Antioxidative Phenolics from the Fresh Leaves of *Ternstroemia japonica*

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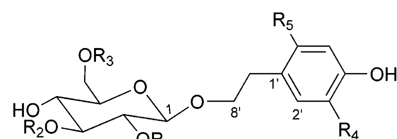
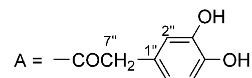
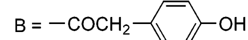
Six new phenylethanoid glucosides, ternstrosides A−F (**1**−**6**), a new kaempferol derivative (**7**), and eight known compounds were isolated from the fresh leaves of *Ternstroemia japonica*. The structures were elucidated by 1D and 2D NMR spectroscopic analyses. Compounds **1**−**7** showed potent antioxidative activity in three different tests, with IC_{50} values in the range 3.26−6.50 μM in the hydroxyl radical (•OH) inhibitory activity test, 33.29−82.21 μM in the total ROS (reactive oxygen species) inhibitory activity test, and 1.14−13.53 μM in the peroxynitrite ($ONOO^-$) scavenging activity test.

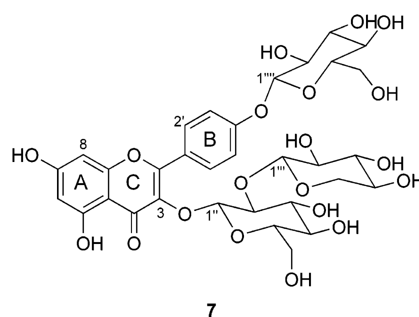
Ternstroemia japonica Thunb. (Theaceae) is a tall evergreen tree that grows in Korea, Japan, China, and the Philippines.[1] Its flowers, fruits, and leaves have been used for the treatment of chest pain and numbness in traditional Chinese medicine.[2] An aldehydic carotenoid[3] and triterpenes such as oleanolic acid, primulagenin A, camelliagenin A, and A_1-barrigenol[4] have been reported from this plant. In our previous studies, eight saponins[5] and four jacaranone derivatives[6] have been isolated from the *n*-BuOH fraction and the EtOAc fraction of the fresh fruits of *T. japonica*, respectively. As a part of our ongoing search for antioxidative constituents from natural sources, we investigated the antioxidative activities of the solvent-partitioned fractions from the MeOH extract of the fresh leaves (Table 4). The *n*-BuOH-soluble fraction of the leaves showed stronger antioxidative activity than that of the fruits (12.15 ± 0.09 μg/mL, IC_{50} in hydroxyl radical inhibitory activity test), and the leaves are a more economical source of the antioxidative material than fruits since they are available any season. From the *n*-BuOH-soluble fraction, we isolated and characterized six new phenylethanoid glucosides, ternstrosides A−F (**1**−**6**), a new kaempferol derivative (**7**), and eight known compounds, 2-(3,4-dihydroxyphenyl)ethyl β-D-glucopyranoside,[7,8] 2-(4-hydroxyphenyl)ethyl β-D-glucopyranoside,[9] kaempferol 3-*O*-β-D-galactopyranoside,[10] quercetin 3-*O*-β-D-glucopyranoside,[11] hyperin,[12] quercitrin,[13] methyl 3,4-dihydroxyphenylethanoate,[14] and jacaranone.[6,15−17] This paper deals with the isolation and structure elucidation of the new compounds (**1**−**7**) and the evaluation of their antioxidative activity.







	R_1	R_2	R_3	R_4	R_5
1	A	H	H	OH	H
2	B	H	H	OH	H
3	H	A	H	OH	H
4	H	H	A	OH	H
5	A	H	H	H	H
6	A	H	H	OH	OH



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Results and Discussion

Ternstroside A (**1**) was isolated as a yellow, amorphous solid. The positive-ion HRFABMS of **1** showed a quasimolecular ion at *m/z* 489.1373 $[M + Na]^+$, suggesting the molecular formula $C_{22}H_{26}O_{11}$. The ^1H NMR spectrum of **1** displayed an anomeric proton resonance at δ 4.39 and aromatic protons at δ 6.49−6.75. The ^{13}C NMR spectrum of **1** also showed the existence of a sugar and aromatic rings. The anomeric C-1 (δ 101.0) was correlated with H-8′a (δ 3.90) and H-8′b (δ 3.47) in the HMBC spectrum. The H-8′a and H-8′b signals were correlated in the COSY spectrum and further correlated with the signal at H-7′ (δ 2.54). In the HMBC spectrum of **1**, H-7′ was correlated with three aromatic carbon signals at δ 130.4 (C-1′), 115.1 (C-2′), and 120.2 (C-6′), as well as C-8′ (δ 70.7). These data suggested that **1** had a phenylethanoid glycoside as a partial structure. The resonance at δ 4.69 was assigned to H-2 of the sugar moiety from the correlation with the anomeric proton (δ 4.39) in the COSY spectrum of **1**. The phenylethanoyl substitution at C-2 was revealed by the HMBC experiment where H-2 was correlated to the ester carbonyl carbon (δ 172.0, C-8″). C-8″ was correlated with H-7″ at δ 3.52, and H-7″ was correlated to the aromatic carbon resonances at δ 125.9 (C-1″), 116.5 (C-2″), and 120.7 (C-6″) in the HMBC spectrum of **1**. The substitution site is supported by the chemical shift of H-2 (δ 4.69), which was shifted downfield compared to that of the corresponding proton of 2-(3,4-dihydroxyphenyl)ethyl β-D-glucopyranoside (δ 3.17). The positions of the hydroxyl groups in the aromatic rings were determined by comparison of the chemical shifts and splitting patterns of the aromatic protons [δ 6.62 (d, J = 2.0 Hz, H-2′), 6.66 (d, J = 8.5 Hz, H-5′), 6.49 (dd, J = 8.5, 2.0 Hz, H-6′); δ 6.75 (d, J = 2.0 Hz, H-2″), 6.70 (d, J = 8.0 Hz, H-5″), 6.59 (dd, J = 8.0, 2.0 Hz, H-6″)] with those of the compounds with the same hydroxylation pattern.[8] The sugar was identified as glucose by acid hydrolysis and analytical HPLC. Therefore, the structure of **1** was defined as 2-(3,4-dihydroxyphenyl)ethyl 2-*O*-(3,4-dihydroxyphenylethanoyl)-β-D-glucopyranoside.

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Table 1. ^1H NMR Data of **1**−**4** (CD_3OD, 500 MHz)[a]

position	**1**	**2**	**3**	**4**
1	4.39 (d, 7.5)	4.41 (d, 8.0)	4.39 (d, 8.0)	4.24 (d, 7.5)
2	4.69 (dd, 9.0, 7.5)	4.71 (dd, 10.0, 8.0)	3.32 (m)	3.16 (dd, 8.5, 7.5)
3	3.50 (m)	3.52 (m)	4.97 (m)	3.30 (m)
4	3.30 (m)	3.39 (m)	3.48 (m)	3.26 (m)
5	3.27 (m)	3.35 (m)	3.34 (m)	3.42 (m)
6a	3.86 (dd, 12.0, 2.0)	3.87 (dd, 12.0, 2.0)	3.87 (dd, 12.0, 2.0)	4.42 (dd, 12.0, 2.0)
6b	3.67 (dd, 12.0, 6.0)	3.71 (m)	3.69 (m)	4.20 (dd, 12.0, 6.0)
2′	6.62[b] (d, 2.0)	6.63 (d, 2.0)	6.70[b] (d, 2.0)	6.68[b] (d, 2.0)
5′	6.66[c] (d, 8.5)	6.68 (d, 8.0)	6.68[c] (d, 8.0)	6.65[c] (d, 7.5)
6′	6.49[d] (dd, 8.5, 2.0)	6.51 (dd, 8.0, 2.0)	6.57[d] (dd, 8.0, 2.0)	6.55[d] (dd, 7.5, 2.0)
7′	2.54 (t, 7.5)	2.57 (m)	2.79 (m)	2.75 (t, 7.5)
8′a	3.90 (dt, 9.5, 7.5)	3.94 (m)	4.04 (m)	3.88 (dt, 9.5, 7.5)
8′b	3.47 (dt, 9.5, 7.5)	3.52 (m)	3.72 (m)	3.62 (dt, 9.5, 7.5)
2″	6.75[b] (d, 2.0)	7.09 (d, 8.5)	6.77[b] (d, 2.0)	6.71[b] (d, 2.0)
3″		6.74 (d, 8.5)		
5″	6.70[c] (d, 8.0)	6.74 (d, 8.5)	6.71[c] (d, 8.0)	6.67[c] (d, 7.5)
6″	6.59[d] (dd, 8.0, 2.0)	7.09 (d, 8.5)	6.62[d] (dd, 8.0, 2.0)	6.56[d] (dd, 7.5, 2.0)
7″	3.52 (m)	3.53 (m)	3.59 (s)	3.45 (s)

[a] Multiplicities and coupling constants (in Hz) are in parentheses. [b,c,d]Assignments with the same superscript in the same column may be interchanged.

Ternstroside B (**2**) was isolated as a yellow, amorphous solid. The positive-ion HRFABMS of **2** showed a quasimolecular ion at *m/z* 473.1429 $[M + Na]^+$, suggesting the molecular formula $C_{22}H_{26}O_{10}$. The ^1H NMR data of **2** were similar to those of **1**, except for the splitting pattern of the aromatic protons, due to the absence of one hydroxyl group. The positions of the hydroxyl groups on the aromatic rings were determined by comparison of the chemical shifts and splitting patterns of the aromatic protons [δ 6.63 (d, J = 2.0 Hz, H-2′), 6.68 (d, J = 8.0 Hz, H-5′), 6.51 (dd, J = 8.0, 2.0 Hz, H-6′); δ 7.09 (d, J = 8.5 Hz, H-2″,6″), 6.74 (d, J = 8.5 Hz, H-3″,5″)] with those of the compounds with the same hydroxylation pattern.[8,9] The corresponding aromatic carbons were assigned from HSQC data of **2**. The position of the aromatic rings with different hydroxylation was deduced from the HMBC spectrum of **2**, where H-7′ (δ 2.57) was correlated to the resonances at δ 130.2 (C-1′), 115.9 (C-2′), and 120.1 (C-6′), and H-7″ (δ 3.53) was correlated to the resonances at δ 125.2 (C-1″) and 130.3 (C-2″,6″). The structure of the phenylethanoid glycoside and the phenylethanoyl substitution at C-2 of the sugar moiety in **2** was deduced in the same manner as for **1**. Thus, compound **2** was identified as 2-(3,4-dihydroxyphenyl)ethyl 2-*O*-(4-hydroxyphenylethanoyl)-β-D-glucopyranoside.

Ternstroside C (**3**) was isolated as a yellow, amorphous solid. The positive-ion HRFABMS of **3** showed a quasimolecular ion at *m/z* 489.1371 $[M + Na]^+$, suggest the molecular formula $C_{22}H_{26}O_{11}$. The ^1H and ^{13}C NMR data of **3** were similar to those of **1**, except for the chemical shifts of the sugar moiety. In the COSY spectrum of **3**, the anomeric proton at δ 4.39 was correlated with H-2 at δ 3.32, which was further correlated with H-3 at δ 4.97. The phenylethanoyl substitution at C-3 was revealed by the HMBC correlation between H-3 and the ester carbonyl carbon (δ 172.6, C-8″). Determination of the substitution site was supported by the chemical shift of H-3 (δ 4.97), which was shifted downfield compared to that of 2-(3,4-dihydroxyphenyl)ethyl β-D-glucopyranoside (δ 3.30). The hydroxylation patterns of the two aromatic rings were determined from the chemical shifts and splitting patterns of the aromatic protons, as in **1**. The sugar residue was identified as glucose by acid hydrolysis, as above. Thus, compound **3** was identified as 2-(3,4-dihydroxyphenyl)ethyl 3-*O*-(3,4-dihydroxyphenylethanoyl)-β-D-glucopyranoside.

Ternstroside D (**4**) was isolated as a yellow, amorphous solid. The HRFABMS spectrum of compound **4** showed a quasimolecular ion at *m/z* 489.1370 $[M + Na]^+$, consistent with the molecular formula $C_{22}H_{26}O_{11}$. The ^1H and ^{13}C NMR data of **4** were similar to those of **1**, except for the chemical shifts of the sugar moiety. In the COSY spectrum, H-6a (δ 4.42) correlated with H-6b (δ 4.20), and both correlated with H-5 (δ 3.42). The downfield shift of the C-6 methylene protons, compared to those of 2-(3,4-dihydroxyphenyl)ethyl β-D-glucopyranoside (δ 3.86 and 3.66), is indicative of substitution of the phenylethanoyl group at C-6. The HMBC correlation from H-6 to C-8″ corroborated the substitution. The hydroxylation patterns of the aromatic rings were determined from the chemical shifts and splitting patterns of the aromatic protons, as in **1**. The sugar residue was identified as glucose, as above. Thus, compound **4** was formulated as 2-(3,4-dihydroxyphenyl)ethyl 6-*O*-(3,4-dihydroxyphenylethanoyl)-β-D-glucopyranoside.

Ternstroside E (**5**) was isolated as a yellow, amorphous solid. The HRFABMS spectrum of **5** showed a quasimolecular ion at *m/z* 473.1424 $[M + Na]^+$, consistent with the molecular formula $C_{22}H_{26}O_{10}$. The ^1H and ^{13}C NMR data of **5** were similar to those of **1**, except for the hydroxylation pattern of the phenylethyl moiety. The hydroxylation pattern on the aromatic rings was determined, as in **2**, on the basis of the ^1H NMR spectrum of **5**, which displayed two sets of spin systems assignable to a 1,3,4-trisubstituted aromatic ring [δ 6.75 (d, J = 2.0 Hz, H-2″), 6.69 (d, J = 8.5 Hz, H-5″), 6.58 (dd, J = 8.5, 2.0 Hz, H-6″)] and a 1,4-disubstituted aromatic ring [δ 6.97 (d, J = 8.5 Hz, H-2′,6′), 6.67 (d, J = 8.5 Hz, H-3′,5′)]. The corresponding aromatic carbons were assigned from the HSQC data of **5**. The structure of phenylethanoid glycoside and the phenylethanoyl substitution at C-2 of the sugar moiety in **5** was deduced in the same manner as for **2**. The signal of H-7″ appeared as a multiplet in the ^1H NMR spectrum of **5**, possibly due to the restricted rotation of the phenylethanoyl residue, which is different from **3** (1,3-disubstituted glucose) and **4** (1,6-disubstituted glucose). The sugar was identified as glucose by analytical HPLC of the acid hydrolyzate, as above. Therefore, **5** was identified as 2-(4-hydroxyphenyl)ethyl 2-*O*-(3,4-dihydroxyphenylethanoyl)-β-D-glucopyranoside.

Ternstroside F (**6**) was isolated as a yellow, amorphous solid. The positive-ion HRFABMS of **6** showed a quasimolecular ion at *m/z* 505.1320 $[M + Na]^+$, suggesting the molecular formula $C_{22}H_{26}O_{12}$. The ^1H NMR data of **6** were similar to those of **1**, except for the splitting pattern of the aromatic moiety. The hydroxylation pattern on the aromatic rings was determined, as above, on the basis of the ^1H NMR spectrum of **5**, which displayed AX and AMX spin systems. Two singlets at δ 6.83 and 6.43 from the AX spin system indicated a 2,4,5-trihydroxyphenyl moiety. The corresponding aromatic carbons were assigned from the HSQC data of **6**. From these observations, the structure of **6** was elucidated as 2-(2,4,5-trihydroxyphenyl)ethyl 2-*O*-(3,4-dihydroxyphenylethanoyl)-β-D-glucopyranoside.

Compound **7** was obtained as a brown powder. The positive LRESIMS of **7** showed a quasimolecular ion at *m/z* 743 $[M + H]^+$, suggesting the molecular formula $C_{32}H_{38}O_{20}$. In the ^1H NMR spectrum of **7**, the resonances at δ 8.13 (2H, d, J = 9.0 Hz, H-2′, 6′), 6.89 (2H, d, J = 9.0 Hz, H-3′, 5′), 6.40 (1H, d, J = 2.0 Hz,

Table 2. ^{1}H NMR Data of **5** and **6** (CD_3OD, 500 MHz)[a]

position	5	6
1	4.38 (d, 8.0)	4.31 (d, 8.0)
2	4.68 (dd, 10.0, 8.0)	4.58 (dd, 9.0, 8.0)
3	3.50 (m)	3.41 (m)
4	3.34 (m)	3.35 (m)
5	3.26 (m)	3.29 (m)
6a	3.85 (dd, 12.0, 2.0)	3.88 (dd, 12.0, 2.0)
6b	3.66 (dd, 12.0, 6.0)	3.68 (dd, 12.0, 6.0)
2′	6.97 (d, 8.5)	6.83[b] (s)
3′	6.67 (d, 8.5)	
5′	6.67 (d, 8.5)	6.43[b] (s)
6′	6.97 (d, 8.5)	
7′	2.57 (t, 7.0)	2.70 (m), 2.60 (m)
8′a	3.91 (dt, 9.5, 7.0)	4.09 (dm,[c] 10.0)
8′b	3.48 (m)	3.79 (dm,[c] 10.0)
2″	6.75 (d, 2.0)	6.46 (d, 2.0)
5″	6.69 (d, 8.5)	6.78 (d, 8.0)
6″	6.58 (dd, 8.5, 2.0)	6.66 (dd, 8.0, 2.0)
7″a	3.48 (m)	3.55 (d, 14.0)
7″b	3.43 (m)	3.36 (m)

[a] Multiplicities and coupling constants (in Hz) are in parentheses. [b]Assignments may be interchanged. [c]Doublet of multiplets.

Table 3. ^{13}C NMR Data of **1−6** (CD_3OD, 125 MHz)

position	1	2	3	4	5	6
1	101.0	101.0	103.1	103.2	102.2	101.6
2	74.3	74.3[a]	76.6	73.8	75.4	75.0[a]
3	74.9	74.9[a]	78.1	76.8	76.1	75.2[a]
4	70.5[d]	70.6	68.6	70.5	71.7	71.4
5	76.8	76.9	72.3	74.2	78.0	77.8
6	61.4	61.5	61.2	63.8	62.6	62.5
1′	130.4	130.2[c]	130.2	130.5	131.1[a]	130.5
2′	115.1[a]	115.9[b]	115.1[a]	115.2	131.0[a]	116.0[b]
3′	144.8[b]	143.4[d]	144.9	144.9[a]	116.1	142.4
4′	143.4[b]	144.2[d]	143.4	143.5[a]	156.7	146.3
5′	115.1[a]	116.1[b]	115.0[a]	115.2	116.1	115.3[b]
6′	120.2[c]	120.1	120.0[b]	120.1[b]	130.8[a]	153.2
7′	35.1	35.3	35.5	35.4	36.1	32.7
8′	70.7[d]	70.6	71.1	70.9	71.9	69.8
1″	125.9	125.2	125.9	125.7	127.0	126.3
2″	116.5[a]	130.3[c]	116.5	116.3	117.6	117.2[b]
3″	144.2[b]	115.1[b]	144.1	144.3[a]	146.2	144.9[c]
4″	145.0[b]	156.2	144.9	145.1[a]	145.4	145.4[c]
5″	116.0[a]	115.1[b]	115.9	116.0	116.2	116.1[b]
6″	120.7[c]	130.3[c]	120.7[b]	120.6[b]	121.8	120.9
7″	40.4	40.1	40.3	40.2	41.6	40.8
8″	172.0	171.9	172.6	172.8	173.0	173.0

[a,b,c,d] Assignments with the same superscript in the same column may be interchanged.

Table 4. Antioxidative Activity of the Solvent Partitions

sample	IC50 (μg/mL)[a]		
	•OH[b]	total ROS[c]	ONOO−[d]
EtOAc	29.24 ± 0.08	>300	30.33 ± 2.54
n-BuOH	4.34 ± 0.11	38.83 ± 0.19	6.05 ± 1.05
H_2O	36.76 ± 0.30	>300	59.47 ± 3.70
control	5.85 ± 0.33[e]	34.11 ± 0.15[e]	1.22 ± 0.05[f]

[a] Values of •OH, total ROS, and ONOO− are expressed as the mean ± standard error of triplicate experiments. [b] Inhibitory activity of hydroxyl radical generation in 1.0 mM H_2O_2 and 0.2 mM $FeSO_4$. [c] Inhibitory activity of total ROS generation in kidney postmicrosomal fraction. [d]Inhibitory activity of authentic peroxynitrite. [e]Trolox was used as a positive control. [f] DL-Penicillamine was used as a positive control.

H-8), and 6.19 (1H, d, J = 2.0 Hz, H-6) and anomeric proton resonances at δ 5.30 (d, J = 7.5 Hz, glc H-1″), 4.80 (xyl H-1‴, overlapped with solvent peak), and 4.15 (d, J = 7.5 Hz, glc H-1⁗) suggested that **7** is a kaempferol triglycoside, which was also corroborated by the ^{13}C NMR data. The sugars were identified as two glucoses and one xylose by acid hydrolysis and analytical HPLC. The inner glucose was identified by the downfield shift of H-1″ (δ 5.30).[18] The attachment position of xylose on the inner glucose was determined by correlation between H-1‴ (δ 4.80) and C-2″ (δ 80.1) in the HMBC spectrum of **7**. This was further supported by the downfield shift of C-2″ and the upfield shift of C-1″ (δ 100.1) by a β-effect.[19] The locations of glycosidic linkages on the aglycone were determined as C-3-OH and C-4′-OH of kaempferol by comparison of chemical shifts with those of kaempferol 3-*O*-β-D-galactopyranoside [C-4′, 158.5 (−2.2); C-1′, 122.7 (+1.1); C-3′, 5, 116.3 (+0.3)].[19] From the above data, compound **7** was deduced as 3-*O*-β-D-xylopyranosyl(1→2)-β-D-glucopyranosyl kaempferol 4′-*O*-β-D-glucopyranoside, which is unprecedented.

Table 5. Antioxidative Activity of Compounds **1−7**

compound	IC50 (μM)[a]		
	•OH[b]	total ROS[c]	ONOO−[d]
1	3.41 ± 0.09	39.26 ± 1.02	1.69 ± 0.02
2	4.66 ± 0.04	62.52 ± 0.51	2.36 ± 0.03
3	3.26 ± 0.01	33.29 ± 0.04	1.14 ± 0.01
4	6.50 ± 0.09	66.87 ± 3.01	3.85 ± 0.02
5	4.72 ± 0.04	54.98 ± 2.85	2.56 ± 0.05
6	4.21 ± 0.02	45.36 ± 0.62	1.54 ± 0.01
7	5.28 ± 0.03	82.21 ± 1.52	13.53 ± 0.07
quercetin	2.39 ± 0.05	29.12 ± 0.04	1.38 ± 0.02
control	2.83 ± 0.02[e]	29.52 ± 0.20[e]	1.05 ± 0.03[f]

[a] Values of •OH, total ROS, and ONOO− are expressed as the mean ± standard error of triplicate experiments. [b] Inhibitory activity of hydroxyl radical generation in 1.0 mM H_2O_2 and 0.2 mM $FeSO_4$. [c] Inhibitory activity of total ROS generation in kidney postmicrosomal fraction. [d]Inhibitory activity of authentic peroxynitrite. [e]Trolox was used as a positive control. [f] DL-Penicillamine was used as a positive control.

We investigated the general antioxidative effects of the compounds to inhibit •OH and total ROS and to scavenge authentic ONOO− for the isolated compounds (Table 5). Free radicals and ROS or RNS (reactive nitrogen species), including H_2O_2, $•O_2^-$, •OH, NO•, and ONOO−, play an important role in the etiology of a variety of human degenerative diseases. These reactive species are formed in the body as a consequence of aerobic metabolism and damage all intracellular components, such as nucleic acids, proteins, and lipids. ROS are also implicated in both aging and various degenerative disorders.[20] Compounds **1−7**, with phenolic hydroxyl groups in their structures, showed strong antioxidative activity in those three tests (Table 5). Compounds **1** and **3**, with (3,4-dihydroxyphenyl)acetyl moieties, showed stronger activity than **2**, with a (4-monohydroxyphenyl)acetyl moiety, in all tests. In the •OH and ROS scavenging activity tests, **1**, with a (3,4-dihydroxyphenyl)ethyl moiety at C-1, showed stronger activity than **5** or **6**, which has a (4-monohydroxyphenyl)ethyl or (2,4,5-trihydroxyphenyl)ethyl moiety, respectively, at the same position. Compound **7** showed less strong activity than quercetin but still significant activities in all tests. It is reported that in flavonoids the presence of an *o*-dihydroxyl substitution on the A- or B-ring is critical for the antioxidative activity.[21,22]

Experimental Section

General Experimental Procedures. HPLC was performed on an LKB Bromma 2248 HPLC Pump with a YMC J'sphere ODS-H80 (JH323, 250 × 10 mm i.d., S-4 μm, 8 nm), using a JASCO RI-1530 detector at a flow rate of 1 mL/min. Sugars were detected by HPLC using an analytical NH_2 column. ^{1}H and ^{13}C NMR spectra were measured on a Varian UNITY INOVA 500 (500 MHz) and a Bruker AC 200 (200 MHz). The chemical shifts are given in δ values (ppm) relative to tetramethylsilane (TMS) or residual solvent peak. FABMS data were recorded on a JEOL JMS 110/110 instrument. Optical rotations were obtained using a JASCO DIP-370 digital polarimeter. UV spectra were obtained in MeOH, using a Shimadzu mini 1240 UV−vis spectrophotometer. IR spectra were recorded on a JASCO FT/IR-410 in a KBr disc. GLC was performed on a Hewlett-Packard HP6890 with an HP-1 column. The fluorescence intensity was monitored by a microplate fluorescence spectrophotometer, Bio-Tek Instruments Inc., Winooski, VT.

Plant Material. The plant material was collected in Busan, Korea, in February 2003 and identified by Prof. K. S. Im, Pusan National University. A voucher specimen is deposited at Natural Product Chemistry Laboratory, Pusan National University, Busan, Korea.

Extraction and Isolation. The fresh leaves of *T. japonica* (1.8 kg) were extracted with MeOH under reflux. The MeOH extract was concentrated under reduced pressure, and the residue (140 g) was suspended in H_2O. The suspension was extracted with EtOAc and then with *n*-BuOH to afford 30 g of an EtOAc-soluble fraction and 27 g of an *n*-BuOH-soluble fraction. A portion (11.7 g) of the *n*-BuOH extract was chromatographed on silica gel (500 g) with a step gradient solvent system of CH_2Cl_2−MeOH (10:1 → 7:1 → 5:1), CH_2Cl_2−MeOH−H_2O (7:3:1, lower phase → 65:35:10, lower phase → 6:4:1), and 100% MeOH as eluants to give fractions 1 to 29. Fractions 5 to 7 (794 mg) were combined and subjected to MPLC on a Sephadex LH-20 column (4 × 30 cm) to afford 10 subfractions, B5-1 to B5-10. Subfraction B5-4 was subjected to HPLC on a C_{18} column with 40% MeOH (1% HOAc) to give **1** (8.3 mg, t_R 23 min), **2** (3.6 mg, t_R 32 min), **3** (14 mg, t_R 20 min), **4** (5.9 mg, t_R 28 min), and **5** (5.4 mg, t_R 35 min). Fractions 8 to 11 (2.13 g) were combined and separated by MPLC on Sephadex LH-20 (4 × 30 cm) to give subfractions B8-1 to B8-27. Subfraction B8-7 was subjected to repeated HPLC with 40% MeOH to yield **1** (124 mg, t_R 23 min), **4** (6 mg, t_R 27 min), **5** (3 mg, t_R 38 min), and **6** (2.4 mg, t_R 19 min). Fraction 24 (1.10 g) was subjected to Sephadex LH-20 column chromatography (4 × 30 cm) and then purified by HPLC on a C_{18} column with 57% MeOH to obtain compound **7** (9.8 mg, t_R 14 min).

Ternstroside A (1): yellow, amorphous solid; $[\alpha]^{24}_D$ −33.5 (*c* 2.30, MeOH); UV (MeOH) λ_{max} (log ϵ) 228 (3.42), 280 (2.38) nm; IR (KBr) ν_{max} 3737, 3610, 2919, 2854, 1739, 1690, 1646, 1515, 1457, 794, 671 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 3; HRFABMS *m/z* 489.1373 $[M + Na]^+$ (calcd for $C_{22}H_{26}O_{11}Na$, 489.1373).

Ternstroside B (2): yellow, amorphous solid; $[\alpha]^{24}_D$ −19.8 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ϵ) 235 (2.51), 257 (1.75) nm; IR (KBr) ν_{max} 3652, 2875, 1714, 1675, 1502, 752, 675 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 3; HRFABMS *m/z* 473.1429 $[M + Na]^+$ (calcd for $C_{22}H_{26}O_{10}Na$, 473.1424).

Ternstroside C (3): yellow, amorphous solid; $[\alpha]^{24}_D$ −31.6 (*c* 0.32, MeOH); UV (MeOH) λ_{max} (log ϵ) 236 (3.80), 290 (2.42) nm; IR (KBr) ν_{max} 3731, 3573, 2841, 1675, 1655, 1465, 682 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 3; HRFABMS *m/z* 489.1371 $[M + Na]^+$ (calcd for $C_{22}H_{26}O_{11}Na$, 489.1373).

Ternstroside D (4): yellow, amorphous solid; $[\alpha]^{24}_D$ −28.2 (*c* 0.29, MeOH); UV (MeOH) λ_{max} (log ϵ) 242 (3.21), 251 (2.17) nm; IR (KBr) ν_{max} 3741, 3620, 2925, 2860, 1703, 1643, 1510, 786, 660 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 3; HRFABMS *m/z* 489.1370 $[M + Na]^+$ (calcd for $C_{22}H_{26}O_{11}Na$, 489.1373).

Ternstroside E (5): yellow, amorphous solid; $[\alpha]^{24}_D$ −36.4 (*c* 1.04, MeOH); UV (MeOH) λ_{max} (log ϵ) 254 (4.10), 310 (3.26) nm; IR (KBr) ν_{max} 3612, 2972, 2861, 1812, 1692, 1492, 765, 683 cm^{-1}; ^1H NMR, see Table 2; ^{13}C NMR, see Table 3; HRFABMS *m/z* 473.1424 $[M + Na]^+$ (calcd for $C_{22}H_{26}O_{10}Na$, 473.1424).

Ternstroside F (6): yellow, amorphous solid; $[\alpha]^{24}_D$ −21.4 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 278 (2.85), 245 (2.41) nm; IR (KBr) ν_{max} 3712, 3652, 2974, 2812, 1712, 1513, 775, 685 cm^{-1}; ^1H NMR, see Table 2; ^{13}C NMR, see Table 3; HRFABMS *m/z* 505.1320 $[M + Na]^+$ (calcd for $C_{22}H_{26}O_{12}Na$, 505.1322).

3-*O*-β-D-Xylopyranosyl(1→2)-β-D-glucopyranosyl kaempferol 4′-*O*-β-D-glucopyranoside (7): brown powder; $[\alpha]^{24}_D$ −15.6 (*c* 0.95, MeOH); UV (MeOH) λ_{max} (log ϵ) 343 (4.35), 304 (4.34), 267 (4.57) nm; IR (KBr) ν_{max} 3365, 1650, 1600, 1560 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 8.13 (2H, d, $J = 9.0$ Hz, H-2′, 6′), 6.89 (2H, d, $J = 9.0$ Hz, H-3′, 5′), 6.40 (1H, d, $J = 2.0$ Hz, H-8), 6.19 (1H, d, $J = 2.0$ Hz, H-6), 5.30 (1H, d, $J = 7.5$ Hz, glc H-1″), 4.80 (1H, d, $J = 7.3$ Hz, xyl H-1‴), 4.15 (1H, d, $J = 7.5$ Hz, glc H-1⁗), 3.41−3.82 (m, H-2″−6″, 2‴−5‴, 2⁗−6⁗); ^{13}C NMR (50 MHz, CD_3OD) δ 179.7 (C-4), 166.3 (C-7), 161.5 (C-5), 158.5 (C-4′), 158.5 (C-9), 158.4 (C-2), 135.0 (C-3), 132.4 (C-2′, 6′), 122.7 (C-1′), 116.3 (C-3′, 5′), 105.6 (C-1‴), 105.4 (C-10), 104.3 (C-1⁗), 101.3 (C-6), 100.1 (C-1″), 94.9 (C-8), 80.1 (C-2), 77.8 (C-3″), 77.6 (C-5⁗), 77.0 (C-3⁗), 75.7 (C-5″), 75.0 (C-3‴), 74.9 (C-2⁗), 74.8 (C-2‴), 71.5 (C-4‴), 71.0 (C-4⁗), 70.1 (C-4″), 68.7 (C-5‴), 66.6 (C-6⁗), 62.6 (C-6″); LRESIMS *m/z* 743 $[M + H]^+$ (calcd for $C_{32}H_{39}O_{20}$, 743).

Acid Hydrolysis for Identification of Sugars. Each compound (1−3 mg) was dissolved in 2 mL of 2 N H_2SO_4−1,4-dioxane (1:1) and heated at 100 °C for 1 h. The reaction mixture was diluted with H_2O, neutralized with Dowex SBR (OH^- form), and filtered. The filtrate was passed through a Sep-Pack C_{18} cartridge eluting with H_2O. The eluate was concentrated and analyzed by co-TLC (Si gel) with the authentic sugars (CH_2Cl_2−MeOH−H_2O, 65:40:8; R_f, 0.51 for glc and 0.49 for xyl). The identity of each sugar was further confirmed by comparison of the retention time with those of standard sugars using HPLC: t_R (min), 6.60 for glc and 5.30 for xyl. HPLC analysis was performed with a Supelcosil LC-NH_2 column (250 × 4.6 mm, 5 μm) eluting with CH_3CN−H_2O (75:25) at a flow rate of 1.0 mL/min.

Stereochemistry of the Sugars. Each compound (1−3 mg) was separately refluxed in 5% aqueous H_2SO_4−1,4-dioxane (1:1, 2 mL) for 1 h. The reaction mixture was passed through Dowex SBR (OH^- form), and the filtrate was concentrated. The resulting residues were passed through a Sep-Pack C_{18} cartridge eluting with H_2O. The filtrate was condensed and heated in L-cysteine methyl ester hydrochloride (5 mg) and pyridine (0.5 mg) for 1 h at 60 °C. The reaction mixture was heated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (0.3 mg) for another 1 h at 60 °C, with the supernatants of the reaction mixtures employed for GLC analysis. Identification of the thiazolidine derivatives of D-glucose and D-xylose was carried out by comparison of their retention times with those of standard samples: t_R (min), 10.45 for D-glucose and 7.37 for D-xylose. GLC analysis was performed with an HP-1 column (30 m, 0.32 mm, 0.25 μm) with an initial temperature of 200 °C for 3 min and then temparature programming to 250 °C at a rate of 5 °C/min.

Evaluation of Antioxidative Activity. (1) Measurement of the Inhibition of Total ROS Generation. Rat kidney homogenates prepared from the kidneys of freshly killed male Wistar rats, weighing 150−200 g, were mixed with or without the suspension of extracts or compounds, which were dissolved in 10% EtOH (final concentration: 0.4%). The mixtures were then incubated with 12.5 mM 2′,7′-dichlorodihydrofluorescein diacetate (DCHF-DA), which was dissolved in 100% EtOH (final concentration: 0.2%), at 37 °C for 30 min. A 50 mM phosphate buffer solution at pH 7.4 was also used. DCHF-DA is a stable compound, which is hydrolyzed by intracellular esterase to yield a reduced, nonfluorescent compound, 2′,7′-dichlorodihydrofluorescein (DCHF). The ROS produced by the homogenates oxidizes the DCHF to highly fluorescent 2′,7′-dichlorofluorescein (DCF). The fluorescence intensity of the oxidized DCF was monitored using a microplate fluorescence spectrophotometer, with excitation and emission wavelengths of 460 and 530 nm, respectively.[23] Trolox was used as a positive control.

(2) Measurement of the Inhibition of Hydroxyl Radical Generation. Extracts or compounds that were dissolved in 10% EtOH (final concentration: 0.4%) were added to 1 mM H_2O_2 and 0.2 mM $FeSO_4$ and incubated at 37 °C for 5 min. Esterase-treated 2 μM DCHF-DA in 100% EtOH was then added, and the changes in fluorescence were monitored on a microplate fluorescence spectrophotometer, with excitation and emission wavelengths of 460 and 530 nm, respectively, for 30 min.[22] Trolox was used as a positive control.

(3) Measurement of $ONOO^-$ Scavenging Activity. The $ONOO^-$ scavenging activity was measured by monitoring the oxidation of DHR 123 using a slight modification of the method reported by Kooy et al.[24] DHR 123 (5 mM) in DMF, which was purged with N_2, was stored as a stock solution at 80 °C. This solution was then placed on ice and kept in the dark prior to the study. The buffer consisted of 90 mM NaCl, 50 mM Na_3PO_4, 5 mM KCl at pH 7.4, and 100 μM diethylenetriaminepentaacetic acid (DTPA), each of which was prepared with high-quality deionized H_2O and purged with N_2. The final concentration of DHR 123 was 5 μM. The background and final fluorescent intensities were measured 5 min after treatment with and without the authentic $ONOO^-$. DHR 123 was oxidized rapidly by the authentic $ONOO^-$, and the final fluorescent intensity of the oxidized DHR 123 was measured using a FL 500 microplate fluorescence reader (Bio-Tek Instruments Inc.) at excitation and emission wavelengths of 480 and 530 nm, respectively. The results are expressed as the mean ± standard error ($n = 3$) for the final fluorescence intensity minus background fluorescence. The effects are expressed as the percent inhibition of DHR 123 oxidation, and DL-penicillamine was used as a positive control.

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