D-proline (0.20 g), the preferential crystallization of D isomer was carried out in the same manner as described above. By repeating these procedures, L and D isomers were successively obtained. Table V shows the results obtained by the successive resolution of *N-n*-butyryl-DL-proline.

Optical Resolution of Other *N*-Acyl-DL-prolines. *N*-Acetyl-DL-proline, *N*-chloroacetyl-DL-proline, and *N*-isobutyryl-DL-proline could also be resolved in the same manner as described above. Although *N*-isobutyryl-DL-proline could not be resolved under stirring, the resolution was successful only when crystallization was induced by inoculation and was continued slowly without stirring or scratching. Conditions and results for the resolutions are summarized in Table IV.

Preparation of Optically Active Proline. *N-n*-Butyryl-L-proline obtained by the above procedure was recrystallized from water. A mixture of optically pure *N-n*-butyryl-L-proline (7.4 g) and 5 N HCl (16 ml) was refluxed for 2 h and then diluted with water (100 ml). The resulting solution was passed through a column of Amberlite IR-120 (40 ml, H^+ form). The column was washed with water and L-proline was eluted from the column with 5% NH_4OH (70 ml). The eluates were concentrated, treated with charcoal, and concentrated again to dryness. The residual crystals were dissolved in methanol (10 ml) and acetone (60 ml) was added to the solution at 5 °C. The precipitated crystals were collected, washed with acetone, and air-dried at 55 °C, giving L-proline (4.3 g), $[\alpha]^{25}D$ −85.3° (*c* 1, water). Anal. Calcd for $C_5H_9NO_2$: C, 52.16; H, 7.88; N, 12.17. Found: C, 51.49; H, 7.88; N, 12.04.

In the same procedure, D-proline was obtained from optically pure *N-n*-butyryl-D-proline, $[\alpha]^{25}D$ +85.5° (*c* 1, water). Anal. Found: C, 51.68; H, 7.95; N, 12.06.

Racemization of Optically Active *N-n*-Butyrylproline. A mixture of *N-n*-butyryl-D-proline (7.4 g) and acetic anhydride (0.38 ml) was heated in a boiling water bath for 20 min after being melted. The reaction mixture was dissolved in water (1.5 ml) and cooled at 5 °C. The precipitated crystals were collected, washed with cold water, and air-dried at 50 °C, giving *N-n*-butyryl-DL-proline (6.9 g), $[\alpha]^{25}D$ 0.0° (*c* 1, water), mp 88–89 °C. Anal. Found: N, 7.51.

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Synthesis and Metabolic Fate of Hesperetin-*3*-^{14}C

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Hesperetin-*3*-^{14}C (3′,5,7-trihydroxy-4′-methoxyflavanone-*3*-^{14}C) of specific activity 6.48 μCi/mg was prepared from acetonitrile-*2*-^{14}C by a five-step reaction sequence (8.0% overall radiochemical yield). The metabolic fate of this flavanone in vivo after oral and intraperitoneal administration to intact, bile duct ligated, and bile duct cannulated rats and in vitro with rat cecal microflora was studied. Forty percent of the radioactivity orally administered to intact rats was expired as $^{14}CO_2$, while virtually no $^{14}CO_2$ was produced upon incubation with cecal microflora. The major labeled metabolites found in both the urine of orally dosed animals and in in vitro incubations were 3-phenylpropanoic acids. It appears that bacterial enzymes were responsible for the metabolism of hesperetin-*3*-^{14}C to labeled phenylpropanoic acids, while mammalian hepatic enzymes mediated their further breakdown to benzoic acids and $^{14}CO_2$.

Flavonoids represent the single most widely occurring group of phenolic compounds found in nature (Seikel, 1964). The flavanone hesperidin (hesperetin 7-β-rutinoside), which was once designated vitamin P because of its effect on blood capillary permeability and fragility, is the predominant flavonoid in lemons and sweet oranges (*Citrus sinensis*). This material is readily converted to the aglycone (hesperetin) upon oral administration to rabbits, rats, and humans (Booth et al., 1958).

Earlier studies dealing with the metabolism of the aglycone have involved oral administration to rats (Booth et al., 1958) and in vitro treatments with rat cecal microflora (Scheline, 1968b). These studies demonstrated both the significant metabolic degradation of hesperetin and the intestinal absorption of this compound and/or its degradation products. The doses of test compound employed in these and related studies ranged from 0.15 to 1.0 g per animal.

We report here the first preparation of radiolabeled hesperetin. This labeled material provided the sensitivity

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and specificity necessary to perform the metabolic study presented herein. This study measured the intestinal absorption of hesperetin and its major metabolites and also determined its extent of degradation when administered to rats and incubated with rat cecal microflora at submilligram levels.

SYNTHETIC PROCEDURES

Liquid scintillation counting was performed on a Packard Tri-Carb Model 2420 instrument. Analytical thin-layer chromatography (TLC) was carried out on prelayered Kieselgel F_{254} plates (E. Merck A. G., Darmstadt, Germany) of layer thickness 0.25 mm. The plates (5 × 20 cm) were developed to 12 cm and examined with a Packard Model 7201 radiochromatogram scanner. All chemicals were reagent grade and were used as received unless otherwise indicated. Anhydrous phloroglucinol (mp 218–220 °C) was prepared from the dihydrate (Aldrich Chemical Company, Milwaukee, Wis.) by vacuum drying (120 °C (0.5 mm)) for 8 h. Hexamethylphosphoramide (HMPA) was distilled from CaH_2 at 20 mm and stored over molecular sieves under argon.

2,4,6-Trihydroxy-(*aceto*-β-^{14}C)-phenone (4). Acetonitrile-2-^{14}C was prepared from barium carbonate-^{14}C (General Electric Co., Pleasanton, Calif., specific activity 56.4 mCi/mmol) by reduction to methanol-^{14}C, reaction with HI to produce methyl-^{14}C iodide, and nucleophilic displacement by NaCN in dimethyl sulfoxide (Murray and Williams, 1958; Smiley and Arnold, 1960). A small amount (~10 mg) was diluted with 195 mg (4.76 mmol) of unlabeled acetonitrile to afford material at an estimated specific activity of 2 mCi/mmol.

A 50-ml, two-necked flask equipped with rubber septum and magnetic stir bar was flushed with Ar and charged with 122 mg (0.89 mmol) of anhydrous $ZnCl_2$. The flask was evacuated and heated strongly with a burner flame until the $ZnCl_2$ was completely fused. Anhydrous ether (7.5 ml) was vacuum transferred into the flask and the mixture was stirred until the waxy solid had dissolved. Acetonitrile-2-^{14}C was next added by vacuum transfer (white precipitate on warming to room temperature) followed by vacuum addition of 5 ml of dry HCl-saturated ether. After stirring for 30 min at room temperature, a clear, colorless, mobile oil had separated.

A solution of 630 mg (5.0 mmol) of anhydrous phloroglucinol (**2**) in 5 ml of ether was added by syringe and the mixture was stirred at room temperature. After several hours, a substantial amount of crystalline solid was present. Stirring was continued for 7 days and the ether was removed by pipet. The solid was broken up with a glass rod, rinsed with ether (2 × 5 ml), and refluxed with 5 ml of H_2O under Ar for 1.5 h. On cooling, a crystalline precipitate formed. The H_2O was removed and extracted with ethyl acetate (4 × 5 ml). The solid was dissolved in ethyl acetate and the two organic solutions were combined, washed with ice–water (4 × 2 ml) and brine (2 × 5 ml), dried over $MgSO_4$, and evaporated to afford 251.4 mg (3.4 mCi, 36% radiochemical yield) of labeled phloroacetophenone (4) as a tan solid.

2-Hydroxy-4,6-dibenzyloxy-(*aceto*-β-^{14}C)-phenone (5). Without additional purification, the product of the foregoing reaction was dissolved in 4 ml of dry HMPA and treated with 828 mg (6.0 mmol) of K_2CO_3 and 760 mg (6.0 mmol) of benzyl chloride. After stirring 6 days under Ar, the reaction mixture was pipetted into 20 ml of H_2O and extracted with ether (1 × 10 ml, 4 × 5 ml). The combined extracts were washed with H_2O (3 × 5 ml), 5% KOH solution (3 × 5 ml), H_2O (3 × 5 ml), and brine (2 × 5 ml), dried over $MgSO_4$, and evaporated to afford 487.7 mg (3.0 mCi, 90% radiochemical yield) of a pale yellow oil. Analysis by TLC (silica gel, $CHCl_3$ elution) showed the product to consist of 85% dibenzyloxy derivative **5** and 15% of the corresponding tribenzyloxy compound.

2-Hydroxy-3′,4,6-tribenzyloxy-4′-methoxychalcone-β-^{14}C (6). The product of the preceding reaction was dissolved in 25 ml of absolute ethanol and placed in a 100-ml round-bottomed flask, and 354 mg (1.46 mmol) of 3-benzyloxy-4-methoxybenzaldehyde (Aldrich Chemical Co.) was added. The mixture was warmed slightly under Ar to obtain a homogeneous solution. After cooling, sodium ethoxide (610 mg, 8.97 mmol) was added in one portion as a solid and the mixture was stirred under Ar at room temperature for 11 days. The yellow suspension was transferred to a separatory funnel containing 100 ml of H_2O and 150 ml of hot (~50 °C) ethyl acetate. The layers were separated and the organic layer was washed with H_2O (3 × 50 ml) and brine (1 × 50 ml), dried over $MgSO_4$, and evaporated to give 911.3 mg of yellow solid. The solid was triturated with 15 ml of boiling ether for 10 min and the ether removed. The residue was rinsed with ether (2 × 5 ml) and the combined ethereal solutions were evaporated to afford 1.16 mCi of material which consisted principally of unreacted starting material. The residual solid (471.3 mg, 1.76 mCi, 58% radiochemical yield), which consisted almost entirely of tribenzyloxychalcone **6** by TLC analysis (silica gel, ether elution), was used without additional purification.

Hesperetin-3-^{14}C (7). The yellow solid **6** was dissolved in 40 ml of acetic acid by heating at 90 °C under Ar. After cooling to 60 °C, 2 ml of 48% aqueous HBr was added and the red solution was stirred and maintained at this temperature for 20 h. The mixture was poured into 200 ml of H_2O and extracted with ethyl acetate (1 × 150 ml). The organic solution was washed with H_2O (2 × 100 ml), 5% aqueous $NaHCO_3$ (2 × 100 ml), H_2O (1 × 100 ml), and brine (1 × 100 ml). After drying over $MgSO_4$, the solvent was evaporated to afford 362.4 mg (1.56 mCi) of partially crystalline tan gum which was dissolved in ethyl acetate and placed on a silica gel column packed in $CHCl_3$. Elution with increasing concentrations of ethyl acetate in $CHCl_3$ afforded 112 mg (0.37 mmol, 725.8 μCi, 41% radiochemical yield) of hesperetin-3-^{14}C as a white crystalline solid with a specific activity of 6.48 μCi/mg. The product was shown to be chemically pure by TLC and radiochemically pure by a radiochromatogram scan of TLC plates in two systems (ether, R_f 0.34; ethyl acetate, R_f 0.57).

The unreacted starting material (containing some chalcone) recovered from the condensation was recycled with unlabeled dibenzyloxyacetophenone and aldehyde to afford a second batch of hesperetin-3-^{14}C of somewhat lower specific activity.

MATERIALS AND METHODS

Animal Experiments. The dosage form was prepared by dissolving hesperetin-3-^{14}C in propylene glycol and diluting with an equal volume of H_2O. The concentration of the final solution was 0.30 mg (1.94 μCi)/ml.

Fourteen female Simonsen/Sprague Dawley rats (Simonsen Laboratories, Gilroy, Calif.) weighing 170–190 g were used. Rats had free access to food (Simonsen white diet) and water (0.9% saline for cannulated animals) with the exception of an overnight fast prior to dosing. Food was returned 8 h after dosing.

Urine and fecal samples were separated and collected at 24-h intervals from intact and bile duct ligated animals housed in stainless steel metabolism cages. Bile ducts were ligated by the method of Cameron and Oakley (1932). Blood samples were obtained from the orbital sinus (Riley,

Table I. Rat Preparation and Dosage

Group	Surgical preparation	No. of animals	Dosage route	Dose administered, mg
I	None	4	Oral	0.29
II	Bile duct ligated	4	Oral	0.29
III	Bile duct ligated	3	Intraperitoneal	0.15
IV	Bile duct cannulated	2	Oral	0.29
V	Bile duct cannulated	1	Intraperitoneal	0.15

1960) at times of 4, 8, 12, 24, 48, 72, and 96 h. Bile samples were collected every 24 h from rats with bile duct cannulae (Siperstein and Chaikoff, 1952). The expired CO_2 from two animals housed in stainless steel metabolism cages enclosed in air-tight plexiglass chambers was collected by pulling room air through the chambers and through two sequentially linked gas-washing bottles containing Carbosorb II (Packard Instrument Co., Downers Grove, Ill.). The solutions were changed at 24-h intervals.

The liver, spleen, kidneys, stomach, small intestine, cecum, large intestine, and a small periuterine fat sample were taken for radioassay after sacrifice. Tissue samples, carcasses, and collected excreta were stored at −20 °C.

Immediately after dosing with the stock solution of hesperetin-*3*-^{14}C (Table I), one rat each from groups I and II was placed in a CO_2 collection chamber. Bile only was obtained from cannulated rats (groups IV and V). Blood was collected from animals in groups I and II. All rats were sacrificed after 96 h.

Radioassay of Biological Samples. Serum (50–200 μl) was added directly to liquid scintillation vials containing 10 ml of Instagel (Packard Instrument Co.). Total serum radioactivity was estimated on the assumptions of a 50% hematocrit and that total blood mass represented 7% of body weight (Bruckner-Kardoss and Wostman, 1974). Urine collections were weighed and for intact rats (group I) 500 μl was placed directly into 10 ml of Instagel. The urine (250 μl) of the ligated animals was treated with an equal volume of 30% aqueous H_2O_2 and, after 1 h, diluted with 10 ml of Instagel.

After weighing, bile samples (200 μl) were mixed with 250 μl of peroxide and allowed to stand 1 h. One milliliter of Soluene (Packard Instrument Co.) was added and, 1 h later, the mixture was diluted with 10 ml of Instagel. Cage washings were weighed and 500-μl aliquots were added to 10 ml of Instagel.

Fecal samples were collected in preweighed plastic bags and soaked overnight at 4 °C in three weights of H_2O. After homogenization, aliquots (0.2–1.0 g) were weighed into Cumbustocones (Packard Instrument Co.) and dried at 60 °C. The contents were rinsed from the gastrointestinal tract with physiological saline and homogenized, and aliquots were weighed into Cumbustocones and dried.

Tissues, with the exception of liver samples which were first pulped, were weighed directly into Cumbustocones and dried.

Samples of tissue, gut contents, and feces were oxidized in a Packard Tri-Carb Model 306 sample oxidizer and the liberated CO_2 was trapped in 10 ml of Oxisorb-2 (New England Nuclear, Boston, Mass.). The solutions were diluted with 10 ml of Oxiprep-2 (New England Nuclear) in liquid scintillation vials.

A Packard Tri-Carb Model 3385 liquid scintillation spectrometer was employed for the radioassay of biological samples. Absolute radioactivity was estimated with efficiency correlation curves based on an automatic external standard.

Detection and Characterization of Urine Metabolites. Each urine sample was divided in half. One-half remained untreated while the other was incubated (37 °C, 18 h) with 2500 units/ml of both β-glucuronidase (bacterial, Type I, Sigma Chemical Co., St. Louis, Mo.) and sulfatase (Type H-1, Sigma Chemical Co.). The pH values of the urine were approximately 7. Unlabeled standards of hesperetin, 3-(3-hydroxyphenyl)propanoic acid, 3-(3-hydroxy-4-methoxyphenyl)propanoic acid, and 3-(3,4-dihydroxyphenyl)propanoic acid were added to each sample prior to preparative TLC in order to facilitate visualization and physical manipulation. The standards were shown to be stable to the enzymatic treatments.

Samples of 50–100 μl were placed on preparative (1 mm silica gel layer, 20 × 20 cm) TLC plates (PQ5F 1000, Quanta/Gram, Quantum Industries, Fairfield, N.J.) which contained fluorescent indicator and were developed with chloroform–methanol–acetic acid (36:6:1, v/v/v) for a distance of 18 cm. The standards were located by fluorescence quenching and 1-cm bands were removed, with the locations of the standards being noted, and placed in liquid scintillation vials containing 10 ml of Instagel. The radioactivity in each band was determined as a percent of the total on each plate.

Cecal Microflora Incubation. The cecal contents from a 200-g female Simonsen/Sprague Dawley rat maintained on the routine laboratory diet (see above) were placed in a 25 × 200 mm screw-cap tube containing fluid incubation medium. The medium had been preflushed (10 min) with an oxygen-free stream of N_2–CO_2 (90:10) and flushing was continued for 10 min following the addition of the cecal contents. The suspension was quickly filtered under N_2–CO_2 through Pyrex glass wool into another test tube. One-milliliter aliquots of the cecal suspension (0.1 g fresh weight/ml) were transferred into pregassed 16 × 125 mm screw-cap (Hungate) tubes fitted with rubber septa and containing the substrate dissolved in 50 μl of ethanol. The tubes and controls (composed of medium and substrate without cecal bacteria) were gassed for 10–15 min using syringe needles, attached to a Burrell Model 75 wrist action shaker, and placed in a 37 °C water bath for 24 h.

In addition to the controls lacking cecal bacteria, a pair of controls containing unlabeled hesperetin, cecal bacteria, and barium carbonate-^{14}C (1.2×10^3 dpm/mg) were incubated (gentle agitation) for 24 h at 37 °C in an orbital shaker.

The medium used (TGYEC) was composed of 0.5% Tryptone (Difco, Detroit, Mich.), 0.5% glucose, 0.5% yeast extract, and 0.05% cysteine hydrochloride in 0.1 M phosphate buffer of pH 7.4 (Scheline, 1968a). Substrate concentrations of 0.05 and 5.0 mg/ml were employed using ethanolic solutions of hesperetin-*3*-^{14}C containing unlabeled hesperetin as carrier. An intermediate concentration of 0.5 mg/ml was established in flasks which allowed anaerobic incubation and collection of CO_2 (Kontes K882300, Kontes Glass Co., Vineland, N.J.).

Following incubation, the contents of the Hungate tubes were acidified (0.2 ml of 12 N HCl) and extracted with ether (2 × 5 ml). The extracts were dried (Na_2SO_4), evaporated under N_2, and resuspended in 1.0 ml of ether. Samples were removed for silica gel TLC (LQ6DF Quanta/Gram) and for liquid scintillation counting. The aqueous incubation mixtures were sampled for counting and TLC.

The center wells of the CO_2 flasks were charged with 0.3 ml of Carbosorb II and the incubation mixtures were acidified (1.0 ml of 4 N HCl). After shaking an additional 14 h, the plastic center wells were transferred to scintil-

Table II. Recovery of Radioactivity after Oral or Intraperitoneal Administration of Hesperetin-3-^{14}C to Rats

Group	% of administered dose[a] Urine	Feces	Bile	Internal organs	Gut content	Expired CO_2[b]	Cage washings	Total recovery[c]
I[e]	33.2 ± 6.5	14.8 ± 6.3	NS[d]	1.4 ± 0.5	<0.1	39.2	0.9 ± 0.1	50.4 ± 11.0
II[e,f]	83.8 ± 5.6	4.1 ± 1.1	NS	0.4 ± 0.1	<0.1	6.3	1.0 ± 0.2	89.3 ± 5.6
III[f,g]	74.4 ± 18.4	1.9 ± 0.7	NS	0.3 ± 0.1	<0.1	NS	2.5 ± 0.7	79.2 ± 18.3
IV[e,h]	NS	NS	56.5 ± 22.4	NS	NS	NS	NS	
V[g,h]	NS	NS	100.7[b]	NS	NS	NS	NS	

[a] Data are group means ± the standard deviation. [b] Data are from single rats. [c] Expired CO_2 is not included. [d] NS = no sample. [e] Oral administration. [f] Bile duct ligated. [g] Intraperitoneal administration. [h] Bile duct cannulated.

lation vials containing 20 ml of Instagel, shaken vigorously, and counted. The incubation mixtures containing labeled substrate were extracted with ether (2 × 25 ml), dried over Na_2SO_4, resuspended, and processed as above. Aqueous mixtures were also counted and analyzed by TLC.

Approximately 10% (50 µl) of the ethereal extracts were applied to 19 channel LQ6DF plates (20 × 20 cm, 0.25 mm layer thickness) and developed to 15 cm with the upper phase of benzene–acetic acid–water (6:7:3, v/v/v). After drying, the individual channels were divided into zones (15 × 1 cm^2), removed from the plate, placed in scintillation vials, and moistened with 0.5 ml of 10% aqueous NH_3 for 15–20 min. Ten milliliters of Instagel was then added followed by vigorous shaking.

RESULTS

Synthetic Considerations. The overall synthetic strategy employed involved the aldol condensation of a protected form of radiolabeled phloroacetophenone (2,4,6-trihydroxyacetophenone) with a protected derivative of isovanillin (3-hydroxy-4-methoxybenzaldehyde). The chalcone thus produced was deprotected and cyclized to the flavanone hesperetin (**7**) in the final step. Scheme I depicts the five-step reaction sequence used.

The key intermediate, radiolabeled phloroacetophenone (**4**), was prepared via a Hoesch reaction. The reaction proceeded by the action of zinc chloride and hydrogen chloride on a mixture of phloroglucinol (**2**) and acetonitrile-2-^{14}C in ether under strictly anhydrous conditions (Gulati et al., 1943; Spoerri and DuBois, 1949). The initially formed ketimine hydrochloride (**3**) was hydrolyzed to **4** by refluxing with aqueous hydrochloric acid.

The sequence continues with the conversion of two of the hydroxyl groups of **4** into benzyl ethers. Previous studies in these laboratories had shown that the aldol condensation proceeds in a significantly higher yield when the hydroxyl groups are so protected (DuBois and Crosby, 1974). Dibenzylation was readily accomplished (90% radiochemical yield) by stirring **4** with excess benzyl chloride and potassium carbonate in hexamethylphosphoramide at room temperature. These conditions were the best of a number that were studied in regard to selectivity for oxygen alkylation.

The aldol condensation of radiolabeled phloroacetophenone derivative **5** with 3-benzyloxy-4-methoxybenzaldehyde proceeded in 58% radiochemical yield through the agency of sodium ethoxide in ethanol. Purification and recovery of unreacted **5** were conveniently carried out by triturating the highly crystalline product with boiling ether. The debenzylation of chalcone **6** with concurrent cyclization to flavanone **7** was carried out with 48% aqueous HBr in acetic acid at 60 °C. Hesperetin-3-^{14}C (**7**) was obtained as a white crystalline solid after silica gel column chromatography. The overall radiochemical yield, based on acetonitrile-2-^{14}C, was 8.0%.

Excretion of Radioactivity. The excretion of radioactivity from all animals by all routes (i.e., urine, bile, feces, and expired carbon dioxide) was maximum during the 24 h following dosing and was essentially complete after 48 h. Serum radioactivity peaked between 4 and 8 h in the intact rats (group I) and during the first 4 h in the ligated animals (groups II and III).

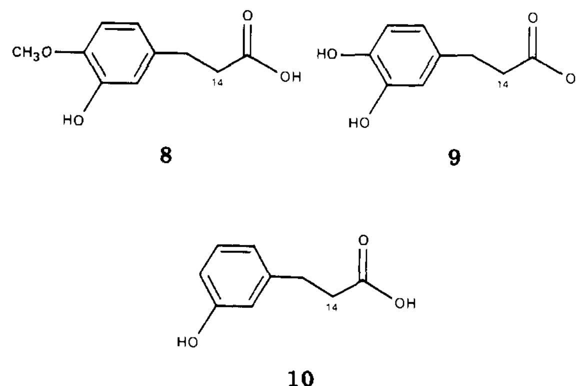
Table III. Chromatographic Distribution of Radioactivity in the Urine of Rats Orally Dosed with Hesperetin-3-^{14}C

	% of total radioact. on TLC plate[a] Remaining at origin	7, R_f 0.90	8, R_f 0.72	9, R_f 0.35	10, R_f 0.62
Before enzymatic deconjugation	51	0	25	10	14
After enzymatic deconjugation	19	0	46	16	19

[a] R_f values refer to PQ5F 1000 plates developed with chloroform–methanol–acetic acid (36:6:1).

The recovery of radioactivity, expressed as a percent of the administered dose, is presented in Table II. The major routes of excretion of radioactivity by orally dosed intact rats (group I) were urine and expired carbon dioxide. The predominant excretory route of the bile duct ligated animals (groups II and III) was urine. The data show that the group II rats expired only 16% as much radiolabeled carbon dioxide as did those rats with intact biliary systems (group I). The majority of the dose appeared in the bile when that material was totally collected (groups IV and V).

Table III presents the chromatographic distribution of the urine radioactivity both before and after enzymatic deconjugation. The chromatographic data show the presence of radiolabeled phenylpropanoic acid metabolites **8**, **9**, and **10** in the urine of the orally dosed intact rats. All



three of these materials were present in both the free and conjugated forms. No free or conjugated hesperetin-3-^{14}C was detected in the urine.

Incubations with Microflora. A very large percentage (95–100) of hesperetin-3-^{14}C and essentially all its deg-

Scheme I

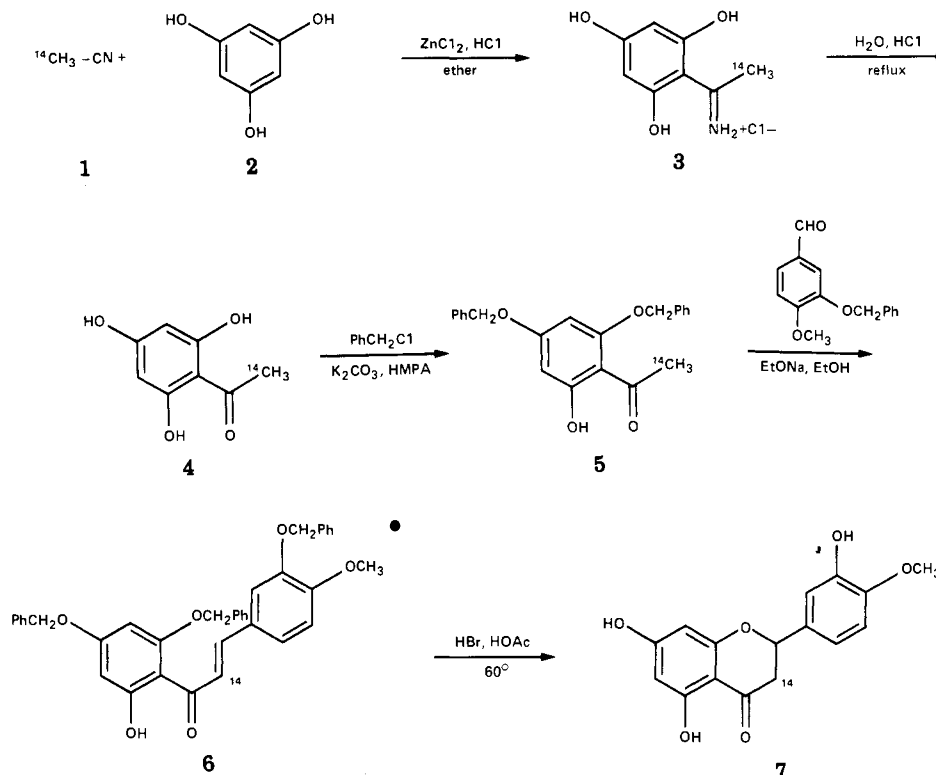


Table IV. Incubation of Hesperetin-3-^{14}C with Rat Cecal Microflora

Sample	Concn, mg/ml	Sp act., μCi/mg	Metabolic products[a,b] 7, R_f 0.35	8, R_f 0.60	9, R_f 0.20	10, R_f 0.47	$^{14}CO_2$
Hesperetin-3-^{14}C	5.0	0.096	93.4	ND[c]	6.5	ND	NT[d]
Control	5.0	0.096	97.8	ND	ND	2.2	NT
Hesperetin-3-^{14}C	0.5	0.32	ND	84.0	ND	15.6	0.37
Control	0.5	0.32	95.4	ND	2.1	2.5	0.02
Hesperetin-3-^{14}C	0.05	6.48	ND	55.5	ND	44.4	NT
Control	0.05	6.48	96.3	3.3	0.3	ND	NT

[a] Data expressed as percent of radioactivity recovered from silica gel fractions. [b] R_f values refer to LQ6DF plates developed with the upper phase of benzene-acetic acid-water (6:7:3). [c] ND = none detected. [d] NT = not tested.

radation products were extracted from the acidified incubations into ether. The quantity of $^{14}CO_2$ produced by gut microbial action was significantly higher than the control, but amounted to an average (n = 2) of only 0.37 mol % of the starting material. The recovery of $^{14}CO_2$ from the barium carbonate-^{14}C controls averaged 75%. The results are summarized in Table IV.

At concentrations of 0.5 mg/ml or below, hesperetin was completely degraded by anaerobic incubation with gut bacteria. The rate of degradation is roughly 3–5 mg per day per g of cecal wet weight. The primary product (ca. 85 mol %) found by TLC analysis at an initial substrate concentration of 0.5 mg/ml was phenylpropanoic acid **8**. A lesser amount of **10** (ca. 15 mol %) was produced. At the lowest concentration tested (0.05 mg/ml), the ratio of these two products was altered. Phenylpropanoic acid **8** accounted for only 55% of the radioactivity, while **10** accounted for about 45%. Curiously, material corresponding in R_f value to dihydroxyphenylpropanoic acid **9** (ca. 6.5 mol %) was observed only at the highest concentration tested and appeared to be the only metabolite present.

DISCUSSION

The present study shows rapid intestinal absorption and subsequent excretion of radioactivity after oral administration of hesperetin-3-^{14}C to rats. The estimated intestinal absorption, based upon radioactivity detected in urine, tissues, and expired carbon dioxide of orally dosed ligated rats, is more than 90% of the dose. This estimate, of course, includes only those metabolites which can be followed by radiotracer methods.

The fact that orally dosed bile duct ligated rats excreted more radioactivity in the urine and less in the expired carbon dioxide than did intact rats suggests enterohepatic circulation of the flavanone and/or its metabolites. Enterohepatic circulation would increase the exposure of the labeled species to the action of degradative intestinal bacterial and hepatic enzymes (Williams et al., 1965).

The primary metabolic product of hesperetin-3-^{14}C found both in the urine of rats after oral administration and after incubation with rat cecal contents is 3-(3-hydroxy-4-methoxyphenyl)propanoic acid (**8**). The labeled flavanone was completely degraded to phenylpropanoic acids, benzoic acids, and carbon dioxide when fed to rats at a dose of 0.3 mg. The lower concentrations of substrate that were incubated with rat cecal contents also resulted in total metabolism. In previous studies reporting urinary excretion of hesperetin, or related compounds, after oral administration to rats (Booth et al., 1958; Griffiths and Smith, 1972), doses of 0.15 to 1.0 g per rat were employed. The total degradation found in this study is quite likely

due to the lower dose mass used.

A significant amount of labeled carbon dioxide was expired by the intact rat which was orally dosed. However, very little radioactive carbon dioxide was produced when hesperetin-*3*-^{14}C was incubated with rat cecal flora. These data suggest that the β oxidation of the propyl chain of the phenylpropanoic acid metabolites is mediated by mammalian and not bacterial enzymes. This has been reported previously by Griffiths and Smith (1972).

Intraperitoneal injection of hesperetin-*3*-^{14}C to a bile duct cannulated rat resulted in excretion of 100% of the administered radioactivity in the bile. These data indicate that no radioactive carbon dioxide was generated because the flavanone did not come into contact with intestinal microflora and undergo metabolism to phenylpropanoic acids. If, as suggested, no phenylpropanoic acids were produced, it appears that the mammalian systems are not capable of carrying out the required acyl (i.e., carbonyl to phloroglucinol ring) cleavage. Scheline (1973) has recently reported similar results for closely related compounds.

For the orally dosed rats, nearly 40% of the radioactivity administered as hesperetin-*3*-^{14}C was expired as carbon dioxide. This means that a minimum of 40% of the dose was metabolized by acyl cleavage and that these products were in turn further metabolized by β oxidation to benzoic acids. This must be considered a minimum value because the degradation of phenylpropanoic acids to benzoic acids will afford labeled acetate that would be included in biochemical pathways other than those resulting in carbon dioxide. At the dose levels employed in these experiments, it is apparent that benzoic acid derivatives, including *m*-hydroxyhippuric acids (Booth et al., 1957), are quantitatively the most significant metabolites of hesperetin-*3*-^{14}C.

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Functional Characteristics of Starches from Proso and Foxtail Millets

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The functional characteristics of proso and foxtail millet starches were determined and compared with those of wheat and rye starches. The millet starches showed higher water binding capacity values and gelatinization temperatures than the wheat starch. With two exceptions, the millet starches produced swelling power values at 90 °C which were similar to those of the wheat starch, but lower than those of the rye starch. Solubilities of the millet starches were lower than those of the wheat starch, except for the starch from one millet variety. Amylograph viscosities of millet starches were higher than those of the wheat starch at all reference points.

Millet is a very important food plant in many parts of the world. In the United States, however, it is a minor cereal crop. Proso (*Panicum milaceum* L.) is the common millet which has been grown since prehistoric times for human use. Foxtail millet (*Setaria italica* (L.) Beauv.) is generally grown for hay or forage (Hinze, 1972).

In the U.S. the proso type is used in feeding rations, as birdseed, and also as a human food. Dehulled proso can be consumed as a puffed cereal or cooked as a hot breakfast cereal. Millet flour can be used as a partial substitute in formulations, which call for wheat flour, to impart a distinct nutlike flavor.

Both proso and foxtail millet are somewhat higher in protein than rice, sorghum, corn, and oats (Matz, 1959). Amino acid compositions of millet varieties have been determined by Mangay et al. (1957), Wilkinson et al. (1968), and Jones et al. (1970). The cereal is higher in ash and fiber compared to other cereals used for human consumption (Hinze, 1972). Very little, however, has been published about the properties of millet starches.

It was the purpose of this investigation to study the functional characteristics of starches isolated from proso and foxtail millet varieties.

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MATERIALS AND METHODS

(A) Sample Identification. Samples selected for this study included six varieties of proso millet: Abarr and