Phenylethanoid Glycosides from *Digitalis purpurea* L.

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**Key Words:** *Digitalis purpurea*, Scrophulariaceae, 2-(3,4-Dihydroxyphenyl)ethyl-O-α-L-rhamnopyranosyl-(1-6)-4-O-[E]-feruloyl]-β-D-glucopyranoside, Purpureaside D (1), 2-(3,4-Dihydroxyphenyl)ethyl-O-α-L-rhamnopyranosyl-(1-6)-O-β-D-glucopyranosyl-(1-3)-4-O-[E]-feruloyl]-β-D-glucopyranoside, Purpureaside E (3), Phenylethanoid glycoside

*Digitalis purpurea* is a biennial herb of the Scrophulariaceae family, and is commonly used to treat congestive heart failure. Moreover, it was also reported that *Digitalis* glycosides have potent anticancer effects. Recently, we isolated five phenylethanoid glycosides from this plant and studied for their effects on the expression of inducible nitric oxide synthase and their ability to induce GST and their protective efficacies against aflatoxin B1 (AFB1)-induced cytotoxicity.

In a continuation of the phytochemical study on *D. purpurea*, we now report the isolation and structural elucidation of two new phenylethanoid glycosides, purpureaside D (1) and purpureaside E (3), together with two known phenylethanol glycosides, scroside D (2), and forsythiaside (4) as well as their antioxidative activity on the NBT superoxide scavenging assay.

The leaves of *D. purpurea* were extracted with MeOH at room temperature for three times. The MeOH extract was suspended in water and then partitioned sequentially with equal volumes of dichloromethane, ethyl acetate, and n-butanol. An ethylacetate soluble fraction of the MeOH extract was purified by silica gel, Sephadex LH 20, and LiChroprep RP-18 column chromatography yielded two new phenylethanoid glycosides, named purpureaside D (1) and purpureaside E (3) together with two known phenylethanoid glycosides, scroside D (2), and forsythiaside (4) (Chart 1). The structure of new compounds were determined to be 2-(3,4-dihydroxyphenyl)ethyl-O-α-L-rhamnopyranosyl-(1-6)-4-O-[E]-feruloyl]-β-D-glucopyranoside (1), 2-(3,4-dihydroxyphenyl)ethyl-O-α-L-rhamnopyranosyl-(1-6)-O-β-D-glucopyranosyl-(1-3)-4-O-[E]-feruloyl]-β-D-glucopyranoside (3), respectively, on the basis of spectral analysis.

Compound 1 was obtained as an yellow amorphous powder, with [α]$_D^{25}$ -191.3°. Its molecular formula was determined to be C$_{36}$H$_{38}$O$_{15}$ by HR-FAB-MS ([M+Na]+ m/z 661.2106, calc.

**Chart 1.** Structures of Compounds 1-4 from *Digitalis purpurea* L.

**Figure 1.** Key HMBC Correlations for Compounds 1 and 3.
<table>
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<tr>
<th></th>
<th>1''</th>
<th>2''</th>
<th>3''</th>
<th>4''</th>
<th>5''</th>
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<tr>
<td>C</td>
<td>δ_H</td>
<td>δ_C</td>
<td>δ_H</td>
<td>δ_C</td>
<td>δ_H</td>
<td>δ_C</td>
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<tr>
<td>1</td>
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<td>36.80</td>
<td>2.69 (m)</td>
<td>35.07</td>
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<tr>
<td>2</td>
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<td>116.51</td>
<td>6.63 (d, 8.0)</td>
<td>115.50</td>
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</tr>
<tr>
<td>3</td>
<td>6.57 (dd, 8.0, 2.0)</td>
<td>121.43</td>
<td>6.50 (dd, 8.0, 2.0)</td>
<td>119.53</td>
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</table>

Table 1. $^{1}$H-NMR (500 MHz) and $^{13}$C-NMR (125 MHz) Spectral Data of Compounds 1 and 3

661.2108). The $^{1}$H- and $^{13}$C-NMR spectral data of 1 were quite similar to those of forsythiside (4) except for the presence of a feruloyl group instead of a caffeoyl group in 4. In the $^{1}$H-NMR spectrum, feruloyl and 3,4-dihydroxy phenylethyl groups confirmed by the six aromatic proton signals between $\delta_H$ 6.57-7.20 for 2 ABX systems, two olefinic protons at $\delta_H$ 6.40 and $\delta_H$ 7.66, a benzylic methylene proton at $\delta_H$ 2.81(2H, m), and two non-equivalent protons at $\delta_H$ 3.73 (1H, dd, $J_H$ = 16.0, 8.0 Hz) and $\delta_H$ 3.90 (-OCH$_3$) were overlapped with other signals.

In CD$_3$OD. $^{b}$In DMSO-$d_6$. $^{c}$Overlapped with other signals.

The configuration of the glucopyranosyl and rhamnopyranosyl were assigned to be β- and α-, respectively, according to the coupling constant of the hydroxyphenylethyl and feruloyl groups, revealed signals for 12 carbons corresponding to the carbohydrate moiety. In the $^{1}$H- and $^{13}$C-NMR spectra, the anomeric protons at $\delta_H$ 4.37 (1H, d, $J_H$ = 8.0 Hz), $\delta_H$ 4.65 (1H, d, $J_H$ = 1.5 Hz) indicated that 1 contained one glucopyranosyl and one rhamnopyranosyl unit. And these findings matched those in the HSQC spectrum, where two corresponding carbon signals appeared at $\delta_C$ 104.65 and 102.44 respectively. The presence of these sugar moieties were further confirmed by the acid hydrolysis of 1 which resulted in the release of D-glucose and L-rhamnose, identified by TLC comparison with the authentic samples. The configuration of the glucopyranosyl and rhamnopyranosyl were assigned to be β- and α-, respectively, according to the coupling constant of the
Table 2. Antioxidative Activities of Compounds 1, 3, and 4 from Digitalis purpurea L.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ (μg/mL)</th>
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<tr>
<td>Purpureaside D (1)</td>
<td>38.10 ± 2.42**</td>
</tr>
<tr>
<td>Purpureaside E (3)</td>
<td>18.35 ± 3.74**</td>
</tr>
<tr>
<td>Forsythiaside (4)</td>
<td>7.98 ± 2.02**</td>
</tr>
<tr>
<td>Allopurinol*</td>
<td>3.38 ± 1.60</td>
</tr>
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</table>

*Used as a positive control. Each value is the mean ± S.D. of three experiments. **p < 0.001 compared with control value.

The antioxidative activities of the isolated compounds were tested by a NBT superoxide scavenging assay. As shown in Table 2, compounds 1 and 3 showed similar antioxidative activities. When comparing the antioxidative activities of compounds 1 and 3 to that of compound 4, compound 4 was more potent than compounds 1 and 3. In order to understand what makes compounds 1 and 3 different from compound 4 in the antioxidative activity, their chemical structures were compared. As shown in Chart 1, the distinct structural difference is that compounds 1 and 3 have a C-4' feruloyl substitutent in sugar part, while compound 4 has a C-4' caffeoyl group in sugar part. These results suggest that the ortho-dihydroxy (catechol) structure play an important role in the antioxidative function as exhibited in flavonoids.

Experimential

General Procedures. Optical rotations were measured using an Autopol-IV polarimeter. UV spectra were obtained on a Shimadzu UV/Visible Spectrophotometer. The IR spectra were measured in KBr pellets using an IMS 85 (Bruker). The NMR spectra were recorded on a Varian Unity Inova 500 spectrometer. HR-FAB-MS was recorded on a JEOL JMS 700 mass spectrometer (KBIS-Gwangju center). TLC and the preparative TLC were carried out on precoated Silica gel 60 F₂₅₄ (Merek, art. 5715) and RP-18 F₂₅₄ plates. Column chromatography was performed on Silica gel 60 (Merek, 40 - 63 and 63 - 200 μm), MCI gel CHP 20P (Mitsubishi Chemical Co., 70-150 μm), and Sephadex LH-20 (Sigma, 25 - 100 μm).

Plant Material. The leaves of Digitalis purpurea L. (Scrophulariaceae) were collected in the Herbarium of College of Pharmacy, Chosun University, Korea, in July 2006 and authenticated by Professor Emeritus Young Hee Moon of College of Pharmacy, Chosun University, Korea. Voucher specimens were deposited in the Herbarium of the College of Pharmacy, Chosun University, Korea (997-17).

Extraction and Isolation. The dried leaves (180 g) of Digitalis purpurea were extracted with MeOH three times at room temperature and 60.6 g of residue were produced. The MeOH extract was suspended in H₂O and partitioned sequentially in CHCl₃, EtOAc, and n-BuOH. The EtOAc fraction (5.5 g) was chromatographed over a silica gel column using a gradient solvent system of CHCl₃-MeOH-H₂O (8:1:0 → 6:1:0 → 4:1:0 → 2:1:0 → 1:1:0:1, MeOH only) to give subfractions, designated as E1-E10. Subfraction E7 (545 mg) was further purified by column chromatography over a Sephadex LH20 eluting with a MeOH to afford seven subfractions (E71-E75). Subfraction E73 (63.9 mg) was purified by RP-18 CC eluting with an i-PrOH:MeOH:H₂O (5:35:65) and Sephadex LH20 CC eluting with MeOH:H₂O (9:1) to give compound 1 (13.08 mg, purpureaside D) and 2 (sorbose D, 3.35mg). Subfraction E92 (204.7 mg) was purified by CC over a Sephadex LH20 to afford seven subfractions (E91-97). Subfraction E92 (30.5 mg) was purified by RP-18 CC eluting with an i-PrOH:MeOH:H₂O (5:30:70) to give compound 3 (purpureaside E, 15.05 mg). Subfraction E94 (514.4 mg) was finally purified by repeated RP-18 CC eluting with an i-PrOH:MeOH:H₂O (5:25:75), which afforded compound 4 (forsythiaside).
side, 25.3 mg).

**Compound 1** (**purpureaside D**): Yellow amorphous powder, [α]$_D$)$_{25}^o$ = 191.3$^o$ (c 0.5, MeOH); UV; nm (log ε) 219 (4.20), 288 (3.55), 328 (4.00); IR (KBr) cm$^-1$: 3400, 1705, 1635, 1593, 1515, 1430, 1155; $^1$H- and $^{13}$C-NMR, see Table 1; HR-FAB-MS m/z 661.2106 [M+Na]$^+$ (calcd for C$_{36}$H$_{39}$O$_{12}$Na: 661.2108).

**Compound 2** (**sericine D**): Yellow amorphous powder, [α]$_D$)$_{25}^o$ $-44.0^o$ (c 0.4, MeOH); $^1$H-NMR (CD$_3$OD, 125 MHz) δ 131.57 (C-1), 117.28 (C-2), 20.39 (C-3), 21.33 (C-4), 18.76 (C-5), 25.25 (C-6), 127.81 (C-1'), 114.89 (C-2'), 75.97 (C-3'), 72.51 (C-4'), 74.91 (C-5'), 67.82 (C-6'), 105.87 (C-1'), 121.43 (C-6), 36.80 (C-7), 72.51 (C-8), 104.63 (C-1'), 3400, 1705, 1635, 1593, 1515.

**Acidic Hydrolysates of Compounds 1, and 3.** Compounds 1 and 3 (3 mg each) were dissolved in 1 N HCl (0.6 mL) and MeOH (0.6 mL), and refluxed at 75°C for 90 min. The reaction solution was evaporated under reduced pressure, and the hydrolysate was extracted with EtOAc (2 mL × 3). The aqueous fraction was neutralized with Ag$_2$CO$_3$ and filtered. The filtrate was then concentrated under reduced pressure.$^{10}$ The residue was compared with a standard sugar using TLC, which revealed the sugar to be D-(+)-glucose ($R_f = 0.26$) and L-(+)-threonine ($R_f = 0.46$) in 1 and 3.

**NBT Superoxide Scavenging Assay.** The NBT superoxide-scavenging assay was carried out using a slight modification of an established method.$^{12}$ The reaction mixture, which was equilibrated at 25°C, contained 20 μL of a 15 mM Na$_2$EDTA solution in a buffer (50 mM KH$_2$PO$_4$/KOH pH 7.4 in ionized water), 50 μL of 0.6 mM NBT in a buffer, 30 μL of a 3 mM hypoxanthine in 50 mM KOH solution, 50 μL of xanthine oxidase solution in a buffer (1 unit in 10 mL buffer) and 100 μL of the sample. The plate reader (Molecular Devices Vmax) took readings every 20 s for 5 min at 570 nm. The control was 100 μL of 5% DMSO solution instead of the sample. Results were expressed as relative percentage inhibition to control, given by [rate of control – rate of sample reaction] / rate of control) × 100. Allopurinol was used as a reference compound.

Acknowledgments. This work was supported by research funds from Chosun University in 2011.

References