New Furofuran and Butyrolactone Lignans with Antioxidant Activity from the Stem Bark of *Styrax japonica*

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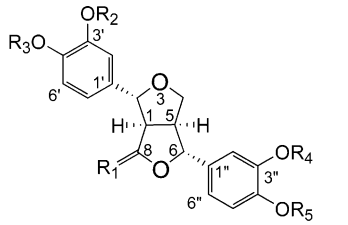
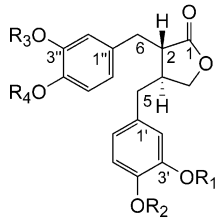
A new furofuran lignan, styraxlignolide B (**1**), and four new dibenzyl-γ-butyrolactone lignans, styraxlignolides C−F (**2**−**5**), were isolated from the EtOAc-soluble fraction of stem bark of *Styrax japonica*. Known compounds, taraxerol (**6**), syringin (**7**), and (−)-pinoresinol glucoside (**8**), were also obtained. The structures of styraxligonolides B−F were determined as 2α-(4′-hydroxy-3′-methoxyphenyl)-6α-(3″,4″-methylenedioxyphenyl)-8-oxo-3,7-dioxabicyclo[3.3.0]octane 4′-*O*-(β-D-glucopyranoside) (**1**), (2*S*,3*S*)-2α-(3″-hydroxy-4″-methoxybenzyl)-3β-(4′-hydroxy-3′-methoxybenzyl)-γ-butyrolactone 4′-*O*-(β-D-glucopyranoside) (**2**), (2*S*,3*S*)-2α-(4″-hydroxy-3″-methoxybenzyl)-3β-(4′-hydroxy-3′-methoxybenzyl)-γ-butyrolactone 4′-*O*-(β-D-glucopyranoside) (**3**), (2*S*,3*S*)-2α-(4″-hydroxy-3″-methoxybenzyl)-3β-(4′-hydroxy-3′-methoxybenzyl)-γ-butyrolactone 4″-*O*-(β-D-glucopyranoside) (**4**), and (2*S*,3*S*)-2α-(3″,4″-dimethoxybenzyl)-3β-(4′-hydroxy-3′-methoxybenzyl)-γ-butyrolactone 4′-*O*-(β-D-glucopyranoside) (**5**) by spectroscopic means including 2D NMR. Compounds **1**−**8** were tested in vitro for antioxidant activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. Styraxlignolide C (**2**), styraxlignolide D (**3**), styraxlignolide E (**4**), and (−)-pinoresinol glucoside (**8**) exhibited weak radical-scavenging activity in the DPPH assay, with IC_{50} values of 380, 278, 194, and 260 μM, respectively.

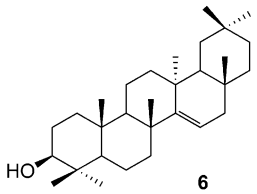
Styrax japonica Sieb. et Zucc. (Styracaceae) is a deciduous tree grown in the southern areas of Korea, Japan, and China. Chemical studies have revealed a variety of pentacyclic triterpenoids,[1] jegosaponins,[2] deacyljegosaponins,[3] and benzofurans.[4] Jegosaponins from the fresh fruits have an antisweet activity.[5] The egonol from the seeds has attracted the attention of synthetic organic chemists, as a result of its activity against human leukemic HL-60 cells.[6] This study describes the structural elucidation of five new furofuran and dibenzyl-γ-butyrolactone lignans from the EtOAc-soluble fraction of *S. japonica*, as well as their relative antioxidant activity toward the DPPH radical.



	R_1	R_2	R_3	R_4	R_5
1	O	CH_3	Glc	$-CH_2-$	
1a	O	CH_3	H	$-CH_2-$	
8	H_2	CH_3	Glc	CH_3	H



	R_1	R_2	R_3	R_4
2	CH_3	Glc	H	CH_3
2a	CH_3	H	H	CH_3
3	CH_3	Glc	CH_3	H
3a	CH_3	H	CH_3	H
4	CH_3	H	CH_3	Glc
4a	CH_3	H	CH_3	H
5	CH_3	Glc	CH_3	CH_3
5a	CH_3	H	CH_3	CH_3



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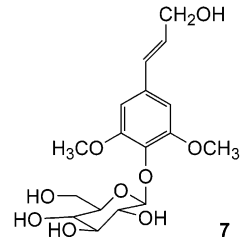

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Results and Discussion

Repeated CC and HPLC of the EtOAc-soluble fraction of the stem bark of *S. japonica* were performed on normal and reversed-phase silica gel, and eight compounds were extracted. The known compounds were identified as taraxerol (**6**),[7] syringin (**7**),[8] and (−)-pinoresinol glucoside (**8**).[9]

Styraxlignolide B (**1**) was obtained as brown plates (MeOH) with a negative optical rotation, $[\alpha]_D^{23}$ −96.8°. The molecular formula of **1** was found to be $C_{26}H_{28}O_{12}$, on the basis of the molecular ion at *m/z* 555.1481 $[M + Na]^+$ in the HRFABMS. The ^1H NMR spectrum showed signals for two methine protons at δ 3.91 (dd, *J* = 9.0, 2.7 Hz) and 3.36 (m), two benzylic oxymethine protons at δ 5.63 (d, *J* = 2.7 Hz) and 5.55 (d, *J* = 2.6 Hz), an oxygenated methylene at δ 4.12 (m) and 4.36 (m), and two 1,3,4-trisubstituted phenyl groups at δ 7.09 (s), 6.88 (d, *J* = 7.9 Hz), and 6.93 (d, *J* = 7.9 Hz); 7.20 (s), 7.55 (d, *J* = 8.9 Hz), and 7.08 (d, *J* = 8.9 Hz) (Table 1), which were assigned to a lignan of the 3,7-dioxobicyclo[3.3.0]octane type, compared with that of the furofuran lignans isolated from *Saussurea medusa*.[10] This observation was further supported by the ^{13}C NMR spectrometric assignments (a carbonyl carbon at δ 177.3, four trisubstituted carbons at δ 84.8, 83.7, 53.3, and 49.7, an oxygenated methylene at δ 73.0, and two aromatic rings) coupled DEPT and HMQC, and HMBC correlations between δ_H 5.63 (H-2) and δ_C 73.0 (C-4)/110.7 (C-2′)/118.3 (C-6′), as well as δ_H 5.55 (H-6) and δ_C 73.0 (C-4)/106.5 (C-2″)/119.8 (C-6″) (Figure 1). The C-8 position

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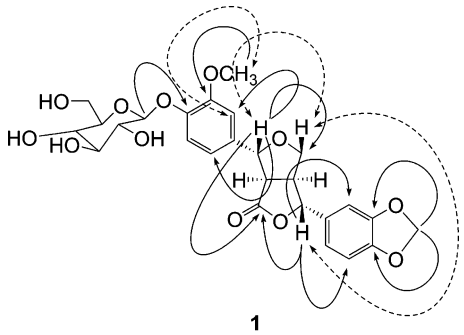
Table 1. ^{1}H NMR (600 MHz) Spectroscopic Data of Styraxlignolides B−F (**1**−**5**)

proton	**1**	**2**	**3**	**4**	**5**
1	3.91 dd (9.0, 2.7)				
2	5.63 d (2.7)	2.77 dd (9.0, 6.6)	2.79 ddd (9.0, 6.6, 5.4)	2.82 ddd (9.0, 6.0, 5.4)	2.70 m
3		2.63 m	2.62 m	2.61 m	2.43 m
4	4.12 m, 4.36 m	3.86 t (8.4), 4.07 t (8.4)	3.87 t (9.0), 4.11 t (8.4)	3.91 t (8.4), 4.16 t (8.4)	3.85 t (8.4), 4.06 t (8.4)
5	3.36 m	2.47 dd (13.8, 9.6), 2.72 dd (13.8, 4.8)	2.51 dd (13.6, 9.0), 2.73 dd (13.8, 5.4)	2.53 m, 2.74 dd (12.6, 5.4)	2.47 m, 2.50 m
6	5.55 d (2.6)	3.04 dd (13.8, 6.6), 3.15 dd (13.8, 4.8)	3.09 dd (13.8, 6.6), 3.16 dd (13.8, 5.4)	3.08 d (6.0, 2H)	2.77 dd (13.7, 6.8), 2.83 dd (13.7, 5.4)
2′	7.09 s	6.81 s	6.78 d (1.8)	6.79 s	6.64 d (1.2)
5′	6.88 d (7.9)	7.52 d (7.8)	7.53 d (8.4)	7.21 d (7.8)	6.95 d (8.2)
6′	6.93 d (7.9)	6.66 d (7.8)	6.69 dd (8.4, 1.8)	6.73 d (7.8)	6.58 dd (8.2, 1.2)
2″	7.20 s	7.28 s	7.01 d (1.8)	7.01 s	6.76 d (1.3)
5″	7.55 d (8.9)	6.97 d (7.8)	7.21 d (7.8)	7.55 d (8.4)	6.84 d (8.2)
6″	7.08 d (8.9)	6.86 d (7.8)	6.95 dd (7.8, 1.8)	6.91 d (8.4)	6.68 dd (8.2, 1.4)
OCH_3-3′	3.68 s	3.80 s	3.77 s	3.83 s	3.69 s
OCH_3-3″			3.81 s	3.77 s	3.69 s
OCH_3-4″		3.76 s			3.69 s
$-CH_2-$	5.99 d (5.7)				
Glc 1‴	4.36 m	4.36 m	4.40 m	4.38 m	3.24 m
2‴	4.12 m	4.15 m	4.38 m	4.36 m	3.21 m
3‴	4.36 m	4.35 m	4.34 m	4.32 m	3.12 m
4‴	4.36 m	4.38 m	4.16 m	4.13 m	3.26 m
5‴	4.36 m, 4.52 m	4.40 m, 4.57 d (12.0)	4.41 m, 4.57 dd (12.0, 1.8)	4.40 m, 4.55 d (12.0)	3.42 dd (11.0, 5.8), 3.64 dd (11.0, 3.8)
6‴					



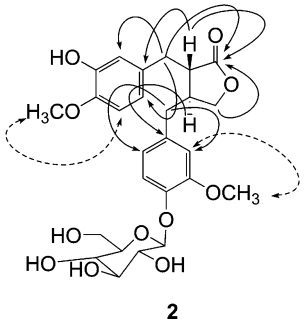


Figure 1. Significant NOE (↔) in the NOESY spectrum and HMBC (→) correlations for compounds **1** and **2**.

could be substituted by a carbonyl group due to a quaternary carbon signal at δ 177.3. This carbonyl group was further supported by the HMBC correlations between δ_H 5.63 (H-2)/δ_H 5.55 (H-6) and δ_C 177.3 (C-8). In addition, the ^{1}H NMR spectrum showed a methoxy group at δ 3.68 and a methylenedioxy group at δ 5.99 (d, J = 5.7 Hz), which correlated with the quaternary carbons at δ 150.1 (C-3′), and 148.6 (C-3″) and 148.2 (C-4″), respectively, in the HMBC, and led to the identification of the position of the methoxy and methylenedioxy groups. The small coupling constants of H-2 (J = 2.7 Hz) and H-6 (J = 2.6 Hz) indicated that both were pseudoaxial protons.[11] This was further confirmed by the NOE effects of the NOESY spectrum between H_{ax}-2 (δ 5.63) and H_{ax}-4 (δ 4.12), as well as between H_{ax}-4 and H_{ax}-6 (δ 5.55). The specific rotation of compound **1** is −96.8°, i.e., near that of (−)-pinoresinol glucoside (**8**) ([α]$^{23}_D$ −85.4°; lit. [α]$_D$ −82.3°), hence confirming the 1*R*,2*R*,5*S*,6*R*-configuration.[9] The CD spectrum of compound **1** ([θ]$_{280}$ −255.9, [θ]$_{235}$ −459.5) and ^{13}C NMR data confirmed **1** was 1*R*,2*R*,5*S*,6*R*-(−)-3,7-dioxabicyclo[3.3.0]-octane. On the other hand, the ^{1}H NMR spectrum of compound **1** contained the signals for an anomeric proton at δ 5.68 and six oxygenated protons due to a hexose unit. Enzymatic hydrolysis of compound **1** yielded a monosaccharide unit, which was identified by co-TLC with an authentic sample. The absolute configuration was determined by gas chromatography to be D-glucose. The configuration of the glycosidic linkage for the glucopyranoside unit was determined to be β on the basis of the $J_{1,2}$ value of the anomeric proton at 7.0 Hz (δ 5.68). Linkage of the D-glucose moiety was determined on the basis of the HMBC correlation between δ_H 5.68 (H-1‴) and δ_C 147.4 (C-4′). Therefore, styraxlignolide B (**1**) was found to be 1*R*,2*R*,-5*S*,6*R*-(−)-2-(4′-hydroxy-3′-methoxyphenyl)-6-(3″,4″-methylenedioxyphenyl)-8-oxo-3,7-dioxabicyclo[3.3.0]octane 4′-*O*-(β-D-glucopyranoside).

The molecular formula of styraxlignolide C (**2**) was found to be $C_{26}H_{32}O_{11}$, on the basis of the molecular ion at *m/z* 543.1842 [M + Na]$^+$ in the HRFABMS. The ^{1}H NMR spectrum displayed signals for two methine protons at δ 2.77 (dd, J = 9.0, 6.6 Hz) and 2.63 (m), an oxygenated methylene at δ 3.86 (t, J = 8.4 Hz) and 4.07 (t, J = 8.4 Hz), two sets of ABX systems of the phenyl protons at δ 6.66−7.52, two benzylic protons at δ 2.47 (dd, J = 13.8, 9.6 Hz) and 2.72 (dd, J = 13.8, 4.8 Hz), and the other two benzylic protons at δ 3.04 (dd, J = 13.8, 6.6 Hz) and 3.15 (dd, J = 13.8, 4.8 Hz) (Table 1). The ^{13}C NMR spectrum, in combination with the DEPT and HMQC spectra, showed signals for a carbonyl carbon, two sp^3 methylenes, two sp^3 methines, six sp^2 quaternary carbons, and six sp^2 methines. This indicated a dibenzylbutyrolactone lignan with a structure similar to matairesinol isolated from *Wikstroemia viridiflora*.[12] Furthermore, the ^{1}H NMR spectrum showed two *O*-methyl groups at δ 3.76 and 3.80, which correlated with the quaternary carbons at δ 150.7 (C-3′) and 148.1 (C-4″), respectively, in the HMBC, which led to the identification of the positions of the two *O*-methyl groups. This finding was further confirmed by the NOE effects between δ 3.80 (C-3′-OCH_3) and 6.81 (H-2′), as well as δ 3.76 (C-4″-OCH_3) and 6.97 (H-5″) in the NOESY spectrum

(Figure 1). In addition, the ^{1}H NMR spectrum of **2** showed an anomeric proton at δ 5.69 and six oxygenated protons. The enzymatic hydrolysis of compound **2** yielded **2a** and glucose. The absolute configuration and linkage of glucose were determined as D using gas chromatography and β on the basis of the $J_{1,2}$ value (7.2 Hz) of the anomeric proton. Sugar moiety connectivity was determined on the basis of the HMBC correlation between δ_H 5.69 (H-1‴) and δ_C 147.2 (C-4′). The EIMS of **2a** showed a molecular ion peak at *m/z* 358 ($C_{20}H_{22}O_5$, 100%) and a prominent fragment peak at *m/z* 137 ($C_8H_9O_2$, 80%), which indicated the presence of a 3(4)-methoxy-4(3)-hydroxybenzyl group on **2a**. Lopes et al.[13] reported that *trans*-dibenzylbutyrolactone tended to show the distinct nonequivalence of the protons of the C-4 methylene group (δ 3.9 and 4.2) in the ^{1}H NMR spectrum. In contrast, in the *cis*-derivative, the hydrogens of the C-methylene group were almost equivalent in the δ 4.0−4.1 range. The ^{1}H NMR spectrum of compound **2** showed the characteristic signals (H-4; δ 3.86 and 4.07) of a *trans*-2,3-dibenzylbutyrolactone lignan. Harmatha et al.[14] studied the optical rotation of the isolated lignans and stereoselective synthetic lignans and concluded that the (2*R*,3*R*)-isomer was levorotatory and the (2*S*,3*S*)-isomer was dextrorotatory. The optical rotation of compound **2** (−8.7°, MeOH, *c* 0.23) is different from that of matairesinol glucoside (−43.2°, *c* 1.3, MeOH),[15] and its aglycone (**2a**; +23.1, *c* 0.29, MeOH) is opposite that of (−)-matairesinol, for which the optical rotation is −44° (*c* 0.62, acetone).[16] Therefore, the absolute configuration of compound **2** is opposite that of (−)-matairesinol and should be 2*S*,2*S*. On the basis of the above evidence, compound **2** was characterized as (2*S*,3*S*)-2α-(3″-hydroxy-4″-methoxybenzyl)-3β-(4′-hydroxy-3′-methoxybenzyl)-γ-butyrolactone 4′-*O*-(β-D-glucopyranoside).

Styraxlignolide D (**3**) was isolated as colorless plates (MeOH), and its molecular formula of $C_{26}H_{32}O_{11}$ was established by the molecular ion peak at *m/z* 543.1840 [M + Na]$^+$ in the HRFABMS. Its UV spectrum exhibited absorbances at 203, 226, and 280 nm that matched those of **2**. Furthermore, the ^{1}H and ^{13}C NMR spectra of compound **3** were quite similar to those of compound **2**. Careful examination of the spectroscpic data, however, revealed several significant differences. The most prominent changes were a higher field shift of H-2″ (δ 7.01, d, J = 1.8 Hz) by 0.27 ppm and lower field shifts of H-5″ (δ 7.21, d, J = 7.8 Hz) and H-6″ (δ 6.95, dd, J = 8.7, 1.8 Hz) by 0.24 and 0.09 ppm, respectively, compared with the corresponding signals of compound **2** (Table 1). In addition, the HMBC spectrum showed correlation between δ_H 3.81 (methoxy proton) and δ_C 149.3 (C-3″, quaternary carbon), which indicated the presence of a 4″-hydroxy-3″-methoxyphenyl group instead of a 3″-hydroxy-4″-methoxyphenyl group in compound **2**. This observation was further supported by an NOE effect between δ_H 3.81 (C-3″-OCH$_3$) and δ_H 7.01 (H-2″) in the NOESY experiment (Figure 1) and the EIMS spectrum of **3a**, which showed a prominent ion at *m/z* 137 ($C_8H_9O_2$, 100%). The enzymatic hydrolysis of compound **3** yielded **3a** and glucose. The linkage and connectivity of the glucose were determined to be β by the $J_{1,2}$ value (7.2 Hz) of the anomeric proton and based on the HMBC correlation between δ_H 5.69 (H-1‴) and δ_C 147.3 (C-4′). The methylene protons of H-4 in the ^{1}H NMR spectrum were nonequivalent (δ 3.87; t, J = 9.0 Hz and 4.11; t, J = 8.4 Hz), indicating two benzyl groups being in a *trans*-relation. The specific rotations of compounds **3** and **3a** were −10.0° and +31.9°, respectively, which were near those of compound **2**; therefore, compound **3** had the 2*S*,3*S*-configuration. Consequently, the structure of compound **3** was determined to be (2*S*,3*S*)-2α-(4″-hydroxy-3″-methoxybenzyl)-3β-(4′-hydroxy-3′-methoxybenzyl)-γ-butyrolactone 4′-*O*-(β-D-glucopyranoside).

The molecular formula of styraxlignolide E (**4**) was assigned to be $C_{26}H_{32}O_{11}$ by HRFABMS and ^{13}C NMR, which was identical to that of styraxlignolide D (**3**). In addition, the NMR spectroscopic data of compound **4** were almost identical to those of compound **3**. However, careful examination of the ^{1}H and ^{13}C NMR spectra of both compounds showed considerable differences in the two phenyl group signals. The signal of H-5′ (δ 7.21, d, J = 7.8 Hz) was shifted to lower field by 0.32 ppm, while that of H-5″ (δ 7.55, d, J = 8.4 Hz) was shifted to higher field by 0.34 ppm, compared to the corresponding proton signals of compound **3**. This indicated that a sugar moiety was bonded at the C-4″ position. This was further supported by the HMBC correlation observed between the proton at δ_H 5.67 (d, J = 6.6 Hz, H-1‴) and the carbon at δ_C 147.4 (C-4″). Furthermore, the aglycone (**4a**) of compound **4** was the same as **3a**. The configuration of glucose was determined to be β by the $J_{1,2}$ value (6.6 Hz) of the anomeric proton. The methylene protons of H-4 in the ^{1}H NMR spectrum were nonequivalent (δ 3.91; t, J = 8.4 Hz and 4.16; t, J = 8.4 Hz), indicating two benzyl groups being in a *trans*-relation. The specific rotations of compounds **4** and **4a** were −10.4° and +30.6°, respectively, and the data resembled those of compound **2**; therefore, compound **4** had the 2*S*,3*S*-configuration. The structure of compound **4** was established as (2*S*,3*S*)-2α-(4″-hydroxy-3″-methoxybenzyl)-3β-(4′-hydroxy-3′-methoxybenzyl)-γ-butyrolactone 4″-*O*-(β-D-glucopyranoside).

Styraxlignolide F (**5**) was also isolated as colorless plates (MeOH). Its molecular formula was deduced to be $C_{27}H_{34}O_{11}$, on the basis of the molecular ion peak at *m/z* 557.1996 [M + Na]$^+$ in the HRFABMS. The ^{1}H NMR spectrum of compound **5** was similar to that of compound **3**, except for a methoxy signal at δ 3.69. This suggested the presence of a methoxy group instead of a hydroxy group. A corresponding change was also observed in the ^{13}C NMR spectrum, in which the methoxy carbon signal at δ 55.4 was present. The position of the methoxy group was confirmed by the HMBC correlation observed between δ 3.69 and 147.4 (C-4″). This observation was further supported by the NOE effect between δ_H 3.69 (C-4″-OCH$_3$) and 6.84 (H-5″) in the NOESY experiment and the EIMS spectrum of the aglycone (**5a**) of compound **5**, which showed a prominent ion peak at *m/z* 151 ($C_9H_{11}O_2$, 100%). Accordingly, the structure of **5** was assigned to be 2-(3″,4″-dimethoxybenzyl)-3-(4′-hydroxy-3′-methoxybenzyl)-γ-butyrolactone 4′-*O*-(β-D-glucopyranoside). Compound **5** had the same absolute configuration, 2*S*,3*S*, as that of compound **3** from their similar optical rotation.

Compounds **1**−**8** were tested for their in vitro antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay. Styraxlignolides C−F (**2**−**4**) and (−)-pinoresinol glucoside (**8**) exhibited weak antioxidant scavenging activity against DPPH, with IC$_{50}$ values of 380, 278, 194, and 260 μM (Table 3), respectively. From this result, lignan compounds from *S. japonica* showed marginal activity, having only one free phenolic group, in contrast to those compounds with an IC$_{50}$ > 500 μM, which do not have free phenolic groups at all.

Experimental Section

General Experimental Procedures. Melting points were measured on a Yanagimoto micro hot-stage melting point apparatus and are uncorrected. Optical rotations were mea-

Table 2. ^{13}C NMR (150 MHz) Spectroscopic Data of Styraxlignolides B−F (**1**−**5**)

carbon	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[b]
1	53.3	179.4	179.5	179.5	178.3
2	83.7	47.0	47.3	47.2	45.5
3		42.2	42.1	42.1	40.7
4	73.0	71.8	71.9	71.9	70.6
5	49.7	38.4	38.4	38.5	36.8
6	84.8	35.1	35.3	35.3	33.6
8	177.3				
1′	134.9	133.5	133.5	130.3	132.5
2′	110.7	114.1	114.1	113.7	113.0
3′	150.1	150.7	150.7	149.3	148.8
4′	147.4	147.2	147.3	147.4	145.2
5′	116.2	117.1	117.1	117.1	115.4
6′	118.3	121.8	121.9	122.4	120.5
1″	134.1	132.2	130.2	133.1	130.5
2″	106.5	118.4	114.3	114.9	113.2
3″	148.6	148.7	149.3	150.5	148.5
4″	148.2	148.1	147.6	147.4	147.4
5″	108.5	113.1	117.2	116.8	111.8
6″	119.8	121.0	123.3	122.9	121.2
OCH_3-3′	55.8	56.5	56.5	56.5	55.6[c]
OCH_3-3″			56.5	56.5	56.4[c]
OCH_3-4″		56.5			55.4[c]
$-CH_2-$	101.8				
Glc 1‴	102.1	103.0	103.0	102.9	100.3
2‴	74.7	75.4	75.4	75.3	76.8
3‴	78.7	79.1	79.1	79.1	73.2
4‴	71.1	71.9	71.8	71.8	69.7
5‴	78.3	79.4	79.4	79.3	76.9
6‴	62.2	63.0	63.0	62.9	60.7

[a] Pyridine-d_5. [b] DMSO-d_6. [c] Interchangeable.

Table 3. DPPH Radical-Scavenging Activity of Compounds **1**−**8** from *S. japonica*

compound	IC_{50} value (μM)
styraxlignolide B (**1**)	>500[b]
styraxlignolide C (**2**)	380
styraxlignolide D (**3**)	278
styraxlignolide E (**4**)	194
styraxlignolide F (**5**)	>500
taraxerol (**6**)	>500
syringin (**7**)	>500
(−)-pinoresinol glucoside (**8**)	260
α-tocopherol[a]	20.1

[a] Positive control. [b] IC_{50} (μM) values were calculated from regression lines using six different concentrations in triplicate.

sured with a JASCO DIP-370 digital polarimeter in $CHCl_3$ or MeOH. UV spectra were recorded on a UV-2450 spectrometer. CD spectra were recorded in MeOH on a JASCO J-715 spectrometer. ^1H and ^{13}C NMR spectra were recorded on Bruker AMX 600 or Varian Unity Inova 400 spectrometers with TMS as an internal standard. Chemical shifts are presented in ppm. FABMS and HRFABMS were measured on a JMS-HX 110/110A spectrometer (JEOL). EI and ESIMASS were measured on a HP5989A DIP mass spectrometer.

Plant Material. The stem bark of *S. japonica* was collected at Jeju (Korea) in July 2002 and dried at room temperature. A voucher specimen (00250) was identified by Dr. Tae-Jin Kim, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, Korea, and deposited at the Plant Extract Bank, KRIBB.

Extraction and Isolation. The stem bark of *S. japonica* (12.0 kg) was extracted with MeOH at room temperature (4 × 5 L) to obtain 1.4 kg of the solid extract. The MeOH extract was suspended in H_2O and extracted successively with hexane (3 × 3 L) and EtOAc (3 × 3 L) to give the hexane- (118 g) and EtOAc-soluble fractions (78 g), respectively. The EtOAc-soluble fraction (78 g) was chromatographed on a silica gel column eluted with a stepwise gradient of $CHCl_3$ and MeOH to yield five fractions (A−E: 28.1 g; 14.6 g; 5.1 g; 19.3 g; 5.0 g). Fraction A was crystallized from $CHCl_3$ to yield compound **6** (87 mg). Fraction B was chromatographed on a silica gel column eluted with $CHCl_3$−MeOH (10:1) to obtain three subfractions (B1−B3: 4.06 g, 8.80 g, 0.67 g). Fraction B2 was subjected to a RP C-18 column (CH_3CN-H_2O, 75:25) to give compound **5** (1.07 g). Column chromatography of fraction C on silica gel ($CHCl_3$−MeOH, 9:1) separated it into three subfractions (C1−C3: 1.12 g, 2.89 g, 0.88 g). Fraction C2 was further purified by preparative HPLC on RP C-18 (YMC-Pack Pro C-18; 250 × 20 mm i.d.; S-5 μm, 12 nm) using a linear gradient of CH_3CN (20% → 50%) in 0.1% TFA to yield compound **1** (20 mg, t_R 37.8 min). Fraction D was chromatographed on a RP C-18 column (CH_3CN-H_2O, 25:75) to yield five subfractions (D1−D5: 2.2 g, 3.6 g, 8.2 g, 2.2 g, 1.8 g). Fraction D2 was chromatographed on a MPLC/RP C-18 (Merck size B, CH_3CN-H_2O, 3:7) to yield compounds **7** (92 mg) and **8** (1.35 g). Fraction D3 was subjected to MPLC/RP C-18 (Merck size B, CH_3CN-H_2O, 3:7) and further purified by preparative HPLC on a RP C-18 column (a linear gradient of CH_3CN, 15% → 55%, 0.1% TFA) to give compounds **2** (690 mg, t_R 48.7 min), **3** (338 mg, t_R 41.6 min), and **4** (250 mg, t_R 44.3 min).

Styraxlignolide B (1): brown plates (MeOH); mp 111−113 °C; $[\alpha]_D^{23}$ −96.8° (*c* 0.31, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (4.74), 228 (4.14), 282 (3.80) nm; CD (*c* 1 M, MeOH) $[\theta]_{280}$ −255.9, $[\theta]_{235}$ −459.5; FABMS *m/z* 555 $[M + Na]^+$; HRFABMS *m/z* 555.1481 $[M + Na]^+$ (calcd for $C_{26}H_{28}O_{12}Na$, 555.1478); ^1H and ^{13}C NMR data, see Tables 1 and 2.

Enzymatic Hydrolysis of 1. Naringinase (100 mg, from *Penicillium decumbens*) was added to a suspension of **1** (5 mg) in 50 mM acetate buffer (pH 5.5), and the mixture was stirred at 37 °C for 5 h. The reaction mixture was extracted with EtOAc (10 mL × 3), and the organic layer was evaporated to dryness. The residue was chromatographed on a preparative-TLC with $CHCl_3$−MeOH (9:1; R_f, 0.62) to give 2-(3′-methoxy-4′-hydroxyphenyl)-6-(3″,4″-methylenedioxyphenyl)-8-oxo-3,7-dioxabicyclo[3.3.0]octane (**1a**, 2 mg) as a white amorphous powder: ^{13}C NMR ($CDCl_3$, 100 MHz) 176.8 (C-8), 148.5 (C-3′), 148.1 (C-3″), 146.7 (C-4″), 145.3 (C-4′), 133.1 (C-1′), 132.2 (C-1″), 119.0 (C-6″), 118.0 (C-6′), 114.4 (C-5′), 108.5 (C-2′), 108.1 (C-5″), 105.7 (C-2″), 101.4 ($-CH_2-$), 84.5 (C-6), 83.4 (C-2), 72.7 (C-4), 56.0 ($-OCH_3$), 53.2 (C-1), 49.9 (C-5); EIMS (rel int) *m/z* 57 (65), 103 (48), 115 (30), 131 (82), 151 (100), 161 (46), 370 $[M]^+$ (89).

The water layer was checked by silica gel TLC (EtOAc−MeOH−H_2O−AcOH, 65:20:15:15). The spot on the TLC plate was visualized by an anisaldehyde−H_2SO_4 reagent. The configuration of glucose was determined by a GC method described previously.[17] The sugar derivative thus obtained showed a retention time of 21.30 min, identical with that of authentic D-glucose.

Styraxlignolide C (2): colorless plates (MeOH); mp 106−108 °C; UV (MeOH) λ_{max} (log ϵ) 204 (4.69), 225 (4.11), 279 (3.70) nm; $[\alpha]_D^{23}$ −8.7° (*c* 0.23, MeOH); FABMS *m/z* 543 $[M + Na]^+$; HRFABMS *m/z* 543.1842 $[M + Na]^+$ (calcd for $C_{26}H_{32}O_{11}Na$, 543.1842); ^1H and ^{13}C NMR data, see Tables 1 and 2.

Enzymatic Hydrolysis of 2. Naringinase (200 mg) was added to a suspension of **1** (20 mg) in 50 mM acetate buffer (pH 5.5), and the mixture was stirred at 37 °C for 5 h. Workup as above (preparative TLC, $CHCl_3$−MeOH, 9:1; R_f, 0.58) gave 2-(4″-methoxy-3″-hydroxybenzyl)-3-(3′-methoxy-4′-hydroxybenzyl)-γ-butyrolactone (**2a**, 12 mg) as a white amorphous powder: ^{13}C NMR ($CDCl_3$, 100 MHz) 178.6 (C-1), 146.6 (C-3′), 145.6 (C-3″), 145.5 (C-4″), 144.4 (C-4′), 130.9 (C-1′), 129.9 (C-1″), 121.3 (C-6′), 120.7 (C-6″), 115.4 (C-2″), 114.4 (C-5′), 111.0 (C-2′), 110.8 (C-5″), 71.2 (C-4), 55.9 ($-OCH_3$), 55.8 ($-OCH_3$), 46.3 (C-2), 41.5 (C-3), 38.3 (C-5), 34.5 (C-6); $[\alpha]_D$ +23.1° (*c* 0.29, $CHCl_3$); EIMS (rel int) *m/z* 94 (15), 122 (17), 137 (80), 341 (6), 358 $[M]^+$ (100).

Styraxlignolide D (3): colorless plates (MeOH); mp 100−102 °C; UV (MeOH) λ_{max} (log ϵ) 203 (4.74), 226 (4.13), 280 (3.71) nm; $[\alpha]_D^{23}$ −10.0° (*c* 0.28, MeOH); FABMS *m/z* 543 $[M + Na]^+$; HRFABMS *m/z* 543.1840 $[M + Na]^+$ (calcd for $C_{26}H_{32}O_{11}Na$, 543.1842); ^1H and ^{13}C NMR data, see Tables 1 and 2.

Enzymatic Hydrolysis of 3. Reaction (20 mg) and workup as above (preparative TLC, $CHCl_3$−MeOH, 9:1; R_f, 0.61) gave 2-(4″-methoxy-3″-hydroxybenzyl)-3-(3′-methoxy-4′-hydroxy-

benzyl)-γ-butyrolactone (**3a**, 13 mg) as a white amorphous powder: ^{13}C NMR ($CDCl_3$, 100 MHz) 178.8 (C-1), 146.7 (C-3″), 146.6 (C-3′), 144.5 (C-4′), 144.4 (C-4″), 129.7 (C-1″), 129.5 (C-1′), 122.0 (C-6′), 121.3 (C-6″), 114.4 (C-5′), 114.0 (C-5″), 111.5 (C-2′), 110.9 (C-2″), 71.3 (C-4), 55.8 (−OCH_3), 55.7 (−OCH_3), 46.5 (C-2), 41.0 (C-3), 38.3 (C-5), 34.6 (C-6); $[\alpha]_D$ +31.4° (*c* 0.32, $CHCl_3$); EIMASS (rel int) *m/z* 94 (30), 122 (32), 137 (100), 358 $[M]^+$ (43).

Styraxlignolide E (4): colorless plates (MeOH); mp 102−104 °C; UV (MeOH) λ_{max} (log ϵ) 204 (4.94), 224 (4.36), 279 (3.69) nm; $[\alpha]_D^{23}$ −10.4° (*c* 0.23, MeOH); FABMS *m/z* 543 $[M + Na]^+$; HRFABMS *m/z* 543.1848 $[M + Na]^+$ (calcd for $C_{26}H_{32}O_{11}Na$, 543.1842); ^{1}H and ^{13}C NMR data, see Tables 1 and 2.

Enzymatic Hydrolysis of 4. Reaction and workup as above (preparative TLC, $CHCl_3$−MeOH, 9:1; R_f, 0.61) gave 2-(3″-methoxy-4″-hydroxybenzyl)-3-(3′-methoxy-4′-hydroxybenzyl)-γ-butyrolactone (**4a**, 9 mg) as a white amorphous powder: ^{13}C NMR ($CDCl_3$, 100 MHz) 178.8 (C-1), 146.7 (C-3″), 146.6 (C-3′), 144.5 (C-4′), 144.4 (C-4″), 129.7 (C-1″), 129.5 (C-1′), 122.1 (C-6′), 121.3 (C-6″), 114.4 (C-5′), 114.0 (C-5″), 111.5 (C-2′), 110.9 (C-2″), 71.3 (C-4), 55.8 (−OCH_3), 55.8 (−OCH_3), 46.5 (C-2), 41.0 (C-3), 38.3 (C-5), 34.6 (C-6); $[\alpha]_D$ +31.8° (*c* 0.29, $CHCl_3$); EIMASS (rel int) *m/z* 94 (26), 122 (30), 137 (100), 358 $[M]^+$ (40).

Styraxlignolide F (5): colorless plates (MeOH); mp 128−130 °C; UV (MeOH) λ_{max} (log ϵ) 204 (4.68), 228 (4.15), 279 (3.68) nm; $[\alpha]_D^{23}$ −24.8° (*c* 0.25, MeOH); FABMS *m/z* 557 $[M + Na]^+$; HRFABMS *m/z* 557.1996 $[M + Na]^+$ (calcd for $C_{27}H_{34}O_{11}Na$, 557.1999); ^{1}H and ^{13}C NMR data, see Tables 1 and 2.

Enzymatic Hydrolysis of 5. Reaction (20 mg) and workup as above (preparative TLC, $CHCl_3$−MeOH, 9:1; R_f, 0.59) gave 2-(3″,4″-dimethoxybenzyl)-3-(3′-methoxy-4′-hydroxybenzyl)-γ-butyrolactone (**5a**, 12 mg) as a white amorphous powder: ^{13}C NMR ($CDCl_3$, 150 MHz) 178.7 (C-1), 149.1 (C-3″), 148.0 (C-4″), 146.6 (C-3′), 144.5 (C-4′), 130.3 (C-1″), 129.8 (C-1′), 121.4 (C-6″), 121.3 (C-6′), 114.5 (C-5′), 112.5 (C-2″), 111.2 (C-2′), 111.0 (C-5″), 71.2 (C-4), 55.9 (−OCH_3), 55.9 (−OCH_3), 55.8 (−OCH_3), 46.6 (C-2), 41.1 (C-3), 38.3 (C-5), 34.6 (C-6); $[\alpha]_D$ +25.71° (*c* 0.33, $CHCl_3$); EIMASS (rel int) *m/z* 107 (20), 137 (52), 151 (100), 372 $[M]^+$ (42).

Taraxerol (6): colorless plates (MeOH); mp 272−724 °C (lit.[16] 282−283 °C); UV ($CHCl_3$) λ_{max} (log ϵ) 228 (2.95) nm; $[\alpha]_D^{23}$ 0° (*c* 0.43, $CHCl_3$); EIMS *m/z* (rel int) 426 $[M]^+$ (88), 189 (33), 204 (100), 287 (35), 303 (38).

Syringin (7): colorless needles (MeOH); mp 188−190 °C; UV (MeOH) λ_{max} (log ϵ) 221 (4.50), 266 (4.19) nm; $[\alpha]_D^{23}$ −16.9° (*c* 0.23, MeOH); ESIMS *m/z* 395 $[M + Na]^+$.

(−)-Pinoresinol glucoside (8): white amorphous powder; UV (MeOH) λ_{max} (log ϵ) 205 (4.69), 229 (4.20), 279 (3.74) nm; CD (*c* 1 M, MeOH) $[\theta]_{280}$ −179.0, $[\theta]_{235}$ −133.9; $[\alpha]_D$ −85.4° (*c* 0.35, MeOH); ESIMS *m/z* 543 $[M + Na]^+$.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical-Scavenging Activity. The DPPH radical-scavenging activity was measured using a method described previously.[18] Briefly, 10 μL of each sample dissolved in DMSO was prepared in 96-well plates, and then 190 μL of 200 μM ethanolic DPPH solution was added. The mixture was incubated at room temperature for 30 min, and the absorbance of the reaction mixture was measured at 517 nm.

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