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Isolation of Luteolin and Luteolin-7-*O*-glucoside from *Dendranthema morifolium* Ramat Tzvel and Their Pharmacokinetics in Rats

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Supporting Information

ABSTRACT: Luteolin and luteolin-7-*O*-glucoside were isolated from the ethanolic extract of *Dendranthema morifolium* Ramat Tzvel. The structures of these analytes were identified by nuclear magnetic resonance (^{1}H and ^{13}C NMR) and mass spectrometry. Ethanolic and water extracts contained luteolin-7-*O*-glucoside at 4.19 and 6.56%, respectively. However, the level of luteolin was only 0.19% in the ethanolic extract, and luteolin was not detected in the water extract. To examine the pharmacokinetics and bioavailability of luteolin and luteolin-7-*O*-glucoside in rats, parallel studies of luteolin (10 mg/kg, iv; and 100 mg/kg, po) and luteolin-7-*O*-glucoside (10 mg/kg, iv; and 1 g/kg, po) were conducted. The analytes were detected by high-performance liquid chromatography coupled with a photodiode array detector. A phenyl-hexyl (150 × 4.6 mm iv; 5.0 μm) column was used to separate the analytes from the biological samples. The pharmacokinetic data demonstrate that the areas under the concentration curves (AUCs) of luteolin were 261 ± 33 and 611 ± 89 (min μg/mL) after luteolin administration (10 mg/kg, iv; and 100 mg/kg, po, respectively). The oral bioavailability of luteolin was 26 ± 6%. The AUCs of luteolin-7-*O*-glucoside were 229 ± 15 and 2109 ± 350 (min μg/mL) after administration of luteolin-7-*O*-glucoside (10 mg/kg, iv; and 1 g/kg, po, respectively). The oral bioavailability of luteolin-7-*O*-glucoside was approximately 10 ± 2%. In the group that received luteolin-7-*O*-glucoside orally, a biotransformed luteolin product was detected, but this product was not detected in the group that received luteolin-7-*O*-glucoside intravenously. The biotransformation ratio of luteolin to luteolin-7-*O*-glucoside (the AUC ratio of metabolite/parent compound) was approximately 48.78 ± 0.12%. These results demonstrate that luteolin-7-*O*-glucoside is primarily hydrolyzed to luteolin in the gastrointestinal tract and then absorbed into the systemic circulation.

KEYWORDS: *bioavailability, herbal medicine, flavonoid, pharmacokinetics, traditional Chinese medicine*

INTRODUCTION

Flos Chrysanthemi, the flower of *Dendranthema morifolium* Ramat Tzvel (*Chrysanthemum morifolium* Ramat.), is known as Ju-Hua in Chinese. This herb is listed in Shen Nong's Herbal (a historical book of Chinese herbs) as a nontoxic, top-grade herb that has been used as an agent for the treatment of headache, vertigo, and sore throat. *Flos Chrysanthemi* is an edible medicinal herb. In addition to its medicinal uses, it is commonly used to make chrysanthemum tea, a popular beverage in Taiwan that quenches fevers and is nourishing to the eyes. *Flos Chrysanthemi* is one of the most popular medicinal plants, and it is widely used as a food supplement, or health food, by many consumers. The *D. morifolium* extract has been shown to contain a wide variety of flavonoids[1−3] and chlorogenic acids.[3] In our preliminary chemical analysis, we found that the flower of *D. morifolium* is a rich source of luteolin and luteolin-7-*O*-glucoside.

The biological activity of luteolin is thought to be associated in part with its antioxidant potential,[4,5] antitumorigenic effects,[6,7] and anti-inflammatory/antiallergic activities.[8] It has been shown to be an inhibitor of protein kinase C[9] and lipoxygenase.[8] It has also been shown to reduce high blood cholesterol through the inhibition of cholesterol uptake in Caco-2 cells. Therefore, luteolin has clear effects on in vivo cholesterol transport.[10] Luteolin also has a radioprotective effect[4] and a protective effect on doxorubicin-induced cardiotoxicity in mice.[11]

The flavonoid aglycones, such as luteolin, were thought to be rapidly absorbed after oral ingestion. However, the blood concentration of luteolin is relatively lower than that of its phase II metabolites, which may due to the extensive first-pass metabolism of flavonoids and glucuronidation mediated by various UDP-glucuronosyltransferases (UGTs) in the liver and intestine. Therefore, the phase II metabolites of glucuronides, sulfates, and the methylated conjugates were found largely in the blood circulation. Luteolin possesses four hydroxyl groups on the flavone groups, which are available for glucuronidation, and the glucuronide metabolites may reduce the antioxidant activity of aglycones.[12] The conjugates of flavonoids excreted

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into bile can be hydrolyzed to aglycones by intestinal bacteria, which have β-glucuronidase and arylsulfatase. The phase II conjugation of luteolin has been proven by the treatment of deconjugation with hydrochloric acid or β-glucuronidase/sulfatase. The concentrations of luteolin of samples hydrolyzed by hydrochloric acid and a mixture of β-glucuronidase and sulfatase were much higher than those in untreated samples.[13] The results suggested that sulfuric acid/glucuronic acid conjugates of luteolin are the major metabolites.[14] In addition, *O*-methylation is one of the metabolic pathways for the production of luteolin via catechol-*O*-methyltransferase (COMT) to produce chrysoeriol (3-*O*-methylation of luteolin) and diosmetin (4-*O*-methylation of luteolin).[15,16]

A previous report indicated that apigenin-7-*O*-glucoside can be enzymatically hydrolyzed to produce apigenin through β-glucosidase.[17,18] However, the metabolic mechanism of luteolin and luteolin-7-*O*-glucoside production is not entirely understood. In the present study, we first uncover that the flower of *D. morifolium* is a rich source of luteolin and luteolin-7-*O*-glucoside. To identify these two compounds from ethanolic and water extracts, the techniques of nuclear magnetic resonance (NMR), ^{1}H NMR and ^{13}C NMR, and mass spectrometry were used. Our hypothesis is that the luteolin-7-*O*-glucoside can be enzymatically hydrolyzed to produce luteolin by intestinal β-glucosidase, and then this is absorbed into the blood for circulation. To examine the pharmacokinetic mechanisms of luteolin and luteolin-7-*O*-glucoside, a parallel set of experiments was designed to study the effects of oral and intravenous administration of luteolin (10 mg/kg, iv; 100 mg/kg, po) and luteolin-7-*O*-glucoside (10 mg/kg, iv; 1 g/kg, po).

MATERIALS AND METHODS

Chemicals and Reagents. Luteolin and luteolin-7-*O*-glucoside were purified and isolated from the ethanolic extract of *D. morifolium* in our laboratory. HPLC grade methanol, acetonitrile, orthophosphoric acid (85%), and dimethyl sulfoxide were purchased from E. Merck (Darmstadt, Germany). Apigenin, apigenin-7-*O*-glucoside, and heparin were provided by Sigma-Aldrich Chemicals (St. Louis, MO, USA) and stored at room temperature. Triple-deionized water from Millipore (Bedford, MA, USA) was prepared for all aqueous solutions.

Isolation of Luteolin and Luteolin-7-*O*-glucoside. The flowers of *D. morifolium* were purchased from a drug store in Taipei, Taiwan, and identified by Dr. Lie-Chwen Lin, National Research Institute of Chinese Medicine, Taiwan. A voucher specimen (NHP-00861) has been deposited in the herbarium of the National Research Institute of Chinese Medicine. The dried flowers of *D. morifolium* (5.8 kg) were extracted with ethanol (40 L × 3), and the combined extract was evaporated under a vacuum for the EtOH extract. The ethanolic extract was partitioned successively between H_2O and EtOAc, followed by *n*-BuOH (each 1.5 L × 3). The resulting EtOAc fraction was separated on a Sephadex LH-20 column using MeOH for elution to produce 11 fractions, EA1−EA11. Fraction EA9 (2.4 g) was further purified on a Sephadex LH-20 column using acetone to give luteolin. The *n*-BuOH extract was suspended in $CHCl_3$. Throughout the filtration steps, the precipitates from the suspension were collected. The collected precipitates were dissolved in MeOH and subjected to Sephadex LH-20 with eluent of MeOH to obtain luteolin-7-*O*-glucoside. These isolated compounds were identified by spectral analysis and compared with the published literature.[19]

Spectroscopic Analysis. ^{1}H and ^{13}C NMR spectra were measured with a Varian Unity Inova-500 spectrometer with deuterated solvents (methanol-d_4, DMSO-d_6; Cambridge Isotope Laboratories, Tewksbury, MA, USA) as internal standards. The electrospray ionization technique used in mass spectrometry (ESIMS) was recorded on a Shimadzu LCMS-8030 mass spectrometer (Shimadzu, Kyoto, Japan).

Luteolin: light yellow powder; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.19 (1H, d, *J* = 2.0 Hz, H-6), 6.42 (1H, d, *J* = 2.0 Hz, H-8), 6.52 (1H, s, H-3), 6.88 (1H, d, *J* = 8.5 Hz, H-5′), 7.36 (2H, m, H-2′, H-6′); ^{13}C NMR (CD_3OD, 125 MHz) δ 95.0 (C-8), 100.1 (C-6), 103.9 (C-3), 105.3 (C-10), 114.1 (C-2′), 116.8 (C-5′), 120.3 (C-6′), 123.7 (C-1′), 147.0 (C-3′), 151.0 (C-4′), 159.4 (C-9), 163.2 (C-5), 166.0 (C-2), 166.4 (C-7), 183.9 (C-4); ESIMS *m*/*z* 287 $[M + H]^+$.

Luteolin-7-O-glucoside: yellow powder; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 5.05 (1H, d, *J* = 7.5 Hz, H-1″), 6.43 (1H, d, *J* = 2.0 Hz, H-6), 6.73 (1H, s, H-3), 6.78 (1H, d, *J* = 2.0 Hz, H-8), 6.89 (1H, d, *J* = 8.5 Hz, H-5′), 7.40 (1H, d, *J* = 2.0 Hz, H-2′), 7.43 (1H, dd, *J* = 8.5, 2.0 Hz, H-6′); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 60.8 (C-6″), 69.7 (C-4″), 73.2 (C-2″), 76.5 (C-3″), 77.3 (C-5″), 94.9 (C-8), 99.7 (C-6), 100.1 (C-1″), 103.3 (C-3), 105.5 (C-10), 113.6 (C-2′), 116.1 (C-5′), 119.4 (C-6′), 121.5 (C-1′), 145.9 (C-3′), 150.1 (C-4′), 157.1 (C-9), 161.2 (C-5), 163.1 (C-7), 164.6 (C-2), 182.1 (C-4); ESIMS *m*/*z* 449 $[M + H]^+$.

HPLC-MS/MS Parameters. The HPLC-MS/MS system consisted of an LCMS-8030 triple-quadrupole mass spectrometer (Shimadzu) equipped with an electrospray ionization interface and coupled to a Shimadzu LC-20AD HPLC system. The HPLC system was equipped with two pumps, a system controller, an autosampler, a column oven, and an online degasser. Chromatographic separation of analytes was carried out using an Acquity BEH C_{18} column (100 × 2.1 mm, particle size = 1.7 mm; Waters, Ireland). The column temperature was maintained at 35 °C.

The mobile phase (A, 0.01% formic acid in deionized water; B, 0.01% formic acid in 100% methanol) was eluted at a flow rate of 0.2 mL/min. The gradient started at 50% of mobile phase B and was linearly run to 90% B over 3 min. Subsequently, the eluent composition was maintained for 3 min before it was decreased to 50% of mobile phase B. Then, the gradient was returned to the initial condition (50% B) in 1 min and equilibrated for 1 min. The total run time was 8 min, and the injection volume was 5 μL. The electrospray ion source was operated in positive ionization mode for the two compounds. The other parameters were as follows: DL temperature, 250 °C; heat block temperature, 400 °C; nebulizing gas flow, 3.0 L/min; drying gas flow, 15 L/min; CID gas, 230 kPa.

Animal Experiments. Adult male Sprague−Dawley rats were purchased from the laboratory Animal Center of National Yang-Ming University (Taipei, Taiwan). All animal experiments followed National Yang-Ming University guidelines and procedures. The protocol for this animal study has been approved by the Institutional Animal Care and Use Committee (IACUC, approval no. 990511) by the Institutional Animal Experimentation Committee of National Yang-Ming University.

Before surgery, the rats were allowed free access to food and water at all times. A conscious, freely moving rat model was used to minimize the stress caused by restraint or anesthesia and to simulate the pharmacokinetics in conscious states. During surgery, the rats were anesthetized with pentobarbital (50 mg/kg, ip); for the rats in the intravenous group, a polyethylene tube was implanted in the right jugular vein for blood sampling and in the left femoral vein for drug administration. The tubes crossed the subcutaneous tissue and were fixed at the dorsal neck region. The polyethylene tubes were filled with heparinized saline (20 units/mL) to prevent coagulation. After surgery, the rats were placed in experimental cages for 1 day until recovery from anesthesia. Food was not given for 12 h, and water was available at any time.

HPLC−Photodiode Array System and Sample Preparation. The HPLC system consisted of chromatographic pumps (LC-20AT; Shimadzu), an autosampler (SIL-20AC; Shimadzu), and a photodiode array detector (SPD-M20A; Shimadzu). All analytical samples were separated using a phenyl-hexyl column (150 mm × 4.6 mm i.d.; particle size = 5 μm, Phenomenex Luna, Torrance, CA, USA). The mobile phase of HPLC analysis consisted of two solvent compositions: 0.05 M NaH_2PO_4 and acetonitrile of 80:20, v/v, and 70:30, v/v, for luteolin and luteolin-7-*O*-glucoside, respectively. The pH of 0.05 M NaH_2PO_4 was adjusted to 2.6 by using orthophosphoric acid (85%). The flow rate for the mobile phase was set at 1.0 mL/min. The

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J. Agric. Food Chem. XXXX, XXX, XXX−XXX

temperature in the autosampler was set at 8 °C, the analytical volume was 20 μL of each sample, and the photodiode array detector was monitored at 350 nm.

The resulting plasma sample (100 μL) was mixed with 200 μL of internal standard solution (0.2 μg/mL dissolved in acetonitrile) for protein precipitation. The mixture was centrifuged at 13000*g* for 15 min at 4 °C. The supernatant was evaporated to dryness at 40 °C using a centrifugal evaporator. The dried residue was reconstituted in 100 μL of 20% acetonitrile and filtered by a 0.22 μm filter. An aliquot (20 μL) of the filtrate was applied to the HPLC system for analysis.

Method Validation of Linearity, Accuracy, and Precision. The process of method validation serves to demonstrate that the specific method used for quantification of an analyte in a particular biological sample is reliable and reproducible. Calibration standards were prepared from stock solutions of luteolin or luteolin-7-*O*-glucoside and then added into blank plasma to construct the spiked samples with concentrations of 1, 5, 10, 25, 50, 75, and 100 μg/mL. The standard stock solution of luteolin or luteolin-7-*O*-glucoside (1 mg/mL) was prepared in methanol, and working standard solutions were diluted using 50% (v/v) methanol. These spiked samples were processed as described under Materials and Methods, HPLC−Photodiode Array System and Sample Preparation.

The calibration curve was constructed from the peak area ratio of luteolin and luteolin-7-*O*-glucoside and their corrected internal standards of apigenin and apigenin-7-*O*-glucoside. The coefficient correlation values (r^2) of all calibration curves were required to be at least 0.995.

Six replications of the calibration curve were measured on the same day (intraday) for six continuous days (interday) to evaluate the precision and accuracy of this analytical method. The measure of accuracy describes the proximity of mean results (observed concentration, C_{obs}) from this analytical method to the true concentrations (nominal concentration, C_{nom}). The accuracy could be presented as bias, calculated as follows: bias (%) = $[(C_{obs} - C_{nom})/C_{nom}] \times 100$. The measure of precision represents the closeness of each individual analytical result. The precision, which could be quantified as relative standard deviation (RSD), was calculated as follows: RSD (%) = [standard deviation (SD)/C_{obs}] × 100.

The mean values for the accuracy and the precision must be within 15% of the actual value, except at the LLOQ, where it must not exceed the value by >20%. The method validation for this study was performed according to the guidance given by the U.S. Food and Drug Administration.[20]

Drug Administration. *Oral Administration of Luteolin or Luteolin-7-O-glucoside.* Pentobarbital sodium solution (50 mg/kg) was used to anesthetize rats via intraperitoneal administration. Surgical sites were shaved and cleaned with 70% ethanol solution. Then, the right jugular vein was catheterized with polyethylene tubing for blood sampling. The catheter was guided under the skin to the dorsal neck region and fixed.[21] After surgery, the rats were allowed to recover in a clean cage for at least 24 h before drug administration.

After the stabilization period, luteolin and luteolin-7-*O*-glucoside dissolved in dimethyl sulfoxide/water (1:1) were orally administered to the rats ($n = 6$) at doses of 100 mg/kg and 1 g/kg, respectively. A 250 μL blood sample was collected from the jugular vein at 0, 5, 15, 30, 60, 90, 120, 180, 240, and 360 min after drug administration. Blood samples were centrifuged at 8000*g* for 10 min at 4 °C to obtain plasma, and the resulting plasma was stored at −20 °C prior to analysis.

Pharmacokinetic Analysis and Statistics. Pharmacokinetic parameters, including maximum plasma concentration (C_{max}), time to reach the maximum concentrations (t_{max}), half-life ($t_{1/2}$), area under concentration−time curve (AUC), and oral clearance (CL), were estimated by a noncompartmental analysis using the pharmacokinetic program WinNonlin Standard Edition version 1.1 (Scientific Consulting, Apex, NC, USA). The bioavailability was calculated according to the following equation: bioavailability (%*F*) = (AUC_{po}/$dose_{po}$)/(AUC_{iv}/$dose_{iv}$) × 100%. All results were expressed as the average mean ± standard deviation (SD). The statistical analyses were calculated using SPSS version 10.0 (SPSS, Chicago, IL, USA). Student's *t* test was used to compare the differences between groups, and a value of $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Isolation and Identification of Luteolin and Luteolin-7-*O*-glucoside. The luteolin-7-*O*-glucoside content was 4.19 and 6.56% in ethanolic and water extracts, respectively. However, the luteolin content was 0.19% in the ethanolic extract, and luteolin was not detected in the water extract. Luteolin was obtained as a light yellow powder. The ^{1}H spectrum of luteolin revealed the presence of a pair of meta-coupled aromatic protons (δ_H 6.19/6.42), an aromatic singlet (δ_H 6.52), and ABC-coupled aromatic protons (δ_H 6.88/7.35/7.36). In combination with the ^{13}C NMR data, which showed signals for a carbonyl group at δ_C 183.9 (C-4), six oxygenated quaternary carbons (δ_C 147.0, 151.0, 159.4, 163.2, 166.0, and 166.4), two sp^2 quaternary carbons (δ_C 105.3 and 123.7), and six sp^2 tertiary carbons (δ_C 95.0, 100.1, 103.9, 114.1, 116.8, and 120.3), and the ESIMS data, we were able to identify the molecules of luteolin[19] (Supporting Information, Figures S1 and S2).

When the ^{1}H spectrum of luteolin-7-*O*-glucoside was compared with that of luteolin, a difference was observed in the aliphatic region where there was an additional glucosyl moiety (δ 5.05/3.17−3.70) in luteolin-7-*O*-glucoside. This region was also associated with the ^{13}C NMR signals of luteolin-7-*O*-glucoside at δ_C 60.8, 69.7, 73.2, 76.5, 77.3, and 100.1 (Supporting Information, Figures S3 and S4). The ESIMS revealed the molecular ion at m/z 447 $[M - H]^-$, which is consistent with the molecular formula $C_{21}H_{20}O_{11}$. Upon further comparison with our previous study,[19] we were able to confirm the chemical structures of luteolin-7-*O*-glucoside and luteolin (Figure 1 and Figure S5).

Optimization of HPLC−Photodiode Array Conditions. An internal standard is a compound that can be used to correct for aliquot taking, instrumental performance, and, in the present study, should be similar in chemical structure to luteolin and luteolin-7-*O*-glucoside. On the basis of U.S. FDA guidelines,[20] an internal standard was used to facilitate quantification of the target analyte(s) during the development of the analysis method. To select a suitable internal standard, we surveyed PubMed and searched the available chemicals in our laboratory, which included apigenin and apigenin-7-*O*-glucoside, baicalein and baicalin, naringenin and naringin, quercetin and rutin, genistein and genistein, daidzein and daidzein, and the flavonoids and their correlated glycosides. Among the compounds tested, apigenin and apigenin-7-*O*-glucoside were found to be the most appropriate internal standards for correlated luteolin and luteolin-7-*O*-glucoside. Apigenin and apigenin-7-*O*-glucoside exhibited an appropriate retention time of within 15 min, a maximum absorbance at the UV wavelength of 350 nm, and a well-resolved peak shape, and they were previously reported to be highly stable.[22]

To optimize efficiency and resolution for liquid chromatography of the compound tested, the stationary phase of reversed C8, C18, and phenyl-hexyl column was examined. Successful separation and quantification of luteolin and luteolin-7-*O*-glucoside from blood, without other endogenous interfering peaks, was achieved using a phenyl-hexyl column in an optimal mobile phase containing 0.05 M NaH_2PO_4 and acetonitrile of 80:20, v/v, and 70:30, v/v, for luteolin and luteolin-7-*O*-glucoside, respectively. The pH value of the mobile phase plays an important role. After replication of the tests, it was found

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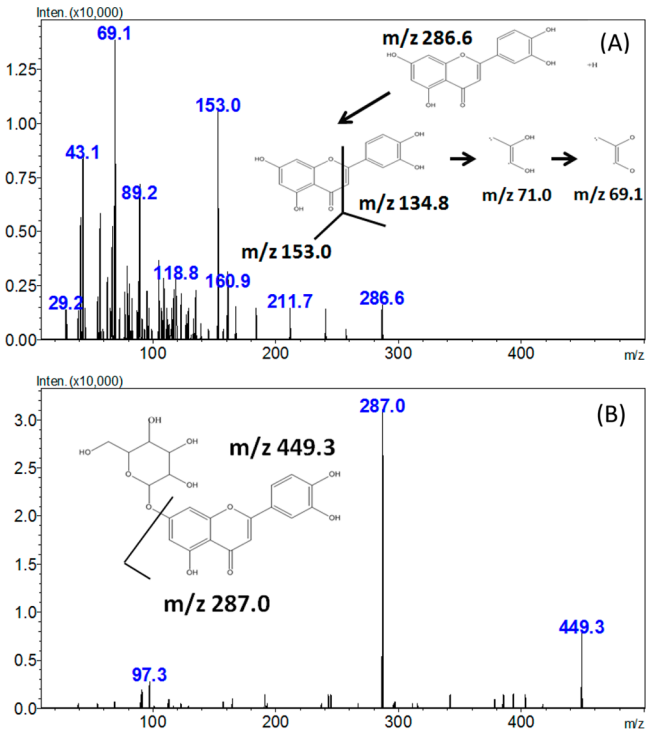

Figure 1. Chemical structures of (A) luteolin and (B) luteolin-7-*O*-glucoside and mass spectra of the analytes and their product ions in UPLC-MS/MS with electrospray in positive ion mode. The mass transitions of luteolin and luteolin-7-*O*-glucoside were *m*/*z* 286.6 → 69.1 and 449.3 → 287.0, respectively.

that the optimal pH value of the phosphate buffers (pH 2.6) could reduce the peak tailing of luteolin, luteolin-7-*O*-glucoside, and the internal standards without significant endogenous interference peaks. These results show that the analytical method used here is valid and reproducible. Our results are consistent with a previous study that the peak tailing of jaceosidin was severe in nonpolar columns.[23]

The analytical method established in this study was validated according to U.S. Food and Drug Administration guidelines for validation of bioanalytical methods.[20] Typical chromatograms of luteolin-7-*O*-glucoside and apigenin-7-*O*-glucoside (internal standard) in rat plasma after luteolin-7-*O*-glucoside administration (1 g/kg, po) are shown in Figure 2.

Validation of HPLC Assay. *Linearity, Accuracy, and Precision.* To evaluate linearity, the concentration of the calibration curve was derived from the peak area ratio of luteolin and luteolin-7-*O*-glucoside and their correlated internal standards, apigenin and apigenin-7-*O*-glucoside. The experimental results showed that the linearity range of luteolin and luteolin-7-*O*-glucoside in rat plasma was 0.125−2 μg/mL, and all of the coefficient correlation values (r^2) were >0.995.

The intraday and interday assays were carried out to evaluate the accuracy (as percent bias) and precision (as percent RSD) of luteolin and luteolin-7-*O*-glucoside (Supporting Information, Tables S1 and S2, $n = 6$). All of the bias and RSD values were <20%, which is the maximum acceptable value. These results revealed that this analytical method is reproducible.[20]

Pharmacokinetics of Luteolin. To describe the preclinical pharmacokinetics of luteolin, a single intravenous bolus of luteolin was administered via the femoral vein at a dose of 10 mg/kg, followed by a series of blood samplings at the designated time points of 5, 15, 30, 60, 90, 120, 180, 240, and 360 min after drug administration. The pharmacokinetic curves representing the changes in luteolin concentrations in the plasma across time are shown in Figure 3. The results suggested that the plasma concentration of luteolin was rapidly distributed and slowly decayed after luteolin administration in rats. The maximum concentration (C_{max}) of luteolin was 7.47 ± 3.78 μg/mL, and this concentration was observed at the first

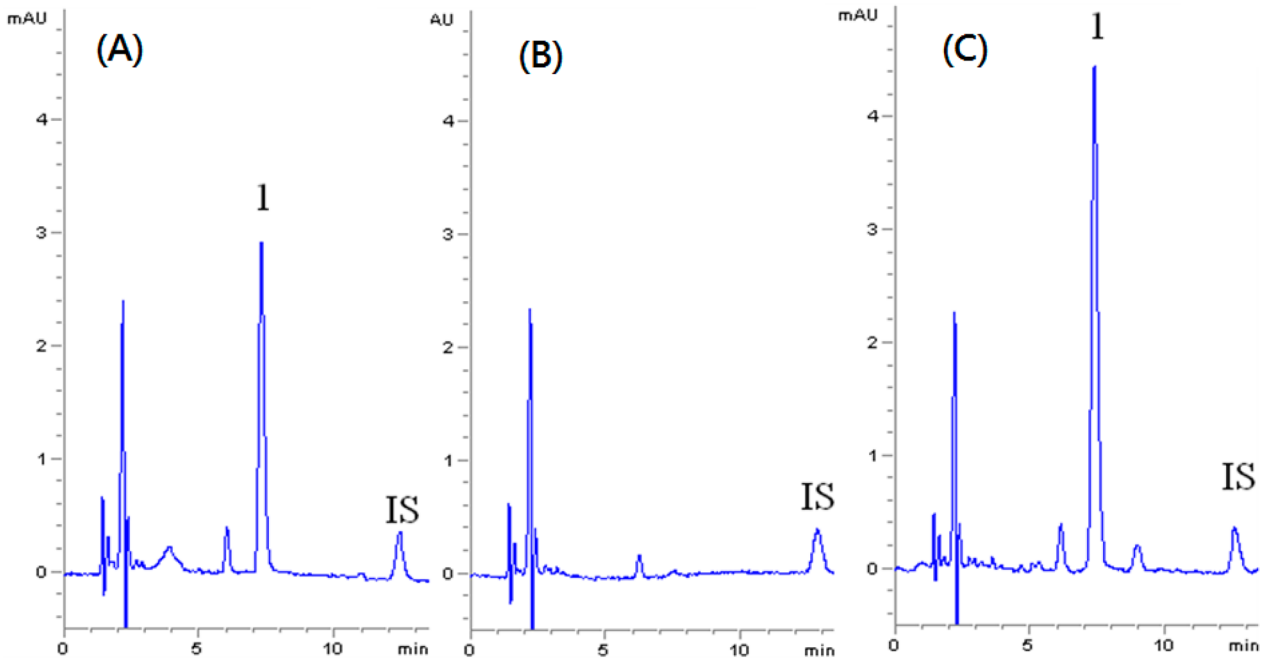

Figure 2. HPLC chromatograms of luteolin-7-*O*-glucoside in rat plasma for po administration of luteolin-7-*O*-glucoside: (A) standard luteolin-7-*O*-glucoside (2.5 μg/mL) and apigenin-*O*-glucoside (0.2 μg/mL); (B) blank plasma; (C) luteolin-7-*O*-glucoside (4.67 μg/mL) after po administration at 60 min. Peaks: 1, luteolin-7-*O*-glucoside; IS, internal standard (apigenin-7-*O*-glucoside).

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J. Agric. Food Chem. XXXX, XXX, XXX−XXX

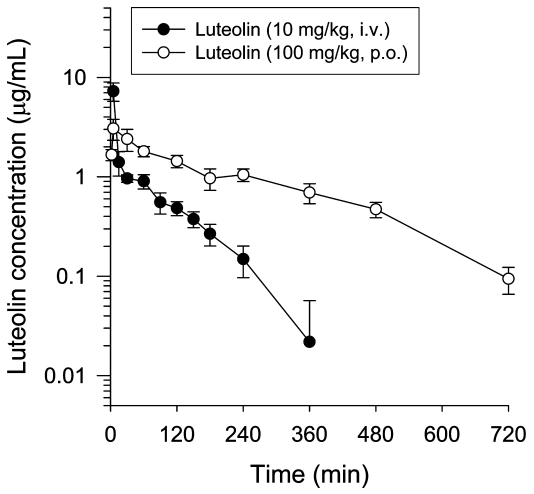

Figure 3. Concentration versus time profiles of luteolin in rat plasma after luteolin administration (10 mg/kg, iv; 100 mg/kg, po). Data are presented as the mean ± standard error, $n = 6$, for each group.

collected time point of 5 min after luteolin administration (10 mg/kg, iv). After this time point, the concentration of luteolin continually decreased up until the time point of 360 min after luteolin administration, at which time the concentration was undetectable.

When luteolin was given orally, the C_{max} of luteolin was 3.07 ± 0.72 μg/mL, and this concentration was observed at 5 min after luteolin administration (100 mg/kg, po). However, the luteolin concentration slowly decreased after oral administration, and the concentration of luteolin could still be detected up to 12 h after oral administration. The elimination half-lives of luteolin were 78 ± 14 and 132 ± 12 min for intravenous and oral administrations, respectively. The oral bioavailability of luteolin was 26 ± 6% on the basis of the doses used and the experimental animal model described above.

The pharmacokinetic profiles of the concentrations of luteolin in rat plasma versus time after oral administration of luteolin (10 mg/kg, iv; 100 mg/kg, po) are shown in Figure 3. There are multiple peaks at a t_{max} of 0.5 and at 4 h after oral administration of luteolin (100 mg/kg, po). This observance of multiple peaks suggested that enterohepatic circulation is a general phenomenon for the flavonoids,[24] such as luteolin and apigenin,[13] naringenin,[24] baicalein,[25] quercetin,[26] genistein,[27] and daidzein.[28] The concentration plateau appears at 1 and 4 h after luteolin intravenous and oral administration, respectively (Figure 3). This phenomenon reflects that the elimination half-time of luteolin by oral administration, 132 ± 12 h (Table 1), is relatively longer than it is by the route of intravenous

Table 1. Pharmacokinetic Parameters of Luteolin (10 mg/kg, iv; and 100 mg/kg, po)[a]

parameter	luteolin (10 mg/kg, iv)	luteolin (100 mg/kg, po)
C_{max} (μg/mL)	7.47 ± 3.78	3.07 ± 0.72
t_{max} (min)		290 ± 94
AUC (μg min/mL)	261 ± 33	611 ± 89
$t_{1/2}$ (min)	78 ± 14	132 ± 12
MRT (min)	66 ± 8	209 ± 12
CL (mL/min/kg)	41.3 ± 4.87	181 ± 25.5
oral bioavailability (%)		26 ± 6

[a]Data are presented as the mean ± SE ($n = 6$).

administration, 78 ± 14 h. Crespy et al. reported that the liver is the active site for the methylation of luteolin for additional biliary excretion and that a transporter system may be involved.[29] Then, the phase II methylated luteolins, chrysoeriol and diosmetin, have been identified, which was proved by the treatment of catechol-*O*-methyltransferase inhibitor, entacapone, in rat.[30,31] In the present study, we did not measure the precise nature of the metabolites from the hepatic conjugation. Consistently, we found that the multiple peaks may support the fate of enterohepatic circulation of luteolin.

With regard to the phase II metabolic mechanism of luteolin, we did not find the chromatographic peaks of metabolites in our analytical system. Luteolin can be found after luteolin-7-*O*-glucoside administration, but luteolin-7-*O*-glucoside was undetectable after luteolin administration (Figures 3 and 4).

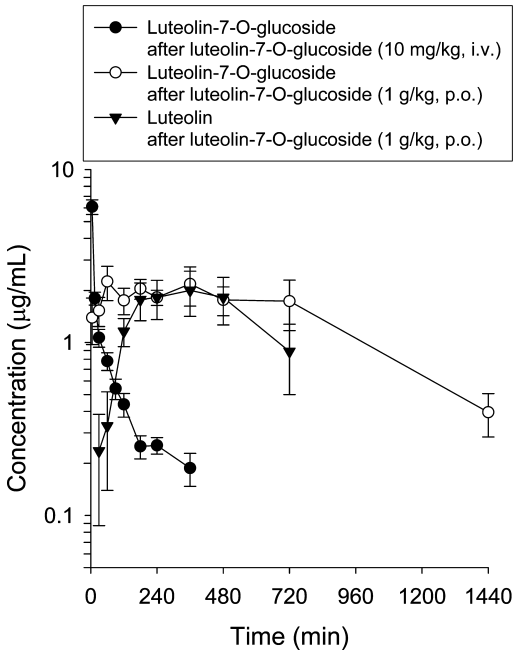

Figure 4. Concentration versus time profiles of luteolin-7-*O*-glucoside and luteolin in rat plasma after luteolin-7-*O*-glucoside administration (10 mg/kg, iv; 1 g/kg, po). Data are presented as the mean ± standard error, $n = 6$.

However, it has been shown that luteolin mainly undergoes conjugation by uridine diphosphoglucuronosyltransferases (UGTs), sulfotransferases (SULTs),[32] and COMT in vivo.[15,16] Because intestinal and hepatic first-pass effects are widely acknowledged to be responsible for low oral bioavailabilities of flavonoids, the oral bioavailabilities of luteolin and luteolin-7-*O*-glucoside were measured and found to be 26 ± 6 and 10 ± 2%, respectively (Tables 1 and 2).

Pharmacokinetics of Luteolin-7-O-glucoside. Luteolin-7-*O*-glucoside was hydrolyzed to luteolin in the gastrointestinal tract and absorbed into the systemic circulation (Figure 4). The AUCs of luteolin-7-*O*-glucoside were 229 ± 15 and 2109 ± 350 (min μg/mL) after administration of 10 mg/kg luteolin-7-*O*-glucoside, iv, and 1 g/kg, luteolin-7-*O*-glucoside, po, respectively (Figure 4). The oral bioavailability of luteolin-7-*O*-glucoside was approximately 10 ± 2%. In the group administered luteolin-7-*O*-glucoside orally, a biotransformed luteolin product was detected, but it was not detected in the

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J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Table 2. Pharmacokinetic Parameters of Luteolin-7-*O*-glucoside (10 mg/kg, iv; and 1 g/kg, po)[a]

parameter	luteolin-7-*O*-glucoside (10 mg/kg, iv)	luteolin-7-*O*-glucoside (1 g/kg, po)	luteolin
C_{max} (μg/mL)	6.09 ± 0.59	3.04 ± 0.76	2.45 ± 0.55
t_{max} (min)		290 ± 94	280 ± 57
AUC (μg min/mL)	229 ± 15	2109 ± 350	1028 ± 253
$t_{1/2}$ (min)	87 ± 7	685 ± 193	
MRT (min)	84 ± 6	527 ± 25	356 ± 32
CL (mL/min/kg)	38.5 ± 2.7	540.2 ± 84.1	
oral bioavailability (%)		10 ± 2	

[a]Data are presented as the mean ± SE (n = 6).

group administered luteolin-7-*O*-glucoside intravenously. These results demonstrate that luteolin 7-*O*-β-glucoside was not readily absorbed alone and that luteolin glucuronides were detected after intestinal absorption.

To clarify the luteolin is the hydrolysis product of luteolin-7-*O*-glucoside, luteolin-7-*O*-glucoside was administered (1 g/kg, po). The concentration versus time curve showed that luteolin was detected in rat plasma after administration of luteolin-7-*O*-glucoside (1 g/kg, po) (Figure 4). It has been reported that intestinal bacteria can hydrolyze glucosides of flavonoids and produce aglycones.[33] Our data demonstrated that both luteolin and luteolin-7-*O*-glucoside can be detected after luteolin-7-*O*-glucoside administration (1 g/kg, po). The oral bioavailability of luteolin-7-*O*-glucoside was 10 ± 2% (Table 2), which suggested that luteolin-7-*O*-glucoside may first be hydrolyzed to luteolin by the bacteria that are present on the surface of the intestinal mucosa. β-Glycosidase is an enzyme that rapidly hydrolyzes glucosides, such as quercetin 3-*O*-glucoside, which had the highest percent of hydrolysis.[34] This phenomenon is consistent with our data that luteolin can be detected at the first collected time point of 5 min after luteolin-7-*O*-glucoside administration (1 g/kg, po). The maximum concentrations (C_{max}) of luteolin and luteolin-7-*O*-glucoside were found at 3.04 ± 0.76 and 2.45 ± 0.55 min after luteolin-7-*O*-glucoside administration (1 g/kg, po).

In conclusion, we first identified that the flower of *D. morifolium* Ramat Tzvel is a highly rich source of luteolin and luteolin-7-*O*-glucoside. We determined that the luteolin-7-*O*-glucoside contents in ethanolic and water extracts were 4.19 and 6.56%, respectively. However, the luteolin content was 0.19% in the ethanolic extract, and luteolin was not detected in the water extract. The oral bioavailability of luteolin was approximately 26 ± 6% after luteolin administration (10 mg/kg, i.v. and 100 mg/kg, p.o.). The oral bioavailability of luteolin-7-*O*-glucoside was approximately 10 ± 2% after luteolin-7-*O*-glucoside administration (10 mg/kg, iv; and 1 g/kg, po). Luteolin is one of the major metabolites present after oral administration of luteolin-7-*O*-glucoside, and we detected a biotransformed luteolin product, although this biotransformed product was not detected in the intravenous administration group. The transformation ratio (the AUC ratio of metabolite/parent compound) of luteolin to luteolin-7-*O*-glucoside was approximately 48.78 ± 0.12%. These results demonstrate that luteolin-7-*O*-glucoside is primarily hydrolyzed to luteolin in the gastrointestinal tract and absorbed into the systemic circulation. These results also imply that people who drink chamomile tea can also retain the benefits from luteolin, due to high transformation ratio of luteolin to luteolin-7-*O*-glucoside.

■ ASSOCIATED CONTENT

Supporting Information

Tables S1 and S2 and Figures S1−S5. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Shim, S. Y.; Park, J. R.; Byun, D. S. 6-Methoxyluteolin from *Chrysanthemum zawadskii* var. *latilobum* suppresses histamine release and calcium influx via down-regulation of FcεRI α chain expression. *J. Microbiol. Biotechnol.* **2012**, *22* (5), 622−627.

(2) Chatterjee, A.; Sarkar, S.; Saha, S. K. Acacetin 7-*O*-β-D-galactopyranoside from *Chrysanthemum indicum*. *Phytochemistry* **1981**, *20*, 1760−1761.

(3) Beninger, C. W.; Abou-Zaid, M. M.; Kistner, A. L. E.; Hallett, R. H.; Iqbal, M. J.; Grodzinski, B.; Hall, J. C. A flavanone and two phenolic acids from *Chrysanthemum morifolium* with phytoxic and insect growth regulating activity. *J. Chem. Ecol.* **2003**, *30*, 579−596.

(4) Shimoi, K.; Masuda, S.; Furugori, M.; Esaki, S.; Kinae, N. Radioprotective effect of antioxidative flavonoids in γ-ray irradiated mice. *Carcinogenesis* **1994**, *15*, 2669−2672.

(5) Seelinger, G.; Merfort, I.; Schempp, C. M. Anti-oxidant, anti-inflammatory and anti-allergic activities of luteolin. *Planta Med.* **2008**, *74* (14), 1667−1677.

(6) Yasukawa, K.; Takido, M.; Takeuchi, M.; Nakagawa, S. Effect of chemical constituents from plants on 12-*O*-tetradecanoylphorbol-13-acetate-induced inflammation in mice. *Chem. Pharm. Bull.* **1989**, *37*, 1071−1073.

(7) Majumdar, D.; Jung, K. H.; Zhang, H.; Nannapaneni, S.; Wang, X.; Amin, A. R.; Chen, Z.; Chen, Z. G.; Shin, D. M. Luteolin nanoparticle in chemoprevention: in vitro and in vivo anticancer activity. *Cancer Prev. Res. (Phila.)* **2014**, *7* (1), 65−73.

(8) Yamamoto, H.; Sakakibara, J.; Nagatsu, A.; Sekiya, K. Inhibitors of arachidonate lipoxygenase from defatted perilla seed. *J. Agric. Food Chem.* **1998**, *46*, 862−865.

(9) Ferriola, P. C.; Cody, V.; Middleton, E., Jr. Protein kinase C inhibition by plant flavonoids. Kinetic mechanisms and structure-activity relationships. *Biochem. Pharmacol.* **1989**, *38*, 1617−1624.

(10) Nekohashi, M.; Ogawa, M.; Ogihara, T.; Nakazawa, K.; Kato, H.; Misaka, T.; Abe, K.; Kobayashi, S. Luteolin and quercetin affect the cholesterol absorption mediated by epithelial cholesterol transporter Niemann−Pick C1-like 1 in Caco-2 cells and rats. *PLoS One* **2014**, *9* (5), No. e97901.

(11) Sadzuka, Y.; Sugiyama, T.; Shimoi, K.; Kinae, N.; Hirota, S. Protective effect of flavonoids on doxorubicin-induced cardiotoxicity. *Toxicol. Lett.* **1997**, *92*, 1−7.

(12) Manach, C.; Morand, C.; Crespy, V.; Demigne, C.; Texier, O.; Regerat, F.; Remesy, C. Quercetin is recovered in human plasma as conjugated derivatives which retain antioxidant properties. *FEBS Lett.* **1998**, *426*, 331−336.

(13) Chen, T.; Li, L. P.; Lu, X. Y.; Jiang, H. D.; Zeng, S. Absorption and excretion of luteolin and apigenin in rats after oral administration

DOI: 10.1021/jf505848z
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

of *Chrysanthemum morifolium* extract. *J. Agric. Food Chem.* **2007**, *55*, 273−277.
(14) Li, L. P.; Jiang, H. D. Determination and assay validation of luteolin and apigenin in human urine after oral administration of tablet of *Chrysanthemum morifolium* extract by HPLC. *J. Pharm. Biomed. Anal* **2006**, *41*, 261−265.
(15) Chen, Z.; Chen, M.; Pan, H.; Sun, S.; Li, L.; Zeng, S.; Jiang, H. Role of catechol-*O*-methyltransferase in the disposition of luteolin in rats. *Drug Metab. Dispos.* **2011**, *39*, 667−674.
(16) Chen, Z.; Kong, S.; Song, F.; Li, L.; Jiang, H. Pharmacokinetic study of luteolin, apigenin, chrysoeriol and diosmetin after oral administration of *Flos Chrysanthemi* extract in rats. *Fitoterapia* **2012**, *83*, 1616−1622.
(17) Németh, K.; Plumb, G. W.; Berrin, J. G.; Juge, N.; Jacob, R.; Naim, H. Y.; Williamson, G.; Swallow, D. M.; Kroon, P. A. Deglycosylation by small intestinal epithelial cell *β*-glucosidases is a critical step in the absorption and metabolism of dietary flavonoid glycosides in humans. *Eur. J. Nutr.* **2003**, *42*, 29−42.
(18) Hostetler, G. L.; Ried, K. M.; Schwartz, S. J. Effects of food formulation and thermal processing on flavones in celery and chamomile. *Food Chem.* **2013**, *141* (2), 1406−1411.
(19) Lin, L. C.; Chou, C. J. Chemical constituents from *Pterocypsela formosana*. *Chin. Pharm. J.* **2002**, *54*, 181−185.
(20) U.S. FDA. *Guidance for Industry, Bioanalytical Method Validation*; U.S. Department of Health and Human Services, Food and Drug Administration Center for Drug Evaluation and Research (CDER): Washington, DC, USA, 2001.
(21) Thrivikraman, K. V.; Huot, R. L.; Plotsky, P. M. Jugular vein catheterization for repeated blood sampling in the unrestrained conscious rat. *Brain Res. Protoc.* **2002**, *10*, 84−94.
(22) Plazonić, A.; Bucar, F.; Males, Z.; Mornar, A.; Nigović, B.; Kujundzić, N. Identification and quantification of flavonoids and phenolic acids in burr parsley (*Caucalis platycarpos* L.), using high-performance liquid chromatography with diode array detection and electrospray ionization mass spectrometry. *Molecules* **2009**, *14* (7), 2466−2490.
(23) Song, W. Y.; Kim, N. J.; Kim, S. Y.; Lee, H. S. Liquid chromatography-tandem mass spectrometry for the determination of jaceosidin in rat plasma. *J. Pharm. Biomed Anal.* **2009**, *49* (2), 381−386.
(24) Ma, Y.; Li, P.; Chen, D.; Fang, T.; Li, H.; Su, W. LC/MS/MS quantitation assay for pharmacokinetics of naringenin and double peaks phenomenon in rats plasma. *Int. J. Pharm.* **2006**, *307* (2), 292−299 (Epub Nov 14, 2005).
(25) Zhang, L.; Lin, G.; Zuo, Z. Involvement of UDP-glucuronosyltransferases in the extensive liver and intestinal first-pass metabolism of flavonoid baicalein. *Pharm. Res.* **2007**, *24*, 81−89.
(26) Chen, X.; Yin, O. Q.; Zuo, Z.; Chow, M. S. Pharmacokinetics and modeling of quercetin and metabolites. *Pharm. Res.* **2005**, *22* (6), 892−901.
(27) Sfakianos, J.; Coward, L.; Kirk, M.; Barnes, S. Intestinal uptake and biliary excretion of the isoflavone genistein in rats. *J. Nutr.* **1997**, *127* (7), 1260−1268.
(28) Blaut, M.; Schoefer, L.; Braune, A. Transformation of flavonoids by intestinal microorganisms. *Int. J. Vitam. Nutr. Res.* **2003**, *73* (2), 79−87.
(29) Crespy, V.; Morand, C.; Besson, C.; Cotelle, N.; Vézin, H.; Demigné, C.; Rémésy, C. The splanchnic metabolism of flavonoids highly differed according to the nature of the compound. *Am. J. Physiol.* **2003**, *284* (6), G980−G988.
(30) Chen, Z.; Chen, M.; Pan, H.; Sun, S.; Li, L.; Zeng, S.; Jiang, H. Role of catechol-*O*-methyltansferase in the disposition of luteolin in rats. *Drug Metab. Dispos.* **2011**, *39*, 667−674.
(31) Chen, Z.; Kong, S.; Song, F.; Li, L.; Jiang, H. Pharmacokinetic study of luteolin, agigenin, chrysoeriol and diosmetin after oral administration of *Flos Chrysanthemi* extracted in rats. *Fitoterapia* **2012**, *83*, 1616−1622.
(32) Shimoi, K.; Okada, H.; Furugori, M.; Goda, T.; Takase, S.; Suzuki, M.; Hara, Y.; Yamamoto, H.; Kinae, N. Intestinal absorption of luteolin and luteolin 7-*O*-glucoside in rats and humans. *FEBS Lett.* **1998**, *438*, 220−224.
(33) Hawksworth, G.; Drasar, B. S.; Hill, M. J. Intestinal bacteria and the hydrolysis of glycosidic bonds. *J. Med. Microbiol.* **1971**, *4*, 451−459.
(34) Zhang, C. P.; Liu, G.; Hu, F. L. Hydrolysis of flavonoid glycosides by propolis *β*-glycosidase. *Nat. Prod Res.* **2012**, *26* (3), 270−273.

DOI: 10.1021/jf505848z
J. Agric. Food Chem. XXXX, XXX, XXX−XXX