JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

# Identification and Urinary Excretion of Metabolites of 5-(Hydroxymethyl)-2-furfural in Human Subjects following Consumption of Dried Plums or Dried Plum Juice

RONALD L. PRIOR,*,†,‡ XIANLI WU,‡ AND LIWEI GU‡

U.S. Department of Agriculture, Agriculture Research Service, Arkansas Children's Nutrition Center, 1120 Marshall Street, Little Rock, Arkansas 72202, and USDA Human Nutrition Research Center on Aging at Tufts, Boston, Massachusetts 02111

5-(Hydroxymethyl)-2-furfural (**I**) is a major breakdown product occurring in solutions with high concentrations of fructose and glucose and is present in many fruit juices, in heat-sterilized parenteral solutions, and in baby cereals. The objective of this study was to characterize and identify 5-(hydroxymethyl)-2-furfural metabolites in human subjects following the consumption of dried plum juice and/or dried plums. Subjects were fasted overnight and blood and urine samples were obtained during the day following consumption. Subjects fed the dried plum juice and dried plums consumed 3944 $\mu$mol (497 mg) and 531 $\mu$mol (67 mg) of **I**, respectively. Four presumed metabolites of **I** were detected in the urine of subjects that consumed dried plum juice. They were tentatively identified using HPLC-MS/MS as (1) *N*-(5-hydroxymethyl-2-furoyl)glycine (**III**), (2) 5-hydroxymethyl-2-furoic acid (**II**), (3) (5-carboxylic acid-2-furoyl)glycine (**IV**), and (4) (5-carboxylic acid-2-furoyl)aminomethane (**V**). Total urinary excretion during the 6 h following the consumption of dried plum juice was 168, 1465, 137, and 75 $\mu$moles on the basis of **II** as a standard for **II**, **III, IV**, and **V**, respectively. The estimated total recovery of **I** metabolites was 46.2% and 14.2% of the **I** dose during the first 6 h after consumption of dried plum juice and dried plums, respectively. **I** seems to be metabolized rapidly to glycine conjugates and other metabolites and excreted in the urine.

**KEYWORDS: Hydroxymethylfurfural; prunes; human subjects**

## INTRODUCTION

Dried plums and dried plum juice contain a number of phytochemicals that have antioxidant activity, particularly chlorogenic acid and its isomers (*1*). During the studies of metabolism of phytochemicals in dried plum or dried plum juice, we observed the appearance of some unknown metabolites in urine following the consumption of dried plum juice. It is known that dried plums and dried plum juice have considerable amounts of 5-(hydroxymethyl)-2-furfural (**I**) **(Figure 1**) (*1*), and our preliminary observations indicated that some of the metabolites that were observed were derived from **I**. **I** is not a phenolic compound and does not exhibit antioxidant activity by in vitro antioxidant capacity assays. **I** is a major breakdown product occurring in solutions high in fructose and glucose. Most of the formation of **I** occurs during heating or autoclaving, but spontaneous breakdown also takes place. **I** also is present in many fruit juices, heat-sterilized parental solutions (*2*), infant formulas (*3*), and baby cereals (*4*). Low pH promotes heat-induced hexose decomposition to furan derivatives. Ulbricht et al. (*5*) estimated that humans may ingest up to 150 mg of **I**/day. Thus, **I** is an important artificial compound in the human diet.

The objective in this study was to identify and quantify, using HPLC-MS/MS, compounds in urine that appeared to be metabolites of **I** following the consumption of dried plums or dried plum juice. To our knowledge, this is the first report of **I** metabolism in human subjects.

## MATERIALS AND METHODS

**Subjects.** Six healthy women (65−70 y; body wt = 66.2 ± 1.1 kg) participated in this study. All study participants were considered in good health on the basis of a medical history questionnaire, physical examination, and normal results of clinical laboratory tests. All of the subjects fulfilled the following eligibility criteria: no history of cardiovascular, hepatic, gastrointestinal, or renal disease; no alcoholism; no antibiotic or supplemental vitamin and/or mineral use at least 4 weeks prior to the start of the study; no smoking. The study protocol was approved by the Human Investigation Review Committee of Tufts University and the New England Medical Center, and written informed consent was obtained from each study participant.

**Study Design.** Each participant was admitted to the Metabolic Research Unit (MRU) at the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts 24 h before the day of sampling. In the evening before the day of sampling, subjects were fasted overnight. In the morning of the sampling day, an intravenous catheter was inserted

* Corresponding author. Tel: 501-354-2747. Fax: 501-364-2818. E-mail: priorronaldl@uams.edu.
† U.S. Department of Agriculture, Agriculture Research Service.
‡ Arkansas Children's Nutrition Center.

10.1021/jf0601113 CCC: $33.50 
Published on Web 04/18/2006

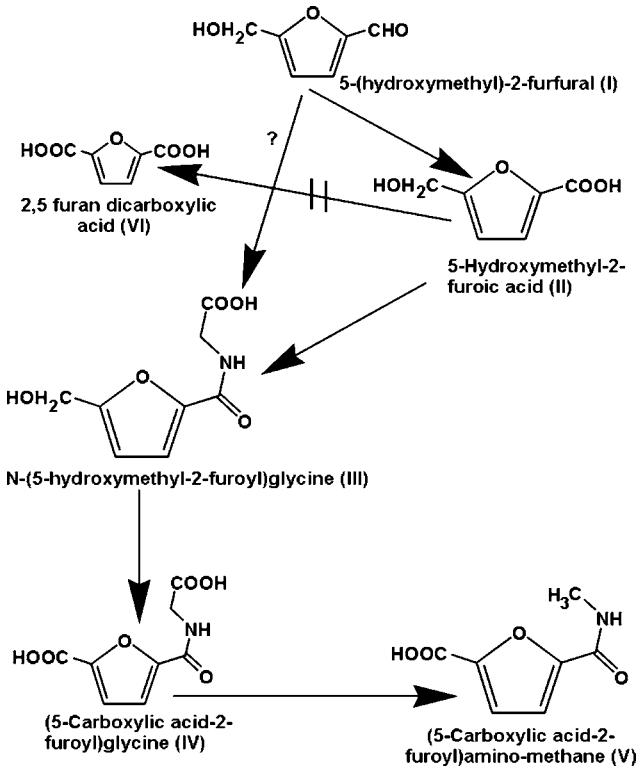


**Figure 1.** Structures of 5-(hydroxymethyl)-2-furfural (**I**) and proposed metabolism in subjects consuming dried plums or dried plum juice.

**Table 1.** Concentrations and Amount Consumed of 5-(Hydroxymethyl)-2-furfural (**I**), Chlorogenic Acid Isomers, $ORAC_{FL}$, and Total Phenolic Components Found in Dried Plum Juice and Dried Plums

| | dried plum juice | | dried plums | |
|---|---|---|---|---|
| component | /mL | consumed | /g | consumed |
| chlorogenic acid, mg | 0.057 | 21.2 | 0.037 | 4.7 |
| chlorogenic isomers, mg | | | | 7 |
| 3-*O*-caffeoylquinic acid | 0.236 | 74.3 | 0.573 | 5.1 |
| 4-*O*-caffeoylquinic acid | 0.224 | 70.6 | 0.329 | 43.1 |
| 5-(hydroxymethyl)-2-furfural, mg | 1.577 | 497 | 0.467 | 61.2 |
| 5-(hydroxymethyl)-2-furfural, mmol | | 3994 | | 486 |
| antioxidant capacity, mmol of TE[a] | 21.3 | 6716 | 82.4 | 10794 |
| tot. phenolics, mg | 3.5 | 1102 | 14.0 | 1835 |

[a] TE: trolox equivalent.

into one forearm. A 15 mL heparin blood sample (zero baseline sample) was obtained from each fasting subject, following which the subjects were given one of three dietary treatments: control, 315 mL of water; 315 mL of dried plum juice; dried plums (131 g blended in 315 mL of water). The amounts of **I**, other major compounds, total phenolics, and antioxidant capacity ($ORAC_{FL}$) (*6*, *7*) consumed are presented in **Table 1**. Additional blood samples were obtained at 0.25, 0.5, 1, 2, 3, 4, and 6 h after consumption of the drink. An interval of at least 2 weeks occurred between each treatment, and the order of administering the treatments was randomized for each subject. Urine samples were collected from these subjects before and at 0−2, 2−4, and 4−6 h after the consumption of each treatment.

A lunch was given following the blood sampling at 4 h. The diets provided to these subjects during their residency in the MRU were designed to be low in flavonoids and other phenolics and **I** but provide the recommended dietary allowance for protein and energy. All meals were prepared under the supervision of a dietitian in the MRU. The consumption of water was not limited. Other foods or beverages were not allowed during the residency.

**Chemicals and Standards.** Ammonium acetate, lithium acetate, and sulfatase (type H-5) were purchased from Sigma Chemical Co. (St. Louis, MO). 5-Hydroxymethyl-2-furoic acid (**II**) and 5-(hydroxymethyl)-2-furoyl glycine (**III**) (**Figure 1**) were provided by SRI International (Menlo Park, CA) through Midwest Research Institute (Kansas City, MO). All other solvents were from Fisher Scientific (Fair Lawn, NJ).

**Analysis of Urine.** *Separation of Urine Metabolites Using HPLC with Electrochemical Detection.* Urine (500 $\mu$L) was added to 500 $\mu$L of ammonium acetate buffer (1 M, pH 6) in a disposable culture tube. Samples to be treated with enzyme were added to ammonium acetate buffer containing the enzyme. The enzyme solution was prepared by adding 1.65 mg of sulfatase (type H-5)/500 $\mu$L of ammonium acetate buffer. The nonenzyme and enzyme-treated samples were placed in a 37 °C water bath for 3 h. After the incubation period the samples are treated with 10 $\mu$L of HCl (20 $\mu$L + 1 mL $H_2O$) (pH < 3) to stop the enzyme reaction.

Analysis of urine was carried out on an ESA-HPLC with a Coularray detector with a 250 mm × 4.6 mm i.d. Zorbax SB-$C_{18}$ column plus a UV detector (ESA Biosciences, Chelmsford, MA). Buffer A in the HPLC gradient contained methanol/acetic acid/water (5/2/93, v/v) with 50 mM lithium acetate (pH 3.8). Buffer B consisted of methanol/acetic acid/water (93/2/5, v/v) with 50 mM lithium acetate (pH 5.5). Both buffers were filtered through Sep-Pak C18 cartridges (Waters, MA) before using. The HPLC gradient consisted of 0% B from 0 to 18 min, 0 to 20% B from 18 to 68 min, and 20 to 70% B from 68 to 98 min. The detector cells were cleaned for 1 min, and then the gradient went from 70 to 0% B in 10 min to prepare for the injection of the next sample. Cell potentials were set at 0, 60, 125, 190, 255, 320, 385, 450, 515, 580, 645, 710, 775, and 840 mv. The initial two cells were used to collect output data from a UV detector set at 260 and 320 nm. Compounds **II** and **III** were quantified against their standards, and **IV** and **V** were estimated using **II** as standard, assuming a similar UV absorption at 260 nm.

*HPLC-MS/MS Analysis.* Numerous conditions were tested in an attempt to find a solvent system that was compatible with the mass detector but also would reproduce the UV profile observed with the UV and Coularray detection system used for quantitating the metabolites. Removing the lithium acetate salt, which is required for the Coularray buffer system, changed the elution pattern of several peaks. However, if we used ammonium acetate in the place of lithium acetate and matched the pH in the mobile phase, we could come close to reproducing the Coularray profile but still have a buffer system that worked quite well with the mass spectrometer. The MS analysis of urine **I** and metabolites was carried out on an HP series 1100 HPLC system including an autosampler, a binary pump, a 250 mm × 4.6 mm i.d. Zorbax SB-$C_{18}$ column, and a diode array detector (Agilent Technologies, Palo Alto, CA). Mass spectrometry was performed with an Esquire-LC mass spectrometer (MS) (Bruker Daltonics, Billerica, MA), an ion trap instrument equipped with an electrospray interface. Using separate injections, column effluent was monitored in either positive or negative ion mode of the MS. The MS was set to perform auto MS/MS. Major MS parameters in positive ion mode were the following: capillary, −3500 V; nebulizer, 40 psi; dry gas, 9 L/min; dry temperature, 330 °C; trap drive, 33.4; end plate offset, −66 V; skim 1, 9.4 V. Major MS parameters in the negative ion mode were the following: capillary, +3500 V; end plate offset, −67.8 V; skim 1, −18.6 V.

*Analysis of Plasma.* Plasma was analyzed using an ESA-HPLC with a Coularray detector plus a UV detector (ESA Biosciences, Chelmsford, MA) and conditions similar to that for urine described earlier. Salicylic acid was used as an internal standard.

## RESULTS AND DISCUSSION

Subjects given dried plum juice consumed 3944 $\mu$mol (497 mg) of **I**, and those given dried plums consumed 486 $\mu$mol (61.2 mg) of **I** (**Table 1**). In our analysis of the data, the focus was on evaluating those peaks appearing in the chromatogram of urine following consumption of dried plum juice that were absent or present at extremely low concentrations in control urine samples. If the urinary excretion of new compounds with significant UV absorption at 260 nm was high, relative to

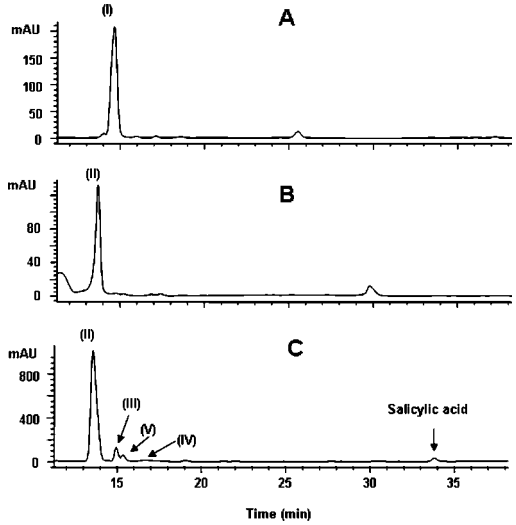


**Figure 2.** Representative HPLC chromatograms of 5-(hydroxymethyl)-2-furfural (**I**) in dried plum juice (A), 5-hydroxymethyl-2-furoic acid (**II**) in serum (B), and of metabolites in urine at 0–2 h at 260 nm (C).

**Table 2.** 5-(Hydroxymethyl)-2-furfural (**I**) Metabolites and Estimated Metabolite Excretion during the First 6 h after Consumption of Water, Dried Plums, or Dried Plum Juice

| | micromol excreted/6 h following meal[a] | | |
|---|---|---|---|
| metabolite | control | dried plum | dried plum juice |
| **IV**[b] | 1.7 ± 1.7 (1/6) | 4.3 ± 2.7 (2/6) | 168 ± 39 (6/6) |
| **II** | 5.5 ± 0.9 (6/6) | 36.8 ± 9.0 (6/6) | 1465 ± 53 (6/6) |
| **III** | 0 (6/6) | 6.3 ± 3.6 (4/6) | 137 ± 32 (6/6) |
| **V**[c] | 17.5 ± 8.2 (3/6) | 21.7 ± 8.0 (5/6) | 75 ± 7 (6/6) |

[a] Calculated quantities (mean ± SEM, $\mu$mol) of all metabolites were based upon **II** as standard. Number of subjects with metabolite detected in urine is shown in parentheses. [b] Tentatively identified as (5-carboxylic acid-2-furoyl)glycine (**IV**). [c] Tentatively identified as (5-carboxylic acid-2-furoyl)aminomethane (**V**).

controls, and changed with time following consumption of dried plum juice, they were considered as possible **I** metabolites. Some of these metabolites could be detected following the consumption of dried plums, but generally these were quite low or nondetectable. This would be predicted if they were metabolites of **I** since the quantity of **I** consumed was more than 8× higher with dried plum juice compared to dried plums (**Table 1**).

Four metabolites of **I** were identified in the urine on the basis of MS data, UV spectra on a diode array detector, information found in the literature, and standards which were obtained for two of the compounds (**Figures 1**−**7**). They are presumed to be **I** metabolites since they were structural derivatives of **I** and did not exist in either dried plum juice or in the control urine. One metabolite which we have been able to identify was an oxidized product of **I** was 5-hydroxymethyl-2-furoic acid (**II**) (**Figure 2**). This was the major metabolite of **I** with 1465 $\mu$mol excreted in the first 6 h after consumption of dried plum juice (**Table 2**) or about 36.9% of the dose of **I**. All six subjects consuming dried plums also had increased excretion of **II** compared to controls (36.8 vs 5.5 umol/6 h). Compound **II** was identified on the basis of mass spectral data and retention time compared to an authentic standard. Compound **II** had an *m*/*z* of 140.8, and a daughter ion was formed having an *m*/*z* of 96.8 (**Table 2**; **Figure 3**). The largest quantities were excreted in the first 2 h after consumption of dried plum juice (**Figure 3**).

Compound **II** was also a significant metabolite which appeared in plasma (**Figure 4**). A peak plasma concentration of approximately 120 $\mu$M was observed after 30 min, and

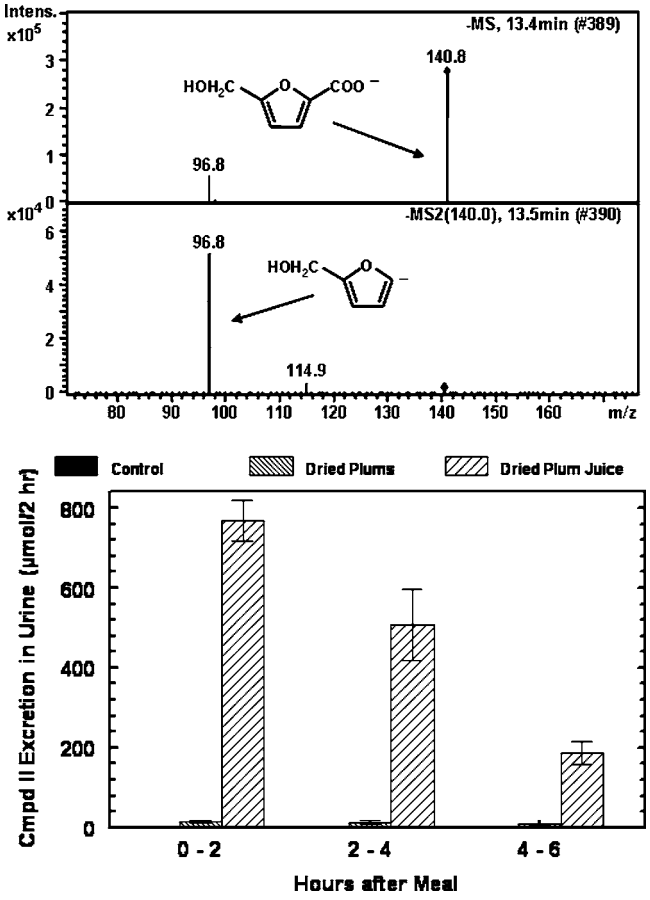


**Figure 3.** MS, MS/MS and urinary excretion of 5-hydroxymethyl-2-furoic acid (**II**) in human subjects following dried plum juice consumption. The quantity of 5-hydroxymethyl-2-furoic acid (**II**) excreted is calculated using **II** as a standard.

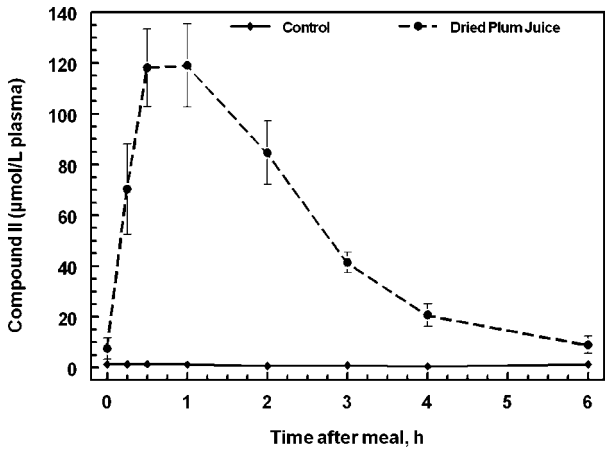


**Figure 4.** Plasma concentrations ($\mu$mol/L) of 5-hydroxymethyl-2-furoic acid (**II**) in human subjects following consumption of dried plum juice. The quantity of 5-hydroxymethyl-2-furoic acid (**II**) in plasma was calculated using 5-hydroxymethyl-2-furoic acid (**II**) as a standard. The area under the plasma curve was 318 $\mu$mol/(L·h).

concentrations began to decline 60 min after consumption of the dried plum juice. By 6 h the plasma levels of **II** were nearly back to baseline.

A second metabolite was also identified on the basis of mass spectrometric data and retention time compared to an authentic standard as a glycine conjugate, 5-(hydroxymethyl)-2-furoyl glycine (**III**) (**Table 2**; **Figures 1** and **5**) with an *m*/*z* of 197.9 and three daughter ions with an *m*/*z* of 168.1, 153.9, and 123.9.

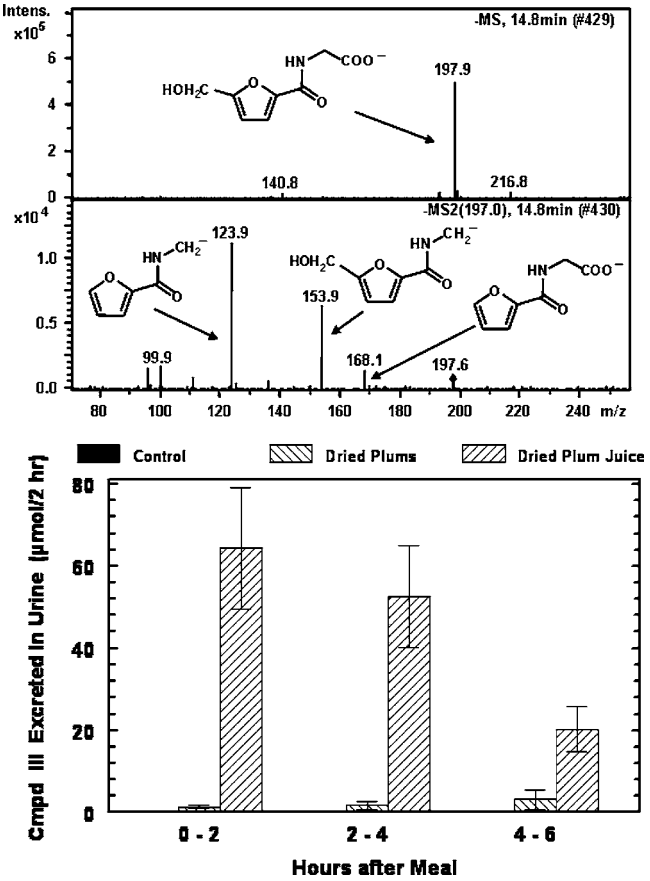


**Figure 5.** MS, MS/MS, and urinary excretion of *N*-(5-hydroxymethyl-2-furoyl)glycine (**III**) in human subjects following consumption of water, dried plums, or dried plum juice. The quantity of **III** excreted was calculated using 5-hydroxymethyl-2-furoic acid (**II**) as a standard.

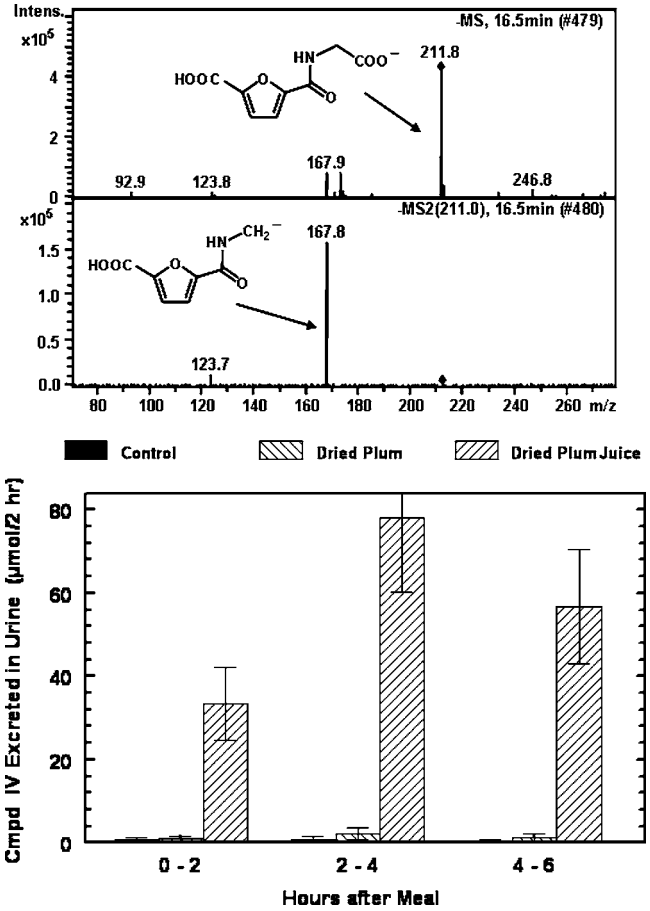


**Figure 6.** MS, MS/MS, and urinary excretion of a metabolite of 5-(hydroxymethyl)-2-furfural (**I**) tentatively identified as (5-carboxylic acid-2-furoyl)glycine (**IV**) in human subjects following consumption of water, dried plums, or dried plum juice. The quantity of **IV** excreted was calculated using 5-hydroxymethyl-2-furoic acid (**II**) as a standard.

This appeared to be a metabolite of **I** with 137 ± 32 $\mu$mol (3.4% of **I** dose) recovered in the urine during the first 6 h (**Table 2**; **Figure 5**). Four of the six subjects that consumed dried plums also had **III** in the urine, but only 6.3 $\mu$mol was recovered in the urine during the first 6 h (**Table 2**). None of the control subjects had detectable levels of **III** in the urine.

A third metabolite was observed to have a parent ion *m*/*z* of 211.9 and two daughter ions with *m*/*z* of 167.9 and 123.9 (**Figure 6**). This metabolite had two daughter ions with the same *m*/*z* as **III** (**Figure 5**). In addition, its UV spectrum was almost identical with that of **III**. All these observations indicated that the structure was very similar to that of **III**. On the basis of this information and its molecular weight, this metabolite was tentatively identified as (5-carboxylic acid-2-furoyl)glycine (**IV**) (**Table 2**; **Figure 6**). The proposed MS/MS fragment of this metabolite is shown in **Figure 6**. The excretion of **IV** was different from the other metabolites in that the amount excreted was highest in the second 2 h period and it declined in the subsequent 2 h period. Total recovery in the urine during the first 6 h after dried plum juice consumption was calculated to be 168 $\mu$mol (**Table 2**) or approximately 4.2% of the **I** dose. Compound **IV** was detected in the urine of 1 control subject and 2 of the subjects that consumed dried plums.

A fourth metabolite had an *m*/*z* of 167.8 and a daughter ion *m*/*z* 123.9 (**Figure 7**). On MS/MS, this compound also showed a strong daughter ion (*m*/*z* 123.9) which also appeared as a major daughter ion of **III** (**Figure 5**) and a minor daughter ion of compound **IV** (**Figure 6**), indicating that it must have the same core structure. From molecular ion to daughter ion, a 44 Da fragment was lost, which was most likely a carboxyl group. Thus, this metabolite has tentatively been identified as 5-hydroxymethyl-2-furoyl)aminomethane (**V**) (**Figure 1**). Compound **V** was excreted in smaller quantities with an average of 75 $\mu$mol excreted (1.8% of **I** dose) in subjects that consumed dried plum juice (**Table 1**). Surprisingly, there were detectable levels of compound **V** in 3 of 6 of the control subjects (**Table 2**).

Godfrey et al. (*8*) identified and quantitated three metabolites, 2,5-furan dicarboxylic acid (**VI**) (**Figure 1**) and compounds **II** and **III** in the urine of rats and mice. Formation of **III** was inversely proportional to dose in rats (*8*, *9*) but not mice. There does not seem to be any evidence for the formation of glucuronide or sulfate conjugates of any of these metabolites in mice or rats or from our data in humans. Incubation of urine samples with a mixture of $\beta$-glucuronidase and sulfatase did not significantly change the area of any of the compound **I** related peaks detected (data not presented). Therefore, we concluded that no glucuronide or sulfate conjugates of **I** were formed. Earlier, Germond et al. (*9*) demonstrated in rats that **I** was completely converted to two metabolites, which were identified by nuclear magnetic resonance (NMR) and MS as **II** and **III**. Administration of high doses of **I** showed a similar rapid elimination but a proportional reduction of the amount of the glycine conjugate produced. From our data, the ratio of **II**/**III** excreted in the urine was 10.7 in subjects consuming dried plum juice and 5.8 in subjects consuming dried plums where **I** intakes were 3944 and 531 $\mu$mol from dried plum juice and dried plums, respectively. This agrees with previous data in rats suggesting that increased **I** intake reduces formation of the

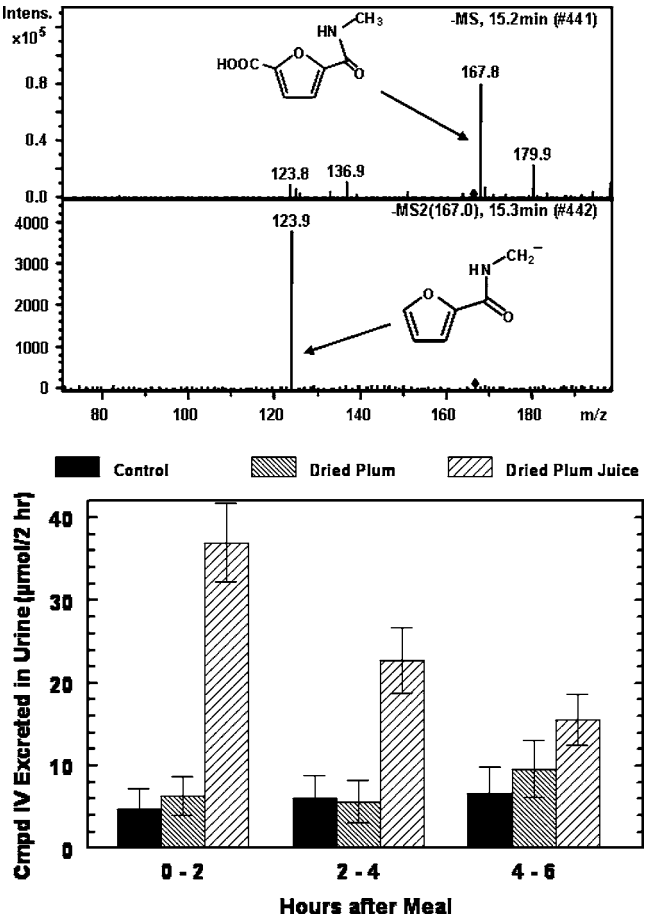


**Figure 7.** MS, MS/MS, and urinary excretion of a metabolite of 5-(hydroxymethyl)-2-furfural (**I**) tentatively identified as (5-carboxylic acid-2-furoyl)aminomethane (**V**) in human subjects following dried plum juice consumption. The quantity of **V** is based upon 5-hydroxymethyl-2-furoic acid (**II**) as a standard.

glycine conjugate (*8*, *9*). We did not find any evidence for the presence of **VI** in the urine of human subjects, which was found in mice and rats (*8*). However, compound **IV** seems to be a metabolite that is present in the human (**Figure 6**) but not in mice or rats. Godfrey et al. (*8*) reported that absorption of **I** was rapid in male rats and mice. Excretion was primarily via the urine in both rats and mice, with 60−80% of the administered dose excreted by this route within 48 h. Germond et al. (*9*) found a higher elimination of **I** or its metabolites in the urine with a recovery of 95−100% after 24 h in rats. We estimated that the recovery of **I** metabolites was 46.2% and 14.2% for dried plum juice and dried plums, respectively, during the first 6 h following the meal.

Compound **I**, a heat-induced decomposition product of hexoses, is present in various foods and drinks (*4*, *10*−*12*). There has been some concern expressed historically concerning possible toxicity issues with **I** (*5*). On the basis of infusion studies with rats and dogs, compound **I** does not appear to be toxic at concentrations ordinarily encountered (e.g., 10 mg/L). However, dried plum juice contained over 1500 mg/L (**Table 1**). The amount of compound **I** consumed by subjects in this study was approximately 7.5 mg/kg (**Table 1**). Dosages of parenterally administered **I** exceeding 75 mg/kg body weight have led to some toxic effects, including increased activity of hepatic enzymes, altered serum-protein fractions, increased relative spleen weight, and hepatic fatty degeneration. After review of available literature, Ulbricht et al. (*5*) concluded that, from a clinical standpoint, the amount of **I** formed as a result of the heat sterilization of parenteral solutions containing hexoses does not seem to pose any significant toxicological problems. Janzowski et al. (*13*) also concluded that **I** does not pose a serious health risk, even though the highest concentrations in specific foods approach the biologically effective concentration range in cell systems. Zhang et al. (*14*) concluded that **I** could act to both initiate and promote formation of aberrant crypt foci in rats, although the doses studied were quite high (1% in the diet or 300 mg/ kg).

Total urinary excretion during the 6 h following the consumption of dried plum juice was 168, 1465, 137, and 75 $\mu$mol (4.2%, 36.9%, 3.4%, and 1.9% of compound **I** dose) on the basis of **II** as a standard for **IV**, **II**, **III**, and **V**, respectively (**Table 2**). Although other compounds appeared in the urine following dried plum or dried plum juice consumption, the group of metabolites presented in this manuscript were considered to be unique to compound **I** since they were absent or very low in the control subjects and in a blueberry treatment group (data not presented), they are structural derivatives of **I** demonstrated by their mass spectral data and UV spectra, excretion following dried plum juice was severalfold higher than that observed with dried plums, and they were all very polar compounds. Possible pathways of metabolism of compound **I** in the human are presented in **Figure 1**. Although it is not clear whether compound **I** can be metabolized directly to **II** and **III**, the urinary excretion of **II** is much greater than **III**; thus, compound **II** is likely a precursor to compound **III**.

Compound **I** seems to be metabolized fairly rapidly to **II** and **III** and other metabolites and excreted in the urine. Compounds **III**−**V** all appear to be glycine conjugate metabolites of **I**. Compounds **IV** and **V** are metabolites observed in the human but not in mice or rats. No evidence was obtained for the formation of **VI** in the human even though it has been observed in rats and mice.

## ACKNOWLEDGMENT

Appreciation is expressed to Dr. Leo Burka for arranging with Midwest Research Institute (Kansas City, MO) to provide purified **II** and **III** for use in these studies. Contributions of Dr. G. Cao in the design of the experiment and of H. Huang in sample analysis are acknowledged. This work was supported in part by a grant from the California Dried Plum Board.

## LITERATURE CITED

(1) Donovan, J. L.; Meyer, A. S.; Waterhouse, A. L. Phenolic composition and antioxidant activity of prunes and prune juice (*Prunus domestica*). *J. Agric. Food Chem.* **1998**, *46*, 1247−1252.

(2) Cook, A. P.; Macleod, T. M.; Appleton, J. D.; Fell, A. F. Reversed-phase high-performance liquid chromatographic method for the quantification of 5-hydroxymethylfurfural as the major degradation product of glucose in infusion fluids. *J. Chromatogr., A* **1989**, *467*, 395−401.

(3) Ferrer, E.; Alegria, A.; Farre, R.; Abellan, P.; Romero, F. High-performance liquid chromatographic determination of furfural compounds in infant formulas. Changes during heat treatment and storage. *J. Chromatogr., A* **2002**, *947*, 85−95.

(4) Fernandez-Artigas, P.; Guerra-Hernandez, E.; Garcia-Villanova, B. Browning indicators in model systems and baby cereals. *J. Agric. Food Chem.* **1999**, *47*, 2872−2878.

(5) Ulbricht, R. J.; Northup, S. J.; Thomas, J. A. A review of 5-hydroxymethylfurfural (HMF) in parenteral solutions. *Fundam. Appl. Toxicol.* **1984**, *4*, 843−853.

(6) Prior, R. L.; Hoang, H.; Gu, L.; Wu, X.; Bacchiocca, M.; Howard, L.; Hampsch-Woodill, M.; Huang, D.; Ou, B.; Jacob, R. Assays for hydrophilic and lipophilic antioxidant capacity (Oxygen Radical Absorbance Capacity ($ORAC_{FL}$)) of plasma and other biological and food samples. *J. Agric. Food Chem.* **2003**, *51*, 3273−3279.

(7) Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In *Methods in Enzymology*; Academic Press: San Diego, CA, 1999; pp 152−178.

(8) Godfrey, V. B.; Chen, L. J.; Griffin, R. J.; Lebetkin, E. H.; Burka, L. T. Distribution and metabolism of (5-hydroxymethyl)furfural in male F344 rats and B6C3F1 mice after oral administration. *J. Toxicol. Environ. Health, Part A* **1999**, *57*, 199−210.

(9) Germond, J. E.; Philippossian, G.; Richli, U.; Bracco, I.; Arnaud, M. J. Rapid and complete urinary elimination of [14C]-5-hydrooxymethyl-2-furaldehyde administered orallly or intravenously to rats. *J. Toxicol. Environ. Health* **1987**, *22*, 79−89.

(10) Eller, K. I.; Pimenova, V. V.; Kon, I. [5-hydroxymethylfurfural as indicator of quality of juices for child nutrition]. *Vopr. Pitan.* **2001**, *70*, 37−39.

(11) Roig, M. G.; Bello, J. F.; Kennedy, J. F.; Rivera, Z. S.; Lloyd, L. L. A reversed-phase HPLC method for measurement of 5-hydroxymethyl furfuraldehyde and furfuraldehyde in processed juices. *Bioseparation* **1992**, *3*, 177−184.

(12) Talcott, S. T.; Percival, S. S.; Pittet-Moore, J.; Celoria, C. Phytochemical composition and antioxidant stability of fortified yellow passion fruit (*Passiflora edulis*). *J. Agric. Food Chem.* **2003**, *51*, 935−941.

(13) Janzowski, C.; Glaab, V.; Samimi, E.; Schlatter, J.; Eisenbrand, G. 5-Hydroxymethylfurfural: assessment of mutagenicity, DNA-damaging potential and reactivity towards cellular glutathione. *Food Chem. Toxicol.* **2000**, *38*, 801−809.

(14) Zhang, X. M.; Chan, C. C.; Stamp, D.; Minkin, S.; Archer, M. C.; Bruce, W. R. Initiation and promotion of colonic aberrant crypt foci in rats by 5-hydroxymethyl-2-furaldehyde in thermolyzed sucrose. *Carcinogenesis* **1993**, *14*, 773−775.

**Received for review January 13, 2006. Revised manuscript received March 23, 2006. Accepted March 23, 2006.**

JF0601113