## Triterpene Saponins with Hyaluronidase Inhibitory Activity from the Seeds of *Camellia sinensis*

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The MeOH extract of the seeds of *Camellia sinensis* (L.) KUNTZE gave twelve new saponins (1–12) along with ten known saponins (13–22). These saponins (1–22) showed stronger hyaluronidase inhibitory activity than the positive control, rosmarinic acid.

Key words triterpene saponin; Camellia sinensis; hyaluronidase inhibitor

Tea is cultivated widely in Asia and has a longstanding reputation for its health-promoting properties. The bioactivity of the constituents of Camellia species has been reported previously, including for antisweet, gastroprotective, gastricemptying inhibitory, gastrointestinal transit accelerating, antioxidative and antimicrobial activity.<sup>1-3)</sup> Although studies on hyaluronidase inhibitory activity of tea saponins have been reported, detailed analysis of these compounds is still lacking.<sup>4)</sup> The hyaluronidase inhibitor rosmarinic acid is already known.<sup>5)</sup> In the course of the studies on the constituents of the seeds of Camellia sinensis, we obtained twelve new saponins (1-12) together with ten known saponins (13-22).<sup>1,2,6-10)</sup> The present report deals with the isolation of these saponins, structural elucidation of the new compounds, and analysis of their hyaluronidase inhibitory activities. As a result, compounds (1–22) were found to have stronger inhibitory activities (IC<sub>50</sub>: 19.3–55.6  $\mu$ M) than rosmarinic acid (IC<sub>50</sub>: 240.1  $\mu$ M).

## **Results and Discussion**

The seeds of *C. sinensis* cultivated in Shizuoka prefecture, Japan, were extracted with MeOH, and the extract was concentrated under reduced pressure and then dissolved in water and extracted with EtOAc. The MeOH eluate, obtained from a Mitsubishi Diaion HP-20 column for the water layer, was subjected to preparative HPLC, affording twelve new compounds (1-12), together with ten known compounds (13-22), which were identified by comparison of NMR data with reported data.

Teaseedsaponin A (1) was isolated as a colorless amorphous powder. The molecular formula was established as  $C_{62}H_{96}O_{28}$ on the basis of the high resolution (HR)-FAB-MS data ([M–H]<sup>-</sup> ion at *m/z* 1287.6034). The <sup>1</sup>H- and <sup>13</sup>C-NMR data were similar to those of foliatheasaponin III (14).<sup>7)</sup> The <sup>1</sup>H-NMR spectrum of 1 indicated the presence of the following functions, seven singlet methyl groups at  $\delta$  0.81, 0.92, 1.08, 1.11, 1.24, 1.28, 1.50 (each 3H, s, 25, 26, 24, 29, 30, 23, 27), a



Chart 1

methylene and three methines bearing an oxygen function at  $\delta$ 3.73, 4.22 (each 1H, d, J=11.0 Hz, 28), 4.40 (1H, d, J=10.0 Hz, 22), 5.84 (1H, brs, 16), 5.93 (1H, d, J=10.0Hz, 21), an olefinic proton at  $\delta$  5.44 (1H, brs, 12), four anomeric protons at  $\delta$  4.91 (1H, d, J=7.5 Hz, GlcA-1), 5.11 (1H, d, J=7.5 Hz, Glc-1), 5.65 (1H, d, J=7.5 Hz, Gal-1), 5.77 (1H, d, J=5.0 Hz, Ara-1), two acetyl methyl at  $\delta$  1.97, 2.50 (each 3H, s, 28-O-Ac-2, 16-O-Ac-2), one angeloyl unit at  $\delta$  1.92 (3H, dq, J=1.5, 1.5 Hz, 21-O-Ang-5), 2.01 (3H, dq, J=7.0, 1.5 Hz, 21-O-Ang-4), 5.91 (1H, qq, J=7.0, 1.5 Hz, 21-O-Ang-3). The <sup>13</sup>C-NMR spectrum of 1 showed signals owing to four carboxyl groups at  $\delta$ 172.1 (GlcA-6), 168.2 (21-O-Ang-1), 169.8 (16-O-Ac-1), 170.4 (22-O-Ac-1). In the <sup>13</sup>C-NMR spectrum, the carbon signals resulting from a sugar chain at C-3 were similar to those of camelliasaponin A<sub>1</sub> (13).<sup>6)</sup> Acid hydrolysis gave D-glucose, D-galactose, L-arabinose and D-glucuronic acid, and the <sup>1</sup>Hdetected heteronuclear multiple-bond connectivity (HMBC) experiment showed that the sugar chain at C-3 was decided to be  $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 3)-[ $\beta$ -D-galactopyranosyl- $(1\rightarrow 2)$ ]- $\beta$ -D-glucuronopyranosyl. Furthermore, an HMBC experiment with 1 showed long-range correlations between H-16 and the carboxyl carbon of the acetyl unit ( $\delta_{\rm C}$  169.8), H-21 and the carboxyl carbon of the angeloyl unit ( $\delta_{\rm C}$  168.2), and H-28 and the carboxyl carbon of the acetyl unit ( $\delta_{\rm C}$  170.4). Consequently, the structure of teaseedsaponin A was determined to be 1.

Teaseedsaponin B (2) was established as  $C_{62}H_{96}O_{28}$  on the basis of HR-FAB-MS data ( $[M-H]^-$  ion at m/z 1287.5997), which was the same as that of **1**. The <sup>1</sup>H- and <sup>13</sup>C-NMR data of **2** were similar to those of **1**, which suggested that **2** had two acetyl groups and one angeloyl group. In the <sup>1</sup>H-NMR spectrum, H-22 shifted to a lower field at  $\delta$  6.12 (1H, d, J=10.0 Hz, 22) compared with that of **1**, suggesting that **2** had an acetyl group at C-22. Furthermore, the positions of three acyl units in **2** were decided by the HMBC experiment, which exhibited long-range correlations between H-16 and the carboxyl carbon of the acetyl unit ( $\delta_C$  167.8), H-21 and the carboxyl carbon of the acetyl unit ( $\delta_C$  170.4). Consequently the structure of teaseedsaponin B was determined to be **2**.

Teaseedsaponin C (3) showed a molecular peak at m/z1187.5849 in negative mode HR-FAB-MS, suggesting a molecular formula of C<sub>58</sub>H<sub>92</sub>O<sub>25</sub>. In the <sup>1</sup>H-NMR spectrum, H-3 shifted to a lower field at  $\delta$  4.12 (1H, dd, J=11.5, 3.5 Hz, 3), and in the <sup>13</sup>C-NMR spectrum, C-3 shifted to a higher field at  $\delta$  83.3 (3), compared with those of 1, suggesting that 3 had a hydroxyl methyl group at C-23. In the <sup>1</sup>H-NMR spectrum, H-16 shifted to a higher field at  $\delta$  4.59 (1H, brs), and in the <sup>13</sup>C-NMR spectrum, C-21 shifted to a higher field at  $\delta$  41.8 (21), compared with those of 2, suggesting that C-16 was not acylated and C-21 had a methylene group. The <sup>1</sup>H-NMR spectrum of **3** showed signals owing to a *cis*-(2)-hexenoic acid unit at  $\delta$  0.85 (3H, t, J=7.5 Hz, Hex-6), 1.37 (2H, sext, J=7.5 Hz, Hex-5), 2.75 (2H, m, Hex-4), 5.93 (1H, dt, J=11.5, 2.0 Hz, Hex-2), 6.12 (1H, dt, J=11.5, 7.5 Hz, Hex-3), on the basis of the homonuclear Hartman-Hahn (HOHAHA) difference spectrum. The HMBC experiment for 3 showed a long-range correlation between H-22 and the carboxyl carbon of the cis-(2)-hexenoic acid unit ( $\delta_{c}$  166.7), and it was determined that **3** had the *cis*-(2)-hexenoic acid unit at C-22. In the <sup>13</sup>C-NMR spectrum, the carbon signals resulting from the sugar chain at C-3 were similar to those of theasaponin A<sub>3</sub> (15).<sup>8)</sup> Acid hydrolysis gave D-xylose, D-galactose, L-arabinose and D-glucuronic acid, and the HMBC experiment showed that the sugar chain at C-3 was decided to be  $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 3)-[ $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 2)]- $\beta$ -D-glucuronopyranosyl. Consequently, the structure of teaseedsaponin C was determined to be **3**.

The molecular formula  $C_{63}H_{98}O_{28}$  was concluded for teaseedsaponin D (4) on the basis of the molecular peak at m/z1301.6156 in the HR-FAB-MS. The <sup>1</sup>H- and <sup>13</sup>C-NMR data for 4 were similar to those of floratheasaponin B,<sup>2)</sup> which suggested that 4 had two angeloyl groups. In addition, in the <sup>13</sup>C-NMR spectrum, C-15 shifted to a higher field at  $\delta$  34.8 (15), compared with those of floratheasaponin B,<sup>2)</sup> suggesting that 4 had a methylene group at C-15. In the <sup>1</sup>H-NMR spectrum at  $\delta$  4.14 (1H, dd, *J*=12.5, 4.5Hz, 3) and <sup>13</sup>C-NMR at  $\delta$  83.0 (3) data for C-3 were similar to those of **3**, which suggested that 4 had a hydroxyl methyl group at C-23. In the <sup>13</sup>C-NMR spectrum, the carbon signals resulting from the sugar chain at C-3 were superimposable on those of **1**, and as a result of acid hydrolysis, the structure of teaseedsaponin D was determined to be **4**.

Teaseedsaponin E (5) was established as  $C_{62}H_{96}O_{27}$  on the basis of the HR-FAB-MS data ( $[M-H]^-$  ion at m/z 1271.6079). The <sup>1</sup>H- and <sup>13</sup>C-NMR data were superimposable on those of 4 except for the sugar moiety. The <sup>13</sup>C-NMR data of the sugar chain at C-3 were similar to that of 3, and the result of acid hydrolysis showed that the structure of teaseedsaponin E was determined to be 5.

Teaseedsaponin F (6) had a molecular formula  $C_{62}H_{98}O_{27}$ , as determined by negative-mode HR-FAB-MS data at m/z1273.6219. The <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of **6** were similar to those of **5**, which suggested that **6** had two acyl groups. The <sup>1</sup>H-NMR spectrum of **6** showed signals resulting from a 2-metyl-butyroyl unit at  $\delta$  0.80 (3H, t, J=7.5Hz, 2Mb-4), 1.10 (3H, d, J=7.0Hz, 2Mb-5), 1.71 (2H, dq, J=7.0, 7.5Hz, 2Mb-3), 2.27 (1H, sext, J=7.0Hz, 2Mb-2), which were similar to those of floratheasaponin C.<sup>2)</sup> Finally, the HMBC spectrum of **6** showed long-range correlations between H-21 and the carboxyl carbon of the angeloyl unit ( $\delta_C$  167.6), and H-22 and the carboxyl carbon of the 2-methyl-butyroyl unit ( $\delta_C$  176.7). It was determined that **6** had an angeloyl unit at C-21 and a 2-methyl-butyroyl unit at C-22. Consequently, the structure of teaseedsaponin F was determined to be **6**.

For teaseedsaponin G (7), the molecular formula  $C_{58}H_{90}O_{25}$  was deduced from HR-FAB-MS data ([M–H]<sup>-</sup> ion at m/z 1185.5696). The <sup>1</sup>H- and <sup>13</sup>C-NMR data were very similar to those of **3**. In the <sup>1</sup>H-NMR spectrum, H-23 shifted to a lower field at  $\delta$  9.85 (1H, s, 23), and in the <sup>13</sup>C-NMR spectrum, C-23 shifted to a lower field at  $\delta$  209.8 (23), compared with those of **3**, suggesting that 7 had an aldehyde group at C-23. Consequently, the structure of teaseedsaponin G was determined to be **7**.

Teaseedsaponin H (8) showed an  $[M-H]^-$  ion peak in HR-FAB-MS at m/z 1273.5846 corresponding to a molecular formula of C<sub>61</sub>H<sub>94</sub>O<sub>28</sub>. The <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of 8 were similar to those of assamsaponin H,<sup>9</sup> which suggested that 8 had two acyl groups. The <sup>1</sup>H-NMR spectrum of 8 showed signals resulting from a *cis*-(2)-hexenoic acid unit at  $\delta$  0.85 (3H, t, *J*=7.5Hz, Hex-6), 1.38 (2H, sext, *J*=7.5Hz, Hex-5), 2.74 (2H, m, Hex-4), 6.05 (1H, dt, *J*=11.0, 1.0Hz, Hex-2), 6.15 (1H, dt, J=11.0, 7.5 Hz, Hex-3), and the positions of two acyl groups in **8** were clarified by the HMBC experiment. Thus, long-range correlations were observed between H-21 and the carboxyl carbon of the *cis*-(2)-hexenoic acid part ( $\delta_{\rm C}$  167.1) and between H-28 and the carboxyl carbon of the acetyl part ( $\delta_{\rm C}$  170.6), and it was determined that **8** had the *cis*-(2)-hexenoic acid part at C-21 and the acetyl part at C-28. Consequently, the structure of teaseedsaponin H was determined to be **8**.

Teaseedsaponin I (9) showed a molecular peak at m/z 1299.6010 in the negative mode HR-FAB-MS, suggesting a molecular formula  $C_{63}H_{96}O_{28}$ . The <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of 9 were similar to those of 4, which suggested that 9 had two angeloyl groups. Furthermore, the <sup>1</sup>H- and <sup>13</sup>C-NMR spectra were shifted to a lower field at  $\delta_{\rm H}$  9.91 (1H, s, 23),  $\delta_{\rm C}$  209.9 (23), compared with those of 4, suggesting that 9 had an aldehyde group at C-23. Consequently, the structure of teaseedsaponin I was determined to be 9.

Teaseedsaponin J (10), whose molecular formula  $C_{62}H_{94}O_{27}$  was determined by negative-mode HR-FAB-MS data at m/z 1269.5904. The <sup>1</sup>H- and <sup>13</sup>C-NMR data were very similar to those of **9** except for the sugar moiety. <sup>13</sup>C-NMR data of the sugar chain at C-3 were similar to those of **7**, and the results of acid hydrolysis showed that the structure of teaseedsaponin J was determined to be **10**.

The molecular formula,  $C_{62}H_{94}O_{27}$  was concluded for teaseedsaponin K (11), on the basis of the molecular peak at m/z1269.5923, which was the same as that of 10. The <sup>1</sup>H- and <sup>13</sup>C-NMR data of 11 were similar to those of 10, which suggested that 11 had two acyl groups. In the <sup>13</sup>C-NMR spectrum, acyl-4 and acyl-5 shifted to a higher field at  $\delta$  14.0 (Tig-4), 12.3 (Tig-5), compared with the angeloyl group, suggesting that 11 had a tigloyl group. In the HMBC of 11, long-range correlations observed between H-21 and the angeloyl carboxyl carbon ( $\delta_C$ 167.9) and between H-22 and the tigloyl carboxyl carbon ( $\delta_C$ 169.4), and it was determined that 11 had an angeloyl group at C-21 and a tigloyl group at C-22. Consequently, the structure of Teaseedsaponin K was determined to be 11.

Teaseedsaponin L (12) showed an [M-H]<sup>-</sup> ion peak in HR-FAB-MS at m/z 1285.5851 corresponding to a molecular formula of C<sub>62</sub>H<sub>94</sub>O<sub>28</sub> In the <sup>1</sup>H-NMR spectrum, H-16 and H-22 shifted to a lower field at  $\delta$  5.59 (1H, brs, 16) and 6.12 (1H, d, J=10.0 Hz, 22), respectively, compared with those of 8, suggesting that 12 had an acetyl group at C-16 and C-22. Furthermore, in the <sup>1</sup>H-NMR spectrum, H-28 shifted to a higher field at  $\delta$  3.46, 3.57 (each 1H, d, J=10.5 Hz, 28), compared with that of 8, suggesting that 12 had a hydroxymethyl group at C-28. In addition, the <sup>1</sup>H-NMR spectrum of **12** showed signals resulting from a *cis*-(2)-hexenoic acid unit at  $\delta$  0.85 (3H, t, J=7.5 Hz, Hex-6), 1.39 (2H, sext, J=7.5 Hz, Hex-5), 2.74 (2H, m, Hex-4), 5.98 (1H, dt, J=11.0, 1.5 Hz, Hex-2), 6.19 (1H, dt, J=11.0, 7.5 Hz, Hex-3). The HMBC experiment for 12 showed long-range correlations between H-16 and the acetyl carbon ( $\delta_{\rm C}$  169.8), H-21 and the *cis*-(2)-hexenoic acid carbon ( $\delta_{\rm C}$  166.6), and H-22 and the acetyl carbon ( $\delta_{\rm C}$  170.5). It was determined that 12 had acetyl groups at C-16 and C-22 and a cis-(2)-hexenoic acid group at C-21. Furthermore, in the <sup>13</sup>C-NMR spectrum, the carbon signals resulting from the sugar chain at C-3 were similar to those of 3, and by acid hydrolysis, the structure of teaseedsaponin L was determined to be 12.

In this study, in order to investigate the biological activities of isolated compounds 1-22, their effects on hyaluronidase

inhibitory activities were measured. Table 3 shows the IC<sub>50</sub> values. As a result, the inhibitory activities of compounds **1–22** were found to be stronger than rosmarinic acid, used as a positive control. Furthermore the inhibitory activity of **5**, **6**, camelliasaponin  $A_1$  (**13**), camelliasaponin  $B_1$  (**16**) and assamsaponin F (**21**) had nearly ten-times stronger hyaluronidase inhibitory activities than rosmarinic acid. As far as we know, this is the first report on oleanane-type triterpene saponin having hyaluronidase inhibitory activity. Moreover, structure–activity relationship was not able to be acquired, there were few data obtained this time. So, we will research the saponin from other plants.

## Experimental

**General Procedures** Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. UV spectra were measured in methanol on a JASCO V-630 spectrophotometer. <sup>1</sup>H-(400 MHz) and <sup>13</sup>C- (100 MHz) NMR spectra were recorded on an  $\alpha$ -400 FT-NMR spectrometer, and chemical shifts are given as  $\delta$  values, with tetramethylsilane (TMS) as an internal standard at 35°C in pyridine- $d_5$ . Inverse-detected heteronuclear correlations were measured using <sup>1</sup>H-detected heteronuclear multiple quantum coherency (HMQC) (optimized for <sup>1</sup> $J_{C-H}$ =145 Hz) and HMBC (optimized for <sup>n</sup> $J_{C-H}$ =8 Hz) pulse sequences with a pulse field gradient. HR-FAB-MS data were obtained on a JEOL JMS 700 mass spectrometer in the negative mode using *m*-nitrobenzyl alcohol as the matrix. Preparative HPLC was performed on a JASCO 800 instrument.

**Plant Material** The seeds of *C. sinensis* were collected at Takakusayama in Shizuoka prefecture, Japan, in October 2010.

Extraction and Isolation The cut seeds of C. sinensis (920 g) were extracted twice with methanol (5 L) under reflux for 3h. The extract was concentrated under reduced pressure to give a residue (111 g). The residue was suspended in hot water (1.5 L) and extracted with ethyl acetate continuously for 8h. After removing EtOAc, the water layer was fractionated using a Mitsubishi Diaion HP-20 column (6.5×28 cm), eluting with water (10L), H<sub>2</sub>O-MeOH (50:50) (5L) and MeOH (3 L). The fraction eluted with MeOH was concentrated under reduced pressure to give a brown residue (13.3 g). The MeOH eluate (4.0 g) was subjected to preparative HPLC [column, Tosoh TSKgel ODS-80Ts, 5.5×180 cm; solvent, 0.1% trifluoroacetic acid (TFA)-CH<sub>2</sub>CN (65:35-57:43) linear gradient, UV 205 nm] to give 30 fractions (Frs. A-Z and a-d). The 61:39 solvent gave Fr. F [theasaponin A<sub>3</sub> (15) (142.3 mg)], the 59:41 solvent gave Fr. R (3) (13.1 mg), the 58:42 solvent gave Fr. V (7) (31.7 mg), the 57:43 solvent gave Fr. Y (5) (49.8 mg) and Fr. a (9) (13.3 mg), the 56:44 solvent gave Fr. b (10) (54.1 mg). Then, Fr. M (37.9 mg) was subjected to semipreparative HPLC [column, Cosmosil 5PE-MS, 2×25 cm; solvent, H<sub>2</sub>O-CH<sub>3</sub>CN (60:40)+TFA (0.05%), UV 205 nm] to give 1 (4.2 mg), 2 (8.7 mg) and 8 (4.3 mg). Fr. X (15.8 mg) was subjected to semipreparative HPLC [column, Cosmosil 5PE-MS, 2×25 cm; solvent, H<sub>2</sub>O-CH<sub>2</sub>CN (57.5:42.5)+TFA (0.05%), UV 205 nm] to give 4 (5.3 mg). Fr. Z (20.6 mg) was subjected to semipreparative HPLC [column, Cosmosil 5PE-MS, 2×25 cm; solvent, H<sub>2</sub>O-CH<sub>3</sub>CN (57.5:42.5)+TFA (0.05%), UV 205 nm] to give 6 (4.9 mg) and 11 (9.4 mg). Fr. U (26 mg) was subjected to semipreparative HPLC [column, Develosil C30-UG-5, 2×25 cm; solvent, H<sub>2</sub>O-CH<sub>3</sub>CN (55:45)+TFA (0.05%), UV 205 nm] to give 12 (16.7 mg). Fr. N (34.0 mg) was subjected to semipreparative HPLC [column, Cosmosil Cholester, 2×25 cm; solvent, H<sub>2</sub>O-CH<sub>2</sub>CN (57.5:42.5)+TFA (0.05%), UV 205 nm] to give camelliasaponin  $A_1$  (13) (12.9 mg) and theasaponin  $E_5$ (20) (6.4 mg). Fr. O (29 mg) was subjected to semipreparative HPLC [column, Cosmosil 5PE-MS, 2×25 cm; solvent, H<sub>2</sub>O- $CH_3CN$  (60:40) + TFA (0.05%), UV 205 nm] to give foliatheasaponin III (14) (5.2 mg). Fr. G (33.0 mg) was subjected to semipreparative HPLC [column, Cosmosil 5PE-MS, 2×25 cm; solvent, H<sub>2</sub>O-CH<sub>3</sub>CN (62.5:37.5)+TFA (0.05%), UV 205 nm] to give camelliasaponin  $B_1$  (16) (7.1 mg) and assamsaponin F (21) (6.9 mg). Fr. H (30 mg) was subjected to semipreparative HPLC [column, Cosmosil 5PE-MS, 2×25 cm; solvent, H<sub>2</sub>O-CH<sub>2</sub>CN (62.5:37.5)+TFA (0.05%), UV 205 nm] to give teasaponin  $E_2$  (17) (4.1 mg) and assamsaponin B (22) (8.3 mg). Fr. D (20mg) was subjected to semipreparative HPLC [column, Cosmosil 5PE-MS, 2×25 cm; solvent, H<sub>2</sub>O-CH<sub>3</sub>CN (62.5:37.5)+TFA (0.05%), UV 205 nm] to give assamsaponin G (18) (8.0 mg). Fr. E (23 mg) was subjected to semipreparative HPLC [column, Cosmosil 5PE-MS, 2×25 cm; solvent, H<sub>2</sub>O-CH<sub>3</sub>CN (62.5:37.5)+TFA (0.05%), UV 205 nm] to give teasaponin  $E_1$  (19) (16.7 mg).

Teaseedsaponin A (1): Colorless amorphous powder.  $[\alpha]_D^{26}$ -6.1 (*c*=0.33, MeOH). UV  $\lambda_{max}$  (MeOH) nm (log  $\varepsilon$ ): 221.5 (3.95). <sup>1</sup>H-NMR: see Table 1. <sup>13</sup>C-NMR: see Table 2. HR-FAB-MS *m/z*: 1287.6034 (Calcd for C<sub>62</sub>H<sub>95</sub>O<sub>28</sub>: 1287.6011).

Teaseedsaponin B (2): Colorless amorphous powder.  $[\alpha]_D^{25}$ -8.5 (*c*=0.74, MeOH). UV  $\lambda_{max}$  (MeOH) nm (log  $\varepsilon$ ): 216.5 (4.03). <sup>1</sup>H-NMR: see Table 1. <sup>13</sup>C-NMR: see Table 2. HR-FAB-MS *m/z*: 1287.5997 (Calcd for C<sub>62</sub>H<sub>95</sub>O<sub>28</sub>: 1287.6011).

Teaseedsaponin C (3): Colorless amorphous powder.  $[\alpha]_D^{25}$ -0.2 (*c*=1.09, MeOH). UV  $\lambda_{max}$  (MeOH) nm (log  $\varepsilon$ ): 218 (4.11), 247 (3.27). <sup>1</sup>H-NMR: see Table 1. <sup>13</sup>C-NMR: see Table 2. HR-FAB-MS *m/z*: 1187.5849 (Calcd for C<sub>58</sub>H<sub>91</sub>O<sub>25</sub>: 1187.5850).

Teaseedsaponin D (4): Colorless amorphous powder.  $[\alpha]_D^{25}$ -1.7 (*c*=0.54, MeOH). UV  $\lambda_{max}$  (MeOH) nm (log  $\varepsilon$ ): 215.5 (4.08), 254.5 (3.13). <sup>1</sup>H-NMR: see Table 1. <sup>13</sup>C-NMR: see Table 2. HR-FAB-MS *m/z*: 1301.6156 (Calcd for C<sub>63</sub>H<sub>97</sub>O<sub>28</sub>: 1301.6167).

Teaseedsaponin E (5): Colorless amorphous powder.  $[\alpha]_D^{25}$ -0.3 (*c*=1.17, MeOH). UV  $\lambda_{max}$  (MeOH) nm (log  $\varepsilon$ ): 212 (4.23). <sup>1</sup>H-NMR: see Table 1. <sup>13</sup>C-NMR: see Table 2. HR-FAB-MS *m/z*: 1271.6079 (Calcd for C<sub>62</sub>H<sub>95</sub>O<sub>27</sub>: 1271.6062).

Teaseedsaponin F (6): Colorless amorphous powder.  $[\alpha]_{26}^{26}$ +3.4 (*c*=0.40, MeOH). UV  $\lambda_{max}$  (MeOH) nm (log  $\varepsilon$ ): 225 (3.98), 255 (3.18). <sup>1</sup>H-NMR: see Table 1. <sup>13</sup>C-NMR: see Table 2. HR-FAB-MS *m/z*: 1273.6219 (Calcd for C<sub>62</sub>H<sub>97</sub>O<sub>27</sub>: 1273.6218).

Teaseedsaponin G (7): Colorless amorphous powder.  $[\alpha]_D^{26}$ +7.3 (*c*=1.0, MeOH). UV  $\lambda_{max}$  (MeOH) nm (log  $\varepsilon$ ): 259.5 (3.03). <sup>1</sup>H-NMR: see Table 1. <sup>13</sup>C-NMR: see Table 2. HR-FAB-MS *m/z*: 1185.5696 (Calcd for C<sub>58</sub>H<sub>89</sub>O<sub>25</sub>: 1185.5694).

Teaseedsaponin H (8): Colorless amorphous powder.  $[\alpha]_D^{25}$  +19.9 (*c*=0.37, MeOH). UV  $\lambda_{max}$  (MeOH) nm (log  $\varepsilon$ ): 208 (4.25). <sup>1</sup>H-NMR: see Table 1. <sup>13</sup>C-NMR: see Table 2. HR-FAB-MS *m/z*: 1273.5846 (Calcd for C<sub>61</sub>H<sub>93</sub>O<sub>28</sub>: 1273.5854).

Teaseedsaponin I (9): Colorless amorphous powder.  $[a]_D^{25}$ +7.1 (*c*=1.31, MeOH). UV (MeOH)  $\lambda_{max}$  (log  $\varepsilon$ ) nm: 211.5 (4.27). <sup>1</sup>H-NMR: see Table 1. <sup>13</sup>C-NMR: see Table 2. HR-FAB-MS *m/z*: 1299.6010 (Calcd for C<sub>63</sub>H<sub>95</sub>O<sub>28</sub>: 1299.6011).

Teaseedsaponin J (10): Colorless amorphous powder.  $[\alpha]_{D}^{2}$ 

615

+12 (*c*=1.09, MeOH). UV  $\lambda_{max}$  (MeOH) nm (log  $\varepsilon$ ): 216 (4.25). <sup>1</sup>H-NMR: see Table 1. <sup>13</sup>C-NMR: see Table 2. HR-FAB-MS *m/z*: 1269.5904 (Calcd for C<sub>62</sub>H<sub>93</sub>O<sub>27</sub>: 1269.5905).

Teaseedsaponin K (11): Colorless amorphous powder.  $[a]_D^{25}$ +5.8 (*c*=0.85, MeOH). UV  $\lambda_{max}$  (MeOH) nm (log  $\varepsilon$ ): 207 (4.31). <sup>1</sup>H-NMR: see Table 1. <sup>13</sup>C-NMR: see Table 2. HR-FAB-MS *m/z*: 1269.5923 (Calcd for C<sub>62</sub>H<sub>93</sub>O<sub>27</sub>: 1269.5905).

Teaseedsaponin L (12): Colorless amorphous powder.  $[\alpha]_D^{25}$ +5.8 (*c*=1.45, MeOH). UV  $\lambda_{max}$  (MeOH) nm (log  $\varepsilon$ ): 210 (4.30), 250 (3.18). <sup>1</sup>H-NMR: see Table 1. <sup>13</sup>C-NMR: see Table 2. HR-FAB-MS *m/z*: 1285.5851 (Calcd for C<sub>62</sub>H<sub>93</sub>O<sub>28</sub>: 1285.5854).

Acid Hydrolysis and Sugar Identification<sup>11</sup> Compounds 1–12 (*ca.* 1 mg) were dissolved separately in 50% TFA (50  $\mu$ L). The solutions were heated at 100°C for 1 h. The reaction mixtures were dried, diluted with H<sub>2</sub>O, and extracted with EtOAc. The H<sub>2</sub>O layers were concentrated to dryness. The residues were stirred with L-cysteine methyl ester hydrochloride in pyridine (20 mg/mL, 50  $\mu$ L) at 60°C for 1 h. *o*-Tolyl isthiocyanate (5  $\mu$ L) was added and heated at 60°C for another 1 h. The reaction mixtures were subjected to HPLC analysis [column: Cosmosil 5C<sub>18</sub>-AR-II, 4.6×250 mm; solvent, H<sub>2</sub>O–CH<sub>3</sub>CN (78:22)+TFA (0.05%), UV 250 nm] to identify the derivatives of D-glucuronic acid ( $t_R$  62.8 min), D-galactose ( $t_R$  19.2 min), and L-arabinose ( $t_R$  25.0 min) from 1–12, D-xylose ( $t_R$  25.8 min) from 3, 5, 6, 7, 10, 11 and 12, D-glucose ( $t_R$  22.2 min) from 1, 2, 4, 8 and 9.

Assay of Inhibitory Effect on Hyaluronidase Inhibitory effects on hyaluronidase were determined by the Morgan-Elson method<sup>12)</sup> as modified by Kakegawa et al.<sup>13)</sup> Thus, samples dissolved in 0.1 mol/L acetate buffer (pH 4.0) (40  $\mu$ L) and hyaluronidase (Type IV-S: derived from bovine testis, 2140 units/ mg solid, Sigma Chemical Co., St. Louis, U.S.A.) in buffer (final concentration: 400 NF units/mL,  $20 \mu$ L) were mixed, and the mixtures were incubated at 37°C for 20min. Next, the compound 48/80 (Sigma Chemical Co., St. Louis, U.S.A.) in buffer (0.1 mg/mL,  $40 \mu$ L) was added as an activator, and the mixtures were allowed to react for 20 min at 37°C. After hyaluronic acid potassium salt, derived from rooster comb (Wako Pure Chemical Industries Ltd., Osaka, Japan) in buffer (final concentration: 0.4 mg/mL,  $100 \mu \text{L}$  had been added, the mixture was incubated at 37°C for 40 min. Then, the reactions were stopped by adding  $40\,\mu\text{L}$  of  $0.4\,\text{mol/L}$  NaOH, and the mixtures were cooled. Boric acid solution (pH 9.1) (40  $\mu$ L) was added to each solution, and the mixtures were boiled for 3 min. The mixtures were cooled using ice, 1.2 mL of a pdimethylaminobenzaldehyde reagent ( $40 \mu L$ ) was added, and the mixtures were allowed to react for 20 min at 37°C. After the reactions had been completed, absorbance at  $\lambda$  595 nm was measured. A blank test was performed in the same fashion as a negative control. The following formula was used to calculate the inhibitory effects on hyaluronidase activity:

rate of inhibiting hyaluronidase activity (%)

$$= [\{(S_{t} - S_{b}) - (C_{t} - C_{b})\} / (S_{t} - S_{b})] \times 100$$

where  $S_t$  is absorbance of the solution containing a study sample at  $\lambda$  595 nm,  $S_b$  is absorbance of the blank solution at  $\lambda$  595 nm,  $C_t$  is absorbance of the control solution at  $\lambda$  595 nm, and  $C_b$  is absorbance of the blank control solution at  $\lambda$  595 nm. The IC<sub>50</sub> value of inhibitory effects on hyaluronidase was determined within the range of the concentrations tested.

		1		2		3	4		
Position	$\delta_{\rm H} \left( J \text{ in Hz} \right)$	HMBC (H to C)	$\delta_{\rm H}(J~{\rm in}~{\rm Hz})$	HMBC (H to C)	$\delta_{\rm H} \left( J \text{ in Hz} \right)$	HMBC (H to C)	$\delta_{\rm H}(J~{\rm in}~{\rm Hz})$	HMBC (H to C)	
3	3.23 (dd, 12.0 4.0)	,	3.24 (dd, 11.5 3.5)	,	4.12 (dd, 11.5, 3.5)	,	4.14 (dd, 12.5 4.5)	,	
5	0.71 (brd, 10.0)		0.72 (brd, 12.0)		1.58 (brd, 11.0)		1.57 (brd, 11.0)		
9	1.64 (br d,		1.64 (over-		1.84 (over-		1.85 (over-		
11	1.84 (over- lapped)		1.85 (over- lapped)		1.85 (over- lapped)		1.86 (over- lapped)		
	1.90 (over- lapped)		1.91 (over- lapped)		1.93 (over- lapped)		1.94 (over- lapped)		
12	5.44 (brs)	9, 14	5.40 (brs)	9, 14	5.39 (brs)	14	5.43 (brs)	9, 14	
15	1.64 (over- lapped) 1.92 (over-		1.62 (over- lapped) 1.89 (over-		1.59 (over- lapped)		1.59 (over- lapped)		
	lapped)		lapped)						
16	5.84 (brs)	1'	5.61 (brs)	1'	4.59 (brs)		4.47 (brs)		
18	2.77 (over- lapped)		3.00 (dd, 13.5 5.0)	5,	3.04 (dd, 14.0, 4.0)		3.08 (over- lapped)		
19	1.42 (over- lapped)		1.41 (over- lapped)		1.32 (over- lapped) 1.86 (over- lapped)		1.40 (over- lapped) 1.86 (over- lapped)		
21	5.93 (d, 10.0)	20, 22, 29, 30, 1"	5.87 (d, 10.0)	22, 29, 30, 1"	mpped)		6.66 (d, 10.0)	20, 22, 29, 30, 1"	
22	4.40 (d, 10.0)	16, 17, 21	6.12 (d, 10.0)	16, 17, 21, 28, 1‴	6.19 (dd, 12.0, 5.5)	1‴	6.28 (d, 10.0)	16, 17, 22, 28, 1‴	
23	1.28 (s)	3, 4, 5, 24	1.29 (s)	3, 4, 5, 24	3.76 (d, 10.5) 4.37 (d, 10.5)		3.75 (d, 10.0) 4.40 (d, 10.0)	3, 5	
24	1.08 (s)	3, 4, 5, 23	1.10 (s)	3, 4, 5, 23	1.07 (s)	3, 4, 5, 23	1.04 (s)	3, 4, 5, 23	
25	0.81 (s)	5, 9, 10	0.79 (s)	1, 5, 9, 10	0.91 (s)	9, 10	0.91 (s)	1, 5, 10	
26	0.92 (s)	7, 8, 9, 14	0.78 (s)	7, 8, 9, 14	0.91 (s)	7, 8, 14	0.90 (s)	7, 8, 9, 14	
27	1.50 (s)	8, 13, 14, 15	1.50 (s)	8, 13, 14, 15	1.82 (s)	8, 13, 14, 15	1.79 (s)	8, 13, 14, 15	
28	3.73 (d, 11.0)	1‴″	3.46 (d, 11.0)	22	3.51 (d, 10.7)		3.40 (d, 11.0)	18, 22	
	4.22 (d, 11.0)		3.60 (d, 11.0)		3.68 (d, 10.7)		3.66 (d, 11.0)		
29	1.11 (s)	19, 20, 21, 30	1.07 (s)	19, 20, 21, 30	1.03 (s)	19, 20, 21, 30	1.07 (s)	19, 20, 21, 30	
30	1.24 (s)	19, 20, 21, 29	1.29 (s)	19, 20, 21, 29	1.27 (s)	19, 20, 21, 29	1.32 (s)	19, 20, 21, 29	
16-0-	(Ac)		(Ac)						
2'	2.50 (s)	1'	2.51 (s)	1'					
21- <i>O</i> - 2"	(Ang)		(Ang)				(Ang)		
3"	5.91 (qq, 7.0, 1.5)		5.99 (qq, 7.0, 2.0)	5″			5.97 (qq, 7.0, 1.5)	5″	
4″	2.01 (dq, 7.0, 1.5)	2", 3"	2.06 (dq, 7.0, 2.0)	2", 3"			2.08 (dq, 7.0, 1.5)	2", 3"	
5"	1.92 (dq, 1.5, 1.5)	1", 2", 3"	1.98 (dq, 2.0, 2.0)	1", 2", 3"			2.01 (dq, 1.5, 1.5)	1", 2", 3"	
6″ 22. C									
22-0-			(Ac) 2.04 (s)	1‴	(Hex) $5.93 (dt, 11.5, 2.0)$	3‴, 4‴	(Ang)		
3‴					6.12 (dt, 11.5, 7.5)	2‴	5.92 (qq, 7.0,	5‴	
4‴					2.75 (m)	2‴, 5‴, 6‴	2.05 (dq, 7.0, 1.5)	2‴, 3‴	
5‴					1.37 (sext, 7.5)	4‴, 6‴	1.91 (dq, 1.5, 1.5)	1‴, 2‴, 3‴	
6‴					0.85 (t, 7.5)	4‴, 5‴	,		
28-0-	(Ac)				~ / /				
2‴″	1.97 (s)	1‴″							

Table 1. <sup>1</sup>H-NMR Spectroscopic Data (400 MHz, Pyridine-d<sub>5</sub>) of Compounds 1–12

May	201	2
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		1		2		3	4		
Position	$\delta_{\rm H} \left( J \text{ in Hz} \right)$	HMBC (H to C)	$\delta_{\rm H} \left( J \text{ in Hz} \right)$	HMBC (H to C)	$\delta_{\rm H} \left( J \text{ in Hz} \right)$	HMBC (H to C)	$\delta_{\rm H} \left( J \text{ in Hz} \right)$	HMBC (H to C)	
GlcA									
1	4.91 (d, 7.5)	3	4.92 (d, 7.5)	3	5.04 (d, 7.5)	3	5.06 (d, 7.5)	3	
2	4.65 (dd, 8.5, 7.5)		4.68 (dd, 8.5, 7.5)		4.59 (over- lapped)		4.63 (dd, 8.0, 7.5)		
3	4.41 (over-		4.41 (over-		4.27 (over-		4.28 (over-		
4	4 54 (over-		4 52 (over-		4 45 (over-		4 48 (over-		
	lapped)		lapped)		lapped)		lapped)		
5	4.51 (over-		4.51 (over-		4.41 (over-		4.40 (over-		
	lapped)		lapped)		lapped)		lapped)		
	5 65 (d. 7 5)	GlcA-2	5 67 (d. 7 5)	GlcA-2	5 83 (d. 75)	GlcA-2	576 (d. 75)	GlcA-2	
2	4.48 (over-	GICA-2	4.51 (over-	GIGA-2	4.49 (over-	GIGA-2	4.50 (over-	GIEA-2	
_	lapped)		lapped)		lapped)		lapped)		
3	4.22 (over-		4.24 (over-		4.31 (over-		4.23 (over-		
	lapped)		lapped)		lapped)		lapped)		
4	4.50 (brs)		4.52 (brs)		4.52 (brs)		4.50 (brs)		
5	4.18 (over- lapped)		4.20 (over- lapped)		4.33 (over- lapped)		4.27 (over- lapped)		
6	4.42 (over-		4.41 (over-		4.35 (over-		4.34 (over-		
	lapped)		lapped)		lapped)		lapped)		
	4.47 (over-		4.46 (over-		4.47 (over-		4.48 (over-		
A	lapped)		lapped)		lapped)		lapped)		
Ara 1	5 77 (d. 5 0)	GlcA-3	578 (d. 50)	GlcA-3	5 72 (d. 6 5)	GlcA-3	578 (d. 50)	GlcA-3	
2	4.72 (dd. 7.0.	OICA-5	4.74 (dd. 7.5.	OICA-5	4.56 (over-	OICA-5	4.77 (dd. 6.5.	OleA-5	
-	5.0)		5.0)		lapped)		5.0)		
3	4.41 (over-		4.43 (over-		4.36 (over-		4.47 (over-		
	lapped)		lapped)		lapped)		lapped)		
4	4.36 (over-		4.37 (over-		4.32 (over-		4.49 (over-		
5	3.73 (over-		3.74 (over-		3.76 (dd. 12.0	).	3.75 (over-		
	lapped)		lapped)		2.0)	,	lapped)		
	4.35 (over-		4.39 (over-		4.38 (over-		4.37 (over-		
Y-1	lapped)		lapped)		lapped)		lapped)		
					5.02(d.7.5)	$\Delta r_{2}$			
2					4 12 (dd 8 0	Ald-2			
2					5.0)				
3					4.02 (dd, 8.0,				
					5.0)				
4					4.24 (m)				
5					5.52 (dd, 11.0 11.0)	,			
					4.42 (over-				
					lapped)				
Gle									
1	5.11 (d, 7.5)	Ara-2	5.13 (d, 7.5)	Ara-2			5.12 (d, 7.5)	Ara-2	
2	4.11 (dd, 11.0 7 5)	,	4.12 (dd, 10.0 7.5)	),			4.13 (over- lanned)		
3	4.09 (over-		4.11 (over-				4.12 (over-		
	lapped)		lapped)				lapped)		
4	4.29 (over-		4.21 (over-				4.18 (over-		
-	lapped)		lapped)				lapped)		
5	5.78 (m)	1	5.82 (m) 4.30 (dd 12.0	)			5.82 (m) 4.28 (over		
U	5.0)	· ·	5.0)	· ·			lapped)		
	4.41 (over-		4.41 (over-				4.47 (over-		
	lapped)		lapped)				lapped)		

D :(:	4	5	(	6		7	8		
Position	$\delta_{\mathrm{H}} \left( J \text{ in Hz} \right)$	HMBC (H to C)	$\delta_{\rm H} \left( J \text{ in Hz} \right)$	HMBC (H to C)	$\delta_{\rm H} \left( J \text{ in Hz} \right)$	HMBC (H to C)	$\delta_{\rm H} \left( J \text{ in Hz} \right)$	HMBC (H to C)	
3	4.12 (over- lapped)		4.12 (dd, 10.5, 5.0)		4.01 (over- lapped)		4.03 (dd, 11.5, 4.5)		
5	1.55 (br d, 11.0)		1.56 (brd, 11.0)		1.38 (over- lapped)		1.39 (over- lapped)		
9	1.82 (over-		1.83 (over-		1.78 (over-		1.77 (over-		
11	lapped) 1.85 (over-		lapped) 1.85 (over-		lapped) 1.77 (over-		lapped) 1.74 (over-		
	lapped)		lapped)		lapped)		lapped)		
	1.97 (over- lapped)		1.95 (over- lapped)		1.98 (over- lapped)		1.96 (over- lapped)		
12	5.43 (brs)	9, 14	5.42 (brs)	14	5.37 (brs)	9, 14	5.43 (brs)	9, 14	
15	1.56 (over- lapped)		1.54 (over- lapped)		1.54 (over- lapped)		1.58 (brd, 15 0)		
	1.87 (over-		1.85 (over-		1.87 (over-		1.85 (br d,		
16	lapped)		lapped)		lapped)		15.0) 4.70 (brs)		
18	4.45 (018) 3.08 (over-		4.43 (01 s) 3.07 (over-		4.37 (018) 3.08 (dd, 14.0,		4.70 (01s) 2.82 (dd, 14.0,		
10	lapped)		lapped)		3.0)		4.0)		
19	l.41 (over- lapped)		lapped)		l.33 (over- lapped)				
	1.84 (over-		1.84 (over-		1.78 (over-				
21	6.66 (d, 10.0)	29, 30, 1"	6.62 (d, 10.5)	20, 22, 29, 30, 1"	1.99 (dd, 11.5,		6.42 (d, 10.0)	22, 29, 30, 1"	
22	6.28 (d, 10.0)	16, 17, 28, 1‴	6.21 (d, 10.5)	16, 17, 21, 28, 1‴	6.10 (dd, 11.5, 5.5)	16, 17, 28, 1‴	4.45 (d, 10.0)	17, 16, 21	
23	3.74 (d, 10.5)	3	3.77 (d, 10.5)	3	9.85 (s)	4, 24	9.90 (s)	4	
24	4.33 (d, 10.5) 1.04 (s)	3 4 5 23	4.38 (d, 10.5) 1.07 (s)	3 4 5 23	1 44 (s)	3 4 5 23	1 44 (s)	3 4 5 23	
25	0.91 (s)	9, 10	0.91 (s)	1, 10	0.84 (s)	1, 5, 10	0.82 (s)	1, 5, 9, 10	
26	0.90 (s)	7, 8, 14	0.90 (s)	7, 8, 9, 14	0.82 (s)	7, 8, 9, 14	0.94 (s)	7, 8, 9, 14	
27	1.79 (s)	8, 14, 15	1.77 (s)	8, 13, 14, 15	1.81 (s)	8, 13, 14	1.77 (s)	8, 14, 15	
28	3.40 (d, 10.5) 3.66 (d, 10.5)	18, 22	3.39 (d, 11.0) 3.66 (d, 11.0)	18, 22	3.49 (d, 10.5) 3.63 (d, 10.5)	17, 18, 22	4.21 (d, 10.0) 4.30 (d, 10.0)	18, 1‴	
29	1.07 (s)	19, 20, 21, 30	1.06 (s)	19, 20, 21, 30	1.05 (s)	19, 20, 21, 30	1.13 (s)	19, 20, 21, 30	
30	1.32 (s)	19, 20, 21, 29	1.30 (s)	19, 20, 21, 29	1.27 (s)	19, 20, 21, 29	1.30 (s)	19, 20, 21, 29	
16- <i>O</i> - 2'									
21-0-	(Ang)		(Ang)				(Hex)		
2″							6.05 (dt, 11.0, 1.0)	1", 4"	
3″	5.97 (qq, 7.0, 1.5)	1", 4", 5"	6.06 (qq, 7.5, 1.5)	5″			6.15 (dt, 11.0, 7.5)	1″	
4″	2.08 (dq, 7.0, 1.5)	1", 2", 3"	2.15 (dq, 7.0, 1.5)	2", 3"			2.74 (m)		
5″	2.00 (br s)	1", 2", 3"	2.03 (dq, 1.5, 1.5)	1", 2", 3"			1.38 (sext, 7.5)	3", 4", 6"	
6"	<i></i>						0.85 (t, 7.5)	4", 5"	
22-0- 2‴	(Ang)		(2Mb) 2.27 (sext, 7.0)	1‴, 3‴	(Hex) 5.94 (dt, 11.5, 2.0)	3‴, 4‴			
3‴	5.92 (qq, 7.0,	1‴, 4‴, 5‴	1.71 (dq, 7.0, 7.5)	4‴, 5‴	6.14 (dt, 11.5, 7.5)	2‴, 4‴, 5‴			
4‴	2.04 (dq, 7.0,	1‴, 2‴, 3‴	0.80 (t, 7.5)	2‴, 3‴	2.76 (m)	3‴, 5‴, 6‴			
5‴	1.91 (br s)	1‴, 2‴, 3‴	1.10 (d, 7.0)	1‴, 2‴, 3‴	1.39 (sext, 7.5)	3‴, 4‴, 6‴			
6‴					0.86 (t, 7.5)	4‴, 5‴			
28- <i>O</i> -							(Ac)	1////	
2							1.99 (S)	1	

May	201	2
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	:	5		6	7		8	
Position	$\delta_{\rm H} \left( J \text{ in Hz} \right)$	HMBC (H to C)	$\delta_{\rm H} \left( J \text{ in Hz} \right)$	HMBC (H to C)	$\delta_{\rm H} \left( J \text{ in Hz} \right)$	HMBC (H to C)	$\delta_{\rm H} (J \text{ in Hz})$	HMBC (H to C)
GlcA								
1	5.03 (d, 7.0)	3	5.03 (d, 8.0)	3	4.82 (d, 7.0)	3	4.84 (d, 7.5)	3
2	4.57 (dd, 9.0, 7.0)		4.59 (dd, 8.0, 8.0)		4.47 (over- lapped)		4.50 (over- lapped)	
3	4.27 (over- lapped)		4.26 (over- lapped)		4.30 (over- lapped)		4.31 (over- lapped)	
4	4.38 (over- lapped)		4.43(over- lapped)		4.43 (over- lapped)		4.47 (over- lapped)	
5	4.37 (over- lapped)		4.40 (over- lapped)		4.45 (over- lapped)		4.47 (over- lapped)	
Gal								
1	5.80 (d, 7.0)	GlcA-2	5.84 (d, 8.0)	GlcA-2	5.70 (d, 7.0)	GlcA-2	5.63 (d, 7.5)	GlcA-2
2	4.50 (over- lapped)		4.48 (over- lapped)		4.40 (over- lapped)		4.43 (over- lapped)	
3	4.30 (over-		4.29 (over- lanned)		4.26 (over-		4.23 (over-	
4	4.52 (brs)		4.51 (brs)		4.48 (brs)		4.52 (brs)	
5	4.29 (over-		4.32 (over-		4.32 (over-		4.28 (over-	
6	(apped)		lapped)		(apped))		(apped)	
0	lapped)		4.55 (over- lapped)		4.51 (over- lapped)		4.31 (over- lapped)	
			4.47 (over-		4.45 (over-		4.48 (over-	
			lapped)		lapped)		lapped)	
Ara			5 50 (1 ( 0)		5 (0 (1 5 0)		5 50 (1 5 0)	
1	5.68 (d, 5.0)	GlcA-3	5.72 (d, 6.0)	GlcA-3	5.69 (d, 5.0)	GlcA-3	5.78 (d, 5.0)	GlcA-3
2	4.33 (dd, 9.0, 5.0)		4.33 (dd, 8.0, 6.0)		4.33 (dd, 8.0, 6.0)		4.73 (dd, 7.0, 5.0)	
3	4.33 (over- lapped)		4.35 (over- lapped)		4.34 (over- lapped)		4.44 (over- lapped)	
4	4.29 (over- lapped)		4.31 (over- lapped)		4.29 (over- lapped)		4.38 (over- lapped)	
5	3.75 (over-		3.75 (dd, 15.0	),	4.34 (over-		3.75 (dd, 11.5	,
	4 35 (over-		4 38 (over-		4 73 (brd		4 43 (over-	
	lapped)		lapped)		10.0)		lapped)	
Xyl	5.02 (1.7.0)		5.01 (1.0.0)		4.00 (1.7.0)			
1 2	5.03 (d, 7.0) 4.11 (dd, 8.0,	Ara-2	5.01 (d, 8.0) 4.11 (dd, 8.0,	Ara-2	4.99 (d, 7.0) 4.10 (dd, 8.5,	Ara-2		
3	4.03 (dd, 8.0,		4.01 (dd, 9.0,		4.02 (dd, 8.5,			
4	(120, 0.0)		9.0) 4.22 (m)		8.3) 4.19 (m)			
5	3.49 (dd, 10.5)	,	4.42 (over-		3.51 (brt,			
	4.37 (over-		3.51 (dd, 11.0	),	4.42 (over-			
Gla	lapped)		10.0)		lapped)			
1							5 10 (d. 7 0)	Ara-2
2							4.11 (dd, 7.0, 7 0)	1110 2
3							4.11 (over-	
4							4.21 (over- lapped)	
5							3.82 (ddd. 8.0	, 6.0, 3.0)
6							4.28 (over- lapped)	·
							4.41 (over- lapped)	

D iii	9	)	1	10	11		12		
Position	$\delta_{\rm H} \left( J \text{ in Hz} \right)$	HMBC (H to C)	$\delta_{\rm H} \left( J \text{ in Hz} \right)$	HMBC (H to C)	$\delta_{\rm H} \left( J \text{ in Hz} \right)$	HMBC (H to C)	$\delta_{\rm H} (J \text{ in Hz})$	HMBC (H to C)	
3	4.05 (dd, 12.0,		4.01 (over-		4.03 (over-		3.96 (dd, 11.9,		
5	4.5)		lapped)		lapped)		4.5)		
5	1.37 (over- lapped)		1.38 (over- lapped)		1.37 (over- lapped)		1.37 (over- lapped)		
9	1.77 (over-		1.77 (over-		1.80 (over-		1.76 (over-		
	lapped)		lapped)		lapped)		lapped)		
11	1.75 (over-		1.78 (over-		1.78 (over-		11 /		
	lapped)		lapped)		lapped)				
					1.95 (over-				
					lapped)				
12	5.41 (brs)	9, 14	5.41 (brs)	9, 14	5.41 (brs)	9, 14	5.38 (brs)	9, 14	
15	1.52 (over-		1.51 (over-		1.54 (over-		1.53 (brd,		
	1 78 (over		1 83 (over		1 85 (over		13.0) 1.81 (brd		
	lapped)		lapped)		lapped)		15.0)		
16	4.46 (brs)		4.45 (brs)		4.44 (brs)		5.59 (brs)	1'	
18	3.11 (over-		3.06 (br d,		3.09 (brd,		2.99 (dd, 14.0,		
	lapped)		10.0)		10.0)		4.0)		
19	1.42 (brd,		1.42 (over-		1.42 (brd,		1.45(over-		
	10.0)		lapped)		10.0)		lapped)		
	1.80 (over-				1.80 (over-				
21	6.66.(d + 10.0)	22 29 30 1"	6 66 (d. 10 0)	20 22 29	6.72 (d 10.5)	22 29 30 1"	5.83 (d. 10.0)	22 29 30 1"	
21	0.00 (u, 10.0)	22, 27, 50, 1	0.00 (u, 10.0)	30, 1"	0.72 (u, 10.5)	22, 29, 50, 1	5.65 (u, 10.0)	22, 27, 50, 1	
22	6.28 (d, 10.0)	16, 17, 21, 28, 1‴	6.27 (d, 10.0)	16, 17, 21, 28, 1‴	6.23 (d, 10.5)	16, 17, 21, 28, 1‴	6.12 (d, 10.0)	16, 21, 28, 1‴	
23	9.91 (s)	3, 24	9.87 (s)	4	9.91 (s)	4	9.95 (s)	4, 24	
24	1.45 (s)	3, 4, 5, 23	1.44 (s)	3, 4, 5, 23	1.46 (s)	3, 4, 23	1.48 (s)	3, 4, 5, 23	
25	0.82 (s)	5, 9, 10	0.88 (s)	1, 9, 10	0.82 (s)	9, 10	0.78 (s)	1, 5, 9, 10	
26	0.83 (s)	7, 8, 9, 14	0.89 (s)	7, 14	0.82 (s)	7, 8, 14	0.72 (s)	7, 9, 14	
27	1.79 (s)	8, 14, 15	1.79 (s)	13, 14, 15	1.79 (s)	13, 14, 15	1.43 (s)	13, 14, 15	
28	3.39 (d, 11.0)	17, 18, 22	3.38 (d, 11.0)		3.36 (d, 11.0)	22	3.46 (d, 10.5)		
	3.62 (d, 11.0)		3.62 (d, 11.0)		3.61 (d, 11.0)		3.57 (d, 10.5)		
29	1.10 (s)	19, 20, 21, 30	1.08 (s)	19, 20, 21, 30	1.11 (s)	19, 20, 21, 30	1.08 (s)	19, 20, 21, 30	
30	1.33 (s)	19, 20, 21, 29	1.33 (s)	19, 20, 21, 29	1.33 (s)	19, 20, 21,29	1.27 (s)	19, 20, 21, 29	
16-0-							(Ac)	17	
21.0	(Ang)		(Ama)		(1 = 2)		2.57 (s)	1	
21-0-	(Ang)		(Ang)		(Ang)		(Hex) 5.08 (dt 11.0	1" 4"	
2							1.5)	1, 7	
3″	5.98 (qq, 7.0, 1.5)	5″	5.97 (qq, 7.0, 2.0)	5″	5.93 (qq, 7.0, 1.5)	4", 5"	6.19 (dt, 11.0, 7.5)		
4″	2.04 (dq, 7.0, 1.5)	2", 3"	2.08 (dq, 7.0, 2.0)	2", 3"	2.05 (dq, 7.0, 1.5)	2", 3"	2.74 (m)	2", 3", 5", 6"	
5″	2.01 (dq, 1.5, 1.5)	1", 2", 3"	2.01 (dq, 2.0, 2.0)	1", 2", 3"	1.99 (dq, 1.5, 1.5)	1", 2", 3"	1.39 (sext, 7.5)	3", 4", 6"	
6″							0.85 (t, 7.5)	4", 5"	
22-0-	(Ang)		(Ang)		(Tig)		(Ac)		
2‴							2.05 (s)	1‴	
3‴	5.92 (qq, 7.0, 1.5)	5‴	5.91 (qq, 7.0, 2.0)	5‴	6.95 (qq, 7.5, 1.5)	1‴			
4‴	2.09 (dq, 7.0, 1.5)	2‴, 3‴	2.04 (dq, 7.0, 2.0)	2‴, 3‴	1.47 (over- lapped)	2‴, 3‴			
5‴	1.90 (dq, 1.5, 1.5)	1‴, 3‴	1.90 (dq, 2.0, 2.0)	1‴, 2‴, 3‴	1.84 (s)	1‴, 2‴, 3‴			
6‴									
28-0-									
2									

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	9	9		10		11	12		
Position	$\delta_{\rm H} \left( J \text{ in Hz} \right)$	HMBC (H to C)	$\delta_{\rm H} \left( J \text{ in Hz} \right)$	HMBC (H to C)	$\delta_{\rm H} \left( J \text{ in Hz} \right)$	HMBC (H to C)	$\delta_{\rm H} \left( J \text{ in Hz} \right)$	HMBC (H to C)	
GlcA									
1	4.85 (d, 7.5)	3	4.82 (d, 7.5)	3	4.84 (d, 7.5)	3	4.82 (d, 7.5)	3	
2	4.50 (over-		4.47 (over-		4.51 (over-		4.51 (over-		
	lapped)		lapped)		lapped)		lapped)		
3	4.30 (over-		4.30 (over-		4.32 (over-		4.33 (over-		
4	lapped)		lapped)		lapped)		lapped)		
4	lapped)		4.42 (Over-		lapped)		lapped)		
5	4.50 (over-		4.44 (over-		4.48 (over-		4.49 (over-		
	lapped)		lapped)		lapped)		lapped)		
Gal									
1	5.63 (d, 7.5)	GlcA-2	5.70 (d, 7.5)	GlcA-2	5.73 (d, 7.5)	GlcA-2	5.73 (d, 7.5)	GlcA-2	
2	4.41 (over-		4.41 (over-		4.44 (over-		4.45 (over-		
2	lapped)		lapped)		lapped)		lapped)		
3	4.32 (over- lanned)		4.27 (over- lanned)		4.29 (over- lanned)		4.30 (over-		
4	4.52 (brs)		4.49 (br s)		4.50 (brs)		4.50 (brs)		
5	4.30 (over-		4.32 (over-		4.35 (over-		4.36 (over-		
	lapped)		lapped)		lapped)		lapped)		
6	4.31 (over-		4.46 (over-		4.47 (over-		4.35 (over-		
	lapped)		lapped)		lapped)		lapped)		
	4.50 (over-						4.50 (over-		
A ro	lapped)						lapped)		
Ala 1	578 (d. 50)	GlcA 3	5 60 (d 6 0)	GloA 3	572 (d. 60)	GleA 3	5.74(d, 5.0)	GlcA 3	
2	4.72 (dd - 5.0)	GICA-5	4 52 (dd 6 0	OICA-5	5.72 (d, 0.0) 4 54 (dd 7 5	OICA-5	4.55 (dd 7.0)	GICA-5	
2	7.0)		8.0)		6.0)		5.0)		
3	4.48 (over-		4.35 (over-		4.36 (dd, 7.5,		4.38 (over-		
	lapped)		lapped)		3.5)		lapped)		
4	4.42 (over-		4.30 (over-		4.31 (over-		4.32 (over-		
-	lapped)		lapped)		lapped)		lapped)		
5	3./4 (dd, 11.5, 2.0)		3.73 (br d, 10.0)		3.75 (dd, 12.5 2 0)	,	3.75 (dd, 11.5,		
	4 36 (over-		4 36 (over-		4 37 (over-		4 38 (over-		
	lapped)		lapped)		lapped)		lapped)		
Xyl									
1			4.99 (d, 7.5)	Ara-2	5.00 (d, 7.5)	Ara-2	5.01 (d, 7.5)	Ara-2	
2			4.09 (dd, 8.5, 7.5)		4.11 (dd, 9.0, 7.5)		4.11 (dd, 8.5, 7.5)		
3			4.00 (dd, 8.5,		4.01 (dd, 9.0,		4.02 (dd, 8.5,		
4			8.5)		9.0) 4.20 (m)		8.5)		
4			4.21 (III) 3.50 (brt		4.20 (III) 3.52 (brt		4.24 (III) 3.52 (brt		
5			11.0)		11.0)		11.0)		
			4.42 (over-		4.42 (over-		4.43 (over-		
			lapped)		lapped)		lapped)		
Gle									
1	5.09 (d, 7.5)	Ara-2							
2	4.09 (dd, 8.0,								
2	7.5) 4.11 (avar								
3	4.11 (over-								
4	4.21 (dd, 10.0,								
	9.0)								
5	3.81 (ddd, 9.0,								
(	8.0, 5.0)								
0	4.28 (over- lanned)								
	4.40 (over-								
	lapped)								

6	2	2
υ	2	4

Carbon	1	2	3	4	5	6	7	8	9	10	11	12
1	38.8	38.8	38.8	38.8	38.8	38.8	38.3	38.2	38.3	38.3	38.2	38.1
2	26.6	26.5	25.5	25.6	25.6	25.5	25.3	25.2	25.2	25.3	25.2	25.3
3	89.5	89.5	83.3	83.0	83.4	83.2	84.4	84.3	84.3	84.4	84.4	84.6
4	40.1	40.0	43.5	43.5	43.5	43.5	55.2	55.1	55.1	55.2	55.2	55.1
5	55.8	55.8	48.4	48.3	48.3	48.2	48.4	48.5	48.5	48.4	48.4	48.5
6	18.2	18.3	18.3	18.2	18.3	18.2	20.4	20.5	20.4	20.4	20.4	20.3
7	33.0	33.1	32.9	32.9	32.9	33.0	32.5	32.5	32.4	32.5	32.4	32.4
8	39.6	39.6	40.2	40.2	40.2	40.2	40.4	40.6	40.8	40.4	40.3	40.3
9	46.2	46.8	47.1	47.0	47.3	47.0	46.9	46.9	46.8	46.8	46.8	46.9
10	36.8	26.8	36.8	36.8	36.8	36.8	36.1	36.0	36.1	36.1	36.1	36.0
11	23.9	23.8	23.9	23.9	24.0	23.9	23.8	23.9	23.8	23.8	23.8	23.7
12	123.2	125.2	122.9	122.9	122.4	123.0	123.0	122.7	123.0	123.0	123.0	124.8
13	141.0	141.0	143.8	142.8	142.8	142.9	143.9	142.8	142.9	142.9	143.9	141.1
14	41.4	41.2	41.8	41.7	41.7	41.7	41.9	41.9	41.7	41.7	41.7	41.4
15	31.0	31.0	35.1	34.8	34.9	34.9	35.0	34.7	34.8	34.8	34.8	31.0
16	71.0	71.5	70.0	68.8	68.8	68.3	69.9	67.7	68.7	68.7	68.4	71.3
17	47.0	46.9	44.9	48.1	48.1	48.3	44.9	47.1	48.1	48.1	48.3	46.7
18	40.2	39.6	41.0	40.2	40.2	40.2	41.0	40.3	40.2	40.2	40.1	39.6
19	47.3	47.3	47.5	47.2	47.1	47.2	47.5	47.4	47.2	47.2	47.2	47.2
20	35.9	36.0	32.1	36.4	36.4	36.4	32.1	36.1	36.4	36.4	36.3	35.9
21	80.1	78.5	41.8	78.8	78.9	78.7	41.9	81.2	78.8	78.7	78.7	78.3
22	70.0	73.4	72.8	73.8	73.8	73.6	72.7	71.2	73.7	73.7	74.1	73.4
23	28.0	28.0	65.0	64.9	65.0	65.0	209.8	209.8	209.9	209.8	209.8	210.1
24	16.8	16.8	13.6	13.6	13.5	13.0	11.1	11.1	11.1	11.1	11.1	11.1
25	15.7	15.6	16.3	16.2	16.3	16.0	15.9	15.9	15.8	15.9	15.8	15.8
26	17.0	16.7	17.0	17.0	17.0	17.0	16.9	17.1	16.9	16.9	16.9	16.8
27	27.1	27.0	27.6	27.6	27.6	27.5	27.6	27.3	27.6	27.6	27.5	26.9
28	65.0	63.8	63.7	63.7	63.7	63.7	63.7	66.8	63.7	63.7	63.6	63.8
29	30.0	29.4	33.5	29.5	29.6	29.5	33.5	29.8	29.6	29.6	29.5	29.4
30	19.9	19.7	25.2	20.3	20.3	20.3	25.3	20.2	20.3	20.3	20.2	19.8
16-0-	(Ac)	(Ac)	20.2	20.5	20.5	20.5	20.0	20.2	20.5	20.5	20.2	(Ac)
10-0-	169.8	169.8										169.8
2'	22.1	22.0										22.0
21.0	(Ang)	$(\Lambda ng)$		(Ang)	(Ang)	(Ang)		(Hev)	(Ang)	(Ang)	(Ang)	(Hev)
1"	(Ang)	(Ang)		(Ang)	(Ang)	(Ang)		167.1	(Ang)	(Ang)	(Ang)	(IICX) 166.6
1 2″	100.2	107.8		107.7	120.0	107.0		107.1	107.7	107.7	107.9	120.2
2 3″	129.1	128.0		129.1	129.0	120.0		140.0	129.1	129.1	129.2	1/0.3
J /"	16.1	156.0		157.0	157.1	16.2		20.0	157.1	157.2	150.5	21.2
4 5″	21.0	21.0		21.0	21.0	21.0		22.6	21.0	21.0	20.0	22.5
S ("	21.0	21.0		21.0	21.0	21.0		12.0	21.0	21.0	20.9	12.0
22.0		$(\Lambda_{\alpha})$	(Haw)	(Ang)	(Ang)	(2) (b)	(Haw)	13.9	(Ang)	(Ang)	(Tia)	13.8
22-0-		(AC)	(Hex)	(Ang)	(Ang)	(2IVID)	(Hex)		(Ang)	(Ang)	(11g)	(AC)
1		1/0.4	100.7	108.5	108.3	1/0./	100.7		108.5	108.5	109.4	170.5
2"		20.9	121.3	129.1	129.2	41./	121.3		129.1	129.1	129.2	20.8
5 ////			149.5	15/.1	15/.2	2/.1	149.5		15/.1	15/.2	13/.0	
4‴ <i>с</i> ‴			31.1	15.8	15.9	11.9	31.2		15.9	15.9	14.0	
5‴ <‴			22.6	20.8	20.8	16.7	22.6		20.8	20.8	12.3	
6‴			13.9				13.9	<i></i>				
28-0-	(Ac)							(Ac)				
1‴″	170.4							170.6				
2‴″	20.7							20.7				

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Table 2. (Continued)

Carbon	1	2	3	4	5	6	7	8	9	10	11	12
GlcA												
1	105.7	105.7	104.1	104.6	103.9	104.1	104.1	104.0	104.0	104.1	104.1	104.3
2	79.2	79.2	78.6	78.8	78.9	78.6	78.3	78.5	78.5	78.2	78.3	78.3
3	84.2	84.3	84.7	85.1	84.0	84.7	84.1	84.5	84.5	84.1	84.2	84.7
4	71.3	71.3	71.0	71.2	71.0	71.0	70.8	70.9	71.0	70.5	70.8	70.8
5	77.2	77.2	77.4	77.3	77.0	77.4	77.3	77.3	77.3	77.5	77.3	77.4
6	172.1	172.2	171.9	172.0	173.0	171.9	172.0	172.0	171.9	172.0	171.9	171.9
Gal												
1	103.8	103.7	103.1	103.5	103.0	103.2	103.3	103.6	103.6	103.3	103.3	103.1
2	73.9	73.9	73.8	73.8	73.8	73.8	73.7	73.8	73.7	73.7	73.7	73.7
3	75.0	75.1	75.3	75.1	75.0	75.3	75.3	75.2	75.2	75.3	75.4	75.4
4	69.9	69.9	70.2	70.0	70.3	70.2	70.6	70.3	70.3	70.8	70.6	70.5
5	76.5	76.5	76.5	76.6	76.4	76.5	76.5	76.6	76.6	76.5	76.5	76.5
6	61.9	61.9	62.0	62.0	62.0	62.0	62.1	62.1	62.2	62.1	62.1	62.2
Ara												
1	101.8	101.8	101.7	101.8	101.8	101.7	101.7	101.8	101.7	101.7	101.7	101.7
2	81.2	81.2	82.3	81.2	82.1	82.3	82.3	81.2	81.3	82.3	82.3	82.3
3	72.5	72.6	73.4	72.4	73.5	73.4	73.4	72.3	72.4	73.4	73.4	73.4
4	67.8	67.7	68.4	67.6	68.4	68.3	68.4	67.8	67.6	68.4	68.4	68.4
5	66.1	65.0	66.1	64.9	66.4	66.0	66.1	64.9	64.9	66.2	66.0	66.1
Xyl												
1			107.0		106.7	107.0	107.0			107.0	107.1	107.1
2			75.9		75.6	75.9	75.9			75.8	75.9	75.9
3			78.3		78.7	78.3	78.2			78.2	78.3	78.3
4			70.8		70.8	70.8	70.8			70.8	70.8	70.8
5			67.5		67.4	67.5	67.5			67.5	67.5	67.5
Glc												
1	105.9	105.9		105.9				106.0	106.0			
2	75.8	75.8		75.8				75.9	75.9			
3	78.4	78.4		78.4				78.5	78.4			
4	71.4	71.5		71.6				71.7	71.6			
5	78.5	78.4		78.4				78.5	78.5			
6	62.6	62.6		62.7				62.8	62.7			

Table 3. Hyaluronidase Inhibitory Activities ( $IC_{50}$ ) of Isolated Compounds (1–22)

Compound	IC <sub>50</sub> (µм)
1	55.2
2	36.4
3	26.6
4	30.2
5	20.0
6	24.2
7	28.8
8	48.7
9	44.9
10	36.8
11	40.5
12	28.5
13	24.9
14	52.0
15	26.4
16	24.1
17	54.9
18	29.7
19	38.7
20	51.5
21	19.3
22	55.6
Rosmarinic acid	240.1

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