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Bioavailability of Citrus Limonoids in Humans

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This study utilizes liquid chromatography/mass spectrometry (LC-MS) to analyze the plasma of four groups of four healthy male and female subjects administered high doses of pure limonin glucoside (0.25−2.0 g in 200 mL of buffered water) for the presence of limonin to establish the absorption, metabolism, and bioavailability of citrus limonoids to humans. The plasma analysis revealed increasing amounts of limonin associated with increasing doses of limonin glucoside among the subject groups in mean maximum concentration amounts ranging from 1.74 to 5.27 nmol/L. A high degree of variability in the analyzed limonin concentration was observed within the subject groups. The mean time to maximum concentration was 6 h. A second compound with MS/MS characteristics identical to limonin was detected in amounts up to 5.13 nmol/L and is hypothesized to be a limonin epimer. Poststudy health evaluation established no ill effects among study subjects consuming high doses of limonin glucoside.

KEYWORDS: Bioavailability; humans; limonoids; citrus

INTRODUCTION

Citrus fruits and juices have long been recognized to contain secondary metabolites including antioxidants (ascorbic acid, flavanones, simple phenolics), folate, and pectin that are important to human nutrition. Limonoids are secondary metabolites in all citrus fruit tissues whose role in human nutrition has not been established. These triterpenoid compounds occur in citrus as either limonoid aglycones or limonoid glycosides. Limonoid glucosides occur in large amounts in citrus fruit and juice with levels in juice reported to be as high as 500 μg/mL (*1*). Amounts of limonoid glucoside in excess of 2 g/L have been reported in citrus processing byproducts (*2*), and it has been estimated that 15 000 tons of limonoid glucosides are available in the byproducts of the citrus processing industry worldwide. Limonoid glycosides, like limonin glucoside (**Figure 1**), are water soluble, tasteless, nonmutagenic (*3*), and have shown no significant toxic effects when fed to hamsters up to 0.5% of the animal diet (*4*). The limonoid aglycones, like limonin (**Figure 1**), occur at low levels in citrus juices. Several of the water insoluble aglycones are responsible for the development of delayed bitterness in citrus juices in concentrations as low as 6 ppm (*5*).

Several studies have established citrus limonoids as having significant biological activity in mammalian systems. In vivo animal tests with mice have shown that citrus limonoids induce glutathione S-transferase activity (*6*) and inhibit forestomach (*7*), oral (*8*), lung (*9*), skin (*3*), and colon (*10*, *11*) tumors in animals. In vitro studies with human breast cancer cells have shown limonoids to be potent inhibitors of the proliferation of estrogen receptor negative and estrogen receptor positive human breast cancer cells in culture (*12*, *13*). In vitro studies have shown selected citrus limonoids, including limonin (**Figure 1**), to effectively reduce the production of medium apo B in cultured human liver cells HepG2 suggesting that the limonoids could contribute to the cholesterol lowering effect of citrus juices (*14*).

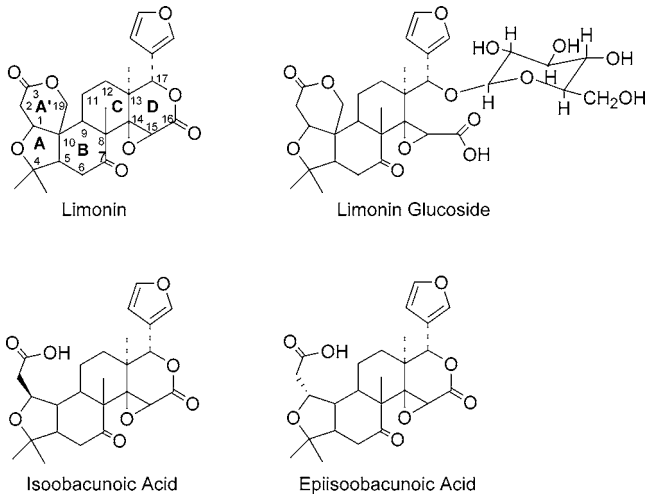

Figure 1. Structures of limonin, limonin glucoside, isoobacunoic acid, and epiisoobacunoic acid.

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The in vivo animal and in vitro cancer cell studies provide evidence that citrus limonoids may act as important chemopreventative agents in mammalian systems. There have been no studies to determine the metabolic fate of citrus limonoids in animals or humans.

Limonin glucoside (**Figure 1**) is the most prominent limonoid glucoside found in citrus juices and is readily available from orange juice or citrus juice processing byproducts (*15*, *16*). In this study, we utilize liquid chromatography coupled to mass spectrometry (LC-MS) to analyze plasma from healthy subjects administered high doses of pure limonin glucoside for the presence of limonoid metabolites, specifically limonin, the limonoid aglycone derived from limonin glucoside. The verification of limonoid metabolites in the plasma of humans establishes the absorption of citrus limonoids by humans and verifies that these biologically active compounds are bioavailable to humans to participate in the improvement of human health and nutrition.

MATERIALS AND METHODS

Subjects and Study Design. The clinical portion of the study was conducted between April and October 2001 at the USDA Western Human Nutrition Research Center (WHNRC), University of California Davis. Candidates recruited from the Davis, CA, area were screened for good health by a medical history questionnaire, physical exam, and standardized blood and urine tests including a complete blood count with leukocyte differential, clinical chemistry panel, urinalysis, and tests for infectious disease. The nine women, body weight 48.0−79.0 kg (mean ± SD = 62.0 ± 9.9), and seven men, body weight 71.8−108.6 kg (mean ± SD = 90.6 ± 12.6) accepted into the study were nonsmokers, age 19−51 years (mean ± SD = 28.9 ± 9.0 years). The study group ethnicity consisted of nine Caucasians, four Hispanics, two Asians, and one East Indian. The study protocol was approved by the Human Subjects Review Committee of the University of California, Davis. All participating subjects signed informed consent before entering the study.

To minimize the possible appearance of dietary limonoids in the blood, the 16 subjects avoided consumption of citrus fruit or juices for 3 days prior to and after the limonoid dose tests. The limonin glucoside solutions administered to the study subjects were prepared in a 0.05 M potassium citrate buffer solution (pH 4.0, sterile water) in concentrations of 1.25, 2.5, 5.0, and 10.0 mg/mL after an overnight fast. Each individual in a subject group consumed 200 mL (0.25, 0.50, 1.0, and 2.0 g, respectively) of the limonin glucoside solutions.

Because data on possible toxicity of purified limonoids to humans were limited, the study protocol required administering the lowest dose of 0.25 g of limonin glucoside to the first four subjects to confirm nontoxicity before continuing the study. The dose was then doubled to 0.5 g for the next four subjects, and this procedure was repeated twice more to a final total of 16 subjects and the highest dose of 2.0 g. Toxicity was evaluated by (i) nursing personnel monitoring subjects for ill effects continuously over the 6 h postdose and at 1 and 7 days postdose and (ii) analyzing blood samples at predose and 1 and 7 days postdose for clinical indicators of toxicity including the liver enzymes alkaline phosphatase and alanine and aspartate aminotransferases.

Sample Collection and Laboratory Methods. After overnight fast, blood samples with ethylenediaminetetraacetic acid anticoagulant were taken by venipuncture before the limonin glucoside dose (predose) and at 1, 3, 6, and 24 h postdose. The blood was immediately processed to remove red cells, and aliquots of plasma were frozen at −70 °C for later analysis for limonoid metabolites. Separate blood samples and random urine samples were taken at predose and 1 and 7 days postdose for clinical measures of health and toxicity. The tests included a complete blood count with leukocyte differential (System 9000 Diff, Serono Baker Diagnostics, Allentown, PA), a serum chemistry panel that included electrolytes, nitrogenous products, glucose, proteins, lipids, and liver enzymes (Synchron LX20, Beckman Coulter, Fullerton, CA), and a dipstick urinalysis (Bayer Corp., Elkhart, IN). Plasma volume was estimated to be 8% of total body water (TBW) where TBW is 54% of body weight (*17*).

Study Standards and Solvents. Pure limonin glucoside was isolated from a mixture of limonoid glucosides obtained from citrus molasses according to our published procedure (*15*). The limonin glucoside was identical in spectral and physical properties to a pure standard available in our laboratory. Pure limonin was available in our laboratory. Podophyllotoxin was purchased from Aldrich Chemical Co., Milwaukee, WI. All chromatographic solvents were high-performance liquid chromatography grade (Fisher, San Jose, CA), and water was distilled and deionized. Tetrahydrofuran (THF) contained no stabilizer.

Preparation and Analysis of Analytical Samples. Podophyllotoxin (10 μL, 1 ng/μL) in THF was added to plasma (1 mL) as an internal standard, and the plasma sample was diluted to 2 mL with 10 mM aqueous ammonium acetate (pH 4.0). The aqueous plasma mixture was applied to a Strata SPE cartridge (Phenomonex, Irvine, CA) (1 mL/30 mg) that had been previously washed with MeOH (2 mL) and 4 mM aqueous ammonium acetate (2 mL). The cartridge was rinsed (in vacuo) with water (2 × 2 mL), and the clarified plasma fraction was eluted into a test tube with MeOH (2 mL). The MeOH fraction was concentrated to dryness under N_2; the dried residue was redissolved in THF (100 μL) and transferred to 300 μL autoinjector microtubes (Waters, Milford, MA). The solution was allowed to evaporate to dryness, and the residue was redissolved in THF (50 μL).

All liquid chromatography was conducted on a Waters 2690 chromatography system with 3 μL injections. Liquid chromatography utilized a Hypersil BDS C-18 (3 μm) reverse phase column (2 mm i.d. × 50 mm) (Keystone, Bellefonte, PA) employing a MeOH/4 mM formic acid gradient (time 0, MeOH:4 mM formic (45:55); time 3 min, MeOH:4 mM formic (30:70); time 4, MeOH:4 mM formic (45:55); 0.4 mL/min, 40 °, total run time = 15 min). All standard solutions were tightly sealed and refrigerated between uses.

Mass spectral analysis was conducted on a ThermoFinnigan LCQ Advantage ion-trap mass spectrometer coupled to the chromatographic delivery system. The mass spectrometer was operated in the electrospray ionization MS/MS mode (capillary temperature, 300 °C; capillary voltage, 20 V; and ion spray voltage, 5.2 kV). Limonin was detected by single ion monitoring (SIM) analysis as a protonated molecule (*m*/*z* 471.2); the mass spectrometer was tuned to maximize a limonin signal in a standard limonin solution (∼1 ng/μL) in the chromatographic solvent. Podophyllotoxin was monitored in the SIM mode at *m*/*z* 397.4. The mass spectrometer was tuned in the MS/MS mode by infusion of limonin and podophyllotoxin solutions (∼1ng/μL, 0.3 mL/min), and the instrument was set to capture the protonated molecules of limonin and podophyllotoxin (mass window = 1.5) and to generate fragment ions (fragmentation energies: limonin, 36.0%, and podophyllotoxin, 38.0%). The resulting product ions were detected by SIM at *m*/*z* 313.1, 351.2, and 379.4 for podophyllotoxin and 425.2 and 367.1 for limonin. MS analysis utilizing the SIM detected product ions of limonin (*m*/*z* 425.2) and podophyllotoxin (internal standard) (*m*/*z* 379.4) was conducted as previously described (*18*) and included bracketed standards and blanks. Five standard solutions of limonin (3.86 pg/μL to 1.5 ng/μL) prepared in THF and containing podophyllotoxin as an internal standard (100 pg/μL) were included before and after plasma sample analysis runs to generate calibration curves for limonin quantification under the same LC-MS conditions as the plasma samples. A blank solution was included between the bracketed standard solutions and the plasma samples.

RESULTS

Calibration curves for all LC-MS analyses of limonin in the plasma samples were linear with correlation coefficients exceeding 0.99. A detection limit of 23 pg (0.05 pmol) was observed for limonin at a 10:1 signal-to-noise ratio.

Poststudy comparisons of blood chemistry values for test subjects were normal and comparable to prestudy values. A poststudy evaluation of the general health of test subjects revealed no adverse effects of limonin glucoside ingestion.

Limonin was detected in the plasma of all but one subject in four subject groups that were administered single bolus 0.25,

Table 1. Plasma Limonin Concentration in Subjects at Different Times after Limonin Glucoside Intake

subject	age	gender	LG dose (g)	body wt (kg)	plasma volume (L)	time (h) 1	3	6[a]	24
1	37	M	0.25	86.7	3.75	ND[b]	ND	1.69 (*6.34*)	ND
2	41	M	0.25	71.8	3.10	ND	ND	1.90 (*5.89*)	ND
3	23	F	0.25	60.3	2.60	ND	ND	0.75 (*1.95*)	ND
4	21	F	0.25	65.8	2.84	ND	ND	2.62 (*7.44*) (1.74, 0.39)	ND
5	19	F	0.50	79.0	3.41	ND	ND	ND	6.14 (*20.93*)
6	31	M	0.50	102.5	4.43	ND	ND	1.20 (*5.32*)	0.68 (*3.01*)
7	22	F	0.50	59.8	2.58	ND	ND	0.92 (*2.37*)	ND
8	28	M	0.50	85.2	3.68	ND	ND	4.90 (*18.03*) (1.77, 1.07)	ND
9	19	M	1.0	106.6	4.61	ND	0.73 (*3.36*)	5.48 (*25.06*)	ND
10	38	F	1.0	51.0	2.20	ND	ND	7.18 (*15.80*)	ND
11	26	F	1.0	69.9	3.02	ND	ND	ND	ND
12	21	M	1.0	100.7	4.35	ND	ND	7.89 (*34.32*) (5.15, 1.77)	1.02 (*4.44*)
13	31	F	2.0	68.9	2.98	0.75 (*2.23*)[c]	0.53 (*1.58*)	5.69 (*16.96*)	0.83 (*2.47*)
14	51	M	2.0	82.9	3.58	1.54 (*5.51*)	7.04 (*25.20*)	0.62 (*2.22*)	ND
15	28	F	2.0	48.0	2.07	1.25 (*2.59*)	0.77 (*1.59*)	8.80 (*18.22*)	ND
16	26	F	2.0	55.5	2.40	ND (0.90, 0.33)[d]	0.63 (*1.51*) (2.24, 1.60)	5.96 (*14.30*) (5.27, 1.70)	1.72 (*4.13*)

[a] One way ANOVA, dose groups 0.25 and 0.50 vs 1.0 and 2.0 not significant ($p = 0.19$) for 6 h only. [b] Not detected at detection limit of 0.05 nmol/L. [c] Concentration in nmol/L (total nanomoles in plasma). [d] (Mean, standard error) for dose group (nmoles/L) assumes 0.05 nmol/L for not detected.

0.50, 1.0, and 2.0 g doses of limonin glucoside (**Table 1**). With two exceptions (subjects 5 and 14), all of the subjects displayed the maximum concentration (C_{max}) of limonin in plasma 6 h (T_{max}) postdose and with the exception of subject 9, only the highest dosed subjects (subjects 13−16) showed the presence of limonin in plasma prior to the 6 h postdose period. Two of the 0.5 g dose subjects (subjects 5 and 6) and three of the 1.0 and 2.0 g dose subjects (subjects 12, 13, and 16) revealed the presence of limonin in plasma at 24 h postdose. The mean C_{max} of limonin of the subject groups increased with the increased doses administered at the 6 h T_{max} (group 1, 1.74 nmol/L; group 2, 1.77 nmol/L; group 3, 5.15 nmol/L; group 4, 5.27 nmol/L). The standard errors of the mean C_{max} of the four subject groups at T_{max} were 0.39, 1.07, 1.77, and 1.70, respectively. An analysis of variance could only be applied to the 6 h T_{max} data because of the large number of nondetectable concentrations at 1, 3, and 24 h postdose. A one way analysis of variance using SAS PROC MIXED (*19*) allowing for incorporation of the heterogeneity of variance of dose groups 0.25 and 0.50 g vs 1.0 and 2.0 g showed no significant variance between the dose groupings ($p = 0.19$). No significant correlation was detected between the detected plasma limonin content and the gender, age, body weight, or plasma volume.

During the LC-MS analysis of the plasma samples of subjects consuming higher levels of limonin glucoside (subjects 10−16), a second peak (**Figure 2**) began to appear with a shorter retention time than limonin but with a protonated molecule and MS/MS product ions identical to those of limonin. The identical nature of this compound's MS/MS character to that of limonin and the chromatographic similarity to limonin suggest this substance to be an epimer of limonin (see Discussion). Therefore, we relied on calibration curves for limonin for the LC-MS quantification of this compound (**Table 2**). This limonin-like material appeared in plasma of all but one (subject 9) of the 1.0 and 2.0 g dose subject groups 1−6 h postdose and was present in low amounts (0.93 nmol/L) in the plasma of subject 11, whose plasma sample showed no limonin. A mean maximum theoretical concentration, 1.24 nmol/L, of this material occurs 1 h postdose in the plasma of subjects administered 1.0 g of limonin glucoside. In the 2.0 g dose group, the theoretical mean maximum concentration for the limonin-like material is 5.13 nmol/L and appears in the 3 h postdose time period. While the material primarily appears in the 1 and 3 h postdose time period for the high dose subjects, it persists into the 6 h postdose time period for two of the 1.0 and 2.0 g dose subjects. An analysis of variance of the 1 and 3 h data applying SAS PROC MIXED revealed that dose, hour, or interaction were not significant ($p > 0.11$) between the groupings.

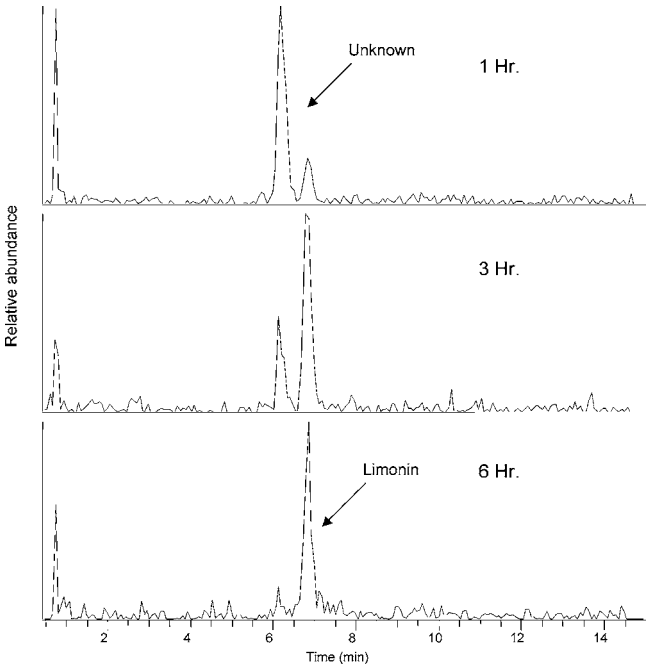

Figure 2. LC-MS analysis of plasma of subject 15 with single ion monitoring of *m/z* 425.2 product ion from MS/MS fragmentation of *m/z* 471.2 protonated molecular ion 1, 3, and 6 h postlimonin glucoside administration.

DISCUSSION

Although in vivo animal tests to assess the antitumor activity of limonoids have been conducted, no information exists about the absorption and metabolic fate of limonoids in mammalian

Table 2. Relative Calculated Plasma Concentration of Uncharacterized Limonoid in Subjects at Different Times after Limonin Glucoside Intake Based on Calibrated Limonin Standard Curves[a]

		time (h)			
subject	LG dose (g)	1	3	6	24
10	1.0	2.07 (*4.55*)[b]	0.77 (*1.69*)	ND[c]	ND
11	1.0	0.66 (*1.99*)	0.93 (*2.81*)	ND	ND
12	1.0	2.18 (*9.48*)	1.37 (*5.96*)	ND	ND
13	2.0	4.25 (*12.67*)	1.42 (*4.23*)	ND	ND
14	2.0	4.97 (*17.79*)	12.60 (*45.11*)	1.75 (*6.26*)	ND
15	2.0	9.67 (*20.02*)	4.13 (*8.55*)	0.05 (*0.10*)	ND
16	2.0	1.01 (*2.42*)	2.37 (*5.69*)	3.22 (*7.73*)	ND

[a] Limonin epimer not detected in subjects 1–9. [b] Concentration in nmol/L (total nanomoles uncharacterized limonoid in plasma). [c] Not detected (amount less than detection limit of instrument).

systems. The goals of this study were to establish that citrus limonoids are absorbed and metabolized in humans and to obtain benchmark information about limonoid metabolism in humans in order to expand our knowledge of the role of these compounds in human health and nutrition.

While citrus limonoids have been tested as antitumor agents in animals, no information exists about biologically active limonoid metabolites. Lacking this information, we decided to utilize existing information about plant secondary metabolites with recognized biological activity in humans as a model for the development of an experimental plan for this study. Polyphenols are aromatic, acidic, polar compounds that commonly occur in fruit and vegetables as glycosides (i.e., flavanone glycosides in citrus). Limonoid glucosides in citrus fruit and juices have aromatic character and are acidic, polar compounds. On the basis of the general chemical comparison and the commonality of their occurrence in citrus sources, information about the metabolism of polyphenols was chosen as the model in developing the experimental approach in this study.

It is recognized that metabolism of polyphenolic substances in animals and humans involves chemical conjugation of the polyphenols (*20*). In the case of polyphenolic glycosides, metabolism in animals and humans usually involves initial glycosidase cleavage of the phenol−glycoside linkage with subsequent reaction/transformation to form the conjugated polyphenols most commonly detected in plasma. It has also been observed that the administration of high doses of polyphenol aglycones to humans can lead to the appearance of free polyphenol aglycones in human plasma (*21*, *22*). These phenomena may be the result of saturation of the polyphenolic metabolic pool. The availability of large quantities of pure limonin glucoside in our laboratory and the research information related to large doses of polyphenolics guided our decision to administer high doses of limonin glucoside to human subjects and to utilize the sensitivity of LC-MS to analyze the plasma of test subjects for the presence of limonin as a biomarker to establish limonoid absorption, metabolism, and bioavailability. No attempt is made in this study to provide comprehensive identification and pharmacokinetic analysis of the limonin glucoside metabolites.

LC-MS analysis of plasma from subjects in four groups administered limonin glucoside in high doses revealed the presence of limonin in low nmol/L amounts in all but one subject. This establishes that limonin glucoside is absorbed and metabolized in humans and that a metabolic product of limonin glucoside (limonin) is bioavailable to humans. Significant variation in the amount of bioavailable limonin was observed among subjects within each group. Differences in lifestyle behavior, diet, and metabolism of the test subjects were not monitored or controlled and may have contributed to the observed variation since gender, age, body weight, and plasma volume were not found to be significant factors. Similar variations have been observed in a study of the bioavailability of naringenin and hesperetin conjugates from orange juice (*23*). The mean detected amount of limonin per subject group closely followed the increase in dose among the groups administered 0.25, 0.50, and 1.0 g of limonin glucoside. The maximum detected amount of limonin in the highest dose group (2.0 g) was only slightly higher than the group administered 1.0 g, but subjects in this group were the only subjects that had detectable plasma limonin in the time periods 1 and 3 h after dosing.

The appearance of a second compound with MS/MS characteristics identical to limonin was remarkable and allows reasonable speculation about the chemical character of this compound and its place in limonin glucoside to limonin metabolism in humans. The exact mass spectral comparison of limonin to the unknown compound strongly suggests that the compound may be a stereoisomer (epimer) of limonin. Limonin related citrus limonoids (isoobacunoic acid and epiisoobacunoic acid (**Figure 1**)) epimeric at C-1 have been identified in citrus (*24*); however, no limonoids epimeric at C-17 have been reported. In the case of the limonin-like unknown (*m*/*z* 471.2) detected in the high dose subject groups in this study, the creation of a C-17 epimer of limonin can be mechanistically rationalized to proceed through glucoside hydrolysis of limonin glucoside followed by lactonization to form limonin and its C-17 epimer (epilimonin) (**Figure 3**). Epilimonin would display the furan ring at C-17 in a β-orientation rather than the α-orientation

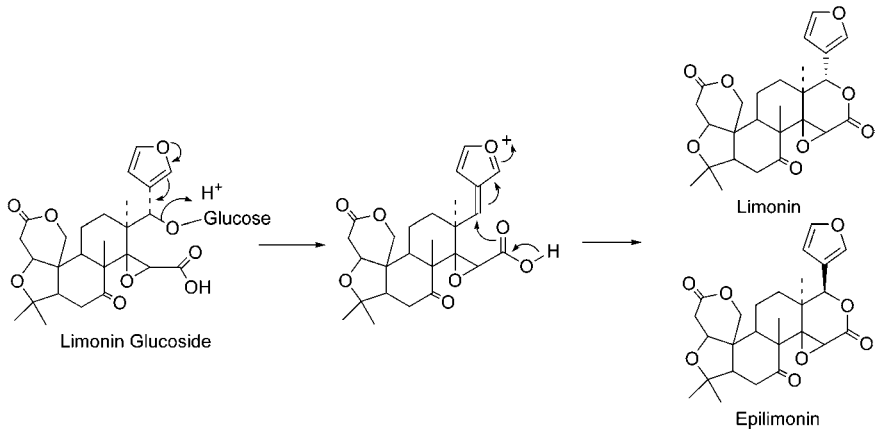

Figure 3. Proposed mechanism for the formation of limonin and epilimonin from limonin glucoside.

of limonin. It would be expected that epilimonin would have the exact mass spectral properties as limonin and slightly different chromatographic properties. On the basis of speculation of the existence of this compound as an epimer of limonin, a theoretical analysis of occurrence of this compound utilizing calibration curves for limonin is presented in **Table 2**. The appearance of larger theoretical amounts of this compound prior to that of limonin in the two highest dose rate subject groups suggests that the compound may emanate from a secondary saturation of the metabolic pool producing limonin. The observed increase in the mean theoretical amount of this compound (1.24−5.15 nmol/L) among the high dose rate (1.0 and 2.0 g) subjects while the mean maximum amount of limonin remains nearly the same (5.15−5.27 nmol/L) would be consistent with this scenario. While the chemical structure of the unknown compound cannot be conclusively related to limonin on the basis of the information available, its appearance in the plasma of the human subjects confirms that another compound with the same molecular weight as limonin is produced in humans administered high doses of limonin glucoside. Previous reports of epimerization of chiral centers of steroidal (*25*), prostaglandin (*26*), imidazole (*27*), and alkaloid (*28*) drug metabolites in humans illustrates epimerization in human metabolism and supports the theory that the epimerization of limonin to epilimonin may proceed in the metabolism of limonin glucoside by humans.

While the detection of limonin in the plasma of human subjects administered limonin glucoside confirms that limonoids are absorbed and metabolized in humans, it does not provide significant evidence of the mode of the absorption and metabolism of these compounds. In the case of both polyphenolic aglycones and glycosides, it is recognized that absorption and metabolism at common dietary levels include glycoside hydrolysis and subsequent conjugation to glucuronides or sulfates with or without methylation (*29*−*31*). At normal dietary levels, phenolic glycosides can appear unchanged in human plasma (*32*) or low levels of the polyphenol aglycones can appear (*22*). In the case of the high dose administration of limonin glucoside to humans, limonin glucoside is found to undergo hydrolysis and cyclization to form the limonoid aglycone limonin and a suspected epimer of limonin. The presence or absence of limonin conjugates or limonin glucoside conjugates in subjects administered high doses of limonin glucoside cannot be confirmed since no conjugates of these compounds have been reported, and at present, no standards or analytical methods exist for their detection or quantification.

Limonoid glucosides are naturally available in orange juices in amounts averaging 320 mg/L, with limonin glucoside representing 56% (180 mg/L) of the average total limonoid glucoside mixture (*1*). The lowest dose of limonin glucoside administered in this study, 0.25 g/200 mL, is comparable to the amount of a natural mixture of all limonoid glucosides in about four glasses of orange juice and is equivalent to the natural amount of limonin glucoside in about seven glasses of orange juice. The mean total plasma level of limonin for the four subjects consuming the lowest dose of limonoid glucoside was 5.04 nmol 6 h after dosing. This level of limonin represents 1.32×10^{-3}% of the potential limonin available in the 0.25 g dose of limonin glucoside administered. The detected level of limonin in the plasma at 6 h for the subjects fed the lowest dose of limonin glucoside indicates that limonin would be bioavailable from orange juice at this consumption level in nutritional as well as pharmacological levels. Because the identity and activity of metabolic conjugates of citrus limonoids have not been established, the level of limonin may not reflect the potential therapeutic concentration of most active limonoid glucoside metabolites. In light of the in vivo and in vitro validation of limonoids as effective antitumor agents, the significance of limonoid aglycones levels in plasma to human health will emerge from further studies that examine the detailed phamacokinetics and metabolic fate of the citrus limonoids in humans and the characterization and quantification of the most bioactive forms of these compounds.

This documentation of the bioavailability of citrus limonoids in humans and the recognized biological activity of these compounds in mammalian systems is consistent with their prominent participation in the matrix of phytochemicals in citrus that contribute to improving human health and nutrition. The potential abundant supply of nontoxic, water soluble limonoid glucosides from citrus processing industrial byproducts endorses the reclamation of these compounds for use as nutraceuticals or as healthful fortifiers in functional foods.

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