Matrix Metalloproteinase-1 Inhibitor from the Stem Bark of *Styrax japonica* S. et Z.

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A bioassay-guided fractionation of the ethyl acetate soluble fraction from the stem bark of *Styrax japonica* S. et Z. (*Styracaceae*) yielded two new lignan compounds, styraxjaponoside A (1) and styraxjaponoside B (2), along with three known compounds, matairesinoside (3), egonol glucoside (4), and dihydrodehydrodiconiferyl alcohol 9′-O-glucoside (5). The structures of compounds 1—5 were determined by spectroscopic method, as well as 1D- and 2D-NMR (HSQC, 1H–1H COSY, and HMBC) spectroscopy. Among them, compound 2 exhibited potent inhibitory activity against matrix metalloproteinase (MMP)-1, and prevented the UV-induced changes in the MMP-1 expression. In addition, compounds 3 and 5 were isolated from this plant for the first time.

**Key words** *Styrax japonica*; *Styracaceae*; lignan glycoside; styraxjaponoside B; matrix metalloproteinase (MMP-1) inhibitor

*Styrax japonica* S. et Z. is a member of the Styracaceae family, which is a shrub found in Central America and Mexico, including South Anatolia. The resin from this species has been used in traditional medicine to treat inflammatory diseases. An earlier investigation on the phytochemical constituents of the *Styrax* species revealed them to be a rich source of benzo furans, benzo furan glycosides, and saponins. Recently, we reported the isolation of pentacyclic triterpenoids from this plant, in addition to their cytotoxic activity. The matrix metalloproteinases (MMPs) are essential for tissue remodeling, as well as the healing cascade under normal physiological conditions. It has been suggested that alterations in collagen, which is the major structural component of the skin, are the cause of changes observed in naturally aged and photaged skin such as skin wrinkling and a loss of elasticity. In an ongoing investigation into MMP-1 inhibitory compounds from *S. japonica*, two new lignan glycosides (1, 2) and three known compounds (3—5) were isolated from the ethyl acetate soluble fraction. This paper reports the isolation and structure determination of the constituents from the stem bark of *S. japonica* as well as the effect on the type I procollagen and MMP-1 expression of compound 2.

**Results and Discussion**

Activity-guided fractionation of the ethyl acetate soluble fraction from the stem bark of *S. japonica* S. et Z. yielded two new lignan glycosides and three known compounds (Fig. 1).

**Fig. 1. Structures of Compounds 1—5 Isolated from *S. japonica***

Styraxjaponoside A (1) was obtained as colorless crystals. The molecular formula of 1 was found to be C_{26}H_{30}O_{13} by HR-FAB-MS spectrometry (m/z 551 [M+H]^+). The UV spectrum exhibited absorption maxima at 234 and 281 nm, which are characteristic absorption bands of a butyrolactone-type lignan. In the IR spectrum, the signals for a hydroxyl (3410 cm^{-1}), γ-lactone carbonyl (1751 cm^{-1}), aromatic ring (1600, 1502 cm^{-1}), and methylenedioxy (925 cm^{-1}) groups were observed. The 1H- and 13C-NMR spectra showed a typical pattern of methylenedioxygenated dibenzylbutyrolactone-type lignan, and the structure of 1 was similar to that of burse hernin, except for the 7,7′-dihydroxy group and glucosyl moiety. Moreover, the anomeric proton at δ 4.86 (1H, d, J=7.5 Hz) suggested the presence of a glucosyl moiety. Based on the coupling constant value of the anomeric proton, the configuration of the glucosidic linkage was determined to be β. On acid hydrolysis, 1 gave D-(-)-glucose, which was identified by co-TLC with standard D-(-)-glucose. Compared with the chemical shifts of H-7 and 7′ (δ 2.51, 4H, br s), and H-8 and 8′ (δ 3.00—2.80, 2H, m) of burse hernin, downfield shifts of H-7 (δ 5.31, 1H, d, J=4.0 Hz), H-7′ (δ 5.36, 1H, d, J=4.0 Hz), H-8 (δ 3.53, 1H, d, J=4.0 Hz), and H-8′ (δ 3.27, 1H, m) were observed in 1, and these chemical shifts strongly suggested that the hydroxy group was located at C-7 and 7′. In the HMBC spectrum of...
1, the carbon signals at δ 149.04 (C-3′) and 148.74 (C-4′) showed a correlation with the proton signal at δ 5.98 (OCH₂O), and the carbon signals at δ 135.93 (C-1) and 133.54 (C-1′) correlated with the proton signals at δ 5.31 (H-7) and 5.36 (H-7′), respectively. Furthermore, the carbon signals at δ 53.86 (C-8) and 50.32 (C-8′) correlated with the proton signals at δ 4.34 and 4.05 (methylene protons, H₂-9). These results indicate that the skeleton of 1 was a methylene-dioxygenated dibenzylbutyrolactone-type lignan. In addition, the ¹H–¹³C long-range correlation between the anomeric proton (δ 4.86, 1H, d, J=7.5 Hz) and the C-4 carbon (δ 146.63) suggest that the sugar was located at C-4. Based on these observations, styraxjaponoside A (1) was determined to be 7,7′-dihydroxybursheermin 4-β-D-glucoside.

Styraxjaponoside B (2) was obtained as colorless crystals. The molecular formula of 2 was found to be C₂₇H₃₃O₁₄ by HR-FAB-MS spectrometry (m/z 557 [M+Na]⁺). The UV spectrum exhibited absorption maxima at 232 and 279 nm, which are characteristic absorption bands of a butyro lactone-type lignan. In the IR spectrum, signals for γ-lactone carboxyl (1760 cm⁻¹) and aromatic ring (1590, 1510 cm⁻¹) were observed. The ¹H- and ¹³C-NMR spectra of 2 were similar to matairesinol,¹¹ butyro lactone-type lignan, except for the glucosyl moiety. Li et al.¹² proposed the reference to the chemical shifts of the cis- and trans-dibenzy lbutyro lactones. The ¹H-NMR spectrum of 2 exhibited the characteristic signals of a trans-8,8′-dibenzy lbut yrolactone lignan, so its relative configuration was determined to be trans. The position of the glucosyl group was confirmed by the HMBC spectrum. A ¹H-¹³C long-range correlation between the C-4′ carbon (δ 146.81) and the anomeric proton (δ 4.86, 1H, d, J=7.5 Hz) was observed. Based on the coupling constant value of the anomeric proton, the configuration of the glucosidic linkage was determined to be β.⁹ On acid hydrolysis,¹⁰ 2 gave d- (+)-glucose, which was identified by co-TLC with standard d- (+)-glucose. Therefore, the structure of styraxjaponoside B (2) was determined to be 4-methoxy matairesinol 4′-β-D-glucoside.

Compounds 3—5 were identified as matairesinol,¹³ egonol glucoside,³ and dihydrodehydroconiferyl alcohol 9′-O-glucoside,¹³ respectively, by comparing the NMR spectral data with those reported in the literature. Compounds 3 and 5 have not been previously isolated from S. japonica.

The in vitro cell cytotoxicity was evaluated according to reference,⁴ and the compounds 1—5 showed no cytotoxicity against the human der mal fibroblasts in the test dose (0.1—10 μM, p<0.001 compared to the control). This study examined the effects of compounds 1—5 on the expression of type I procollagen, and the MMP-1 proteins in cultured human der mal fibroblasts. The human dermal fibroblasts were treated with 10 μM for 48, 72, and 96 h, and the type I procollagen and MMP-1 expression levels were then determined in the culture media by Western blot analysis. Epigallocatechin-3-gallate (EGCG) was used as a positive control throughout the experiments (Tables 1, 2). Antioxidants, such as EGCG, Vitamin C, and E inhibit the MMP-1 expression level in human der mal fibroblasts.¹⁴ EGCG is a major constituent polyphenol in green tea and commonly used for preventing UV-induced adverse skin reaction such as sunburn and pho toaging.¹⁵,¹⁶ Compound 2 increased the type I procollagen protein expression level by 518.9±18.0% (p<0.05, n=5) at 10 μM (Table 1), and decreased the MMP-1 protein expression level significantly in a dose-dependent manner by an average of 62.1±8.3% (p<0.05, n=5) at 10 μM, compared with the vehicle-treated control cells (Table 1). In order to determine if 2 has any inhibitory effects on the UV-induced changes in the MMP-1 protein expression level, human der mal fibroblasts were irradiated with 100 mJ/cm² of UV light with or without treatment of 2 (0.1, 1, 10 μM). The UV-induced MMP-1 protein expression level was significantly inhibited by 63.5±17.6% (p<0.05, n=5) at 10 μM by a pre-treatment with 2 in the cultured human der mal fibroblasts in a dose-dependent manner (Table 2). Throughout the experiment, 2 exhibited almost equivalent effects on type I procollagen and MMP-1 expression to that of EGCG, which is used as a positive control.

In conclusion, styraxjaponoside B (2) can be used for the treatment and prevention of the skin aging processes, based on the following results: 1) Topical application of 2 in the human der mal fibroblasts increases the type I procollagen expression level and decreases the MMP-1 expression level. 2) Styraxjaponoside B (2) prevents the UV-induced changes in the MMP-1 expression level.

### Table 1. The Effects of 2 on the Type I Procollagen and MMP-1 Expressions

<table>
<thead>
<tr>
<th>Conc. (μM)</th>
<th>Compd.</th>
<th>Type I procollagen</th>
<th>MMP-1</th>
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<tbody>
<tr>
<td>0</td>
<td>EGCG</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.1</td>
<td>156.4±6.44 (C-1′)</td>
<td>126.1±12 (b)</td>
<td>76.4±9.48 (b)</td>
</tr>
<tr>
<td>1</td>
<td>296.9±2.36 (C-1′)</td>
<td>205.3±16 (b)</td>
<td>58.2±6.64 (b)</td>
</tr>
<tr>
<td>10</td>
<td>515.5±13.47 (C-1′)</td>
<td>519.8±18 (b)</td>
<td>43.2±8.30 (b)</td>
</tr>
</tbody>
</table>

(a) EGCG (epigallocatechin-3-gallate) was used as a positive control. (b) p<0.05 compared with the control. Each value represents a mean±S.E.M. (n=5).

### Table 2. The Effect of 2 on the UV-Induced in the MMP-1 Expression in the Human Dermal Fibroblasts

<table>
<thead>
<tr>
<th>Conc. (μM)</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>EGCG</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>0.1</td>
<td>61.2±11.6</td>
</tr>
<tr>
<td>1</td>
<td>62.2±9.07</td>
</tr>
<tr>
<td>10</td>
<td>22.9±5.31</td>
</tr>
</tbody>
</table>

(a) The cells were pretreated with compound prior to UV irradiation (100 mJ/cm²) and harvested 72 h later. (b) None UV. (c) Used as a positive control. (d) p<0.05 compared with the control. Each value represents a mean±S.E.M. (n=5).
Experimental

General Procedures The melting points were determined using a Fisher Scientific 307N0043 melting point apparatus and are uncorrected.

The optical rotations were measured using an Autopol-IV polarimeter (Rudolph Research Flangers). UV spectra were obtained on a Shimadzu UV/Visible spectrophotometer (UV-1601PC). The IR spectra were measured in KBr pellets using an IKS 85 (Bruker). The NMR spectra were recorded on a Varian Unity Inova 500 spectrometer. The HR-FAB-MS was recorded on a JEOL JMS 700 mass spectrometer. TLC and preparative TLC were carried out on precoated silica gel 60 F254 (Merck, art. 7575) and RP-18 F8s (Merck, art. 15389) plates. TLC of 1–5 was performed on precoated silica gel 60 F254 and RP-18 F8s plates, which were developed with CHCl3–MeOH–Me2CO–H2O (17 : 3 : 3 : 0.3, solvent A) and MeOH–H2O (30 : 3 : 2 : 0.3). Gel filtration column chromatography (Sephadex LH-20, Sigma, 25–100 μm) silver carbonate (Ag2CO3, Aldrich Co.) and (±)-glucose (C6H12O6, Sigma) were used as a neutralization substance and standard sugar on acidic hydrolysis, respectively. Epigallocatechin-3-gallate (EGCG, m.w. 458.4) was used as a positive control and obtained from Pharmadou (Kyoto, Japan).

Plant Material The stem bark of S. japonica was collected from Jogyasan, Suncheon, Chonnam, Korea, in September 2002. A voucher specimen is deposited in the Herbarium of the College of Pharmacy, Chosun University, Korea (CSU-964-17).

Extraction and Isolation The air-dried stem bark of S. japonica (654 g) was cut and extracted with MeOH (31×3) at 80 °C for 4 h (×3). The MeOH extract (120 g) was suspended in water (1.3 l) and then partitioned sequentially with equal volumes of dichloromethane, ethyl acetate, and n-butanol. Each fraction was evaporated in vacuo to yield the residues of CH2Cl2 (13 g), EtOAc (12 g), n-BuOH (75 g), and water (2.9 g) extract. Among these fractions, the ethyl acetate extract had a strong MPM-1 expression inhibition effect; the MPM-1 expression decreased to 68% at 10 μg/ml compared with the control. The ethyl acetate extract (4 g) was subjected to column chromatography on silica gel (178.30 (C-9), 150, 1510, 1510 (aromatic ring)) and pure 4-(25). The spectral data were identical with those reported in the literature.13)

Acidic Hydrolysis of 1 and 2 Compounds 1 and 2 were dissolved in 1 N HCl (1 ml) and MeOH (1 ml) and refluxed at 75 °C for 90 min. The reaction solution was evaporated under reduced pressure, and the hydrolysate was extracted with EtOAc (3 ml×3). The aqueous fraction was neutralized with Ag2CO3, filtered, and the filtrate was concentrated to yield 1,2-D-glucoside (150 mg, MeOH, UV λmax (MeOH) nm (log ε): 233 (1.66), 279 (1.10). IR (EtOH) UV λmax (EtOH) nm (log ε): 233 (2.41), 281 (3.81). IR (KBr) νcm−1 (cm−1): 3600–2300 (OH), 1755 (-lactone CO), 1595, 1510 (aromatic ring). The spectral data were identical with those reported in the literature.15

Human Fibroblast Cell Culture The primary cultures of the dermal fibroblasts were established from human adult foreskins in Dulbecco’s modified eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS), 2 μg glutamine, penicillin (100 U/ml), and streptomycin (100 μg/ml) in a 37 °C humidified incubator containing 5% CO2.

Statistical Analysis The data was analyzed using a Student’s t-test. The results are presented as a means±S.E.M. All the p values quoted were two-tailed, and a p value 0.05 was considered significant.

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References and Notes


