Aglycones and Sugar Moieties Alter Anthocyanin Absorption and Metabolism after Berry Consumption in Weanling Pigs

Xianli Wu, Hoy E. Pittman, III, Steve Mckay,* and Ronald L. Prior


ABSTRACT To investigate the absorption and metabolism of anthocyanins (ACNs) with different aglycones and sugar moieties, weanling pigs (11.4 ± 3.8 kg) were fed, in a single meal, a freeze-dried powder of chokeberry, black currant, or elderberry at a single dose of 229, 140, or 228 μmol total ACN/kg body weight (BW), respectively. These berries provided ACNs with differences in aglycone as well as some unique differences in the sugar moieties. The relative proportions of the different metabolites depended upon concentrations, quantities consumed, and types of glycoside of ACNs in the berry. Delphinidin ACNs were not metabolized to any measurable extent. Cyanidin ACNs were metabolized via methylation and glucuronidation as well as by formation of both derivatives on the same ACN molecule. ACNs with either a di- or trisaccharide attached to them were excreted in the urine primarily as the intact form. Over 80% of the ACN compounds containing rutinose or sambubiose, which were excreted in the urine from black currant, elderberry, or Marion blackberry, were excreted as the intact molecule. The limited metabolism of these ACNs that did occur was via methylation. ACN monoglycosides other than the glucoside were metabolized via methylation and/or glucuronide formation. The monoglucuronide that formed represented a small proportion of the metabolites relative to the methylated or the mixed methylated and glucuronide forms of ACNs. The data clearly demonstrate that the aglycone and the sugar moieties can alter the apparent absorption and metabolism of ACNs. J. Nutr. 135: 2417–2424, 2005.

KEY WORDS: • anthocyanin • chokeberry • elderberry • black currant • metabolism

Anthocyanins (ACNs) are water-soluble glycosides and acetylglycosides of anthocyanidins, which are polyhydroxyl and polymethoxyl derivatives of the 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). Intakes of ACNs have been estimated to be as high as 180–215 mg/d in the United States (3), but solid data are lacking because of the limited ACN food composition data. The targeted berries in this project are sometimes referred to as the “purple berries,” and their ACN content was characterized in detail recently (4). Among them, black currant contained 14 ACNs, and the 4 major ones were delphinidin-3-rutinoside (Dp-3-rut, 44.8%), cyanidin-3-rutinoside (Cy-3-rut, 23.5%), delphinidin-3-glucoside (Dp-3-glc, 25.1%), and cyanidin-3-glucoside Cy-3-glc (6.6%); chokeberry contained 8 ACNs, and the 4 major ACNs were cyanidin-3-galactoside (Cy-3-gal, 65.6%), Cy-3-glc (2.5%), cyanidin-3-arabinoside (Cy-3-arab, 28.3%), and cyanidin-3-xylloside (Cy-3-xyl, 3.7%); elderberry contained 7 ACNs, and Cy-3-glc (54%), cyanidin-3-sambubioside (Cy-3-sam, 39.7%), and cyanidin-3-sambubioside-5-glucoside (Cy-3-sam-5-glc, 6%) were the 3 major ACNs. The total ACNs in these 3 berries were 562 mg/100 g fresh weight (FW) for black currant, 1480 mg/100 g FW for chokeberry, and 1374 mg/100 g FW for elderberry, respectively. Several health-related effects of black currants were reported including antioxidant, anticancer, effects on endothelial vasorelaxation, and effects on influenza and herpes virus activity (5–11). In a recent double-blind, crossover, randomized, controlled trial that tested the effect of Ribes nigrum (black currant) ACNs on dark adaptation, a significant lowering of the dark adaptation threshold at 30 min after a single 50-mg dose of extract occurred (12). Other results from rigorous clinical trials do not support the hypothesis that ACNs from Vaccinium myrtillus (bilberry) improve normal night vision (13). Chokeberries (Aronia melanocarpa) are high in antioxidants and were shown to be potent inhibitors of the growth of colon cancer–derived HT-29 cells (14,15), and chokeberry juice was effective in lowering blood glucose in fasting diabetic subjects (16). Other health effects have also been demonstrated in various animal (17–19) and human...
studies (20). Elderberry (Sambucus nigra) was reported to have antiviral properties as well as stimulate the healthy immune system (21), and may be effective in the treatment of influenza A and B (22,23).

ACNs from elderberry were reported to be absorbed and excreted intact in humans (24,25). Since that time, several studies have confirmed that in humans and animal models, all ACNs studied are absorbed intact (26–35). More recently it was shown that ACNs can be methylated (36) and conjugated with glucuronide (26–28,37) and in some cases sulfate (34).

Little attention has been paid to the effects of the specific ACN aglycone or sugar moiety on apparent absorption and metabolism of ACNs. Nielsen et al. (35) reported that a larger proportion of the ACN rutinoside than of the glucoside was absorbed in both humans and in rabbits, but the aglycone (Cy vs. delphinidin) itself did not influence absorption. This difference was likely observed previously (29,38), but was not addressed by the authors.

A number of studies examined ACN absorption and metabolism in rats; however, rats may metabolize ACNs differently from humans. For instance, protocatechuic acid, a degradation product of Cy, was observed in the plasma of rats, but not humans (36); in addition, the aglycone of Cy (37) was observed in the jejunum of rats (36), but was not reported in any data from humans. Using weanling pigs as an animal model, we found based upon the data presented in this article compared with previous data (26,27) that they provide a good representation of what we know occurs metabolically in humans (28). The objectives of these studies were to determine the effects of the aglycone (delphinidin vs. Cy) and sugar moiety (glucose, galactose, arabinose, xylose, rutinoside, and sambubioside) on ACN absorption and metabolism using black currants, chokeberries, and elderberries in weanling pigs.

MATERIALS AND METHODS

Chemicals and materials. The 3-O-β-glucoside of pelargonidin, Cy, peonidin, delphinidin, petunidin, and malvidin (6 mixed ACN standard, HPLC grade) was obtained from Polyphenols Laboratories. Methanol was obtained from Fisher Scientific; formic acid from Aldrich Chemical; and 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. The compositions of black currant (Ribes nigrum, “Ben Alder”), chokeberry (Aronia melanocarpa), and elderberry (Sambucus nigra) were described previously (4).

Animals and study design. All animal protocols were approved by the UAMS Animal Care and Use Committee. Healthy pigs (Hampshire/Duroc Cross; n = 9, 21 d old) were purchased from a local swine producer; they were brought to the Arkansas Children’s Nutrition Center animal facility and allowed to adapt for 7 d before surgery. On d 8, surgery was performed using isoflurane as an anesthetic; 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 solution was injected into the HPLC-electrospray ionization (ESI)/MS/MS system for analysis of ACNs. ACN 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 ACNs 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 electrospray MS was performed with an Esquire-LC Mass Spectrometer (Bruker Daltonics). Experimental conditions were the same as those described previously (25).

Statistics. All data with a sample number ≥ 3 were expressed as mean ± SEM if not stated otherwise. The charts were made by Sigma Plot 2001 (SPSS) or SlideWrite Plus (Advanced Graphics Software).

RESULTS AND DISCUSSION

Data presented on ACN metabolism after a meal from chokeberry, black currant, or elderberries provide some unique differences in some of their sugar moieties. In addition, black currant is one of the few berries that contains a high proportion of delphinidin ACNs (~70%) (4). Previous studies identified and characterized the ACNs in these berries (4), and the current studies have focused on absorption and metabolism of these ACNs in weanling pigs. Identification and peak assignment of ACNs and their metabolites were based on the comparison of their retention time and MS data with standards and published data (4,28,39). In previous studies (28), we identified 2 methylated metabolites of Cy that shared the same MS and MS/MS data with an m/z of 463 and aglycones with an m/z of 301. On the basis of retention times, one was identified as peonidin-3-glucoside, the 3’ methylated form of Cy-3-glc. The other metabolite was designated as isopeonidin-3-glucoside, which would be the 4’ methylated form of Cy-3-glc. The same metabolite was observed in the current studies and we have used the same terminology. Previous studies showed that catechol-O-methyl transferase (COMT) methylates ACNs in the enzymatic synthesis of malvidin-3-glucoside and its isomer from petunidin-3-glucoside (40).

Composition of anthocyanins in chokeberry and urinary excretion. A chromatogram of the ACNs in chokeberry and a representative chromatogram of a urine sample 2–4 h after consuming a meal containing chokeberry are presented in supplemental Figure 1. Because Cy was the only major anthocyanidin, the ACNs in chokeberry provided an opportunity to evaluate the effects of the different monoglycosides on apparent absorption and metabolism of Cy glucosides. A total of 18 different ACN-based compounds, including 4 major original ACNs and 14 metabolites, were identified in the urine based upon absorbance at 520 nm or aglycones with an m/z of 287, 301, or 303 after MS/MS. Kay et al. (31) identified only 11 ACN-based compounds in the urine of humans after chokeberry consumption.

The highest percentage of the dose of the intact Cy glyco-
Identification of original anthocyanins and their metabolites and their recovery in urine of weanling pigs after chokeberry consumption

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Retention time (min)</th>
<th>m/z</th>
<th>Compound</th>
<th>Dose, Parent ACN (µmol/kg BW)</th>
<th>ACN in urine (nmol/kg BW)</th>
<th>Recovery in urine (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.6</td>
<td>609</td>
<td>477/433/301 Isopeobelin-3-arabinoside monoglucuronide</td>
<td>—</td>
<td>0.45 ± 0.09</td>
<td>0.0007 ± 0.0001</td>
</tr>
<tr>
<td>2</td>
<td>14.9</td>
<td>639</td>
<td>477.1/301 Peonidin-3-galactoside monoglucuronide</td>
<td>—</td>
<td>5.41 ± 0.96</td>
<td>0.0036 ± 0.0006</td>
</tr>
<tr>
<td>3</td>
<td>17.8</td>
<td>609</td>
<td>477/433/301 Peonidin-3-arabinoside monoglucuronide</td>
<td>—</td>
<td>5.31 ± 0.75</td>
<td>0.0082 ± 0.0012</td>
</tr>
<tr>
<td>4</td>
<td>21.3</td>
<td>609</td>
<td>477/301  Peonidin-3-xylloside monoglucuronide</td>
<td>—</td>
<td>1.27 ± 0.20</td>
<td>0.0151 ± 0.0024</td>
</tr>
<tr>
<td>5</td>
<td>23.9</td>
<td>449</td>
<td>287.2 Cyanidin-3-galactoside</td>
<td>150.1 ± 10.2</td>
<td>89.48 ± 15.32</td>
<td>0.0596 ± 0.0102</td>
</tr>
<tr>
<td>6</td>
<td>26.2</td>
<td>449</td>
<td>287 Cyanidin-3-glucoside</td>
<td>5.7 ± 0.4</td>
<td>2.10 ± 0.42</td>
<td>0.0369 ± 0.0073</td>
</tr>
<tr>
<td>7</td>
<td>26.9</td>
<td>463</td>
<td>287 Cyanidin monoglucuronide</td>
<td>—</td>
<td>6.21 ± 0.92</td>
<td>0.1090 ± 0.0162</td>
</tr>
<tr>
<td>8</td>
<td>28.5</td>
<td>419</td>
<td>287.3 Cyanidin-3-arabinoside</td>
<td>64.7 ± 4.4</td>
<td>19.56 ± 3.59</td>
<td>0.0302 ± 0.0055</td>
</tr>
<tr>
<td>9</td>
<td>30.5</td>
<td>463</td>
<td>301.1 Isopeobelin-3-galactoside</td>
<td>—</td>
<td>10.44 ± 1.67</td>
<td>0.0071 ± 0.0011</td>
</tr>
<tr>
<td>10</td>
<td>31.1</td>
<td>463</td>
<td>301.1 Peonidin-3-galactoside</td>
<td>—</td>
<td>35.73 ± 5.98</td>
<td>0.0028 ± 0.0040</td>
</tr>
<tr>
<td>11</td>
<td>33.9</td>
<td>477</td>
<td>301.2 Isopeobelin monoglucuronide</td>
<td>—</td>
<td>4.61 ± 0.49</td>
<td>0.0031 ± 0.0003</td>
</tr>
<tr>
<td>12</td>
<td>34.9</td>
<td>477</td>
<td>301.1 Peonidin monoglucuronide</td>
<td>—</td>
<td>23.52 ± 2.52</td>
<td>0.0157 ± 0.0017</td>
</tr>
<tr>
<td>13</td>
<td>35.6</td>
<td>433</td>
<td>301.2 Isopeobelin-3-arabinoside</td>
<td>—</td>
<td>3.18 ± 0.58</td>
<td>0.0049 ± 0.0009</td>
</tr>
<tr>
<td>14</td>
<td>36.7</td>
<td>433</td>
<td>301.3 Peonidin-3-arabinoside</td>
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<td>11.29 ± 2.04</td>
<td>0.0175 ± 0.0032</td>
</tr>
<tr>
<td>15</td>
<td>36.8</td>
<td>463</td>
<td>287.2 Cyanidin monoglucuronide</td>
<td>—</td>
<td>3.15 ± 0.69</td>
<td>0.0376 ± 0.0083</td>
</tr>
<tr>
<td>16</td>
<td>38.2</td>
<td>419</td>
<td>287 Cyanidin-3-glucoside</td>
<td>8.4 ± 0.6</td>
<td>3.05 ± 0.10</td>
<td>0.0077 ± 0.0001</td>
</tr>
<tr>
<td>17</td>
<td>46.2</td>
<td>433</td>
<td>301 Isopeobelin-3-xylloside</td>
<td>—</td>
<td>0.66 ± 0.12</td>
<td>0.0012 ± 0.0001</td>
</tr>
<tr>
<td>18</td>
<td>47.8</td>
<td>433</td>
<td>301 Peonidin-3-xylloside</td>
<td>—</td>
<td>1.20 ± 0.27</td>
<td>0.0143 ± 0.0032</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>228.8 ± 5.9</td>
<td>223.55 ± 33.66</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 7; parent anthocyanins present in chokeberry are in italics.
2 Recovery of metabolites was calculated on the basis of their most likely parent compounds. Metabolites of Cy-3-gal were assumed to include peaks #2, #9, #10, #11, and #12; Cy-3-arab metabolites were assumed to include peaks #1, #3, #13, and #14; Cy-3-xyl metabolites were assumed to include peaks #4, #17, and #18; and Cy-3-glu metabolites were assumed to include peaks #7 and #15.

Side recovered in the urine was from Cy-3-gal (0.060%) compared with Cy-3-xyl (0.038%), Cy-3-gluc (0.037%), or Cy-3-arab (0.030%) (Table 1). Metabolites of the other Cy glycosides accounted for 0.053, 0.031, and 0.037% of the dose of galactoside, arabinoside, and xyloside, respectively. Both methylated and glucuronidated conjugates of these Cy glycosides were observed. Due to possible low concentrations, there were no methylated metabolites of Cy-3-glc from chokeberry detected in the urine. Of the total ACN-based compounds in the urine, Cy-3-gal accounted for 60.7% of the total, which was similar to the 55.3% found previously in 2 human subjects (31). However, Kay et al. (31) observed higher amounts of glucuronide conjugates than we observed (10.6% vs. 2.8%). The dose of chokeberry ACN/kg body weight used in our study was ~5.6 times that used by Kay et al. (31). This dose difference may explain the differences in glucuronide conjugate synthesis as will be discussed later. Of the 11 ACNs they detected, only one original ACN (Cy-3-gal) was found in the intact form. From the retention time, “Peak 1,” which had a suspicious molecular weight, was most likely a monoglucuronide of an ACN. They also observed metabolites with an m/z of [447 (3 compounds), 491 and 493] that we did not observe. The later compound was identified as malvidin-3-galactoside/glucoside. This would represent an oxidative modification, which we also did not observe and was not reported previously.

Composition of anthocyanins in black currant and urinary excretion. A chromatogram of the ACNs in black currants and a representative chromatogram of a urine sample 2–4 h after consuming a meal containing black currants are presented in a supplemental Figure 2. A total of 11 different ACN-based compounds were detected in the urine after black currant feeding. Remarkably, no metabolites of either Dp-3-glu or Dp-3-rut were detected in the urine after a meal of black currant. However, Ichiyang et al. (41) reported the formation of small amounts of the 4’-O-methyl delphinidin 3-glucoside in rats administered a dose of pure Dp-3-glc, which was 6 times the amount given in this study. Moreover, in our study, much more Dp-3-rut (~3.2 times) than Dp-3-glc was recovered in the urine (Table 2). Similarly, more intact Cy-3-rut than intact Cy-3-glc (~3.9 times) was recovered in the urine after the meal. Of the total metabolites of Cy-3-glc or Cy-3-rut excreted in the urine, 69.3% were metabolites from Cy-3-glc and only 16.0% were metabolites from Cy-3-rut, but the total excreted in the urine as a percentage of the dose was slightly greater from Cy-3-rut than from Cy-3-glc (Fig. 1, Table 2). This same pattern of metabolism and excretion was observed with Cy-3-glc and Cy-3-rut in blackberry (28). Tavlera et al. (32) observed that Cy-3-rut absorption from the stomach of rats was lower, only about one third of the apparent absorption of ACN monoglycosides (glucoside or galactoside). The absorption and excretion of ACN rutinosides were reported to be slightly delayed compared with glucosides (29). The rutinoside moiety appears to decrease Cy metabolism and thus possibly increase overall stability in the body or alternatively it could increase apparent urinary excretion.

Composition of anthocyanins in elderberry and urinary excretion. A chromatogram of the ACNs in elderberry and a representative chromatogram of a urine sample 2–4 h after consuming a meal containing elderberry are presented in supplemental Figure 3. In urine, a total of 14 ACN-based compounds were identified (Table 3). Like the other 2 berries, only methylated and glucuronidated metabolites were formed from the original ACNs. Among the 3 major ACNs, the largest molecule, Cy-3-sam-5-glc, had the highest recovery in urine, followed by Cy-3-sam. Even though the dose of Cy-3-sam was lower than that of Cy-3-glc (79 vs. 139 µmol/kg BW), ~3.2 times as much Cy-3-sam as Cy-3-glc was excreted in the
Identification of original anthocyanins and their metabolites and their recovery in urine of weanling pigs after black currant consumption

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Retention time</th>
<th>MS m/z</th>
<th>MS/MS m/z</th>
<th>Compound</th>
<th>Dose, Parent ACN</th>
<th>ACN in urine</th>
<th>Recovery in urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min</td>
<td></td>
<td></td>
<td></td>
<td>μmol/kg BW</td>
<td>nmol/kg BW</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>22.1</td>
<td>465</td>
<td>303</td>
<td>Delphinidin-3-glucoside</td>
<td>35.1 ± 2.0</td>
<td>7.77 ± 2.51</td>
<td>0.0221 ± 0.0071</td>
</tr>
<tr>
<td>2</td>
<td>24.3</td>
<td>611</td>
<td>465/303</td>
<td>Delphinidin-3-rutinoside</td>
<td>62.7 ± 3.6</td>
<td>44.43 ± 13.37</td>
<td>0.0708 ± 0.0213</td>
</tr>
<tr>
<td>3</td>
<td>26.2</td>
<td>449</td>
<td>287</td>
<td>Cyanidin-3-glucoside</td>
<td>9.2 ± 0.5</td>
<td>2.09 ± 0.69</td>
<td>0.0227 ± 0.0075</td>
</tr>
<tr>
<td>4</td>
<td>26.9</td>
<td>463</td>
<td>287</td>
<td>Cyanidin monoglucuronide</td>
<td>—</td>
<td>0.36 ± 0.12</td>
<td>0.0039 ± 0.0013</td>
</tr>
<tr>
<td>5</td>
<td>28.9</td>
<td>595</td>
<td>449/287</td>
<td>Cyanidin-3-rutinoside</td>
<td>32.9 ± 1.9</td>
<td>29.29 ± 7.49</td>
<td>0.0891 ± 0.0228</td>
</tr>
<tr>
<td>6</td>
<td>31.7</td>
<td>463</td>
<td>301</td>
<td>Isopeonidin-3-glucoside</td>
<td>—</td>
<td>0.46 ± 0.09</td>
<td>0.0050 ± 0.0010</td>
</tr>
<tr>
<td>7</td>
<td>32.7</td>
<td>463</td>
<td>301</td>
<td>Peonidin-3-glucoside</td>
<td>—</td>
<td>0.30 ± 0.08</td>
<td>0.0033 ± 0.0008</td>
</tr>
<tr>
<td>8</td>
<td>33.3</td>
<td>477</td>
<td>301</td>
<td>Isopeonidin monoglucuronide</td>
<td>—</td>
<td>1.38 ± 0.20</td>
<td>0.0150 ± 0.0022</td>
</tr>
<tr>
<td>9</td>
<td>34.3</td>
<td>477</td>
<td>301</td>
<td>Peonidin monoglucuronide</td>
<td>—</td>
<td>2.23 ± 0.32</td>
<td>0.0241 ± 0.0034</td>
</tr>
<tr>
<td>10</td>
<td>34.7</td>
<td>609</td>
<td>463/301</td>
<td>Isopeonidin-3-rutinoside</td>
<td>—</td>
<td>3.01 ± 0.73</td>
<td>0.0091 ± 0.0022</td>
</tr>
<tr>
<td>11</td>
<td>35.8</td>
<td>609</td>
<td>463/301</td>
<td>Peonidin-3-rutinoside</td>
<td>—</td>
<td>2.63 ± 0.70</td>
<td>0.0080 ± 0.0021</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>139.9 ± 8.1</td>
<td>93.94 ± 25.64</td>
<td>0.0671 ± 0.0155</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 7; parent anthocyanins present in black currant are in italics.
2 Recovery of metabolites was calculated based on their most likely parent compounds. Cy-3-glc metabolites were assumed to include peaks #4, #6, #7, #8, and #9. Cy-3-rut metabolites were assumed to include peaks #10 and #11.

Plasma concentration of anthocyanins and pharmacokinetic data. Concentrations of ACN compounds in plasma after black currant consumption were very low. The lower dose and predominance of delphinidins in black currant and all Cy monoglycosides in chokeberry may explain these observations. A representative chromatogram of ACNs in plasma after elderberry feeding is presented in supplemental Figure 3. Concentrations of Cy-3-sam were much higher than Cy-3-glc in plasma (Fig. 2), expressed as nmol/L per dose (μmol/kg). The area under the plasma concentration curve for Cy-3-sam and Cy-3-glc was 6.07 and 1.18 nmol/L per dose (μmol kg⁻¹), respectively. The differential disposition of Cy-3-sam (Pk #6) and Cy-3-glc (Pk #7) is also evident in the altered ratio of Pk #6 to Pk #7 (see supplemental Figure 4) in the urine compared with the berry. Combining what we observed in this study and in our previous study (26), it is clear that the molecular size significantly influenced pharmacokinetic behavior in plasma. Both Cy-3-rut and Cy-3-sam had a higher concentration/dose and a slower elimination rate. Two possibilities could explain these results; first, Cy diglycosides are absorbed more readily than Cy monoglycosides. However, based upon unpublished data (Wu and Prior, unpublished), the recovery of ACNs within the gut after a meal suggests just the opposite effect with much more Cy-3-glc disappearing than Cy diglycosides. Second, Cy diglycosides are less readily metabolized and consequently more stable than those of monoglycosides (37,42). Which of these explanations might be correct remains an open question.

Comparison of anthocyanin metabolism in different berries. In the current studies, we were able to examine in more detail the metabolism of ACNs in weanling pigs whose metabolic pattern seems to be similar to that of humans. Figure 3 presents the relation between the level of intake of Cy-3-glc from 4 different berry sources [3 in this study and 1 (Marion blackberry) from Wu et al. (4)] and urinary excretion. At the high intake of Cy-3-glc (139 μmol/kg BW) from elderberry, approximately equal percentages of glucuronidated and methylated forms of Cy-3-glc were recovered in the urine, whereas at the lowest dose of Cy-3-glc (5.7 μmol/kg BW from chokeberry), no methylated products of Cy-3-glc (either peonidin-3-glc or isopeonidin-3-glc) were observed (see Fig. 3; Tables 1 and 3); only Cy glucuronidated conjugates were observed after chokeberry consumption, which accounted for 0.109% of the Cy-3-glc dose (Fig. 3; Table 1). The lack of methylated products from Cy-3-glc in chokeberry may result from the increased absorption and excretion of the intact forms of ACNs from elderberry in humans. By comparing their chromatograms under the same separation conditions, the ACNs, including parent ACNs and their metabolites, excreted in pig urine were very similar to those in the urine of humans after elderberry consumption (see supplemental Figure 4). Results from human subjects were described in a previous paper (27). This suggests that weanling pigs are a good animal model for studying the bioavailability of ACNs and possibly other plant secondary metabolites.

![Image of chromatogram](image-url)

**FIGURE 1** Effects of ACN aglycone and sugar moiety (glucose vs. rutinose) on total recovery of delphinidin or cyanidin ACNs in urine of weanling pigs after a meal of black currant berries. Values are means ± SEM, n = 7, of the total excreted (as a percentage of the dose) with the proportions of the parent ACN compound (ACN-3-X), methylated (Methyl), glucuronidated (GLUD) or both methylated and glucuronidated conjugates (Methyl & GLUD) indicated in each bar.
competition for reaction sites on COMT by Cy-3-gal and Cy-3-arab, which were present at much higher concentrations. Methylation of ACNs by COMT was shown previously (40). However, in the current study, after a meal of black currant berries with a relatively small dose of Cy-3-glc (9.3 μmol/kg BW), methylated as well as glucuronidated conjugates were observed. Formation of glucuronidated conjugates was lowest after elderberry consumption and highest after chokeberry consumption (Figs. 3, 4). From these data, there appears to be an inverse relation between the formation of the methylated forms of Cy-3-glc and the glucuronidated conjugates of Cy-3-glc. After elderberry consumption, the formation of glucuronidated conjugates of Cy was observed only with Cy-3-glc but not the Cy-3-sam or Cy-3-sam-5-glc forms. Although the dose of total ACNs from elderberry was similar to that from chokeberry, the amount of Cy-3-glc consumed was much higher from elderberry (139.3 vs. 5.7 μmol/kg BW), which apparently greatly altered the metabolism of Cy-3-glc with lower total urinary recovery of Cy-3-glc and its metabolites from elderberry as a percentage of the dose; however, methylated, glucuronidated, and mixed forms were observed after elderberry consumption but only glucuronidated conjugates after chokeberry consumption. The low amount of Cy-3-glc in the chokeberry meal and detection sensitivity may have accounted for the lack of detection of methylated forms.

The effect of the Cy glycoside on its metabolism is illustrated in Figure 4. Formation of glucuronidated forms of ACNs occurred with all monoglycosides (galactose, arabinose, xylose, and glucose). However, with the di- or triglycoside forms of Cy, the ACN was not metabolized to the glucuronide form.
and the relative amount of methylated and glucuronidated forms was diminished such that no metabolic products were observed with Cy-3-sam-5-glc. Another factor potentially affecting this observation is the amount of ACN in the berry. Cy-3-sam-5-glc concentrations in elderberry were low relative to Cy-3-glc and Cy-3-sam.

The percentages of metabolites relative to the total ACNs excreted in the urine after a meal of chokeberry, elderberry, or black currant are presented in Figure 5. The proportion of metabolites was highest for chokeberry (48.9%) followed by elderberry (30.3%) and black currant (24.6%). Chokeberry contains all Cy-3-monoglycosides, whereas elderberry contains only 61% Cy-3-monoglycosides and black currant contains only 22% as Cy-3-monoglycosides compared with more complex di- or triglycosides. It seems clear from these data that di- or triglycosides connected to the anthocyanidin decrease the metabolism of the ACN such that more is excreted in the urine in the intact form. This was also observed in a previous study with Marion blackberry (28). In contrast, pelargonidin is metabolized extensively such that >90% of the pelargonidin compounds excreted in the urine were metabolites and not the parent compound (28). Thus, both aglycone and glycoside can alter ACN metabolism.

Monoglucuronides of Cy-3-glc, peonidin-3-glc, peonidin-3-gal, peonidin-3-arab, peonidin-3-xyl, and isopeonidin-3-glc and -gal were observed. In these cases, the glucuronide was likely on the 4’ position of the B ring of the aglycone, although this cannot be stated definitively. Fleschhut et al. (37) demonstrated that glucuronidation could occur at any free hydroxyl position in the Cy molecule, which are the hydroxyl positions at C5 and C7 of the A-ring and at the C3’ and C4’ of the B ring. Only one form of monoglucuronide of Cy, peonidin, or isopeonidin was observed. The position of the monoglucuronide of Cy, peonidin, and isopeonidin could not be determined from the MS information alone, but it was most likely at the 3 position of the flavylum ring, which was shown to result in a stable end product (37). The exact mechanism whereby the glucuronide could be formed at the C3 position of the A ring is an open question (4). There are 2 proposed pathways explaining the formation of monoglucuronide, which is shown in Figure 6 with cyanidin monoglucuronide as an example. The first possibility is that the glucuronide is formed directly from Cy-3-glc by UDP-glucose dehydrogenase; the second possibility requires the formation of the Cy aglycone. Theoretically, if the first pathway is correct, the ratio of Cy-3-glc to Cy monoglucuronide should be fairly fixed. If the second pathway is correct, then this ratio may vary depending upon the cyanidin ACN composition. In our previous studies (25,26), there has always been a lower amount of the monoglucuronide than of Cy-3-glc in urine because the Cy-3-glc

![FIGURE 4](image4.png)

Effects of glycoside moiety on cyanidin aglycone on total urine recovery in weanling pigs after a meal of different berries. Values are means (n = 7) of the total excreted (as a percentage of the dose) with the proportions of the parent ACN compound (Cy-3-X), methylated (Methyl), glucuronide (GLUD), or both methylated and glucuronide conjugates (Methyl and GLUD) indicated in each bar. Doses of ACNs (μmol/kg BW) are shown within the parentheses above the error bars.

![FIGURE 5](image5.png)

Urinary ACNs as metabolites (percentage of total ACNs excreted; SEM of total excreted indicated by error bars) in weanling pigs fed a single meal of chokeberry, elderberry, or black currant containing the following amounts of ACNs: chokeberry (229 μmol/kg BW; n = 7); elderberry (228 μmol/kg BW; n = 7); or black currant (140 μmol/kg BW; n = 7).

![FIGURE 6](image6.png)

Chromatograms of Cy-3-glc (Peak #1) and Cy monoglucuronide (Peak #2) in the urine of the same weanling pig after a meal of chokeberry (229 μmol/kg BW), elderberry (228 μmol/kg BW), or black currant (140 μmol/kg BW). The ratios between Cy-3-glc and Cy monoglucuronide and the 2 proposed pathways for the formation of Cy monoglucuronide are also displayed.
was almost always the major ACN in those berries. However, in this study, after the consumption of chokeberry, in which Cy-3-glc is a minor ACN even though only Cy ACNs were found, there was a larger amount of Cy monoglucuronide than Cy-3-glc in urine. The ratio of Cy-3-glc to Cy monoglucuronide in the urine of the same pig was 0.5, 8.5, and 19.0 after a meal of chokeberry, black currant, or elderberry, respectively (Fig. 6). The greater amount of Cy monoglucuronide than Cy-3-glc in urine after chokeberry consumption suggests that the glucuronide may also be formed from Cy glycosides other than Cy-3-glc. Thus, the formation of the glucuronide in not likely to be by a mechanism in which the glucoside serves merely as a substrate for UDP-glucose dehydrogenase to form the corresponding monoglucuronide as was suggested earlier (4); more likely, the glycoside is cleaved, forming the aglycone, which is then glucuronidated. However, we have never observed the presence of the aglycone in plasma or urine of pigs or humans, which casts some doubt on this interpretation (25,36), although it was found in the jejunal fluid of rats after consumption of Cy-3-glc (36). The stability of the aglycone at a neutral pH in the blood or tissues may be short-lived, or the aglycone is glucuronidated very quickly and the aglycone does not accumulate, thus explaining why it has not been detected in plasma or urine.

From these studies of ACN metabolism, we can conclude the following: 1) Delphinidin is not metabolized to any measurable extent, although methylation was observed in rats given a much higher dose than what was given in our studies (41). 2) ACNs with either an attached di- or triglycoside are excreted in the urine primarily as the intact ACN molecule. Steric hindrance from the diglucoside may prevent formation of the glucuronide. Furthermore, these glycosides may prevent degradation of these ACNs to other unknown metabolites because increased quantities relative to the dose are recovered in the urine. From 80–90% of the excreted ACN compounds from black currant, elderberry, or Marion blackberry (28) that exist as the rutinoside or sambubioside were excreted as the intact molecule. The small amount of metabolism that did occur was via methylation. 3) Cy is metabolized via methylation and glucuronide formation as well as by formation of both metabolites on the same ACN molecule. The relative proportions of the different metabolites may depend upon concentrations in the berry, quantities consumed, and the glycoside moiety. The total ACN composition undoubtedly has effects on metabolism that cannot be clearly delineated because of the complexities of some of the berries; consequently, our results must be interpreted in the context of the composition of the complete berry. 4) Other ACN monoglycosides in addition to the glucoside are metabolized via methylation and/or glucuronide formation. Formation of the monoglucuronide represents a small proportion of the metabolites relative to the methylated or the mixed methylated and glucuronidated forms of ACNs.

LITERATURE CITED


