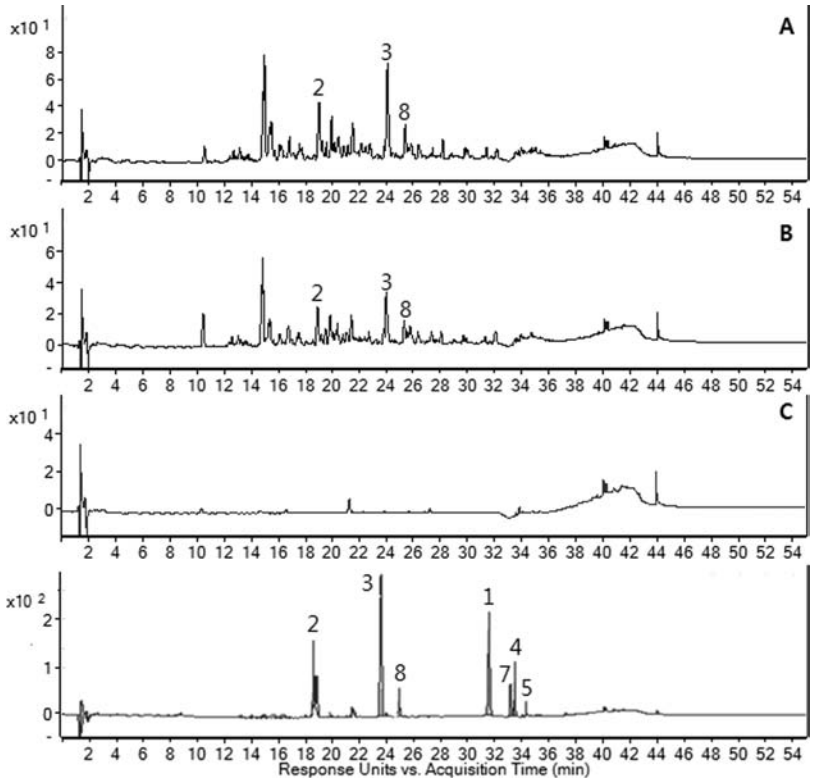

Figure 2. HPLC profiles of flavonoids extracted from (A) the transgenic rice cultivar of *Oryza sativa* spp. *japonica* cv. Hwa-Young, C1/R-S transgenic rice (C1/R-S rice), (B) a hybrid C1/R-S rice/HY, and (C) Hwa-Young (HY) and seven flavonoids (**1**−**5**, **7**, **8**). HPLC analysis was carried out on a C18 column (Agilent, Eclipse Plus C18, 1.8 μm, 100 × 2.1 mm) with gradient elution with solvent A (0.1% formic acid) and B (MeOH/acetonitrile = 1:1). The concentration of B was 5−30% at 0−30 min, 30−100% at 30−40 min, 100−100% at 40−54 min, and 100−2% at 54−55 min. The flow rate was 0.2 mL/min, the injection volume was 2 μL, and a photodiode array detector was used at 280 nm.

Compound **3** was obtained as a light yellow powder and showed absorbance bands due to a hydroxyl group (3390 cm^{-1}) and aromatic double bonds (1644, 1464 cm^{-1}) in the IR spectrum. The compound was very similar to compound **2** in the MS and NMR data, with the exception of the position of the sugar. The connection between the glucopyranosyl unit and the C-4′ hydroxy of the aglycon was verified by a cross-peak observed between the anomer proton signal at δ_H 5.71 (H-1″) and the oxygenated olefin quaternary carbon signal at δ_C 148.3 (C-4′) in the HMBC spectrum. The molecular formula was determined to be $C_{22}H_{24}O_{12}$ from the pseudomolecular ion peak $[M - H]^-$ at m/z 479.1189 in the high-resolution negative FAB/MS (calcd for $C_{22}H_{23}O_{12}$, 479.1190). Thus, compound **3** was determined to be 3′-*O*-methyltaxifolin-4′-*O*-β-D-glucopyranoside, a new dihydroflavonol glycoside.

Compound **7** was obtained as a yellow amorphous powder and showed absorbance bands due to hydroxyl (3334 cm^{-1}), conjugated ketone (1704 cm^{-1}), and aromatic double bond (1596 cm^{-1}) groups in the IR spectrum. The molecular formula was determined to be $C_{28}H_{32}O_{17}$ from the pseudomolecular ion paek $[M - H]^-$ at m/z 639.1566 in the high-resolution negative FAB/MS (calcd for $C_{28}H_{31}O_{17}$, 639.1561). In the ^{1}H NMR spectrum, three olefin methine proton signals at δ_H 7.81 (1H, br s, H-2′), 7.68 (1H, br d, J = 8.0 Hz, H-6′), and 6.88 (1H, d, J = 8.0 Hz, H-5′), due to a 1,2,4-trisubstituted benzene ring, and two olefin methine proton signals at δ_H 6.69 (1H, br s, H-6) and 6.41 (1H, br s, H-8), due to a 1,2,3,5-tetrasubstituted benzene ring, were observed. Additionally, one methoxy signal at δ_H 3.89 (3H, s) was also observed. These data suggested that compound **7** had a 5,7,3′,4′-tetrahydroxy-flavonol moiety.[25,26] In addition, two anomer proton signals appeared at δ_H 5.08 (1H, d, J = 7.6 Hz, H-1″) and 4.45 (1H, d, J = 7.6 Hz, H-1‴), and oxygenated methines and methylene signals of two hexose moieties appeared at δ_H 3.98−3.24 (12H). The ^{13}C NMR spectrum exhibited 28 carbon signals. For the aglycone carbon signals, one conjugated ketone signal at δ_C 177.5 (C-4); seven oxygenated olefin quaternary signals at δ_C 164.3 (C-5), 162.1 (C-7), 157.6 (C-9), 150.0 (C-4′), 148.5 (C-2), 148.4 (C-3′), and 136.0 (C-3); two olefin quaternary signals at δ_C 123.9 (C-1′) and 106.2 (C-10); five olefin methine signals at δ_C 122.9 (C-6′), 116.3 (C-5′), 112.6 (C-2′), 100.1 (C-6), and 95.8 (C-8); and one methoxy signal at δ_C 56.7 (3′-OCH_3) were observed. The methoxy proton signal at δ_H 3.89 was correlated with the oxygenated olefin quaternary carbon signal at δ_C 148.4 (C-3′) in the HMBC spectrum, confirming the position of the methoxy group. The presence of a β-cellobiosyl moiety was suggested by the chemical shifts of two hexose carbon signals such as two hemiacetals at δ_C 104.6 (C-1‴) and 101.3 (C-1″); eight oxygenated methines at δ_C 80.2 (C-4″), 78.1 (C-5‴), 77.9 (C-3‴), 76.8 (C-5″), 76.2 (C-3″), 74.9 (C-2‴), 74.5 (C-2″), and 71.4 (C-4‴); and two oxygenated methylene signals at δ_C 62.5 (C-6‴) and 61.6 (C-6″); as well as the coupling constant of two anomer proton signals (δ_H 5.08, 1H, d, J = 7.6 Hz, H-1″; 4.45, 1H, d, J = 7.6 Hz, H-1‴). The connection between the glucopyranosyl unit (C-1″) and the C-7 hydroxy of the aglycon was verified by the cross-peak observed between the anomer proton signal at δ_H 5.08 (H-1″) and the oxygenated olefin quaternary carbon signal at δ_C 162.1 (C-7) in the HMBC spectrum. Therefore, compound **7** was identified as isorhamnetin-7-*O*-β-D-cellobio-

Table 1. Quantitative Analysis of Major Flavonoids (2, 3, 8) in the Extracts of the Transgenic Rice Cultivar of *Oryza sativa* spp. *japonica* cv. Hwa-Young, C1/R-S Transgenic Rice (C1/R-S rice), Hwa-Young (HY), and Hybrid C1/R-S Rice/HY

				content (μg/g)		
peak	RT (min)	calibration curve[a]	R^2	C1/R-S rice	C1/R-S rice/HY	HY
2	18.5	$y = 4.95317x - 22.59272$	0.9999	483.8	297.4	nd[b]
3	23.4	$y = 13.17325x - 17.55221$	1.0000	289.8	144.7	nd[b]
8	24.6	$y = 1.87351x - 13.70389$	0.9999	654.8	372.9	nd[b]

[a] y = area units, x = concentration in standard solution (ppm). [b] nd = not detected

side (brassicin-4″-*O*-β-D-glucopyranoside), a new flavonol glycoside.[27]

Compound **8** was obtained as a yellow amorphous powder and showed absorbance bands due to hydroxyl (3327 cm^{-1}) and aromatic double bond (1595 cm^{-1}) groups in the IR spectrum. The molecular formula was determined to be $C_{28}H_{32}O_{17}$ from the pseudomolecular ion peak $[M - H]^-$ at m/z 639.1566 in the high-resolution negative FAB/MS (calcd for $C_{28}H_{31}O_{17}$, 639.1561). Compound **8** was very similar to compound **7** in the spectroscopic data of MS, ^{1}H NMR, and ^{13}C NMR, except for the position of two glucosyl moieties. Compound **8** showed two anomer proton signals at δ_H 6.10 (1H, d, J = 7.2 Hz, H-1″) and 6.08 (1H, d, J = 7.2 Hz, H-1‴) and two anomer carbon signals at δ_C 101.8 (C-1″) and 101.7 (C-1‴). Moreover, from the ^{13}C NMR chemical shifts and the coupling constant of two anomer proton signals as J = 7.2 Hz and J = 7.2 Hz, both are β-D-glucopyranose. The connection of the two glucopyranosyl units with C-7 and C-4′ hydroxy groups of the aglycone was verified by the cross-peaks observed between the anomer proton signal at δ_H 6.10 (H-1″) and the oxygenated olefin quaternary carbon signal at δ_C 163.7 (C-7), as well as between the anomer proton signal at δ_H 6.08 (H-1‴) and the oxygenated olefin quaternary carbon signal at δ_C 149.2 (C-4′) in the HMBC spectrum, respectively. Thus, compound **8** was elucidated as brassicin-4′-*O*-β-D-glucosyranoside, a new flavonol glycoside.

HPLC Analysis of Flavonoids in C1/R-S Rice, C1/R-S Rice/HY, and HY Extracts. The flavonoid content and composition in C1/R-S rice, C1/R-S rice/HY, and HY were determined using an HPLC experiment. Figure 2 shows the separation of seven flavonoid standards (**1**–**5**, **7**, and **8**) on the chromatogram. The major flavonoids (**2**, **3**, and **8**) were calibrated. Among C1/R-S rice, C1/R-S rice/HY, and HY, the most prominent peaks in the profiles of secondary metabolites were detected in C1/R-S rice, and all seven flavonoids were identified. Table 1 shows the quantification of major flavonoids (**2**, **3**, and **8**). C1/R-S rice had higher flavonoid contents than the hybrid C1/R-S rice/HY, but none of the flavonoids were detected in the normal cultivar HY. In particular, compound **8** was highest in C1/R-S rice (654.8 μg/g) compared to C1/R-S rice/HY (372.9 μg/g).

Radical-Scavenging Activities of Flavonoids. Vitamin C standard curves relating the concentration of vitamin C to the amount of absorbance reduction caused by vitamin C were obtained using the ABTS assay and the DPPH assay. The radical-scavenging capacities of rice grains are summarized in Table 2. The highest antioxidant capacity was shown by C1/R-S rice [for the ABTS assay, 59.2 ± 0.8 mg of vitamin C equiv/g of variety (mg VCE/g); and for the DPPH assay, 18.9 ± 2.1 mg VCE/g, respectively], whereas the HY × C1/R-S rice hybrid and the HY cultivar showed a lower capacity than C1/R-S rice [for the ABTS assay, 30.0 ± 1.3 mg (HY × C1/R-S rice hybrid) and 13.0 ± 0.3 mg (HY) VCE/g; for the DPPH assay, 10.5 ± 0.3 mg (HY × C1/R-S rice hybrid) and 5.9 ± 0.6 mg (HY) VCE/g, respectively].

Table 2. Radical-Scavenging Capacity of Alcohol Extracts from the Transgenic Rice Cultivar of *Oryza sativa* spp. *japonica* cv. Hwa-Young, C1/R-S Transgenic Rice (C1/R-S Rice), Hwa-Young (HY), and Hybrid C1/R-S Rice/HY and Flavonoids Isolated from C1/R-S Rice Grains

compd/variety	ABTS radical (mg VCE/g)[a]	DPPH radical (mg VCE/g)[a]
1	1263.6 ± 18.5	93.1 ± 2.1
2	833.3 ± 36.5	78.6 ± 1.6
3	598.8 ± 0.4	11.5 ± 1.1
4	1088.7 ± 25.1	297.4 ± 10.5
5	607.5 ± 28.4	16.5 ± 1.8
6	517.9 ± 9.9	56.4 ± 3.2
7	256.8 ± 1.8	75.0 ± 5.5
8	250.9 ± 15.9	49.5 ± 1.1
C1/R-S rice	59.2 ± 0.8	18.9 ± 2.1
HY	13.0 ± 0.3	5.9 ± 0.6
C1/R-S rice/HY	30.0 ± 1.3	10.5 ± 0.3

[a] Milligrams of vitamin C equivalents (VCE)/g of variety.

Furthermore, the radical-scavenging activities of all of the flavonoids (**1**–**8**) isolated from C1/R-S rice grains for the DPPH and ABTS radicals were determined (Table 2). In the ABTS free radical scavenging assay, compounds **1** and **4** showed slightly higher activity (**1**, 1263.6 ± 18.5 mg VCE/g; **4**, 1088.7 ± 25.1 mg VCE/g), and compound **2** showed slightly lower activity (833.3 ± 36.5 mg VCE/g) than vitamin C. Moreover, compounds **3**, **5**, and **6** also showed significant activity (**3**, 598.8 ± 0.4 mg VCE/g; **5**, 607.5 ± 28.4 mg VCE/g; **6**, 517.9 ± 9.9 mg VCE/g). The lowest activity was detected for compounds **7** and **8** (**7**, 256.8 ± 1.8 mg VCE/g; **8**, 250.9 ± 15.9 mg VCE/g). In the DPPH radical scavenging assay, compound **4** showed the highest activity (297.4 ± 10.5 mg VCE/g), and compound **1** had the next highest activity (93.1 ± 2.1 mg VCE/g). Compounds **2**, **6**, **7**, and **8** showed similar activities (**2**, 78.6 ± 1.6 mg VCE/g; **6**, 56.4 ± 3.2 mg VCE/g; **7**, 75.0 ± 5.5 mg VCE/g; **8**, 49.5 ± 1.1 mg VCE/g). The lowest activities were detected for compounds **3** and **5** (**3**, 11.5 ± 1.1 mg VCE/g; **5**, 16.5 ± 1.8 mg VCE/g).

These results showed distinct structure–activity relationships. By comparison of the scavenging potency of dihydroflavonols **1**, **2**, **3**, and **6** for the DPPH and ABTS radicals, aglycone compound **1** showed higher activity than its monoglucosides **2**, **3**, and **6**. Furthermore, the ABTS scavenging activity of flavonol monoglucosides **4** and **5** was 3–4 times higher than those of flavonol diglucosides **7** and **8**. Subsequently, the DPPH scavenging activityies of flavonol glucosides **4** and **7** were higher than those of **5** and **8** because the 4′-hydroxy groups of the latter two were blocked. Flavonol **4** showed higher activity than dihydroflavonol **2** because of the

double bond at C2 and C3. Therefore, it is clear that the double bond at C2 and C3 in the C ring, the free hydroxyl group at C-4′ of the B ring, and the number of attached sugars are key factors showing the potency of the radical-scavenging activity.

C1/R-S rice had very high flavonoid contents and radical-scavenging activities for DPPH and ABTS compared to the normal cultivar HY and hybrid C1/R-S rice/HY. Four new flavonoids, along with four known ones, were isolated from the grains of C1/R-S rice and extensively investigated for their chemical structure. Most flavonoids showed radical-scavenging activities that varied according to the structural variety. Therefore, this study not only revealed the effective constituents contained in C1/R-S rice but also indicated that the flavonoids obtained here may contribute to the development of antioxidative drugs.

AUTHOR INFORMATION

Corresponding Author

*(N.-I.B.) Phone: +82-31-201-2610. Fax: +82-31-204-8116. E-mail: nibaek@khu.ac.kr.

Funding

This study was supported by a grant from the Next-Generation Bio-Green 21 Program (No. PJ009574012013), Rural Development Administration, Republic of Korea.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Fatland, B. L.; Ke, J.; Anderson, M. D.; Mentzen, W. I.; Cui, L. W.; Allred, C. C.; Johnston, J. L.; Nikolau, B. J.; Wurtele, E. S. Molecular characterization of a heteromeric ATP-citrate lyase that generates cytosolic acetyl-coenzyme A in *Arabidopsis*. *Plant Physiol.* **2002**, *130*, 740−756.

(2) Beecher, G. R. Overview of dietary flavonoids: nomenclature, occurrence and intake. *J. Nutr.* **2003**, *133*, 32485−32545.

(3) Kaul, T. N.; Middleton, E., Jr.; Ogra, P. L. Antiviral effect of flavonoids on human viruses. *J. Med. Virol.* **1985**, *15*, 71−79.

(4) Koleckar, V.; Brojerova, E.; Rehakova, Z.; Kubikova, K.; Cervenka, F.; Kuca, K.; Jun, D.; Hronek, M.; Opletalova, V.; Opletal, L. In vitro antiplatelet activity of flavonoids from *Leuzea carthamoides*. *Drug Chem. Toxicol.* **2008**, *31*, 27−35.

(5) Harborne, J. B.; Williams, C. A. Advances in flavonoid research since 1992. *Phytochemistry* **2000**, *55*, 481−504.

(6) Choi, C. W.; Choi, Y. H.; Cha, M. R.; Kim, Y. S.; Yon, G. H.; Kim, Y. K.; Choi, S. U.; Kim, Y. H.; Ryu, S. Y. Antitumor components isolated from the heartwood extract of *Dalbergia odorifera*. *J. Korean Soc. Appl. Biol. Chem.* **2009**, *52*, 375−379.

(7) Ko, Y. J.; Oh, H. J.; Ahn, H. M.; Kang, H. J.; Kim, J. H.; Ko, Y. H. Flavonoids as potential inhibitors of retroviral enzymes. *J. Korean Soc. Appl. Biol. Chem.* **2009**, *52*, 321−326.

(8) Hollman, P. C. H.; Katan, M. B. Absorption, metabolism and health effects of dietary flavonoids in man. *Biomed. Pharmacother.* **1997**, *51*, 305−310.

(9) Murota, K.; Terao, J. Antioxidative flavonoid quercetin: implications of its intenstinal absorption and metabolism. *Arch. Biochem. Biophys.* **2003**, *417*, 12−17.

(10) Romanová, D.; Vachálková, A.; Cipák, L.; Ovesná, Z.; Rauko, P. Study of antioxidant effect of apigenin, luteolin and quercetin by DNA protective method. *Neoplasma* **2001**, *48*, 104−107.

(11) Yang, H. L.; Chen, S. C.; Senthil Kumar, K. J.; Yu, K. N.; Lee Chao, P. D.; Tsai, S. Y.; Hou, Y. C.; Hseu, Y. C. Antioxidant and anti-inflammatory potential of hesperetin metabolites obtained from hesperetin-administered rat serum: an ex vivo approach. *J. Agric. Food Chem.* **2012**, *60*, 522−532.

(12) Shin, Y. M.; Park, H. J.; Yim, S. D.; Baek, N. I.; Lee, C. H.; An, G.; Woo, Y. M. Transgenic rice lines expressing maize C1 and R-S regulatory genes produce various flavonoids in the endosperm. *Plant Biotechnol. J.* **2006**, *4*, 303−315.

(13) Yi, G.; Baek, N. I.; Park, D. S.; Park, S. K.; Manigbas, N. L.; Cho, J. G.; Ahn, J. G.; Woo, Y. M. Characterization of a hybrid population from a cross between a black waxy rice cultivar and transgenic rice with high levels of flavonoids in the endosperm. *Philipp. J. Crop Sci.* **2009**, *34*, 68−77.

(14) Cho, J. G.; Lee, D. Y.; Lee, J. Y.; Lee, D. G.; Lee, Y. H.; Kim, S. Y.; Kim, S. H.; Baek, N. I. Acyclic diterpenoids from the leaves of *Capsicum annuum*. *J. Korean Soc. Appl. Biol. Chem.* **2009**, *52*, 128−132.

(15) Wu, Q.; Cho, J. G.; Lee, D. S.; Lee, D. Y.; Song, N. Y.; Kim, Y. C.; Lee, K. T.; Chung, H. G.; Choi, M. S.; Jeong, T. S.; Ahn, E. M.; Kim, G. S.; Baek, N. I. Carbohydrate derivatives from the roots of *Brassica rapa* ssp. *campestris* and their effects on ROS production and glutamate-induced cell death in HT-22 cells. *Carbohydr. Res.* **2013**, *372*, 9−14.

(16) Kim, D. O.; Lee, K. W.; Lee, H. J.; Lee, C. Y. Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. *J. Agric. Food Chem.* **2002**, *50*, 3713−3717.

(17) Wang, X.; Zhou, H.; Zeng, S. Identification and assay of 3′-*O*-methyltaxifolin by UPLC−MS in rat plasma. *J. Chromatogr., B* **2012**, *911*, 34−42.

(18) Alberto Marco, J.; Sanz-Cervera, J. F.; Yuste, A.; Carmen Oriola, C. Sesquiterpene lactones and dihydroflavonols from *Andryala* and *Urospermum* species. *Phytochemistry* **1994**, *36*, 725−729.

(19) El-Ghazooly, M. G.; El-Lakany, A. M.; Abou-Shoer, M. I.; Aly, A. H. Chemical constituents of *Helichrysum conglobatum* growing in Egypt. *Nat. Prod. Sci.* **2003**, *9*, 213−219.

(20) Park, Y. K; Lee, C. Y. Identification of isorhamnetin 4′-glucoside in onion. *J. Agric. Food Chem.* **1996**, *911*, 34−42.

(21) Al-Oqail, M. M.; Al-Rehaily, A. J.; Hassan, W. H. B.; Ibrahim, T. A.; Ahmad, M. S.; Ebada, S. S.; Proksch, P. New flavonol glycosides from *Barbeya oleoides* Schweinfurth. *Food Chem.* **2012**, *132*, 2081−2088.

(22) Liu, X.; Ma, T.; Wang, M.; Chen, B.; Wang, H. The scavenging effect of phenolic compounds from *Celastrus angulatus* maximum on hydroxyl free radicals. *Tianran Chanwu Yanjiu Yu Kaifa* **1999**, *11*, 11−14.

(23) van den Berg, R.; Haenen, G. R. M. M.; van den Berg, H.; Bast, A. Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. *Food Chem.* **1999**, *66*, 511−517.

(24) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Lebensm.-Wiss. Technol.* **1995**, *28*, 25−30.

(25) Mabry, T. J.; Markham, K. R.; Thomas, M. B. *The Systematic Identification of Flavonoids*; Springer-Verlag: New York, 1970; pp 141−164.

(26) Markham, K. R.; Geiger, H. Proton nuclear magnetic resonance spectroscopy of flavonoids and their glycosides in hexadeuterodimethylsulfoxide. In *The Flavonoids, Advances in Research Since 1986*; Harborne, J. B., Ed.; Chapman & Hall: London, UK, 1994; pp 441−497.

(27) Veitch, N. C.; Grayer, R. J.; Irwin, J. L.; Takeda, K. Flavonoid cellobiosides from *Salvia uliginosa*. *Phytochemistry* **1998**, *48*, 389−393.