Pelargonidin Is Absorbed and Metabolized Differently than Cyanidin after Marionberry Consumption in Pigs\textsuperscript{1,2}

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ABSTRACT Weaning pigs (7.9 ± 1.7 kg) were fed a freeze-dried powder of marionberry (MB) by stomach tube to study the absorption and metabolism of anthocyanins. Four major anthocyanins (ACNs) were found in MB: cyanidin-3-glucoside (Cy-3-glc, 78\%), cyanidin-3-rutinoside (Cy-3-rutin, 20\%), pelargonidin-3-glucoside (Pg-3-glc, 0.4\%), and 1 unknown acylated cyanidin-based ACN (UACy, 1.5\%). In the urine, the 4 original ACNs and 11 metabolites were identified and quantified. The main metabolites were glucuronidated and/or methylated forms of the original anthocyanins. Total recovery of the 4 original ACNs plus their related metabolites was 0.087 ± 0.034% for Cy-3-glc, 0.084 ± 0.026% for Cy-3-rutin, 0.583 ± 0.229% for Pg-3-glc and 0.036 ± 0.011% for UACy (mean ± SD, n = 3), respectively. For the individual ACNs, the amount of metabolites recovered from Cy-3-rutin was lower than that of the original intact Cy-3-rutin, whereas the amounts of metabolites from Cy-3-glc and Pg-3-glc in the urine were much higher than their original forms. In pig plasma, the 2 original ACNs, Cy-3-glc and Cy-3-rutin, and a trace of 1 metabolite (cyanidin monoglucuronide) were detected. The plasma concentration:dose ratio of Cy-3-rutin was higher than that of Cy-3-glc. Different aglycones and/or sugar moieties may influence the absorption and metabolism of ACNs. Cy-3-glc and Cy-3-rutin had similar apparent excretion rates relative to dose, whereas Pg-3-glc had a much higher total urinary excretion than cyanidin-based anthocyanins. Most of Cy-3-glc and Pg-3-glc were excreted in the form of metabolites, whereas most of the Cy-3-rutin was excreted in its original unmetabolized form. Urinary recovery of the acylated anthocyanin was lower than that of nonacylated anthocyanins.


KEY WORDS: • anthocyanin • marionberry • absorption • metabolism • HPLC-ESI/MS/MS

Anthocyanins (ACNs)\textsuperscript{4} are water-soluble glycosides and acylglycosides of anthocyanidins, which are polyhydroxy and polymethoxyl derivatives of a 2-phenylbenzopyrylium (flavylium) cation (1). They are widely distributed in foods of plant origin, especially in fruits and vegetables with dark red and blue colors (2). Numerous epidemiologic and clinical trails showed that fruits and vegetables, many of which are rich in anthocyanins, may be related to the decreased incidence of many chronic and degenerative diseases, including heart disease, cancer, and aging (3–5). Antioxidant mechanisms were suggested as potential means of disease prevention (6,7). An- thocyanins, may be related to the decreased incidence of many chronic and degenerative diseases, including heart disease, cancer, and aging (3–5). Antioxidant mechanisms were suggested as potential means of disease prevention (6,7). Anthocyanins are strong antioxidants in vitro (8). In most cases, fruits and vegetables with a high anthocyanin content were shown to have higher antioxidant capacity than other fruits and vegetables (9). Whether anthocyanins are effective anti- oxidants in vivo remains an open question primarily because of the relatively low apparent absorption of anthocyanins compared with other phenolic compounds (6).

To understand the potential mechanisms whereby anthocyanins produce health benefits, an understanding of their bioavailability and metabolism is critical. Although several recent studies of the bioavailability of anthocyanins exist (10–32), we still know little about the metabolism of anthocyanins and what might account for some rather large differences in metabolites observed and urinary excretion rates. In a previous report, we detected for the first time in humans the intact glucuronide and methylated forms of anthocyanins in the urine of subjects consuming elderberry or blueberry (12); this was since confirmed in other studies (24,31,32).

Marionberries (MBs) are a cross between the Chehalem and Olallieberry blackberries and are grown exclusively in Oregon. MBs were chosen for study because of their high concentrations of anthocyanins, which can be accounted for in primarily 2 anthocyanins, cyanidin-3-glucoside (Cy-3-glc) and cyanidin-3-rutinoside (Cy-3-rutin) (rutinoside: 6-O-α-L-rhamnosyl-β-D-glucose). Minor amounts of pelargonidin-3-glucoside (Pg-3-glc) (2) are also present, which was also of interest because this anthocyanin is not present in many other

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fruits other than strawberries. The objective of this study was to investigate the effects of anthocyanin aglycone and sugar moiety on the absorption and metabolism of anthocyanins in a pig model.

MATERIALS AND METHODS

Chemicals and materials. The 3-O-β-glucoside of pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin (6 mixed anthocyanin standard, HPLC grade) was obtained from Polyphenols Laboratories. Methanol was obtained from Fisher Scientific; formic acid from Aldrich Chemical; trifluoroacetic acid (TFA) from Sigma Chemical. Sep-Pak Vac RC (500 mg) C18 Cartridges for solid-phase extraction (SPE) were purchased from Waters.

Experimental materials. A dry powder obtained by freeze-drying MB was used in this study and was provided by the Oregon Raspberry Nutrition Center producer and brought to the Arkansas Children’s Nutrition Center animal facility and allowed to adapt for a period of 7 d before surgery. On d 8, surgery was performed using isoflurane as anesthetic during which a catheter (silastic tubing, 100 cm long; i.d., 1.02 mm; o.d., 2.16 mm, Dow Corning) was implanted into the femoral artery. The catheter was filled with heparinized saline (1000 U/L) and was flushed with saline every other day and filled with heparinized saline. After surgery, the pigs were allowed 7 d to recover. Four days before administration of the marionberry powder, the pigs were fed a purified diet (see supplemental data) that was free of any polyphenolic or flavonoid-like compounds. At the time of blood sampling, the pigs weighed 7.9 ± 1.7 kg.

The pigs were placed in a metabolic cage and food deprived overnight with water freely available before the experiment. A baseline urine sample was collected in the morning. MB freeze-dried powder was mixed with water (1:3, wt:wt) and was given via gastric intubation. The dose of total anthocyanins from MB was 74.2 ± 11.6 mg/kg body weight (BW). Immediately before feeding, a 0-h blood sample was taken from the catheter. Urine samples were collected from pigs before and between 0 and 2, 2 and 4, and 4 and 24 h after consumption of the MB. Blood was drawn from the catheter at 1, 2, and 4 h after feeding. The urine and blood samples were treated with 0.44 mol/L TFA as reported previously (11,12). Both urine and plasma samples were stored at −70°C until analysis.

Sample preparation. Treated urine sample (6 mL: 5 mL urine plus 1 mL 0.44 mol/L TFA) or 2.4 mL of treated plasma sample (2 mL plasma plus 0.4 mL of 0.44 mol/L TFA) were passed through a Sep-Pak C18 SPE cartridge as described previously (10–12). After SPE treatment, the acidic methanol solutions of urine and blood samples were completely dried with a SpeedVac (SC210A, ThermoSavant) and redissolved in 500 or 200 μL of a 5% formic acid:methanol solution. After filtration with a syringe filter (0.22 μm, Phenomenex), the solution was injected into the HPLC-electrospray ionization (ESI)/MS/MS system for analysis of anthocyanins. Anthocyanin standards were dissolved in acidic methanol to make calibration solutions for quantification and identification purposes.

Analysis of anthocyanins in urine and plasma. The analysis of anthocyanins in urine was carried out on an Agilent series 1100 HPLC system including an autosampler, a binary pump, Zorbax SB-C18 column (4.6 × 250 mm) and a diode array detector (Agilent Technologies). Low-resolution ESI-MS was performed with an Esquire-LC Mass Spectrometer (Bruker Daltonics). Experimental conditions were as described previously (12).

Statistics. All data with a sample number ≥ 3 were expressed as means ± SD. The charts were made by Sigma Plot 2001 (SPSS).

RESULTS

Composition and anthocyanins in MB freeze dried powder. The MB powder contained 0.27% crude fat, 7.0% protein, 1.4% crude fiber, 2.85% ash, 60.3% carbohydrate, and 13.26 kJ/g. Two major and 2 minor anthocyanins were found in MB (Fig. 1A); their concentrations were 16.6 mg/g Cy-3-glc (Pk# 3), 4.1 mg/g Cy-3-rutin (Pk# 5), 0.12 mg/g Pg-3-glc (Pk# 6), and 0.38 mg/g acylated cyanidin-based anthocyanin (UACy, exact structure unknown, Pk# 15). The total antioxidant capacity of the MB measured as oxygen radical absorbance capacity with fluorescein as fluorescent probe was 465 μmol Trolox equivalents/g freeze-dried weight. With the elution gradient used, the retention times of the nonacylated anthocyanins were all <40 min. The unknown anthocyanin,
according to its MS spectral data (MS m/z 593, MS/MS m/z 287) and retention time (RT = 45.8 min), was predicted to be an acylated anthocyanin of cyanidin hexoside.

Identification of chromatographic peaks in urine. There were no anthocyanin peaks detected in urine samples collected before berry feeding. In urine collected from 3 pigs after feeding, 15 anthocyanins were detected by MS spectra (Fig. 1B). Peaks 3, 5, 6 and 15 were the original anthocyanins existing in MB as determined by comparing their retention time and MS spectral data with that of MB anthocyanins. They were Cy-3-glc, Cy-3-rutin, Pg-3-glc, and UACy, respectively. All other peaks were likely metabolites from those 4 original anthocyanins. Their possible structures (Fig. 2) were determined by MS spectra (see supplemental data) and by comparing retention times and MS data to those of standard anthocyanins.

Metabolites of Cy-3-glc in urine. Eight peaks in pig urine were tentatively attributed to be metabolites of Cy-3-glc. They were peak numbers 1, 2, 4, 8, 9, 10, 11, and 13 (Fig. 1B). Peak 4, which was close to peak 3 (Cy-3-glc), had a molecular weight of 463 (MS+ m/z 463) and its MS/MS data indicated that its aglycone was cyanidin (m/z 287). The difference between m/z 463 and m/z 287 is 176, which is predicted to be a glucuronic acid residue. This peak was identified as cyanidin monoglucuronide. We do not know the exact position of the glucuronide residue from the experimental methods that were used.

Peaks 8 and 9 shared the same MS and MS/MS data. Their molecular weights were m/z 463 and their aglycones were m/z 301, respectively. From our previous report (12), we knew that one of them was peonidin-3-glucoside, a methylated form of Cy-3-glc. By comparison with the anthocyanin standards, it was clear that peak 9 was peonidin-3-glucoside. Because cyanidin has only 1 catechol structural unit in the B-ring, and the catechol structure is necessary for catechol-O-methyl transferase (COMT; EC 2.1.1.6) activity (33), peak 8 should have the aglycone with the same formula as peonidin, but with the methoxyl group in the 4’ position in the B-ring instead of the 3’ position as in peonidin. We named this anthocyanidin, isopeonidin. Hence peak 8 was identified as isopeonidin-3-glucoside. Using our current HPLC experimental conditions, the retention time of the 4’ methylated cyanidin (isopeonidin) was shorter than that of 3’ methylated cyanidin (peonidin). Peak 10 coeluted with peak 9. It had a molecular weight m/z of 477 and an aglycone m/z of 301. Peak 11 had the same MS and MS/MS data as peak 10. Most likely, these 2 peaks were glucuronides of peonidin or isopeonidin. Corresponding to the retention times of peaks 8 and 9, peak 10 was identified as isopeonidin monoglucuronide and peak 11 was peonidin monoglucuronide. Two peaks reported in a recent paper (32) also had the same MS spectral data as peaks 10 and 11, but the authors suggested that they were peonidin monoglucuronides with glucuronidation at a different hydroxyl site on the peonidin skeleton. From our results, because we saw both peonidin- and isopeonidin-3-glucoside, these 2 peaks were more likely to be glucuronides of these 2 isomers.

Peaks 13 and 14 also coeluted from the column. Peak 13 had the same MS and MS/MS data as peak 4, but the retention time was much longer than that of peak 4 (Fig. 1B). Thus, it was a much less polar compound than peak 4, cyanidin monoglucuronide. It is not known whether it is a different mono-

![FIGURE 2](http://www.nutrition.org)

FIGURE 2 Structures of anthocyanins in MB (marked with asterisk) and selected metabolites. Rutinose is 6-O-α-L-rhamnosyl-α-glucose. The term “isopeonidin” is a term not previously used, but was used in this manuscript to refer to the 4’ methylated instead of the 3’ methylated metabolite which is peonidin.

* MS spectra of metabolites from Cy-3-glc is given in supplemental data Figure 1A, MS spectra of metabolites from Cy-3-rutin and Pg-3-glc are given in supplemental data Figure 2, both of which are posted online with manuscript at http://www.nutrition.org.

* Extracted ion chromatogram (EIC) extracted at m/z 463, m/z 477, and m/z 609, are provided as supplemental data Figure 3 posted online with this manuscript at http://www.nutrition.org.
tute. Based on the MS and MS/MS data, peak 12 was either peonidin or isopeonidin rutinoside. There was another small peak (peak 14) that was eluted a little later (RT = 37.6 min) which had the same molecular weight as peak 12. We were able to determine the molecular weight from the MS, but could not see its daughter ions from MS/MS due to its low concentration. From the EIC (see supplemental data), we could clearly see 2 peaks with an EIC of m/z 609, which were similar in pattern to another 2 pairs, peaks 8 and 9, and peaks 10 and 11. Combining the information, it was reasonable to predict that peak 12 was isopeonidin-3-rutinoside, whereas peak 14 was peonidin-3-rutinoside.

Metabolites of Pg-3-glc in urine. There was only 1 peak, number 7 (Fig. 1B), that was considered to be a metabolite of Pg-3-glc. Peak 7 had a molecular weight of 447 (MS m/z: 447) and an aglycone of pelargonidin (MS/MS, m/z: 271). This peak was identified as pelargonidin monoglucuronide. The proposed profile of absorbed anthocyanins and their metabolites as observed in pig urine is illustrated in Figure 3.

Peak identification in plasma. In plasma after MB feeding, only Cy-3-glc (Pk 3, Fig. 1C), cyanidin monoglucuronide (Pk 4, Fig. 1C), and Cy-3-rutin (Pk 5, Fig. 1C) were detected and identified by their retention times and MS spectra (Fig. 1C).

Quantification of anthocyanins in urine and plasma. Anthocyanins in urine and plasma were quantified on the basis of their corresponding anthocyanidin-3-glucoside (Table 1). For coeluted peak pairs 9 and 10, 11 and 12, 13 and 14, the percentage of the peak area that was attributed to each peak was determined by the peak area of EIC (see supplemental data).

Comparison of urinary anthocyanin recovery for individual anthocyanins and their metabolites. The urinary excretions of 3 known original anthocyanins and their metabolites during 3 time periods, 0–2 h, 2–4 h, and 4–24 h, were calculated (Fig. 4). The mean recovery of the original anthocyanins and their related metabolites from 3 pigs is compared in Figure 5. The recovery of metabolites was calculated on the basis of the original anthocyanin from which they were most likely converted.

Plasma concentration of anthocyanins and pharmacokinetic data. The concentration and concentration:dose curves of Cy-3-glc and Cy-3-rutin of 2 of 3 pigs are presented in Figure 6. Selected pharmacokinetic parameters were also calculated (Table 2). We could not collect plasma from the first pig because the catheter became inoperable during the experiment.

DISCUSSION

A number of reports were published in last few years concerning the bioavailability of anthocyanins in both humans and experimental animals (10–12,14–27,29,30). From these studies, it is clear that anthocyanins can be absorbed intact and can be excreted as glucuronide and/or methylated conjugates. Our initial report regarding the glucuronide conjugates and methylated forms of cyanidin-based anthocyanins in humans (12) was confirmed (24,31,32). Only recently have we begun to understand the possible pathways of metabolism of anthocyanins. The reported urinary excretion of anthocyanins differed substantially with no apparent explanation (12,13,24).

Although some reports suggested that differences in anthocyanin aglycones and sugar moieties (27) may influence the absorption/metabolism of anthocyanins, few studies were conducted to study these effects. In this study, our first goal was to identify and quantify as many anthocyanin metabolites as possible. We used the neonatal pig as a model because pigs seemed to have a metabolic pattern similar to humans and we could give a higher dose of anthocyanins, thus allowing for the detection of more of the metabolites. In addition, we wanted to study the effect of different aglycone and sugar moieties on the absorption and metabolism of anthocyanins.

Glucuronide conjugates and/or methylation of the catechol structure of the aglycone were 2 major types of metabolites of anthocyanins observed in this paper (Fig. 3). Methylation is an established pathway in the metabolism of flavonoids (34). This transformation occurs in the liver and is catalyzed by COMT (35). The catechol structural unit is necessary for COMT activity (33). Using the pig as a model in this study, we...
Identified both 3-O-methyl and 4’-O-methyl esters of cyanidin in pig urine, indicating that both the 3’ and 4’ hydroxyl group in the B-ring of cyanidin could be conjugated with a methyl group. However, the 3-O-methyl ester was the predominant form observed. This is consistent with previous studies showing that methylation tends to occur at the 3’-O-position of flavonoids with 3’,4’-dihydroxylation in the B ring (34). COMT requires S-adenosylmethionine (SAM) as a methyl donor. In rat liver and kidney, an increase in the ratio of S-adenosylhomocysteine (SAH) to SAM was observed after a large dose (1 g/kg) of anthocyanins from elderberry, suggesting that SAM may serve as the methyl donor and be converted to SAH during the metabolism of anthocyanins (36).

Formation of the glucuronide was the other major metabolic form observed. Both the anthocyanidin and anthocyanidin glycosides were observed to be conjugated. The mono-
glucuronide of both Cy-3-glc and Pg-3-glc was observed, similar to our previous report in humans (12). However, no other investigators have demonstrated the presence of glucuronide metabolites of anthocyanidin glycosides (32). There are 2 likely causes for this: first, the concentrations of the glucuronide forms of the anthocyanidin glycoside are lower than that of anthocyanidin glucuronide; and second, due to their high polarity, they are eluted early from the reverse-phase HPLC column, and may not have been identified because of this early elution. From our results, except for 1 unknown glucuronide that seems to be formed from cyanidin (peak 13), all other anthocyanidins or anthocyanins, including intermediate metabolites, have a single glucuronide conjugate.

From our studies (12) as well as those of others (16,22,23,25,27–29) it appeared that the anthocyanin aglycone and sugar moieties may influence the absorption and metabolism of anthocyanins. The proportion of the total anthocyanins recovered in the urine as metabolites or intact anthocyanins was different based on different anthocyanins (Fig. 5). Of the 2 major original anthocyanins, recovery of Cy-3-rutin was almost 3 times higher than that of Cy-3-glc (0.0771 vs. 0.0268% in Table 1). Others (27) also showed that Cy-3-rutin had a higher excretion rate than Cy-3-glc, although not to the same extent as in our data. The recovery of total metabolites from Cy-3-glc was higher than that of the intact Cy-3-glc, whereas the recovery of total metabolites from Cy-3-rutin was much lower than that of its intact original form, Cy-3-rutin. The total urinary excretion rates (original form plus all metabolites) of Cy-3-rutin and Cy-3-glc were about the same (0.087% of Cy-3-glc vs. 0.084% of Cy-3-rutin). This indicated that for anthocyanins with the same aglycone, such as Cy-3-rutin and Cy-3-glc, their apparent total absorption might be similar, but less Cy-3-rutin is metabolized compared with Cy-3-glc. Cy-3-glc and Pg-3-glc, the 2 original anthocyanins with different aglycones but the same sugar moiety, had significant differences in apparent absorption but relatively the same metabolic pattern with a much higher conversion to metabolites. Total urinary recovery of Pg-3-glc and its metabolites (0.583%) was much higher than that of Cy-3-glc (0.087%). In a recent paper, Felgines et al. (24) also found that the urinary excretion of Pg-3-glc and its metabolites was quite high (1.80% of dose) and that the excretion of metabolites was much higher than the parent compound. The major proportion of strawberry Pg-3-glc was excreted as a pelargonidin monoglucuronide. The ratio of excretion of Pg-3-glc to pelargonidin monoglucuronide in our results was ~1:10.

One unknown acylated anthocyanin in MB, which appeared to be based upon cyanidin (Fig. 1B, Table 1), was also detected in urine. No apparent metabolites from this anthocyanin were detected. The total recovery (original forms) of this unknown anthocyanin (0.036%) was much lower than that of either cyanidin-3-glucoside (0.087%) or cyanidin-3-rutinoside (0.084%) (Fig. 5). Thus, this acylated anthocyanin had a lower apparent urinary recovery but apparently was not metabolized as extensively.

Felgines et al. (24) also reported that a sulfoconjugate of pelargonidin was recovered in human urine after strawberry consumption. However, although a high dose of total anthocyanins (~74 mg/kg BW, mainly cyanidin-based anthocyanins) was given to pigs, we were not able to detect sulfate conjugates of cyanidin in pig urine. The concentration of pelargonidin was likely too low for detection of the sulfoconjugate of pelargonidin because it was <1% of the total anthocyanin dose. These observations seem to point to a unique characteristic of pelargonidin in terms of absorption and me-

### TABLE 2

**Plasma pharmacokinetic parameters of 2 anthocyanins in 2 pigs after MB feeding**

<table>
<thead>
<tr>
<th>Pig no.</th>
<th>Anthocyanin</th>
<th>( C_{\text{max}}^1 ) nmol/L</th>
<th>( C_{\text{max}}:\text{Dose} ) (nmol/L)/μmol</th>
<th>( t_{\text{max}} ) h</th>
<th>AUC (0–4 h) nmol·h/L</th>
<th>AUC (0–4 h):Dose (nmol·h/L)/μmol</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Cy-3-glc</td>
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<td>0.041</td>
<td>1.0</td>
<td>76.9</td>
<td>0.074</td>
</tr>
<tr>
<td></td>
<td>Cy-3-rutin</td>
<td>17.8</td>
<td>0.070</td>
<td>1.0</td>
<td>49.1</td>
<td>0.193</td>
</tr>
<tr>
<td>3</td>
<td>Cy-3-glc</td>
<td>29.5</td>
<td>0.057</td>
<td>1.0</td>
<td>52.6</td>
<td>0.066</td>
</tr>
<tr>
<td></td>
<td>Cy-3-rutin</td>
<td>13.1</td>
<td>0.067</td>
<td>1.0</td>
<td>33.9</td>
<td>0.172</td>
</tr>
</tbody>
</table>

\( C_{\text{max}}^1 \) = peak plasma concentration measured.

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**FIGURE 6** Concentration (A) and concentration/dose curve (B) of the 2 major anthocyanins, cyanidin-3-glucoside and cyanidin-3-rutinoside, in pig plasma after MB feeding.
tabolism. Pelargonidin-based anthocyanins are perhaps the least widely distributed anthocyanins among the 6 common anthocyanins (1). From the report of Felgines et al. (24) and our results, it is interesting to see potential differences in pelargonidin compared with other anthocyanidins. Pelargonidin has a very high total excretion rate (original form and all metabolites) and can easily be conjugated with glucuronic acid and sulfate, which may relate to its chemical structure. Pelargonidin has only 1 hydroxyl group in the B-ring (Fig. 2) which means that it cannot be methylated. To be effectively excreted from the body in the urine, increased biotransformation may be required compared with the other 5 common anthocyanin-based anthocyanins. This may lead to the formation of a high proportion of monoglucuronide and sulfate conjugates.

In our previous paper, we proposed 2 possible pathways that described the formation of monoglucuronide (12). Our current study provides additional evidence relevant to this process. We observed that both peonidin and isopeonidin had only 1 potential position for methylation. Differences of aglycone and sugar moiety significantly influenced the absorption/metabolism of anthocyanins. Cyanidin-3-glucoside and cyanidin-3-rutinoside seemed to have a similar total urinary excretion relative to dose but different metabolic or elimination rates. A large portion (69%) of the cyanidin-3-glucoside excreted in the urine was transformed into metabolites, whereas cyanidin-3-rutinoside tended to remain in its original form in the urine (81%). Compared with cyanidin-3-glucoside, pelargonidin-3-glucoside had a very high total absorption/excretion rate. Total urinary recovery of Pg-3-glc and its metabolites (0.583% of dose) was nearly 7-fold higher than total Cy-3-glc. Similar to Cy-3-glc, most of the Pg-3-glc was excreted in a metabolized form (monoglucuronide, 91%). Of the 2 major anthocyanins in MB, Cy-3-rutin was found to be more stable and appeared to be cleared at a slower rate from plasma than that of Cy-3-glc. Additional studies are warranted to investigate the absorption/metabolism behavior of different aglycones and sugar moieties.

LITERATURE CITED


