JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Isolation of Radiolabeled Isoflavones from Kudzu (*Pueraria lobata*) Root Cultures

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Isoflavones have potential for preventing and treating several chronic health conditions, such as osteoporosis, cardiovascular disease, and cancer. In this study, radiolabeled isoflavones were recovered from kudzu (*Pueraria lobata*) root cultures after incubation with uniformly labeled ^{14}C-sucrose in the culture medium for 21 days. Approximately 19% of administered label was recovered in the isoflavone-rich dried extracts of kudzu root cultures (90.2 μCi/g or 3.3 MBq/g extract). HPLC-PDA analysis revealed the predominant isoflavones isolated from kudzu root cultures to be puerarin, daidzin, and malonyl-daidzin. The average concentration of the major isoflavone puerarin in kudzu root cultures was 33.6 mg/g extract, with a specific activity of 63.5 μCi/g (2.3 MBq/g). The isolated isoflavones were sufficiently ^{14}C-labeled to permit utilization for subsequent in vivo metabolic tracking studies.

KEYWORDS: Kudzu; *Pueraria lobata*; isoflavones; puerarin; daidzein

INTRODUCTION

Numerous epidemiological studies and several clinical trials have indicated the benefits of a family of phytochemicals known as isoflavones for human health (*1–3*). The leguminous plant kudzu (*Pueraria lobata* (Willd.) Ohwi) has been identified as a significant source of unique and physiologically beneficial isoflavones (*4, 5*). Kudzu is regarded as a noxious weed in the U.S., but it has been used for centuries in traditional Chinese medicine in the form of Puerariae radix (0.02−2%), an isoflavone-rich extract derived from roots of *P. lobata*, to treat a variety of conditions including migraine, hypertension, pain, allergies, angina, and alcoholism (*6*). The predominant isoflavones in kudzu have been identified as daidzein and genistein and their *C*- and *O*-glycosides, most notably the *C*-glycoside puerarin (**Figure 1**) (*5*).

After ingestion, it is notoriously difficult to study the pharmacodynamics and pharmacokinetics in vivo of plant secondary metabolites such as isoflavones. One reason for this is the underlying complications of selectively monitoring one or a few compounds when they are ingested as part of a complex diet. Once compounds are absorbed, it can be challenging to distinguish the recently ingested compounds from the background levels already present in a given organ or tissue, and of course, ingested compounds will be rapidly broken down into a series of diverse metabolites (*7*). Radiolabeling can permit the monitoring of absorption, transport, metabolic distribution and kinetics, and accumulation in various organs over time.

Plant tissue culture is an ideal vehicle for radiolabeling phytochemicals. Culture protocols can be adapted to grow cells or organs that specifically produce the compounds of interest, which are then simpler to extract and isolate than those obtained from whole plants or plant parts (*8*). Dramatically, greater

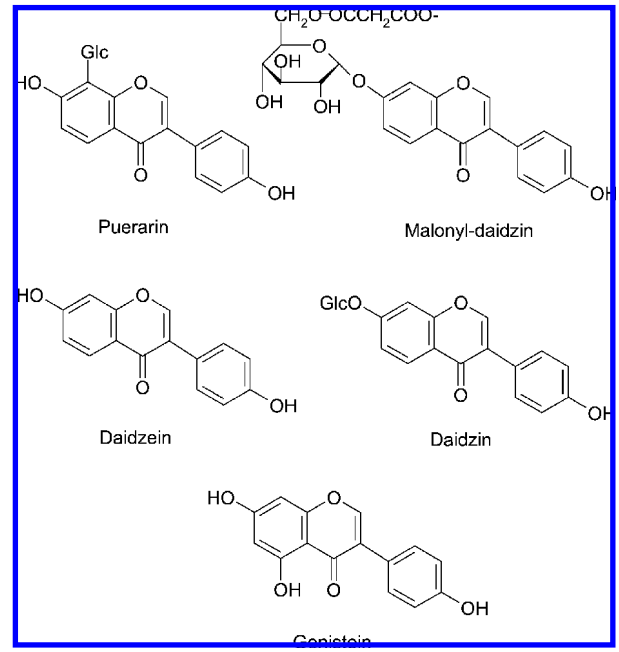

Figure 1. Structures of common kudzu isoflavones. Glc = glucose.

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10.1021/jf801413z CCC: $40.75
Published on Web 08/09/2008

product yield in a short period of time is possible with in vitro cultures, relative to cultivated plants, because they can be oriented to secondary product accumulation rather than growth (*8*). By introducing isotopically labeled compounds in a carbon source (^{14}C-sucrose) or precursor (^{14}C-phenylalanine) into the culture medium, accumulated radiolabeled secondary products can be harvested (*9, 10*).

Previous studies have produced biolabeled plant secondary compounds in vitro with good yields from a variety of species. Krisa et al. (*11*) reported ^{13}C-phenylalanine incorporation into anthocyanins in grape (*Vitis vinifera*) cell cultures with 65% incorporation. Vitrac et al. (*9*) introduced ^{14}C-L-phenylalanine to grape cell cultures to produce a range of radiolabeled polyphenolic compounds, with incorporation yields of 15% reported. In a subsequent study, the authors administered ^{14}C-*trans*-resveratrol obtained from grape cell cultures to mice through gastric intubation and tracked and quantified its absorption and distribution in vivo (*12*). Yousef et al. (*10*) used ^{14}C-sucrose to incorporate the ^{14}C label into a range of anthocyanins, proanthocyanins, and other flavonoids in cell cultures of two species, ohelo (*Vaccinium pahalae*) and grape (a *Vitis* hybrid, Bailey Alicant A), with recovery of 15% of the administered label in the various flavonoids. Similar to the study by Vitrac et al. (*12*), these labeled flavonoids were administered in a rat model, and serum time-course absorption was monitored (*13*). Campbell et al. (*14*) reported the production of ^{14}C-phytoene from tomato (*Lycopersicon esculentum*) cell suspension cultures using ^{14}C-sucrose as the labeled precursor, with a total recovery of 17.5% of the administered label in the cells, and used the radiolabeled phytoene to investigate its uptake and metabolism by prostate tumor cells.

Recently, we established that kudzu cultured root tissues were a superior source of isoflavone *C*-glycosides as compared to cell (callus or suspension) cultures (*15*). In this study, we incorporated ^{14}C label into the culture medium of kudzu root cultures in order to accumulate radiolabeled kudzu bioactive isoflavones for use in metabolic tracking (pharmacokinetics and pharmacodynamics) studies.

MATERIALS AND METHODS

Kudzu Root Cultures. Seed explants of kudzu (*Pueraria lobata*) from wild sources in Illinois were obtained in October 2003 from the Binkley Rd population in Johnston City (Williamson County), IL. This Illinois accession was identified by Terry Esker (Natural Heritage Biologist, Illinois Department of Natural Resources) and a voucher specimen (voucher number 227615) was deposited in the Illinois Natural History Survey herbarium.

Seeds were scarified by scraping three times across the seed coat with a knife. Seeds were agitated for 10 h in 100 mL of tap water containing 2 drops of polyoxyethylene sorbitan monolaurate (Tween-20) (Sigma-Aldrich, St. Louis, MO), surface sterilized in 0.9% sodium hypochlorite for 30 min, and rinsed three times with sterile distilled water. Seeds were placed into test tubes on 15 mL one-half-strength Murashige and Skoog (MS) basal medium (*16*) and germinated within 14 d. Seedlings were dissected to provide explants to initiate root cultures.

Root cultures were initiated using 2 cm-long root segments obtained from 4-week old in vitro kudzu seedlings. Roots excised from these seedlings were cultured in 40 mL of liquid MS medium supplemented with 1.0 mg/L 1-naphthaleneacetic acid (NAA) (Sigma-Aldrich) in 250 mL flasks (*17*). Cultures were maintained in the dark on a rotary shaker (150 rpm) at 25 °C.

During initial root culture protocol development, traditional subculture methods (transferring divisions or sections of actively growing peripheral roots to fresh media) resulted in inconsistent growth responses and cultures often failed to grow or survive. Consistent root productivity was achieved by selecting the most vigorous cultures 3 weeks after division and replacing all but 5 mL of the spent media with 35 mL of fresh media on 3-week intervals. After two additional growth cycles, the cultures were once again divided in half using a scalpel and placed in fresh media. Newly initiated root cultures were established through 7−10 subculture cycles before use in labeling runs.

^{14}C Labeling of Kudzu Root Cultures. Uniformly labeled ^{14}C-sucrose with a specific activity of 10 mCi/mmol or 29 mCi/g (374 MBq/mmol or 1092 MBq/g) in a crystalline solid form (ICN Biomedicals Inc., Irvine, CA) was used as the source of label delivered to the root cultures. ^{14}C-sucrose stock solutions were prepared in sterile double-distilled H_2O (pH 5.7), and the stock solutions were filter sterilized prior to incorporation into the media. Concentrated root culture media containing all components except ^{14}C-sucrose were prepared by bringing media to 90% of the final volume. The media were dispensed and autoclaved at 32 mL per 250 mL flask.

After replacement with fresh media in established cultures (removal of all but 3 mL spent medium followed by transfer of 32 mL of fresh, concentrated medium to root cultures in 250 mL flasks), 5 mL of stock [^{14}C]sucrose solution was added to kudzu root cultures to bring the final media volume to 40 mL. The final ^{14}C label introduced was 6.7 μCi/mL (0.2 MBq/mL) for all kudzu root cultures. Cultures were placed in an enclosed polyacrylic labeling chamber built atop a gyrotary shaker, designed to provide for the safe containment of respired ^{14}C-labeled CO_2 produced by the root cultures (*18*). Kudzu cultures were incubated in the enclosed chamber for 21 d at 22 ± 2 °C in the dark, with the gyrotary shaker set to 160 rpm.

Isoflavone Extraction from Kudzu Root Cultures. Root cultures were harvested and emptied into a Buchner funnel containing #4 Whatman filter paper connected to a suction filtration flask. Roots were separated from the medium and rinsed with distilled deionized water. Roots were drained to the point where liquid was no longer expressed from the Buchner funnel, and fresh tissue weight (FW) was recorded. The roots were placed in a blender (Turbo-twister, Hamilton Beach/Proctor-Silex, Inc., Southern Pines, NC) with enough solvent (80% methanol (MeOH); Fisher Scientific, Pittsburgh, PA) to obtain a 1:5 ratio (w/v) of root tissue to solvent and were blended for 2 min. The root debris was separated from the extract using a Buchner funnel and #4 Whatman filter paper, and the extract was collected. The extraction was repeated on the root debris two times using the same procedure with an additional volume (250 mL) of solvent. The third extraction was allowed to extract overnight at 4 °C, and the combined extracts from the first two extractions were stored at −20 °C. Two final extractions were performed on the root debris with additional volumes (250 mL) of solvent in order to remove the majority of the ^{14}C label and labeled compounds from the root tissue. These final extractions were then filtered, as described above. The solvent extracts of all five extractions were combined and filtered with #1 Whatman filter paper, and the resulting extract was stored at −20 °C until removal of solvents.

Following extraction, MeOH and most of the water were removed under reduced pressure at <40 °C using a rotoevaporator. The samples were transferred to 100 mL round-bottom flasks and stored at −80 °C for freeze-drying. Frozen samples were loaded into a freeze-dryer at −57 °C and 133 × 10^{-3} M bars pressure until completely dried. The dried material was then collected, weighed, placed in 20 mL glass vials, and stored at −20 °C.

^{14}C-Label Enrichment in Dry Extracts and Specific Activities of Isoflavones. To measure the radioactivity in the roots and extracts of roots, a known amount of the sample (5 mg or 100 μL) was added to BioSafe II liquid scintillation cocktail (20 mL) (Research Products International Corp., Mount Prospect, IL), and the components were thoroughly vortexed. Radioactivity was estimated using a Beckman multipurpose liquid scintillation counter model LS-6500 (Beckman-Coulter, Inc., Fullerton, CA). The amount of ^{14}C label recovered in each tissue or extract was calculated by converting the counts per min (CPM) into μCi of label radioactivity (*10*). Briefly, the disintegration-per-minute value from scintillation counting was multiplied by the final volume/weight of the extract, and this value was divided by the volume/weight of the sample measured. The resulting value then was divided by a coefficient constant (2 220 000) to get μCi/mg or mL.

The specific activities for isoflavone compounds were estimated by dividing the amount of ^{14}C label in μCi by compound mass for each

corresponding peak in mg (specific activity = μCi/mg). The concentrations of isoflavone compounds (mg) were estimated by using commercial standards, as described below. To measure ^{14}C counts in each peak (μCi), the collected mobile phase volume (0.8 mL) corresponding to each peak was added to 20 mL of Biosafe II liquid scintillation cocktail and read by liquid scintillation counter. The specific activity values were reported as μCi/g for each individual isoflavone in the dry extract.

HPLC-PDA Analysis of Radiolabeled Isoflavones. HPLC separation and quantification of kudzu isoflavones were achieved using a 250 mm × 4 mm i.d., 5 μm, Supelcosel LC$_{18}$ reversed phase column (Supelco, Bellefonte, PA). Analysis was performed with 1100 HPLC series system (Agilent Technology Inc., New Castle, DE) with a quadratic pump (G1311A), degasser (G1322A), autosampler (G1313A), fraction collector (G1364C), and temperature controlled column compartment (G1316A) of 24 °C. The wavelength absorption was monitored at 262 nm using photodiode array UV/vis detector (PDA). The injection volume was 25 μL for samples and standards with a flow rate of 1.5 mL/min. Solvent A was 90% double-distilled H_2O, 10% acetonitrile (ACN) (Fisher Scientific), and 0.1% trifluoroacetic acid (TFA) (Sigma-Aldrich), and solvent B was 90% ACN, 10% double-distilled H_2O, and 0.1% TFA. The mobile phase gradient system was 0, 70%, 100%, 0%, and 0% of solvent B at 0, 30, 35, 40, and 45 min, respectively. Primary testing showed that major isoflavone compounds corresponded to peaks with retention times of 4.3, 5.3, 6.7, 7.6, 9.3, 11.2, 12.6, and 15.8 min. In comparison with commercial references and HPLC-MS data, these peaks were associated with hydroxy-puerarin, puerarin, daidzin, genistin, malonyl-daidzin, malonyl-genistin, daidzein, and genistein, respectively. The fraction collector was set to collect the isoflavone peaks separately. To quantify the isoflavones in kudzu dry extract, three concentrations of external standards were used. The standards were prepared in 80% MeOH at the following concentrations: puerarin at 75, 150, and 300 μg/mL; daidzin at 50, 100, and 200 μg/mL; malonyl-genistin and genistin at 10, 20, and 40 μg/mL; genistein and daidzein at 3, 6, and 12 μg/mL. All data were processed using Chemstation Software for LC 3D systems (Rev. A.10.02, Agilent Technology, Inc.). Standards for puerarin, daidzein, and genistein were purchased from Sigma-Aldrich for isoflavone identification and quantification. Standards for daidzin, genistin, and malonyl-genistin were purchased from LC Laboratories (Woburn, MA). Isoflavones for which standards were not commercially available were quantified with related standards using a method previously described (*19*).

HPLC-ESI/MS Analysis of Radiolabeled Isoflavones. High-performance liquid chromatography electrospray ionization-mass spectrophotometer (HPLC-ESI-MS) data were obtained for identification of kudzu isoflavones using an LCQ Deca XP mass spectrometer in positive ESI mode (*m*/*z* 150−1000) attached to a photodiode array (PDA) detector (UV: 262 mm) (Thermo Finnigan Corp., San Jose, CA). Analyses were performed according to procedures developed by Yousef et al. (*10*) with minor modifications. The HPLC separations were carried out on a 150 mm × 2.1 mm i.d., 5 μm, VYDAC C$_{18}$-reversed phase column (Western Analytical, Murrieta, CA). The mobile phase solvent consisted of 95% double-distilled H_2O and 5% ACN with 0.1% formic acid (Fisher Scientific) (solvent A) and 95% ACN and 5% double-distilled H_2O with 0.1% formic acid (solvent B). A step gradient of 0%, 70%, 100%, and 0% of solvent B was used at 0, 30, 35, and 40 min, respectively. Extract samples were prepared by dissolving 20 mg in 4 mL of 80% MeOH (5 mg/mL) and filtering through a 0.45 μm nylon syringe filter (Fisher Scientific). The flow rate was 200 μL/min and 5 μL injection volumes were used. The column was equilibrated with solvent A for 10 min between samples at the same flow rate.

Statistical Analysis. The radiolabeling experiment was conducted in three runs, with each run containing eight cultures. The results are expressed as means ± standard deviations (SD) from 24 independent measures. Statistical analyses were performed using SAS (Version 9.13, SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Culture Growth and ^{14}C-Label Partitioning. Kudzu root cultures grown for 21 d in the labeling chamber reached a final average fresh weight of 13.5 g per flask, an average increase of 20.5% in weight. The cultures accumulated 598.0 μCi (22.1 MBq) (26.0% of the administered [^{14}C]sucrose label), in an average of 102.0 g per run of cultured biomass (5.9 μCi/g or 0.2 MBq/g fresh weight), whereas 27.7% of the ^{14}C label remained in the filtered media (**Table 1**). By scintillation counts of samples from CO_2 traps, loss of ^{14}C in the form of CO_2 from respiration accounted for 653.9 μCi (24.2 MBq) (28.0%) of the label. The final recovery of the ^{14}C label from the root cultures into the isoflavone-rich dried extract was 438.6 μCi (16.2 MBq) (18.7%). The overall labeling efficiency was 171.4 μCi/g (6.3 MBq/g) in the dry extract. Of the label recovered in the dry extract, actual incorporation into the isoflavone compounds was found to be approximately 17.8%; the remainder was incorporated into various other compounds in the extract.

Table 1. Percent Distribution of ^{14}C in Root Tissue, Media, and Dried Extract of Kudzu Root Cultures

	final ^{14}C distribution[a]
sample	% of ^{14}C-sucrose dose
roots	26.0 ± 7.6
media	27.7 ± 4.7
respiration (CO_2)	28.0 ± 1.2
dried isoflavone extract	18.7 ± 5.0

[a] Values are mean percentages ± SD of total administered radioactivity in three runs (2300 μCi ^{14}C dose per run), n = 8 per run. Cultures were incubated with ^{14}C label for 21 d.

Tissue samples from both the interior and exterior of the kudzu root culture mass were analyzed for incorporation of the ^{14}C label to test for possible differential distribution of the label within the root mass. It was found that ^{14}C incorporation in the peripheral root tissue was almost twice that in the interior root tissue (0.20 μCi/g or 7.4 kBq/g vs 0.11 μCi/g or 4.1 kBq/g FW tissue, respectively).

Chemical Composition of Isoflavonoid-Rich Fractions. Isoflavones present in radiolabeled kudzu root culture extracts were identified by HPLC-PDA and HPLC-ESI/MS using several criteria, including comparison of their UV, retention time (t_R), and major ion molecular weight (MW) values in comparison with commercial standards and with isoflavones previously reported in extracts of kudzu root (*5, 20*).

The predominant isoflavones accumulated by kudzu root cultures were the glycosides puerarin, daidzin, and malonyl-daidzin (**Figure 2**). This isoflavone profile was consistent with our previous analysis of unlabeled kudzu root cultures (*15*). Peak identities were assigned based on the t_R values of known standards, the MW values of the major ion for each peak, and the order and identity of peaks reported in previous studies of kudzu root extracts; MS data were consistent with these reports (**Figure 3**) (*5, 15, 20*). Other isoflavones found in the radiolabeled kudzu root cultures included additional glycosides of puerarin and genistein and the aglycones daidzein and genistein in smaller quantities.

The isoflavones in the dried extracts of kudzu root cultures were quantified by HPLC-PDA to estimate the concentration of each isoflavone in the dry extract. This step was crucial in determining the potency of radiolabeled kudzu root cultures as a source of labeled, bioactive isoflavones. The most abundant isoflavone accumulated in the ^{14}C-labeled kudzu root cultures was puerarin, with an average concentration of 33.6 mg/g extract (equivalent to 2.6 mg FW) (**Table 2**). The glycosides of daidzein also were abundant in the root cultures, specifically daidzin and malonyl-daidzin, at average concentrations of 32.2 mg/g (1.5

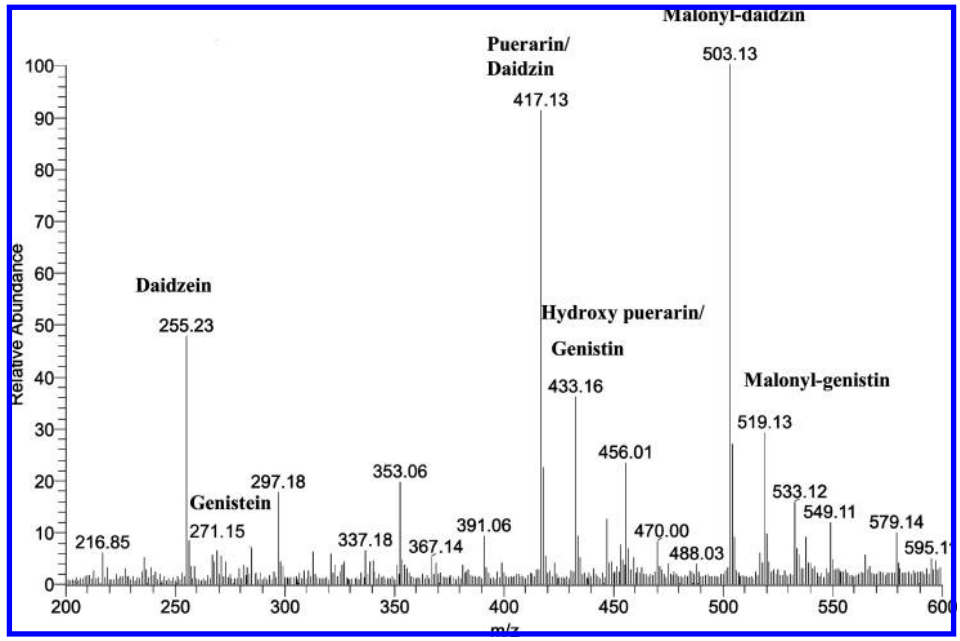

Figure 2. HPLC-ESI/MS spectrum (positive mode) for isoflavones in radiolabeled kudzu (*Pueraria lobata*) root culture 80% MeOH extract.

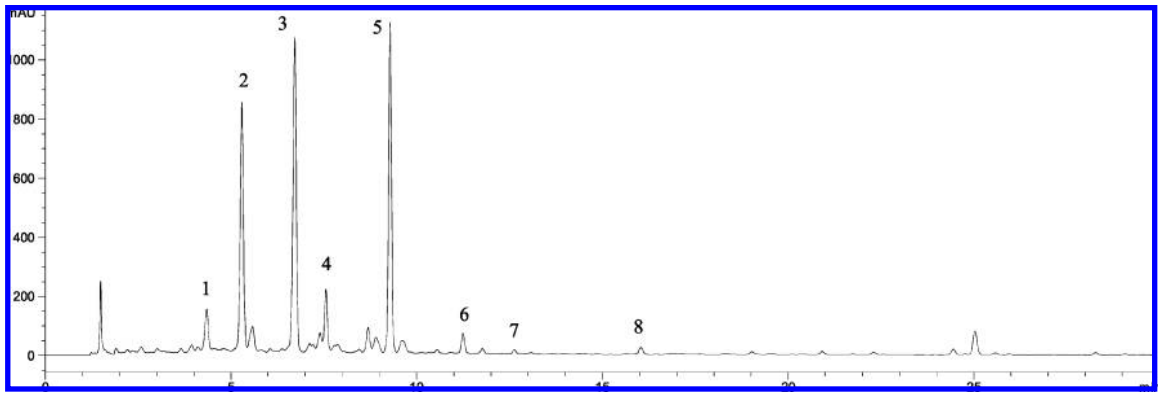

Figure 3. Typical RP-HPLC-PDA/UV chromatogram for isoflavones in radiolabeled kudzu (*Pueraria lobata*) root culture 80% MeOH extract monitored at UV 262 nm. Peak identification: (1) hydroxy-puerarin, (2) puerarin, (3) daidzin, (4) genistin, (5) malonyl-daidzin, (6) malonyl-genistin, (7) daidzein, (8) genistein.

Table 2. Concentrations and Specific Activities of Isoflavone Found in Kudzu Root Culture Extract as Measured by HPLC and Scintillation Counting

isoflavone	concentration (mg/g extract)[a]	specific activity (μCi/g extract)[a]	label recovery (% label in extract)[a]
hydroxy-puerarin	14.4 ± 5.0	168.3 ± 87.9	2.8 ± 0.4
puerarin	33.6 ± 8.1	63.5 ± 28.9	2.9 ± 0.5
daidzin	32.2 ± 5.8	112.8 ± 19.8	5.1 ± 0.6
genistin	1.3 ± 0.5	660.1 ± 149.2	1.3 ± 0.2
malonyl-daidzin	29.1 ± 8.5	85.4 ± 11.2	3.6 ± 1.0
malonyl-genistin	4.5 ± 0.4	146.3 ± 37.3	0.5 ± 0.1
daidzein	0.9 ± 0.6	192.5 ± 63.6	0.4 ± 0.1
genistein	0.6 ± 0.3	185.9 ± 75.9	0.5 ± 0.1
total	116.6 ± 15.1	1611.8 ± 387.6	17.8 ± 2.8

[a] Values are means ± SD of isoflavone content, specific activity, and label recovery across three runs; $n = 8$ for each.

mg/g FW) and 29.1 mg/g dried extract (1.3 mg/g FW), respectively. Concentrations for other isoflavones detected in kudzu root cultures and the levels of ^{14}C incorporation are presented in **Table 2**.

Genistin, one of the less predominant isoflavones, was found to have the most ^{14}C enrichment as measured by specific activity (660.1 μCi/g or 24.4 MBq/g). The most predominant kudzu isoflavones, puerarin and daidzin, however, had lower specific activities of 63.5 μCi/g (2.3 MBq/g) and 112.8 μCi/g (4.2 MBq/g), respectively. By contrast, when measured by label recovery in the extract (% label in extract per isoflavone), the percentage of label present in the various isoflavones appeared proportional to the concentrations at which those isoflavones were present (**Table 2**) (correlation, $R = 0.90$). Daidzin, for example, had the highest overall label incorporation at 5.1% of the label recovered in the dried extract, while the other two predominant kudzu isoflavones malonyl-daidzin and puerarin had the next highest total label incorporation at 3.6 and 2.9%, respectively. Genistin, which had the highest specific activity, accounted for only 1.3% of the recovered label. The reasons behind the higher label incorporation in daidzin and the observed high specific activity of genistin as compared to other isoflavones remain subjects for future investigation.

The extent of ^{14}C-label incorporation into the root tissue depended on the nature of the material. These untransformed kudzu root cultures grow slowly and incorporated only one-quarter of the introduced label into the tissue. The location of the tissue (peripheral or interior root mass) affected the percent label incorporation. Nearly two times the amount of label was

incorporated in the peripheral root tissue surrounding the exterior of the root mass as compared to the interior root tissue. This difference in label incorporation likely reflects the growth pattern of the root cultures, as most new root growth occurs around the periphery of the root mass and that newer peripheral tissue utilized more of the ^{14}C-sucrose for growth (rather than for secondary metabolite biosynthesis) than the older, interior tissue.

The degree of label incorporation into the root tissue (26%) (**Table 1**) was in the range of the recently reported label incorporation in grape (*Vitis vinifera*), ohelo (*Vaccinium pahalae*), and tomato (*Lycopersicon esculentum*) cell cultures (*9, 14, 18*), for which 17−34% radiolabeling efficiency was reported. Moreover, the amount of label recovered in the dried isoflavone-rich kudzu extract was similar to that recovered in flavonoid-rich fractions of labeled grape and ohelo cell cultures. This degree of labeling efficiency is noteworthy given that kudzu root cultures grow more slowly and add less new biomass per growth cycle compared to cell suspension cultures like those used in previous labeling studies (2.3 g of average new biomass per root culture vs 5.8 g of average new biomass per kudzu root-derived suspension culture, per growth cycle; data not shown).

The major isoflavones identified in the root cultures of kudzu were the glycosides puerarin, daidzin, and malonyl-daidzin (**Figures 2** and **3**). This finding is consistent with previous analyses of kudzu root cultures (*15*). These three isoflavones were present in large quantities compared to the other identified compounds, as determined by both HPLC-PDA/UV and HPLC-ESI/MS analyses. The identification of puerarin as the major isoflavone in these cultures is significant because of the reported beneficial physiological effects of puerarin in various in vitro and in vivo studies (*21–23*). As an isoflavone *C*-glycoside, puerarin has greater chemical stability than the easily hydrolyzed *O*-glycosides like daidzin and genistin. Isoflavone *O*-glycosides normally are hydrolyzed to their respective aglycones in the gut by intestinal glycosidases and hydrolases before they are absorbed; the aglycones appear in the serum at peak concentration 4−7 h after ingestion and are more bioactive than their respective *O*-glycosides, which appear maximally 8−11 h postingestion (*24*). The isoflavone *C*-glycosides, however, do not have to be hydrolyzed in order for absorption to occur; they are resistant to hydrolysis by intestinal enzymes and are absorbed mostly intact (*25*). Therefore, it is interesting that puerarin demonstrates significant biological activity as an intact glycoside despite the greater bioactivity usually associated with isoflavone aglycones.

The abundant puerarin, daidzin, and malonyl-daidzin compounds in the labeled kudzu root culture extract had specific activities of 63.5, 112.8, and 85.4 μCi/g, respectively. Although puerarin and malonyl-daidzin had lower specific activities compared to other isoflavones examined, they incorporated the largest percentage of the ^{14}C label. Stilbenes and anthocyanins and catechins had specific activities of 875 μCi/g and 260−350 μCi/g, respectively (*9*), and phytoene had 407 Bq/μg, or 0.11 μCi/g (*14*) in previous reports.

Sucrose, which was the source of ^{14}C label in this work, is used in many biosynthetic pathways in plant cells, and therefore, the label was incorporated into a variety of compounds and cell constituents other than isoflavones. The nature of the labeled sucrose and sucrose utilization in plant cells suggests the label is randomly incorporated into compounds, rather than specifically as from a source like acetate. Kudzu root cultures have proven superior to kudzu root-derived cell suspension cultures for isoflavone production (*15*), however, their slower growth rate would likely result in less uptake and incorporation of the labeled precursor as compared to cell suspension cultures.

Because of their slow growth, the root cultures were allowed to grow for three cycles (9 weeks) with refreshment of media every 3 weeks before addition of the ^{14}C-sucrose to the media. Therefore, isoflavones synthesized during this initial growth period would create a pool of pre-existing, unlabeled isoflavones, leading to a dilution of the specific activity of the recovered isoflavones. However, the total label actually incorporated into each isoflavone was commensurate with the concentration of that isoflavone. That is, we saw a larger amount of label incorporated into the isoflavones that were most abundant; the greatest label recovery was in daidzin, malonyl-daidzin, puerarin, and hydroxy-puerarin. Currently, the major kudzu isoflavones from this work are being tested in preliminary metabolic tracking studies (unpublished results), and we are evaluating a strategy for using fast-growing transformed hairy roots as the vehicle for labeled isoflavone synthesis.

In conclusion, we have biosynthesized and isolated ^{14}C-labeled isoflavones from kudzu (*Pueraria lobata*) using a novel tissue culture system. Kudzu root cultures provide a unique and renewable source of bioactive isoflavones. This study is the first to report, to our knowledge, the accumulation of a diverse profile of biolabeled isoflavones from kudzu root cultures after uniformly labeled [^{14}C]sucrose was introduced to the medium. Major isoflavones including puerarin, daidzin, and malonyl-diadzin were isolated and quantified in this tissue culture system. The biolabeled isoflavones will be employed in studies featuring an animal model and isotope-tracking methodology (*12*) to assess the pharmacodynamics and pharmacokinetics of the kudzu extracts and their purified isoflavones, for a better understanding of their beneficial physiological effects.

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Received for review May 6, 2008. Revised manuscript received June 24, 2008. Accepted June 25, 2008. This work was supported in part by National Center for Complementary and Alternative Medicine-sponsored Purdue-UAB Botanicals Center for Dietary Supplement Research (2 P50 AT-000477-06).

JF801413Z