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# Anti-Complement Activity of Norlignans and Terpenes from the Stem Bark of *Styrax japonica*

Original Paper

**Abstract**

A new norlignan, styraxlignolide A (**1**), and two new terpenes, styraxosides A (**2**) and B (**3**), were isolated from the MeOH-soluble fraction of *Styrax japonica* Sieb. et Zucc. (Styracaceae) stem bark, together with two known compounds, egonol (**4**) and masutakeside I (**5**). The new compounds were determined as 5-(3″-hydroxypropyl)-7-methoxy-2-(3′,4′-dimethoxyphenyl)-benzofuran 3″-*O*-[β-D-xylopyranoside-(1→6)-β-D-glucopyranoside] (**1**), 3β,7β-dihydroxy-4α,4β,8β,10β,14α-pentamethyl-5α-gon-16-en-2-one 3-*O*-[β-D-glucopyranoside-(1→2)-β-D-glucopyranoside] (**2**), and 3β,17β-dihydroxy-28-norolean-12-en-16-one 3-*O*-[α-L-rhamopyranoside-(1→2)-β-D-glucuronopyranoside] (**3**) by spectroscopic means including 2D-NMR. The five compounds were tested *in vitro* for anti-complement activity against the complement system. Compounds **1**, **3**, **4**, and **5** displayed inhibitory activity in the anti-complement assay, with $IC_{50}$ values of 123, 65, 33, and 166 μM, respectively. Compound **1a** and camellenodiol (**3a**), obtained from acid hydrolysis of **1** and **3**, respectively, did not affect the hemolytic activity of human serum against sensitized erythrocytes. This shows that a sugar seems to play a role of enhancing significantly anti-complement activity.

**Key words**
*Styrax japonica* · Styracaceae · anti-complement activity · norlignan · octanordammarane noroleanane · egonol



## Introduction

The complement system is activated either by a cascade mechanism of the classical pathway (CP), an alternative pathway (AP), or the mannan-binding lectin (MBL)-associated serine protease (MBL/MASP) pathway [1]. The thirty odd complement fragments that make up the system include proteolytic pro-enzymes, non-enzymatic components that form functional complexes, co-factors, regulators, and receptors [2]. The proteolytic cascade allows for significant amplification since each proteinase molecule activated at one step can generate multiple copies of activated enzyme later in the cascade which, in turn, cleave non-enzymatic components, such as C3, C4, and C5. The larger fragments derived from C3, C4, and C5 (i.e., C3b, C4b, and C5b) are involved in biological effector functions, such as opsonization, phagocytosis, and immunomodulation. However, the smaller molecules, C3a, C4a, and C5a, designated anaphylatoxins, induce the release of mediators from mast cells and lymphocytes, which cause a variety of inflammatory diseases, and may be fatal if they occur after organ transplantation [2], [3]. Therefore, modulation of complement activity should be beneficial in the therapy of inflammatory diseases.

*Styrax japonica* Sieb. et Zucc. (Styracaceae) is a deciduous tree grown in the Southern areas of Korea and Japan. The pericarps are used as washing soap, cough medicine, and as a piscicidal agent [4]. Previous phytochemical studies performed on this plant included the isolation of jegosaponins and benzofurans [5], [6], [7], [8]. Jegosaponins from fresh fruits display an anti-sweet activity [4], and egonol from the seeds has attracted the

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attention of synthetic organic chemists, due to its activity against human leukemic HL-60 cells [9], [10]. In this study, we report the isolation, structural elucidation, and anti-complement activity of lignans and terpenes isolated from the stem bark of *S. japonica*.

## Materials and Methods

### General experimental procedures

Optical rotations were measured with a JASCO DIP-370 digital polarimeter in MeOH. UV spectra were recorded on a Shimadzu UV-2450 spectrometer. $^1$H- and $^{13}$C-NMR spectra were recorded on a Bruker AMX 600 spectrometer. FAB-MS were measured on a JMS-HX 110/110A spectrometer (JEOL).

### Plant material

The stem bark of *Styrax japonica* Sieb. et Zucc. (Styracaceae) was collected at Jeju (Korea) in July 2002. A voucher specimen verified by Dr. Tae-Jin Kim of Korea Research Institute of Bioscience and Biotechnology (KRIBB), Korea, is deposited at the Plant Extract Bank (00250), KRIBB, Korea.

### 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 a solid extract. The MeOH extract was suspended in $H_2O$ and extracted with hexane (3×3 L) and EtOAc (3×3 L), successively, to give the hexane- (118 g) and EtOAc-soluble fractions (78 g), respectively. The resulting $H_2O$ solution was passed through a Diaion HP-20 column (10.0×85.0 cm) eluting with $H_2O$ (8 L), 50% aqueous MeOH (8 L), and MeOH (8 L), respectively. The MeOH-soluble fraction (145 g) was chromatographed on an RP C-18 column (6.0×90.0 cm) eluted with 50% aqueous MeOH (4 L), 25% aqueous MeOH (4 L), and MeOH (4 L) to yield five fractions (Fr. A – E: 95 g, 6 L; 35 g, 2.5 L; 8.2 g, 1.5 L; 4.6 g, 1 L; 1.2 g, 1 L). Fr. B was chromatographed on RP C-18 eluted with 50% aqueous MeOH (6.0×60 cm, 6 L) to yield five sub-fractions (Fr. B1 – B5 : 2.0 g, 0.8 L; 1.3 g, 0.8 L; 1.2 g, 1.0 L; 28.5 g, 2.6 L; 1.6 g, 0.8 L). Fr. B4 was crystallized from MeOH to yield **1** (12.92 g). The mother liquor of Fr. B4 (14.0 g) was subjected to an RP C-18 column (5.0×50 cm, 65% aqueous $CH_3CN$) to obtain three sub-fractions (Fr. B41 – B43 : 3.12 g, 0.5 L; 8.29 g, 3.5 L; 2.18 g, 1.0 L). Fr. B41 was further chromatographed on silica gel (3.0×50 cm, $CHCl_3$/MeOH, 8.5 : 1.5, 3 L) to give **4** (1.4 – 1.9 L, 2.02 g). Fr. C was chromatographed on a RP C-18 column (4.5×60 cm, 70% aqueous $CH_3CN$, 6.0 L) to yield five sub-fractions (Fr. C1 – C5 : 0.2 g, 0.5 L; 0.6 g, 0.5 L; 1.2 g, 1.5 L; 5.2 g, 2.5 L; 0.8 g, 1.0 L). Fr. C4 was crystallized from MeOH to yield **5** (2.64 g). Fr. C3 was chromatographed on an MPLC/RP C-18 (Merck size B, 70% aqueous $CH_3CN$) to give **2** (110 – 140 mL, 172 mg). Fr. D was subjected to an RP C-18 column (3.0×50 cm, 35% aqueous MeOH, 2 L) to obtain three sub-fractions (Fr. D1 – D3 : 1.4 g, 0.4 L; 1.0 g, 0.5 L; 2.1 g, 1.1 L). Fr. D2 was further subjected to MPLC/RP C-18 (B size, 60% aqueous $CH_3CN$) to give **3** (55 – 80 mL, 60 mg).

*Styraxlignolide A* (**1**): white amorphous powder. UV (MeOH): $\lambda_{max}$ (log $\varepsilon$) = 217 (4.39), 312 (4.38) nm; $[\alpha]_D^{22}$: –53.7° (*c* 0.8, MeOH); FAB-MS: *m/z* = 659 $[M + Na]^+$; HR-FAB-MS: *m/z* = 659.2311 $[M + Na]^+$ (calcd. for $C_{31}H_{40}O_{14}Na$: 659.2316); $^1$H- and $^{13}$C-NMR data: see Table **1**.

*Enzymatic hydrolysis of* **1**: Naringinase (200 mg, from *Penicillium decumbens*) 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. The reaction mixture was extracted with EtOAc (10 mL×3) and the organic layer was evaporated to dryness. The residue was separated by preparative TLC with $CHCl_3$/MeOH (9 : 1; R*f* = 0.55) to give 5-(3″-hydroxypropyl)-7-methoxy-2-(3′,4′-dimethoxyphenyl)-benzofuran (**1a**, 10 mg) as white amorphous powder. $^1$H- and $^{13}$C-NMR data, see Table **1**.

*Styraxoside A* (**2**): white amorphous powder. UV (MeOH): $\lambda_{max}$ (log $\varepsilon$) = 202 (3.82) nm; $[\alpha]_D^{26}$: –11.0° (*c* 0.1, MeOH); FAB-MS: *m/z* = 693 $[M + Na]^+$; HR-FAB-MS: *m/z* = 693.3463 $[M + Na]^+$ (calcd. for $C_{34}H_{54}O_{13}Na$: 693.3462). $^1$H- and $^{13}$C-NMR data, see Table **2**.

*Styraxoside B* (**3**): white amorphous powder. UV (MeOH): $\lambda_{max}$ (log $\varepsilon$) = 201 (3.77) nm; $[\alpha]_D^{26}$: –43.7° (*c* 0.9, MeOH); FAB-MS: *m/z* = 787 $[M + Na]^+$; HR-FAB-MS: *m/z* = 787.4240 $[M + Na]^+$ (calcd. for $C_{41}H_{64}O_{13}Na$: 787.4245). $^1$H- and $^{13}$C-NMR data, see Table **2**.

*Enzymatic hydrolysis of* **3**: Naringinase (200 mg) was added to a suspension of **3** (20 mg) in 50 mM acetate buffer (pH 5.5) and the mixture was stirred at 37 °C for 5 h. Work-up as mentioned above (preparative TLC, hexane/EtOAc, 3 : 1; R*f* = 0.6) gave 3$\beta$,17$\beta$-dihydroxy-28-norolean-12-en-16-one (**3a**, 8 mg) as white amorphous powder. $^{13}$C-NMR ($CDCl_3$; 150 MHz): $\delta$ = 15.3 (C-25), 15.6 (C-24), 17.3 (C-26), 18.2 (C-6), 23.6 (C-11), 23.7 (C-30), 27.0 (C-27), 27.2 (C-2), 28.1 (C-23), 30.3 (C-22), 30.8 (C-20), 32.4 (C-29), 32.7 (C-7), 36.4 (C-10), 37.0 (C-21), 38.4 (C-1), 38.8 (C-4), 39.8 (C-8), 42.9 (C-15), 46.7 (C-9), 47.1 (C-19), 47.4 (C-14), 52.5 (C-18), 55.2 (C-5), 76.5 (C-17), 78.9 (C-3), 125.6 (C-12), 140.4 (C-13), 213.6 (C-16).

*Egonol* (**4**): white amorphous powder. UV (MeOH): $\lambda_{max}$ (log $\varepsilon$) = 219 (4.26), 316 (4.25) nm.

*Masutakeside I* (**5**): white amorphous powder. UV (MeOH): $\lambda_{max}$ (log $\varepsilon$) = 217 (4.48), 312 (4.49) nm; $[\alpha]_D^{22}$: –41.3° (*c* 0.1, MeOH).

### Determination of sugars in 1 – 3

Each sample (2 mg) was refluxed with 2 N HCl/dioxane (1 : 1, 2 mL) for 2 h. The mixture was extracted with EtOAc (5 mL×3). The residual water layer was neutralized with Amberlite MB-3 and dried to obtain a residue. The residue was trimethylsilylated by the method of Hara [10], and analyzed by GC (column, DB-1, 0.25 mm i. d.×30 m; column temperature, 50 – 230 °C, 15 °C/min, 230 °C for 18 min; carrier gas, He). The sugar derivatives obtained exhibited a retention time of 21.30 min, identical with that of authentic D-glucose (L-glucose; 22.00 min).

### Anti-complement assay

A diluted solution of normal human serum (complement serum, 80 $\mu$L) was mixed with a gelatin veronal buffer ($GVB^{2+}$, 80 $\mu$L) with sample dissolved in DMSO. The mixture was pre-incubated at 37 °C for 30 min, and sensitized erythrocytes (sheep red blood cells, 40 $\mu$L) were added. After incubation under the same conditions, the mixture was centrifuged (4 °C, 1500 rpm) and the optical density of the supernatant (100 $\mu$L) was measured at 405 nm

Table **1** $^1$H- (600 MHz) and $^{13}$C- (150 MHz) NMR data of compounds **1** (pyridine-$d_5$) and **1a** ($CDCl_3$)[a]

| Position | 1 | | 1a | |
|---|---|---|---|---|
| | $^1$H, multi (J in Hz) | $^{13}$C | $^1$H, multi (J in Hz) | $^{13}$C |
| 2 | | 157.6 | | 156.8 |
| 3 | 7.21 s | 101.6 | 7.28 s | 100.7 |
| 4 | 7.15 br. s | 113.4 | 7.00 (d, 1.2) | 112.7 |
| 5 | | 138.9 | | 137.9 |
| 6 | 6.85 br. s | 108.6 | 6.66 (d, 1.2) | 107.8 |
| 7 | | 145.9 | | 145.2 |
| 8 | | 143.5 | | 143.0 |
| 9 | | 132.2 | | 131.6 |
| 1′ | | 124.5 | | 124.0 |
| 2′ | 7.63 (d, 2.0) | 109.6 | 7.39 (d, 1.8) | 108.7 |
| 3′ | | 151.0 | | 150.0 |
| 4′ | | 151.0 | | 149.6 |
| 5′ | 7.04 (d, 8.0) | 113.0 | 6.95 (d, 8.3) | 111.8 |
| 6′ | 7.68 (dd, 8.0, 2.0) | 118.8 | 7.47 (dd, 8.3, 1.8) | 118.5 |
| 1″ | 2.89 m | 33.3 | 2.81 (t, 7.2) | 32.8 |
| 2″ | 2.06 m | 33.0 | 1.97 (dt, 15.0, 6.3) | 35.1 |
| 3″ | 3.73 m, 4.26 m | 69.4 | 3.74 (t, 6.3) | 62.7 |
| $OCH_3$-7 | 3.95 s | 56.4 | 4.06 s | 56.5 |
| $OCH_3$-3′ | 3.76 s | 56.3[b] | 3.94 s[b] | 56.5[b] |
| $OCH_3$-4′ | 3.76 s | 56.5[b] | 4.00 s[b] | 56.4[b] |
| Glc 1‴ | 4.83 (d, 8.0) | 105.2 | | |
| 2‴ | 4.04 m | 75.4 | | |
| 3‴ | 4.23 m | 78.8 | | |
| 4‴ | 4.25 m | 71.6 | | |
| 5‴ | 4.11 m | 77.7 | | |
| 6‴ | 4.36 (dd, 11.4, 5.6)<br>4.88 (dd, 11.4, 1.8) | 70.5 | | |
| Xyl 1⁗ | 5.03 (d, 7.0) | 106.5 | | |
| 2⁗ | 4.07 m | 76.0 | | |
| 3⁗ | 4.22 m | 79.1 | | |
| 4⁗ | 3.69 m | 72.1 | | |
| 5⁗ | 4.38 (dd, 11.0, 2.8) | 67.7 | | |

[a] Assignments made on the basis of COSY, HMQC, and HMBC.
[b] Chemical shift value assignments may be interchangeable.

[11]. Rosmarinic acid was employed as positive control [11], [12]. The purity of the compounds used for the assay was above 95% (determined by HPLC).

## Results and Discussion

The molecular formula of styraxlignolide A (**1**) was deduced as $C_{31}H_{40}O_{14}$, based on the molecular ion at *m/z* = 659.2311 $[M + Na]^+$ on HR-FAB-MS. The spectrum of this compound contained signals for a hydroxypropanol group at $\delta$ = 4.26 (1H), 3.73 (1H), 2.89 (2H) and 2.06 (2H), an independent olefinic proton at $\delta$ = 7.21, and two olefinic protons at $\delta$ = 7.15 and 6.85, which were assigned to a hydroxypropylbenzofuran moiety in the $^1$H-NMR spectrum (Table **1**), as compared with that of benzofurans isolated from *Styrax* species [13]. The HMBC correlation between at $\delta_H$ = 2.89 (H-1″) and $\delta_C$ = 138.9 (C-5)/$\delta_C$ = 113.4 (C-4)/$\delta_C$ = 108.6 (C-6) implies that the hydroxypropyl moiety ($C^{1''}H_2$-$C^{2''}H_2$-$C^{3''}$H-OH) is linked to benzofuran at C-5 (Fig. **1**). Furthermore, the presence of a three-substituted phenyl group was established by ABX type signals at $\delta$ = 7.68 (dd; 8.0, 2.0), 7.63 (d; 2.0) and 7.04 (d; 8.0) in the $^1$H-NMR spectrum. This observation was confirmed by signals for three $sp^2$ quaternary carbons and three $sp^2$ methines in the $^{13}$C-NMR spectrum. The $^1$H-NMR spectrum additionally displayed three methoxy groups at $\delta$ = 3.95 and 3.76 (×2), which correlated with quaternary carbons at $\delta$ = 145.9 (C-7), 151.0 (C-3′ and C-4′), respectively, in HMBC, and indicated the positions of the three methoxy groups. Long-range correlations between at $\delta_H$ = 7.21 (H-3) and $\delta_C$ = 124.5 (C-1′), and $\delta_H$ = 7.63 (H-2′)/$\delta_H$ = 7.68 (H-6′) and $\delta_C$ = 157.6 (C-2) led to the assignment of an aglycone moiety in **1**, namely 5-(3″-hydroxypropyl)-7-methoxy-2-(3′,4′-dimethoxyphenyl)-benzofuran.

On the other hand, the $^1$H-NMR spectrum contained signals for two anomeric protons at $\delta$ = 4.83 and 5.03, and ten oxygenated protons due to a pentose unit and a hexose unit in combination with the $^{13}$C-NMR data. On enzymic hydrolysis, **1** yielded monosaccharide units, which were identified by co-TLC (EtOAc:MeOH:$H_2O$:AcOH, 65 : 20 : 15 : 15) with authentic samples, as glucose and xylose. Their absolute configurations were determined as D-glucose and D-xylose, by using gas chromatography.

Table **2** $^{1}$H- (600 MHz) and $^{13}$C- (150 MHz) NMR data of compounds **2** and **3** in pyridine-$d_5$[a]

| Position | 2 | | 3 | |
|---|---|---|---|---|
| | $^{1}$H, multi (J in Hz)[1)] | $^{13}$C | $^{1}$H, multi (J in Hz)[1)] | $^{13}$C |
| 1 | 2.21 (d, 11.4), 2.54 (d, 11.4) | 51.6 | 0.83 m, 1.30 m | 38.7 |
| 2 | | 207.8 | 1.85 m, 2.29 (dd, 10.0, 3.1) | 26.7 |
| 3 | 4.51 s | 83.2 | 3.36 (dd, 13.7, 4.0) | 88.9 |
| 4 | | 45.0 | | 39.4 |
| 5 | 2.69 m | 44.4 | 0.74 (d, 11.2) | 55.9 |
| 6 | 1.88 m, 1.98 m | 30.7 | 1.32 m, 1.47 (d, 11.2) | 18.3 |
| 7 | 4.31 m | 69.2 | 1.21 m, 1.36 m | 33.2 |
| 8 | | 45.1 | | 40.1 |
| 9 | 1.58 m | 47.5 | 1.53 (dd, 10.6, 6.7) | 46.9 |
| 10 | | 41.7 | | 36.9 |
| 11 | 1.14 m, 1.43 m | 24.3 | 1.89 m | 23.8 |
| 12 | 1.33 m, 1.56 m | 23.9 | 5.98 s | 123.3 |
| 13 | 2.71 m | 48.3 | | 142.6 |
| 14 | | 53.6 | | 48.3 |
| 15 | 2.20 (d, 15.3), 2.78 (d, 15.3) | 42.9 | 1.92 (d, 13.2), 3.69 (d, 13.2) | 43.4 |
| 16 | 5.74 br. s | 133.7 | | 215.3 |
| 17 | 5.62 br. s | 130.8 | | 76.4 |
| 18 | 1.36 s | 15.1 | 3.09 (dd, 14.7, 3.4) | 52.8 |
| 19 | 0.93 s | 25.2 | 1.33 m, 1.67 (t, 13.9) | 48.1 |
| 20 | 1.47 s | 29.1 | | 31.0 |
| 21 | 1.18 s | 17.1 | 1.78 m, 1.92 m | 37.2 |
| 22 | 1.11 s | 17.7 | 1.78 (dd, 12.9, 4.0), 2.50 (d, 12.9) | 31.6 |
| 23 | | | 1.25 s | 28.0 |
| 24 | | | 1.20 s | 17.1 |
| 25 | | | 0.85 s | 15.4 |
| 26 | | | 1.23 s | 17.7 |
| 27 | | | 1.33 s | 27.2 |
| 28 | | | | |
| 29 | | | 0.88 s | 32.7 |
| 30 | | | 0.96 s | 23.6 |
| 1′ | 4.94 (d, 7.5) | 104.8 | 5.05 (d, 7.4) | 105.6 |
| 2′ | 4.37 (t, 8.1) | 83.2 | 4.40 (t, 7.4) | 77.4 |
| 3′ | 4.25 m | 78.1 | 4.38 m | 79.3 |
| 4′ | 4.11 m | 71.4 | 4.54 (t, 9.3) | 73.7 |
| 5′ | 3.78 m | 78.2 | 4.65 (t, 9.3) | 77.6 |
| 6′ | 4.27 m, 4.46 m | 62.6 | | 172.6 |
| 1″ | 5.37 (d, 7.6) | 105.9 | 6.60 s | 101.8 |
| 2″ | 4.08 m | 77.1 | 4.86 (d, 1.7) | 72.4 |
| 3″ | 4.21 (d, 9.0) | 77.9 | 4.69 (9.3, 1.7) | 72.5 |
| 4″ | 4.29 m | 71.6 | 4.35 m | 73.7 |
| 5″ | 3.90 m | 78.3 | 4.80 m | 69.7 |
| 6″ | 4.40 m, 4.42 m | 62.6 | 1.73 (d, 6.2) | 18.7 |

[a] Assignments made on the basis of COSY, HMQC, and HMBC.

The configurations of the glycosidic linkages for the glucopyranoside and xylopyranoside units were determined as $\beta$ on the basis of the $J_{1,2}$ values of the anomeric protons of 8.0 Hz ($\delta$ = 4.83) and 7.0 Hz ($\delta$ = 5.03). Sugar connectivity was determined on the basis of correlations between at $\delta_H$ = 4.83 (H-1‴) and $\delta_C$ = 69.4 (C-3‴), and $\delta_H$ = 5.03 (H-1⁗) and $\delta_C$ = 70.5 (C-6‴), supporting the presence of a primeverose moiety (-Glc$^6$-Xyl) [14].

Styraxoside A (**2**) was obtained as a white amorphous powder, $[\alpha]_D$: –11.0°. A molecular formula of $C_{34}H_{54}O_{13}$ was established using HR-FAB-MS, with a mass of *m/z* = 693.3463 $[M + Na]^+$. The $^{1}$H-NMR spectrum contained signals for isolated methylene protons at $\delta$ = 2.54 (d, 11.5) and 2.21 (d, 11.5), and methine at $\delta$ = 4.51 with geminal and angular methyls ($\delta$ = 1.47, 1.18, 0.93) on the other cyclohexane ring (Table **2**), supporting the presence of a $C^1H_2$-$C^2O$-$C^3H$-OH moiety, compared with that of yucalexin B-9 isolated from cassava root [15]. The existence of this moiety was further supported by diaxial coupling between H-1*ax* ($\delta$ = 2.54) and H-3*ax* ($\delta$ = 4.51), and *W*-type coupling between H-3*ax* and H-21-methyl in the COSY spectrum, as well as long-range correlations between at $\delta_H$ = 2.54/4.51 and $\delta_C$ = 207.8 (C-2) in the HMBC spectrum (Fig. **2**). The compound exhibited sig-

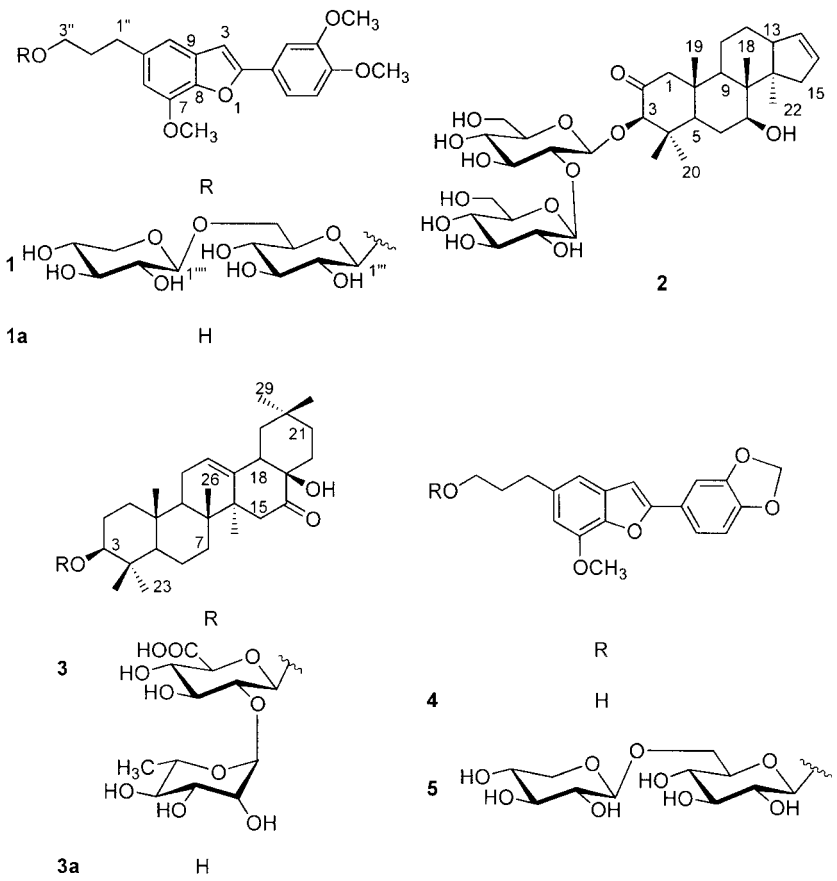

Fig. **1** Chemical structures of compounds from *S. japonica*.

nals for a methine ($\delta$ = 2.69), a methylene ($\delta$ = 1.98, 1.88) and a hydroxymethine ($\delta$ = 4.31), indicating the presence of a $C^5H$-$C^6H_2$-$C^7H$-OH spin system. Diaxial coupling between H-5 and H-7, and correlations between H-6 ($\delta$ = 1.98, 1.88) and H-5/H-7 were observed in the COSY and ROESY spectra, respectively. Furthermore, the $^1$H-NMR and COSY spectral data disclosed two olefinic protons, a methine, and a methylene, suggesting the presence of a spin system $C^{13}H$ ($\delta$ = 2.71)-$C^{17}H$ ($\delta$ = 5.62) = $C^{16}H$ ($\delta$ = 5.74)-$C^{15}H_2$ ($\delta$ = 2.78, 2.20) with a $\delta$ = 2.78 signal exhibiting additional *W*-type coupling to the C-14 methyl substituent. The presence of a double bond at C-16(17) was further supported by the low value of the couplings between the H-16 and H*ax*-15, and H-17 and H*ax*-13 protons, with dihedral angles of approximately 110° in each case. In addition, a methine ($\delta$ = 1.58) coupled to a methylene ($\delta$ = 1.43, 1.14) that was further linked to a second methylene ($\delta$ = 1.56, 1.33), was suggestive of a molecular sequence of $C^9H$-$C^{11}H_2$-$C^{12}H_2$, in which the $\delta$ = 1.58 (H-9) signal displayed *W*-type coupling to the H-18 methyl group. The partial structures were linked using an HMBC spectrum. NMR studies led to the assignment of the aglycone moiety in **2** as 3$\beta$,7$\beta$-dihydroxy-4$\alpha$,4$\beta$,8$\beta$,10$\beta$,14$\alpha$-pentamethyl-5$\alpha$-gon-16-en-2-one, derived by the loss of the C-17 side-chain from a dammarane triterpene [16]. Acid hydrolysis of **2** with 2 N HCl-dioxane produced a sugar component, which was identified as D-glucose. The configurations of the glycosidic linkage for glucopyranoside units were determined as $\beta$ on the basis of the $J_{1,2}$ values of the anomeric protons of 7.5 Hz ($\delta$ = 4.94) and 7.6 Hz ($\delta$ = 5.37). The sugar connectivity was established from correlations between $\delta_H$ = 4.94 (H-1′) and $\delta_C$ = 83.2 (C-3), and $\delta_H$ = 5.37 (H-1″) and $\delta_C$ = 83.2 (C-2′), consistent with the presence of a -Glc$^2$-Glc moiety (Fig. **1**). This is the first isolation of a trioxygenated octanordammarane-type saponin from a natural source.

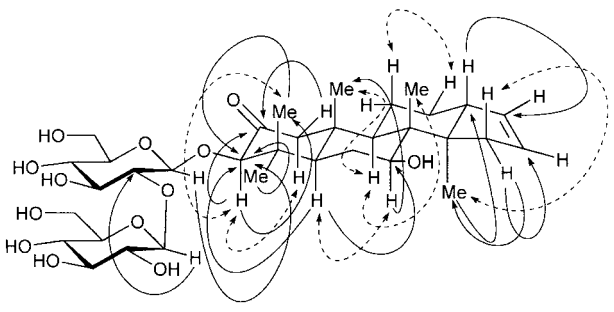

Fig. **2** Significant COSY (<—>) and HMBC (→) correlations for compound **2**.

Styraxoside B (**3**) was obtained as a white amorphous powder with an $[\alpha]_D^{22}$ of –43.7°. HR-FAB-MS of **3** displayed a quasi-molecular ion at *m/z* = 787.4240 [M + Na]$^+$ giving the molecular formula $C_{41}H_{64}O_{13}$. The $^1$H-NMR spectrum of **3** contained seven tertiary methyls at $\delta$ = 0.85 – 1.33, as well as a hydroxy methine at $\delta$ = 3.36 and a trisubstituted double bond at $\delta$ = 5.98 (Table **2**). The $^{13}$C-NMR spectrum of **3** contained signals for secondary ($\delta$ = 88.9) and tertiary ($\delta$ = 76.4) carbinol carbons, and a double bond at $\delta$ = 142.6 and 123.3. Detailed examination of the NMR spectral data, compared with that of camellioside A isolated from *Camellia japonica* (Fig. **1**), disclosed that **3** was a 28-norolean-12-en-16-one triterpene [17]. The $\beta$-orientation of the hydroxy groups at C-3 and C-17 was deduced from the multiplicity of H-3 (dd, *J* = 10.0, 3.1 Hz) and the chemical shift of C-17 ($\delta$ = 76.4), compared with that of camellenodiol isolated from

Table **3** Inhibitory effects of the compounds **1** – **5** isolated form *S. japonica* and analogues (**1a** and **3a**) on complement system of classical pathway

| *Compound* | *$IC_{50}$ (μM)* |
|---|---|
| Styraxlignolide A (**1**) | 123 |
| **1a** | > 200 |
| Styraxoside A (**2**) | > 200 |
| Styraxoside B (**3**) | 65 |
| **3a** | > 200 |
| Egonol (**4**) | 33 |
| Masutakeside I (**5**) | 166 |
| Rosmarinic acid[a] | 182 |

[a] This compound was used as positive control [11], [12].

*Celimisia petriei* [18]. On enzymic hydrolysis, **3** yielded the monosaccharide units D-glucuronic acid and L-rhamnose. The configuration of the glycosidic linkage for the glucuronopyranoside was determined as $\beta$, on the basis of the $J_{1,2}$ value of 7.4 Hz (at $\delta$ = 5.05) and of the rhamnopyranoside unit as $\alpha$ on the basis of the small $J_{1,2}$ value (*anti*-diequatorial) [19]. Sugar connectivities were resolved from correlations between $\delta_H$ = 5.05 (H-1′) and $\delta_C$ = 88.9 (C-3), and $\delta_H$ = 6.60 (H-1″) and $\delta_C$ = 77.4 (C-2′) in the HMBC experiment. In view of the evidence obtained, the structure of **3** was established as 3$\beta$,17$\beta$-dihydroxy-28-norolean-12-en-16-one 3-*O*-[$\alpha$-L-rhamnopyranoside-(1→2)-$\beta$-D-glucuronopyranoside].

Compounds **1** – **5** were tested for anti-complement activity. Compounds **1**, **3**, **4**, and **5** inhibited the hemolytic activity of the complement system with $IC_{50}$ values of 123, 65, 33, and 166 $\mu$M, respectively. On the other hand, **2** was incapable of inhibiting complement activity. The aglycones **1a** and **3a**, obtained from **1** and **3**, were additionally evaluated for anti-complement activity. However, compounds **1a** and **3a** were inactive in our assay system. A comparison of compounds **3** (**1**) and **3a** (**1a**) further confirmed that the sugar of the saponin is necessary to enhance the anti-complement activity of human serum against erythrocytes. This finding is consistent with the anti-complementary properties of kaikasaponin III and soyasaponin I from *Pueraria lobata*, as well as their hydrolytic analogues [20]. The most interesting structure-activity correlation was observed in the case of **1a**, a dimethoxy derivative of egonol (**4**). Of all the compounds tested, **4** was the most active, displaying an $IC_{50}$ value of 33 $\mu$M. In contrast, **1a** was completely inactive in this assay system. Our findings strongly suggest that the methylenedioxy group on the norlignan plays an important role in inhibiting the hemolytic activity of human serum against erythrocytes.

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