Phenolic Glycosides with Antioxidant Activity from the Stem Bark of *Populus davidiana*

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Received May 29, 2006

Phytochemical study on the EtOAc-soluble fraction of the stem bark of *Populus davidiana* resulted in the isolation of 10 phenolic glycosides (**1**−**10**), which were identified on the basis of physicochemical and spectroscopic analyses. Among these, three new compounds, populosides A−C (**1**−**3**), were determined to be 2-coumaroylmethyl-4-hydroxyphenyl-*β*-D-glucopyranoside, 2-coumaroylmethylphenyl-*β*-D-glucopyranoside, and 2-feruoylmethylphenyl-*β*-D-glucopyranoside, respectively. Compounds **1**−**10** were tested for their radical scavenging activity against an azo radical, $ABTS^{\bullet+}$. Of these, populosides A−C (**1**−**3**), populoside (**4**), grandidentatin (**8**), salireposide (**9**), and coumaroyl-*β*-D-glucoside (**10**) exhibited antioxidant activity in this assay.

The genus *Populus* belonging to the Salicaceae family comprises more than 100 species, which are distributed in temperature zone and subtropical regions.[1] Among these, *Populus davidiana* Dode [*P. tremela.* L. var. *davidiana* (Dode) Schneid.] is distributed throughout Korea, Northern China, and Siberia.[2] The plant has been used traditionally for treatment of various diseases, including diarrhea, paralysis, pulmonary disease, pox, and variola.[2] Some phenolic glycosides and flavonoids have been isolated from this plant.[3,4] Previous phytochemical studies have also revealed the presence of phenolic glycosides,[5−10] flavanoids,[11] and organic acids[12] in other species of the genus *Populus*. As a part of our ongoing search for bioactive compounds from natural sources, phytochemical investigation of the EtOAc-soluble fraction of a MeOH extract of *P. davidiana* has resulted in the isolation of three new phenolic glycosides along with seven known ones. This paper deals with the isolation, structure elucidation, and scavenging activity against the $ABTS^{\bullet+}$ radical of these compounds.

Repeated chromatography of the EtOAc-soluble fraction of the MeOH extract on silica gel, YMC gel, Sephadex LH-20, and C_{18} columns led to the isolation of phenolic glycosides (**1**−**10**). Seven of these were known compounds identified as populoside (**4**),[13−15] tremulacin (**5**),[16] tremuldin (**6**),[16] salicin (**7**),[17,18] grandidentatin (**8**),[17] salireposide (**9**),[19,20] and coumaroyl-*β*-D-glucoside (**10**),[21] by comparison of physicochemical (mp, $[\alpha]_D$) and spectroscopic (UV, IR, ^1H and ^{13}C NMR) data with published values.The molecular formula of **1** was deduced to be $C_{22}H_{24}O_{10}$, on the basis of the peak at *m*/*z* 471.1263 $[M + Na]^+$ (calcd for $C_{22}H_{24}O_{10}Na$, 471.1267) in the HRFABMS. The IR spectrum showed absorption bands at 3350, 1680, 1600, 1510, and 1460 cm^{-1}, characteristic of hydroxyl, a ketone, and aromatic groups. The ^1H NMR spectrum revealed proton signals at *δ* 6.71 (d, *J* = 3.0 Hz, H-3), 6.65 (dd, *J* = 3.0, 8.4 Hz, H-5), and 7.0 (d, *J* = 8.4 Hz, H-6), indicating the presence of a 1,3,4-trisubstituted phenolic ring. In addition, a pair of oxygenated methylene protons at *δ* 5.25 and 5.30 (each H, d, *J* = 13.2 Hz, H-7) were evident in the ^1H NMR due to an oxygenated methylene group (δ_C 61.2) in the HMQC spectrum, which correlated with aromatic carbons (δ_C 148.3) in HMBC. Furthermore, the ^1H NMR spectrum of **1** showed AA′BB′ spin system protons at δ_H 6.80 (2H, d, *J* = 8.4 Hz, H-13, 15) and 7.58 (2H, d, *J* = 8.4 Hz, H-12, 16), as well as two *trans*-olefinic protons at *δ* 6.48 (1H, d, *J* = 16.2 Hz, H-9) and 7.66 (1H, d, *J* = 16.2 Hz, H-10). The ^{13}C NMR of **1** disclosed two pairs of overlapped carbon signals at δ_C 130.9 (C-12, 16) and 116.2 (C-13, 15), together with two quaternary carbons at δ_C 125.6 (C-11) and 160.4 (C-14). These observations suggested the presence of a acylated *p*-coumaric moiety for **1**,[22] which was further confirmed by the $^1H-^1H$ COSY couplings between H-9/H-10, H-12/H-16, and H-13/H-15 and by the relevant $^{13}C-^1H$ long-range correlations observed in the HMBC spectrum. Linkage of the acylated *p*-coumaric moiety with gentisyl alcohol was determined on the basis of the HMBC correlation between oxygenated methylene protons (*δ* 5.25 and 5.30) and the carbonyl carbon (*δ* 167.1). Enzymatic hydrolysis of **1** yielded gentisyl alcohol, coumaric acid, and *β*-D-glucose. The configuration of the glycosidic linkage for the glucopyranoside unit was determined to be *β* form on the basis of the $J_{1,2}$ value of the anomeric proton at 7.2 Hz (*δ* 4.86). Thus, the structure of compound **1**, named populoside A, was assigned as 2-coumaroylmethyl-4-hydroxyphenyl-*β*-D-glucopyranoside.

Compound **2** was obtained as white needles. Its IR spectrum also revealed absorption bands at 3400, 1690, 1600, and 1520 cm^{-1}, characteristic of hydroxyl, a ketone, and aromatic groups. Similar to **1**, the ^1H NMR together with ^{13}C NMR patterns of **2** disclosed a sugar moiety that was deduced to a *β*-D-glucopyranosyl unit, a benzoyl moiety, and a coumaroyl moiety. Hence, this evidence suggested a skeleton similar to **1**. However, the ^1H NMR presented four protons signals at *δ* 7.16 (d, *J* = 8.4 Hz, H-6), 7.29 (td, *J* = 7.8, 1.2 Hz, H-5), 7.03 (t, *J* = 7.2 Hz, H-4), and 7.33 (br d, *J* = 7.8 Hz, H-3) (Table 1). These were indicative of the presence of an *ortho*-disubstituted aromatic ring in **2**, instead of the 1,3,4-trisubstituted phenolic ring of **1**, which was supported by correlations in the $^1H-^1H$ COSY. This observation was consistent with the molecular formula $C_{22}H_{24}O_9$, as established by the peak 455.1313 $[M + Na]^+$ (calcd for $C_{22}H_{24}O_9Na$, 455.1318) from HRFABMS. Furthermore, enzymatic hydrolysis of **2** yielded salicyl alcohol, coumaric acid, and D-glucose. Therefore, compound **2** was characterized as 2-coumaroylmethylphenyl-*β*-D-glucopyranoside, named populoside B.

Compound **3**, named populoside C, was isolated as white needles. The IR and ^1H and ^{13}C NMR spectra of **3** were quite similar to those of **2**, except for the presence of a methoxy group (δ_H 3.81, δ_C 56.2). The HMBC spectrum demonstrated that this methoxy group was located on a feruoyl moiety, indicating an acylated ferulic

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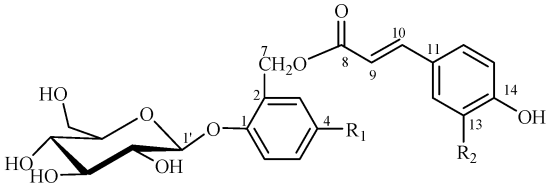
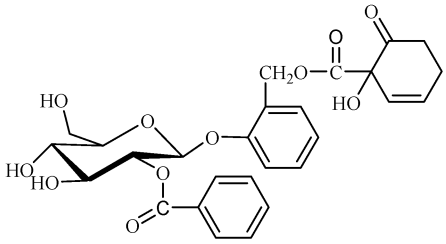
10.1021/np060237u CCC: $33.50
Published on Web 09/02/2006

Chart 1



	R_1	R_2
1	OH	H
2	H	H
3	H	OCH_3
4	H	OH



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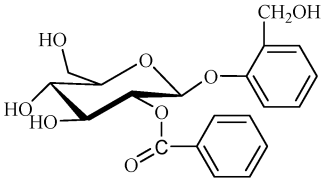

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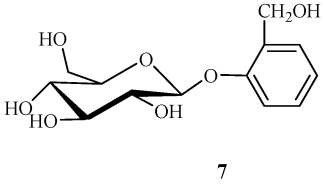

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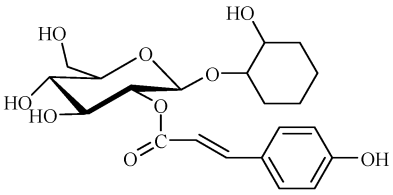

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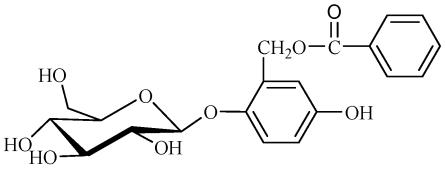

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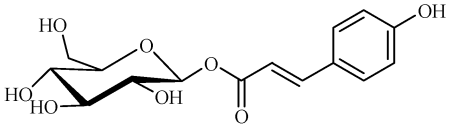

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Table 1. ^{1}H NMR Spectroscopic Data (δ) of Compounds **1**−**4**

proton	**1**[a]	**2**[a]	**3**[a]	**4**[b]
3	6.71 d (3.0)[c]	7.33 br d (7.8)	7.35 br d (7.8)	7.34 br d (7.6)
4		7.03 t (7.2)	7.03 t (7.8)	7.04 t (7.6)
5	6.65 dd (3.0, 8.4)	7.29 td (7.8, 1.2)	7.31 td (7.8, 1.2)	7.28 td (7.6, 1.5)
6	7.0 d (8.4)	7.16 d (8.4)	7.17 d (7.8)	7.23 d (7.6)
7	5.19 d (13.2)	5.25 d (13.2)	5.26 d (13.2)	5.29 d (13.2)
	5.22 d (13.2)	5.30 d (13.2)	5.31 d (13.2)	5.41 d (13.2)
9	6.45 d (15.6)	6.48 d (16.2)	6.56 d (15.6)	6.32 d (16.0)
10	7.60 d (15.6)	7.66 d (16.2)	7.62 d (15.6)	7.59 d (16.0)
12	7.56 d (8.4)	7.58 d (8.4)	7.35 br d (7.8)	7.36 d (2.0)
13	6.80 d (8.4)	6.80 d (8.4)		
15	6.80 d (8.4)	6.80 d (8.4)	6.80 d (7.8)	6.77 d (8.0)
16	7.58 d (8.4)	7.58 d (8.4)	7.13 dd (1.2, 8.4)	6.95 dd (2.0, 8.0)
1	4.63 d (7.2)	4.86 d (7.2)	4.87 d (7.2)	4.93 d (7.2)
2′−5′	3.17−3.33 m	3.17−3.33 m	3.18−3.34 m	3.30−3.52 m
6′	3.71 dd (3.6, 12.0)	3.71 dd (3.6, 12.0)	3.71 dd (3.6, 12.0)	3.87 dd (1.6, 12.0)
	3.49 m	3.49 m	3.49 m	3.68 m
OCH_3			3.81 s	

[a] Spectra recorded at 600 MHz in DMSO-d_6. [b] Spectra recorded at 400 MHz in MeOH-d_4. [c] J values (in Hz) in parentheses.

group for **3** instead of the coumaroyl group of **2**. This evidence led to the conclusion that **3** was 2-feruoylmethylphenyl-β-D-glucopyranoside. The structure was further supported by the molecular formula $C_{23}H_{26}O_{10}$, determined by the molecular peak at *m*/*z* 485.1419 $[M + Na]^+$ in the HRFABMS, and the result obtained from enzymatic hydrolysis of compound **3**. To our knowledge, this is the first isolation of these three compounds, populosides A−C, from a natural source.

Compounds **1**−**10** were evaluated for their scavenging activity against the $ABTS^{\bullet+}$ radical. As the results presented in Table 3 indicate, all compounds possessing hydroxyl phenolic group, **1**−**4** and **8**−**10**, exhibited higher scavenging activities than that of BHT used as a positive control. However, only compound **1** showed higher quenching ability (TEAC = 2.07) than that of caffeic acid (TEAC = 1.98). Compounds **5**−**7** had very weak activities, most likely due to the lack of the phenolic groups.

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler micro-hotstage. UV spectra were obtained with a Beckman Du-650 UV−vis recording spectrophotometer. IR spectra were obtained on a Jasco Report-100 type spectrometer from KBr disc. MS were carried out with a JEOL JMS-HX/HX110A tandem mass spectrometer. ^{1}H NMR (300, 400, and 600 MHz) and ^{13}C NMR (75, 100, and 150 MHz) were recorded on Bruker DRX300 and JEOL 400 spectrometers (the chemical shifts were referenced to δ using TMS as an internal standard). Two-dimensional (2D) NMR experiments (HMBC, HMQC, and COSY) were recorded on a Bruker Avance 500 spectrometer.

Plant Material. The stem bark of *Populus davidiana* Dode was collected from Yangu, Kangwon, Korea, in August 2002, and identified by one of authors, K.B. A voucher specimen (CNU 255) has been deposited at the Herbarium in the College of Pharmacy, Chungnam National University.

Table 2. ^{13}C NMR Spectroscopic Data (δ) of Compounds **1**−**4**

carbon	**1**[a]	**2**[a]	**3**[a]	**4**[b]
1	148.3	155.6	155.6	157.0
2	127.4	125.7	125.7	127.2
3	115.2	129.1	129.2	130.4
4	152.7	122.3	122.3	123.0
5	115.6	129.7	129.7	130.6
6	118.0	115.4	115.4	115.1
7	61.3	61.2	61.2	62.5
8	167.1	167.1	167.1	169.3
9	114.5	114.6	114.9	116.5
10	145.6	145.5	145.8	147.2
11	125.5	125.6	126.1	127.7
12	130.9	130.9	111.7	115.0
13	116.3	116.2	149.9	149.6
14	160.3	160.4	148.4	146.7
15	116.3	116.2	116.0	116.6
16	130.9	130.9	123.8	123.5
1′	102.9	101.5	101.5	102.9
2′	73.8	73.8	73.8	74.9
3′	77.3	77.6	77.6	78.2
4′	70.2	70.2	70.2	71.3
5′	76.9	77.0	77.0	78.0
6′	61.1	61.2	61.2	62.6
OCH_3			56.2	

[a] Spectra obtained for **1**−**3** at 150 MHz in DMSO-d_6. [b] Spectra obtained for **4** at 100 MHz in MeOH-d_4.

Extraction and Isolation. The dried and milled stem bark of *P. davidiana* (4.8 kg) was extracted with hot MeOH three times (3 × 20 L). After filtration and evaporation of the solvent under reduced pressure, the combined crude methanolic extract (1.3 kg) was suspended in H_2O and then successively partitioned with hexane and ethyl acetate (EtOAc) to afford hexane-soluble (47 g) and EtOAc-soluble (770 g) fractions. The EtOAc-soluble fraction was subjected to silica gel column chromatography using $CHCl_3$ and MeOH mixtures of increasing polarity (80:1 to 1:1) to yield seven fractions (E1−E7). Fraction E4 was subjected to silica gel column chromatography using mixtures of $CHCl_3$ and acetone of increasing polarity (50:1 to 1:2) to give eight subfractions (E4.1−E4.8). Subfraction E4.6 was subjected to a silica gel column eluting with $CHCl_3$−MeOH−H_2O (80:20:1) to yield compounds **5** (1.3 g) and **6** (820 mg). Fraction E5 was applied to a silica gel column and eluted with $CHCl_3$−MeOH−H_2O mixtures of increasing polarity (15:1:0.1 to 1:2:0.1) to yield seven subfractions (E5.1−E5.7). The subfraction E5.4 was further chromatographed on a YMC column using MeOH−H_2O (1:9, 3:7, 1:1, and 1:0) as eluted solvent to give four subfractions (E5.4.1−E5.4.4). Subfraction E5.4.1 was purified by a silica gel column with $CHCl_3$−MeOH (8:1) and then an C_{18} column using MeOH−H_2O (2:3) to give compound **7** (260 mg). Fraction E5.4.2 was subjected to a silica gel column eluting with $CHCl_3$−MeOH−H_2O (12:1:0.1) to obtain compounds **8** (540 mg) and **9** (48 mg). Fraction E5.4.3 was chromatographed on a silica gel column eluted with $CHCl_3$−MeOH (15:1), and then a collected subfraction was further chromatographed on an C_{18} column eluted with MeOH−H_2O (1:1.5) to yield compounds **2** (268 mg) and **3** (138 mg). Fraction E5.7 was chromatographed on a silica gel column using $CHCl_3$−MeOH−H_2O (6:1:0.1) as eluted solvent system to give five subfractions (E5.7.1−E5.7.5). Subfractions E5.7.1, E5.7.2, and E5.7.3 were further subjected to C_{18} columns eluted with MeOH−H_2O (1:1) and finally crystallized from MeOH−H_2O (1:1) to yield crystal needles of compounds **1** (132 mg), **4** (350 mg), and **10** (235 mg), respectively.

Populoside A (1): white crystal needles (MeOH); mp 162−163 °C; $[\alpha]_D^{25}$ −2.67 (*c* 0.05 MeOH); UV (MeOH) λ_{max} (log ϵ) 229. 0 (4.29), 316 (4.42) nm; IR (KBr) ν_{max} cm^{-1} 3350, 1680, 1600, 1510, 1460, 1200, 1080, 980, 870, 820; FABMS *m/z* 471.0 [M + Na]$^+$; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m/z* 471.1263 [M + Na]$^+$ (calcd for $C_{22}H_{24}O_{10}Na$, 471.1267).

Enzymatic Hydrolysis of 1. Glucosidase (200 mg, from almond) was added to a suspension of **1** (50 mg) in 50 mM phosphate buffer, which was adjusted to pH 7.0 with NaOH, and the mixture was stirred at 37 °C for 48 h. The reaction mixture was extracted with EtOAc (20 mL × 3), and the organic layer was evaporated to dryness. The residue was chromatographed using preparative TLC with $CHCl_3$−MeOH (8:1), giving gentisyl alcohol [10 mg, white needle, ^1H NMR (400 MHz, MeOH-d_4) δ 4.67 (2H, br s, H-7), 6.72 (1H, d, J = 3.0 Hz, H-3), 6.58 (1H, dd, J = 3.0, 8.4 Hz, H-5), 6.65 (1H, d, J = 8.4 Hz, H-6)] and coumaric acid [7 mg, gray-yellow powder, ^1H NMR (400 MHz, MeOH-d_4) δ 6.35 (1H, J = 16.0 Hz, H-8), 6.74 (2H, J = 8.0 Hz, H-2, 6), 7.37 (2H, J = 8.0 Hz, H-3, 5), 7.35 (1 H, J = 16.0 Hz, H-7)].

The water layer was checked by silica gel TLC (EtOAc−MeOH−H_2O−AcOH, 65:20:15:15), and the TLC plate was visualized using anisaldehyde−H_2SO_4 reagent. The configuration of glucose was determined by a GC method described previously.[23] The obtained sugar derivative showed a retention time that was identical with that of authentic D-glucose.

Populoside B (2): white crystal needles (MeOH−H_2O); mp 187−188 °C; $[\alpha]_D^{25}$ −3.13 (*c* 0.05 MeOH); UV (MeOH) λ_{max} (log ϵ) 205.0 (4.23), 321.0 (4.22) nm; IR (KBr) ν_{max} cm^{-1} 3400, 2910, 1690, 1600, 1520, 1240, 1080, 760; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; FABMS *m/z* 455.0 [M + Na]$^+$; HRFABMS *m/z* 455.1313 [M + Na]$^+$ (calcd for $C_{22}H_{24}O_9Na$, 455.1318).

Enzymatic Hydrolysis of 2. Glucosidase (200 mg, from almond) was added to a suspension of **2** (50 mg) in 50 mM phosphate buffer adjusted to pH 7.0 with NaOH, and the mixture was stirred at 37 °C for 48 h. Workup as above (preparative TLC, $CHCl_3$−MeOH, 8:1) gave salicyl alcohol [8 mg, white needles, ^1H NMR (400 MHz, MeOH-d_4) δ 4.60 (2H, br s, H-7), 6.72 (1H, d, J = 8.0 Hz, H-6), 7. 03 (1H, td, J = 7.7, 1.8 Hz, H-5), 6.75 (1H, td, J = 7.3, 1.1 Hz, H-4), 7.21 (1H, br d, J = 7.7 Hz, H-3)] and coumaric acid [12 mg, gray-yellow powder, ^1H NMR (400 MHz, MeOH-d_4) δ 6.25 (1H, J = 16.0 Hz, H-8), 6.66 (2H, J = 8.0 Hz, H-2, 6), 7.27 (2H, J = 8.0 Hz, H-3, 5), 7.25 (1H, J = 16.0 Hz, H-7)].

Populoside C (3): white crystal needles (MeOH−H_2O); mp 109−111 °C; $[\alpha]_D^{25}$ −1.80 (*c* 0.05 MeOH); UV (MeOH) λ_{max} (log ϵ) 219 (4.29), 328.0 (4.30) nm; IR (KBr) ν_{max} cm^{-1} 3380, 1690, 1620, 1590, 1520, 1450, 1285, 1240, 1180, 1080, 980, 750; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; FABMS *m/z* 485.0 [M + Na]$^+$; HRFABMS *m/z* 485.1419 [M + Na]$^+$ (calcd for $C_{23}H_{26}O_{10}Na$, 485.1424).

Enzymatic Hydrolysis of 3. Glucosidase (200 mg, from almond) was added to a suspension of **3** (50 mg) in 50 mM phosphate buffer adjusted to pH 7.0 with NaOH, and the mixture was stirred at 37 °C for 48 h. Workup as above (preparative TLC, $CHCl_3$−MeOH, 8:1) gave salicyl alcohol [6 mg, white needles, ^1H NMR (400 MHz, MeOH-d_4) δ 4.60 (2H, br s, H-7), 6.72 (1H, d, J = 8.0 Hz, H-6), 7.03 (1H, td, J = 7.7, 1.8 Hz, H-5), 6.75 (1H, td, J = 7.3, 1.1 Hz, H-4), 7.21 (1H, br d, J = 7.7 Hz, H-3)] and ferulic acid [9 mg, white needles, ^1H NMR (400 MHz, MeOH-d_4) δ 6.34 (1H, d, J = 16.0 Hz, H-8), 6.97 (1H, dd, J = 4.0, 8.0 Hz, H-2), 6.76 (1H, d, J = 8.0 Hz, H-3), 7.11 (1H, d, J = 4.0 Hz, H-6), 7.32 (1H, d, J = 16.0 Hz, H-7), 3.87 (3H, s, OCH_3)].

Populoside (4): white crystal needles (MeOH−H_2O); mp 169−170 °C; $[\alpha]_D^{25}$ −3.27 (*c* 0.05 MeOH); UV (MeOH) λ_{max} (log ϵ) 220.0 (4.23), 333 (4.21) nm; IR (KBr) ν_{max} cm^{-1} 3400, 1700, 1600, 1370, 1280, 1160, 1100, 1050, 760; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively.

Table 3. Scavenging Activities of Isolates (**1**−**10**) against $ABTS^{\bullet+}$ Radical

compound	TEAC[a,b]
1	2.07 ± 0.02
2	1.13 ± 0.02
3	1.55 ± 0.01
4	1.67 ± 0.03
5	0.12 ± 0.01
6	0.24 ± 0.01
7	0.21 ± 0.01
8	1.27 ± 0.02
9	1.01 ± 0.02
10	0.78 ± 0.01
caffeic acid[c]	1.98 ± 0.03
BHT[c]	0.80 ± 0.01

[a] The values of scavenging activity against $ABTS^{\bullet+}$ were expressed as Trolox equivalent antioxidant capactity (TEAC), which is the concentration (mM) of Trolox having the same activity as 1 mM of sample. [b] Values are mean ± SD of three experiments. [c] Reference substances.

$ABTS^{\bullet+}$ Radical Scavenging Assay. To analyze the antioxidant activity, a common stable radical chromogen, 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) was used. $ABTS^{\bullet+}$ radical

cation was produced by reacting ABTS with potassium persulfate.[24] In brief, ABTS was dissolved at a 7 mM concentration in 10 mL of H_2O and ABTS radical cation was produced by adding 400 μL of 60 mM $K_2S_4O_8$ (final concentration 2.45 mM). The mixture was stored in the dark at room temperature for 16 h. For the study of antioxidant compounds, the $ABTS^{\bullet+}$ solution was diluted with H_2O to give an absorbance of 0.700 $\pm$ 0.020 at 734 nm. For the scavenging assay, 990 μL of diluted $ABTS^{\bullet+}$ solution was added to 10 μL of test compounds or Trolox standards (final concentration 0−20 μM) in MeOH, and the absorbance was read at ambient temperature exactly 6 min after the initial mixing. Stock solutions of the compounds were prepared so that they produced 10−80% inhibition of the blank absorbance. Appropriate solvent blanks were run in each assay. The percentage decrease of the absorbance at 734 nm was calculated and plotted as a function of the concentration of the antioxidants and of Trolox for the standard reference data. To calculate the Trolox equivalent antioxidant coefficient (TEAC), the slope of the plot of the percentage inhibition of absorbance versus concentration for the antioxidant was divided by the slope of the plot of Trolox. All determinations were carried out in triplicate.

Acknowledgment. The research was supported by a grant from Biogreen Project (2006). We are grateful to Korea Basic Science Institute (KBSI) for supplying the NMR spectra.

Supporting Information Available: ^1H and ^{13}C NMR and HMBC spectra for populosides A−C (**1**−**3**) are available free of charge via the Internet at http://pubs.acs.org.

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NP060237U