Hyaluronidase Inhibitors from *Keiskea japonica*

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An extract of *Keiskea japonica* Miq. showed an inhibitory effect on hyaluronidase activity. From the extract, four new phenylpropanoids, two new maltol glycosides, two new monoterpene glycosides, and two new phenolic compounds were isolated together with 19 known compounds. Among these constituents, two phenylpropanoids and a flavone glucuronide were revealed as hyaluronidase inhibitors.

Key words *Keiskea japonica*; Lamiaceae; hyaluronidase inhibitor; phenylpropanoid

Keiskea japonica, a herbaceous perennial with white flowers that belongs to the family Lamiaceae, grows in the mountainous areas of western Japan.[1)] As the ice that attaches to old stems of *Keiskea japonica* Miq. can produce artistic formations, the plant is named "Shimobashira" in Japanese.

We have searched for hyaluronidase inhibitory extracts of Lamiaceae plants and reported phenylpropanoids and flavone glucuronides.[2—4)] An extract of *K. japonica* also showed inhibitory activity (IC_{50} 608 μg/mL) and 29 compounds (**1**—**29**) were isolated (Figs. 1, 2) from an 80% acetone extract of the plant. Known compounds were identified from spectroscopic data as 3′-*O*-methyl-rosmarinic acid (**14**),[5)] isovitexin (**19**),[6)] vicenin-2 (**20**),[7)] maltol 6′-*O*-(5-*O*-*p*-coumaroyl)-(1→6)-*β*-D-apiofranosyl-*β*-D-glucopyranoside (**21**),[8)] (*Z*)-3-hexenyl *β*-D-glucopyranoside (**23**),[9)] perilloside E (**25**),[10)] benzyl *β*-D-glucopyranoside (**26**),[11)] (6*S*,9*S*)-roseoside (**27**),[12)] (−)-(1*R*,2*R*)-5′-*β*-D-glucopyranosyloxyjasmonic acid (**28**),[13)] and (3*R*)-*O*-*β*-D-glucopyranosyloxy-5-phenylvaleric acid (**29**).[14)] Other known compounds were directly compared with those isolated previously and identified as caffeic acid (**11**),[15)] clinopodic acid A (**12**),[16)] rosmarinic acid (**13**),[16)] luteolin (**15**),[15)] chrysoeriol (**16**),[15)] apigenin 7-*O*-*β*-D-glucuronopyranoside (**17**),[3)] acacenin

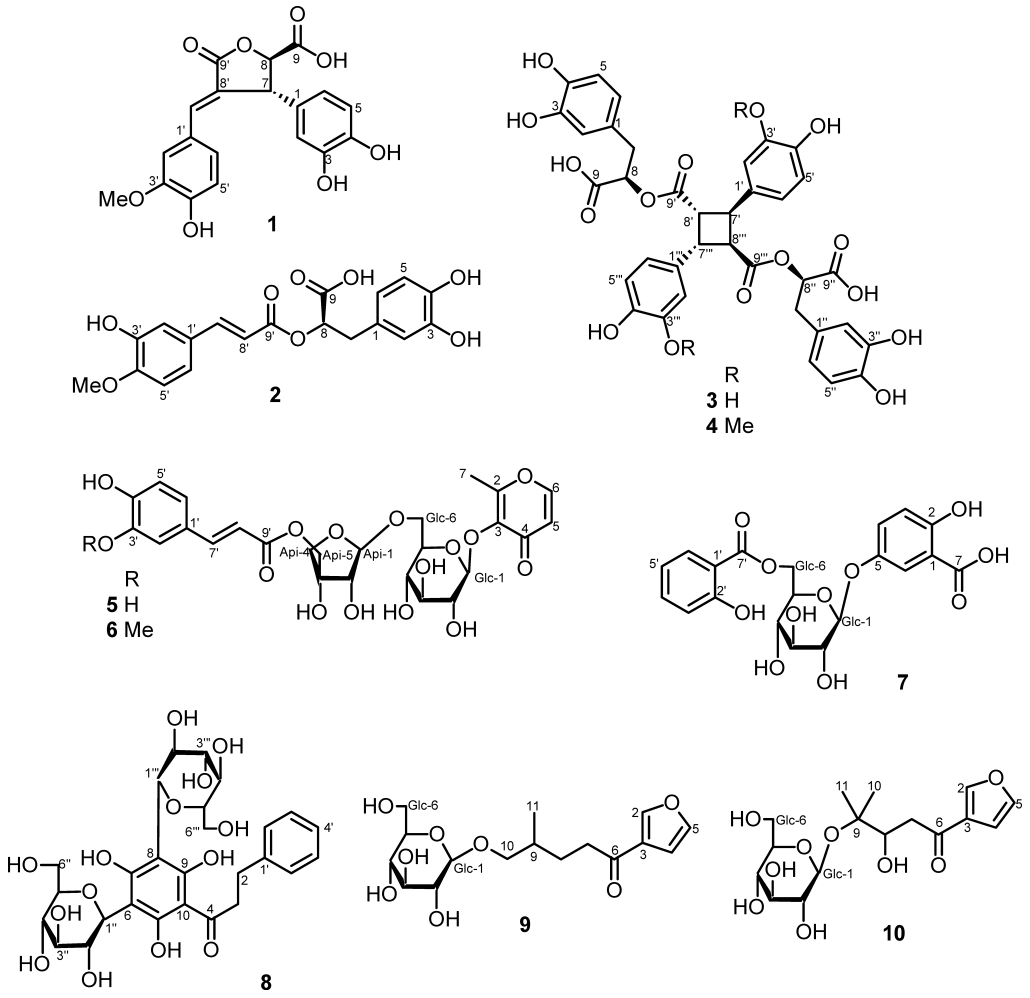

Fig. 1. Structures of **1**—**10**

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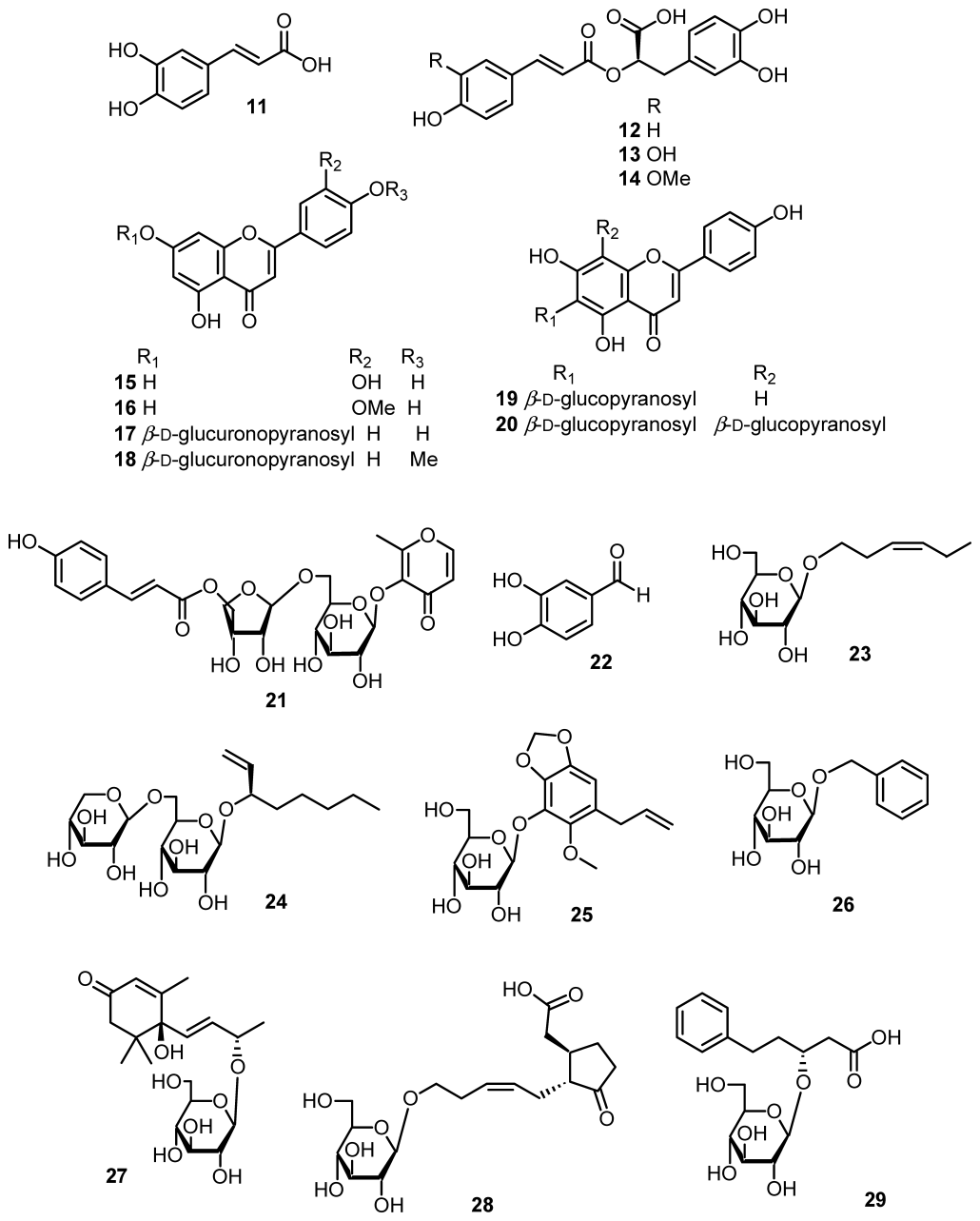

Fig. 2. Structures of **11**—**29**

7-*O*-*β*-D-glucuronopyranoside (**18**),[2] protocatechualdehyde (**22**),[15] and 3-*O*-[*β*-D-xylopyranosyl-(1-6)-*β*-D-glucopyranosyl]-(3*R*)-1-octen-3-ol (**24**).[17]

Compounds **1** and **3**—**10** were new (Fig. 1), and **2** was isolated as a natural product for the first time. **1**—**4** and **11**—**14** were phenylpropanoids and **17** and **18** were flavone glucuronides (Fig. 2).

In the ^{1}H-NMR spectrum of shimobashiric acid A (**1**), two sets of ABX proton signals at (δ 6.77, d, J=2.0 Hz; 6.77, d, J=8.5 Hz; 6.67, dd, J=8.5, 2.0 Hz) and (δ 6.89, d, J=2.0 Hz; 6.75, d, J=8.5 Hz; 6.99, dd, J=8.5, 2.0 Hz), and an olefinic proton signal at δ 7.68 (d, J=2.0 Hz) were observed in the aromatic region. In the aliphatic region, there were two proton signals at δ 4.55 (br s) and 4.78 (overlapped) and a methoxy proton signal at δ 3.56 (3H, s). The methoxy proton signal correlated with H-2′ (δ 6.89) in the nuclear Overhauser effect (NOE) spectrum, and showed the presence of a methoxy group at C-3′. The ^{13}C-NMR spectrum showed 19 carbon signals, and high resolution (HR)-FAB-MS [*m*/*z* 373.0928 (Calcd for $C_{19}H_{17}O_8$: 373.0923)] indicated the molecular formula of **1** to be $C_{19}H_{16}O_8$. The H-7 proton signal (δ 4.55, br s) was long range coupled with aromatic carbons at δ 133.7 (C-1), 115.1 (C-2), and 119.4 (C-6) and an olefinic carbon at δ 122.5 (C-8′) and two carbonyl carbons at δ 173.7 (C-9) and 174.5 (C-9′) in the heteronuclear multiple bond correlation (HMBC) spectrum (Fig. 3). The olefinic proton signal at δ 7.68 (H-7′) was long range coupled with aromatic carbons at δ 126.9 (C-1′), 114.3 (C-2′), and 128.0 (C-6′) and an aliphatic carbon at δ 49.5 (C-7). In the ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum, H-7 correlated with δ 4.78 (H-8) corresponding to an oxygenated carbon at δ 83.6 (C-8). H-8 was long range coupled with two carboxyl carbons at δ 173.7 and 174.5, which suggested that **1**

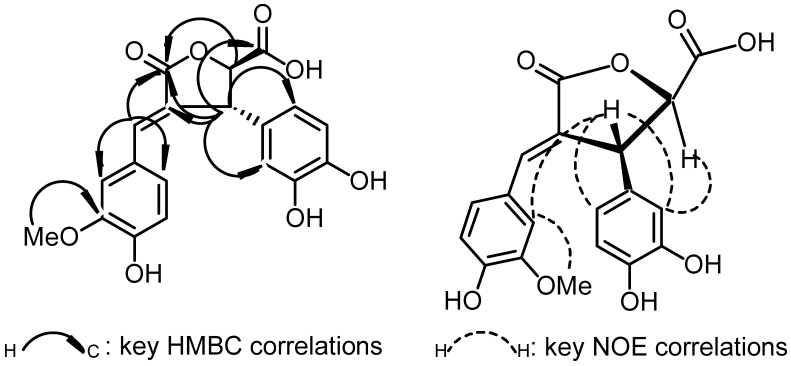

Fig. 3. Key HMBC and NOE Correlations for **1**

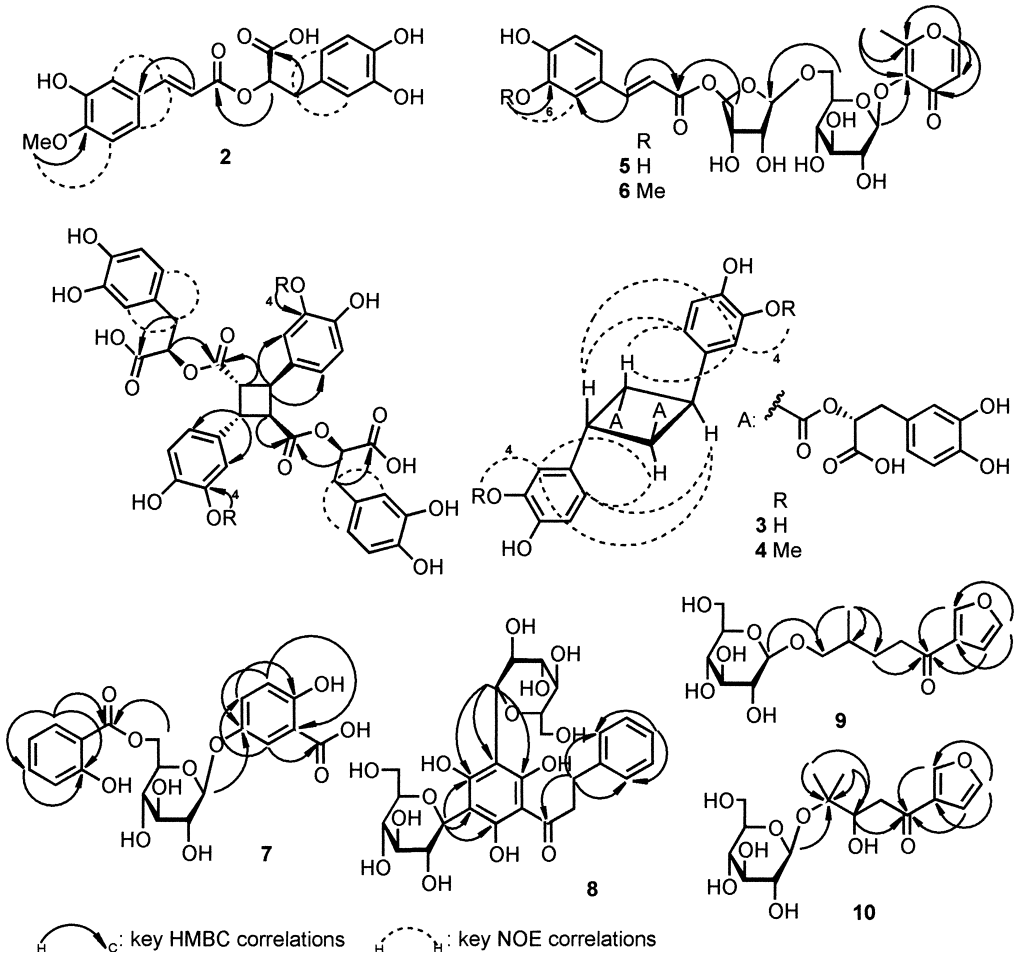

Fig. 4. Key HMBC and NOE Correlations for **2**—**10**

has a five-member ring as shown in Fig. 1. This planar structure represents a state in which C-7 is linked with C-8′ in **14**. In the NOE spectra (Fig. 3), H-7 was correlated with H-2′ and H-6′ showing that C-7′ and C-8′ had an *E*-configuration. The relative configurations of C-7 and C-8 were determined as *rel*-(7*R*,8*R*) based on the NOE correlation between H-8 and H-2 (Fig. 3). For the phenylglycine methyl ester (PGME) method to determine absolute configurations of *α*-oxy-*α*-monosubstituted acetic acid including a *γ*-lactone, (*S*)- and (*R*)-PGME amides of **1** (**1a**, **b**) were synthesized. The difference in ^{1}H-NMR chemical shifts between **1a** and **b** [$\Delta\delta=\delta(S)-\delta(R)$] suggested C-8 to have an *S*-configuration.[18] However, this result conflicted with 8*R*-configurations in rosmarinic acid derivatives. Examples of known absolute configuration about the PGME method for *α*-oxy-*α*-monosubstituted acetic acids have been too small to be conclusive. Hence, absolute configurations of **1** were not determined.

^{1}H- and ^{13}C-NMR spectra of shimobashiric acid B (**2**) are shown in the experimental section. They were similar to those of rosmarinic acid (**13**) except for the existence of a methoxy signal. HR-FAB-MS suggested that the molecular formula of **2** was $C_{19}H_{18}O_8$. The methoxy proton signal at δ 3.90 (3H, s) correlated with an aromatic proton at δ 7.00 (1H, d, J=8.5 Hz, H-5′) in the NOE spectrum (Fig. 4). These data showed that **2**

was 4′-methoxy rosmarinic acid. The absolute configurations of C-8 was determined to be *R* from the retention time of the PGME amide derivative of 3-(3,4-dihydroxyphenyl)-2-hydroxypropanoic acid, which was obtained by acidic hydrolysis of **2,** in (*S*)-2-phenylglycine methyl ester (see Experimental).[2)]

The molecular formula of shimobashiric acid C (**3**) was established as $C_{36}H_{32}O_{16}$ based on HR-FAB-MS [*m/z* 743.1597 (Calcd for $C_{36}H_{32}O_{16}Na$: 743.1587)]. In the ^{1}H-NMR spectrum, four sets of ABX aromatic protons at δ 6.54 (1H, d, *J*=2.0 Hz, H-2), 6.62 (1H, d, *J*=8.0 Hz, H-5), 6.34 (1H, dd, *J*=8.0, 2.0 Hz, H-6), 6.64 (1H, d, *J*=2.0 Hz, H-2′), 6.57 (1H, d, *J*=8.0 Hz, H-5′), 6.31 (1H, dd, *J*=8.0, 2.0 Hz, H-6′), 6.60 (1H, d, *J*=2.0 Hz, H-2″), 6.65 (1H, d, *J*=8.0 Hz, H-5″), 6.43 (1H, dd, *J*=8.0, 2.0 Hz, H-6″), 6.72 (1H, d, *J*=2.0 Hz, H-2‴), 6.63 (1H, d, *J*=8.0 Hz, H-5‴), 6.41 (1H, dd, *J*=8.0, 2.0 Hz, H-6‴) were observed. The H-2 and H-6 protons were correlated with methylene and methine protons at δ 2.50 (overlapped, H-7), 2.58 (1H, dd, *J*=14.0, 6.0 Hz, H-7), and 4.43 (1H, t, *J*=6.0 Hz, H-8) in the NOE spectra (Fig. 4). The H-2″ and H-6″ protons were also correlated with another methylene and methine protons at δ 2.71 (1H, dd, *J*=14.0, 7.0 Hz, H-7″), 2.76 (1H, dd, *J*=14.0, 6.0 Hz, H-7″), and 4.58 (1H, t, *J*=6.0 Hz, H-8″) in the NOE spectra. The ^{13}C-NMR spectrum showed that the presence of four carbonyl carbons at δ 170.2, 170.2, 171.1, and 170.0, eight aliphatic carbons, and 24 aromatic carbons. These results suggested that **3** has four phenylpropanoid units including two 3-(3,4-dihydroxyphenyl)-2-hydroxypropanoic acid moieties. Although the ^{1}H- and ^{13}C-NMR data (in D_2O) of **3** were similar to those of sagerinic acid,[19)] they were not identical. The ^{1}H^{1}H COSY spectrum showed that four methine protons at δ 4.05 (1H, dd, *J*=10.5, 7.0 Hz, H-7′), 3.76 (1H, dd, *J*=10.5, 7.0 Hz, H-8′), 4.13 (1H, dd, *J*=10.5, 7.0 Hz, H-7‴), and 3.67 (1H, dd, *J*=10.5, 7.0 Hz, H-8‴) construct a cyclobutane ring. In the HMBC spectrum (Fig. 4), H-2′ and 6′ were correlated with C-7′ (δ 39.7), and H-2‴ and H-6‴ were correlated with C-7‴ (δ 40.8). These results showed that **3** was a dimer of two rosmarinic acid moieties as shown in Fig. 1. In the NOE spectra, the H-2′ and -6′ protons were correlated with H-8′ and 7‴; H-2‴ and -6‴ protons were correlated with H-7′ and 8‴, which suggested the conformation revealed in Fig. 1.[19)] The conformation of cyclobutane ring was same as that of α-truxillic acid,[20)] and the two sets of ^{1}H-, ^{13}C-NMR chemical shift values for "1—9 and 1′—9′" and "1″—9″ and 1‴—9‴" are interchangeable. The absolute configuration of both C-8 and C-8″ was determined to be *R* as in the case of **2**.

The ^{1}H- and ^{13}C-NMR spectra of shimobashiric acid D (**4**) were similar to those of **3** (in CD_3OD). HR-FAB-MS [*m/z* 771.1899 (Calcd for $C_{38}H_{36}O_{16}Na$: 771.1900)] showed that the molecular formula of **4** was $C_{38}H_{36}O_{16}$, which was C_2H_4 more than that of **3**, indicating the presence of two methoxy groups. Two methoxy proton signals δ 3.83 (3H, s) and 3.84 (3H, s) were correlated with the aromatic carbons at δ 148.8 and 148.9 in the HMBC spectrum, respectively. In the NOE spectra (Fig. 4), the methoxy protons were correlated with δ 6.80 (1H, d, *J*=2.0 Hz. H-2′) and 6.91 (1H, d, *J*=2.0 Hz, H-2‴), respectively, suggesting 3-methoxy-4-hydroxyphenyl moieties. Additionally the H-2′ and -6′ protons were correlated with H-8′ and 7‴; H-2‴ and -6‴ protons were correlated with H-7′ and 8‴, which suggested the conformation of cyclobutane ring was same as that of α-truxillic acid.[20)] Hence, the structure of **4** was determined to be that shown in Fig. 1. The absolute configurations of both C-8 and C-8″ were determined as *R*, as in the case of **2**.

The ^{1}H- and ^{13}C-NMR spectra of shimobashirasides A (**5**) and B (**6**) were almost superimposable onto those of **21**,[8)] except for the aromatic region. For **5**, ABX aromatic proton signals at δ 7.04 (1H, d, *J*=1.5 Hz, H-2′), 6.78 (1H, d, *J*=8.0 Hz, H-5′), and 6.95 (1H, dd, *J*=8.0, 1.5 Hz, H-6′) suggested a caffeoyl group, instead of the *p*-coumaroyl group in **21**. HR-FAB-MS [*m/z* 583.1646 (Calcd for $C_{26}H_{31}O_{15}$: 583.1662)] supported this conclusion. For **6**, there were ABX system aromatic proton signals at δ 7.20 (1H, d, *J*=2.0 Hz, H-2′), 6.82 (1H, d, *J*=8.0 Hz, H-5′), and 7.08 (1H, dd, *J*=8.0, 2.0 Hz, H-6′) and a methoxy proton at δ 3.90 (3H, s), which correlated with the H-2′ proton in the NOE spectrum (Fig. 4). These data suggested that **6** has a feruloyl group. Again HR-FAB-MS [*m/z* 597.1804 (Calcd for $C_{27}H_{33}O_{15}$: 597.1819)], supported this conclusion. Sugar identifications suggested that both **5** and **6** have D-glucose and D-apiose.[8,21)] The coupling constant of Glc-1 [**6**: δ 4.77, d, *J*=7.5 Hz (Glc-1)] and chemical shifts of ^{13}C-NMR [**5**: δ 105.4, 75.0, 78.5, 71.4, 77.5 (Glc-1-5) and δ 110.6 (Api-1); **6** δ 110.6 (Api-1)] showed that the anomeric carbons of these sugars have β-configurations.[8,22)]

Shimobashiraside C (**7**) was revealed to have the molecular formula $C_{20}H_{20}O_{11}$ based on HR-FAB-MS [*m/z* 437.1090 (Calcd for $C_{20}H_{21}O_{11}$: 437.1083)]. In the ^{1}H-NMR spectrum, ABX system proton signals at δ 6.72 (1H, d, *J*=9.0 Hz, H-3), 7.19 (1H, dd, *J*=9.0, 3.0 Hz, H-2), and 7.41 (1H, d, *J*=3.0 Hz, H-6) and ABCD system proton signals at δ 6.95 (overlapped, H-3′), 7.54 (1H, m, H-4′), 6.97 (overlapped, H-5′), and 7.77 (1H, dd, *J*=7.5, 2.0 Hz, H-6′) were observed in the aromatic region. In the HMBC spectrum (Fig. 4), H-4 was long range coupled with an aromatic carbon at C-2 (δ 156.4), H-6 was coupled with the carbonyl carbon at δ 171.3, and an anomeric proton at δ 4.84 (1H, d, *J*=7.5 Hz, H-Glc-1) was coupled with C-5 (δ 149.1). These data showed that an aglycone moiety of **7** was a gentisic acid, which was similar to the gentisic acid 5-*O*-β-D-glucopyranoside.[23)] ^{1}H–^{1}H COSY and ^{13}C-NMR spectra showed a sugar moiety that was a 6-acylated glucose. The sugar analysis[21)] and the coupling constant of H-Glc-1 suggested that the glucose was β-D-glucopyranose. H-Glc-6 protons at δ 4.33 (1H, dd, *J*=11.5, 7.5 Hz) and 4.61 (1H, dd, *J*=11.5, 2.0 Hz) were shifted downfield relative to the H-6 in 5-*O*-β-D-glucopyranoside.[23)] The H-6 protons were long range coupled with a carbonyl carbon at δ 168.6 (C-7′) in the HMBC spectrum. The ABCD spin system protons and an oxygenated aromatic carbon at δ 160.2 suggested that the acyl moiety of **7** was a salicylic acid. Hence, the structure of **7** was identified as shown in Fig. 1.

^{1}H- and ^{13}C-NMR spectra of shimobashiraside D (**8**) were similar to those of phlorein 6,8-bis-*C*-β-D-glucopyranosides[7)] except for signals of the B-ring in the dihydrochalcone moiety. A spin system at δ 7.23 (4H, m, H-2′,3′,5′,6′) and 7.14 (1H, m, H-4′) suggested that **8** has a phenyl moiety instead of the *p*-hydroxyphenyl moiety in 6,8-bis-*C*-β-D-glucopyranosides. HR-FAB-MS [*m/z* 583.2032 (Calcd for $C_{27}H_{35}O_{14}$: 583.2027)], supported the conclusion.

The molecular formula of shimobashiraside E (**9**) was determined as $C_{16}H_{25}O_8$ based on HR-FAB-MS [*m/z* 345.1548 (Calcd for $C_{16}H_{25}O_8$: 345.1550)]. In the ^{1}H- and ^{13}C-NMR spectra, coupling patterns of aromatic protons of the furan ring signal at δ 8.36 (1H, br s, H-2), 6.77 (1H, d, *J*=1.0 Hz,

H-4), and 7.57 (1H, dd, J=1.5, 1.0 Hz, H-5), aromatic carbon signals at δ 150.0 (C-2, having a corresponding proton at δ 8.36), 128.8 (C-3), 109.3 (C-4), and 145.8 (C-5, having a corresponding proton at δ 7.57), and carbonyl carbon signal at δ 198.3 (C-6) suggested that **9** was a perillaketone type monoterpene as shown in Fig. 1.[24] An anomeric proton at δ 4.25 (1H, d, J=8.0 Hz, H-Glc-1), and the ^{1}H–^{1}H COSY spectrum showed a glucose moiety. The sugar analysis[21] and the coupling constant of H-Glc-1 suggested that the glucose was β-D-glucopyranose. The anomeric proton was long range coupled with an oxygenated carbon at δ 75.5 (C-10). These results suggested that **9** was a glucoside of perillaketone and had the structure shown in Fig. 1. The absolute stereochemistry of C-9 is unclear.

The molecular formula of shimobashiraside F (**10**) was determined as $C_{16}H_{25}O_9$ based on HR-FAB-MS [m/z 361.1504 (Calcd for $C_{16}H_{25}O_9$: 361.1499)]. In the ^{1}H- and ^{13}C-NMR spectra, signals of a furan ring, carbonyl carbon, and glucose moiety in **10** were almost superimposable onto those of **9**. Compound **10** has a hydroxyl moiety at C-8 revealed by an oxygenated proton signal at δ 4.16 (1H, dd, J=9.0, 3.0 Hz, H-8) and the ^{1}H–^{1}H COSY spectrum. The anomeric proton at δ 4.54 (1H, d, J=8.0 Hz, H-Glc-1) was long range coupled with the quaternary carbon signal at δ 80.8 (C-9). From these data, the structure of **10** was determined as shown in Fig. 1. The absolute stereochemistry of C-8 is still unknown.

The hyaluronidase inhibitory activity measured for compounds **3**, **9**—**18**, and **20**—**29** is shown in Table 1. Phenylpropanoids and flavone glucuronides showed similar activities (IC_{50} **3**: 594 μM, **14**: 737 μM, **18**: 267 μM) to inhibitors in previous reports.[2—4] All these active compounds in Lamiaceae plants have carboxylic acid in phenylpropanoid oligomers or glucuronic acid in flavonoid glycoside. Carboxylic acids in limited moieties are suggested to be a key functional group for hyaluronidase inhibitory activity, and the level of activity seems to depend on the number of carboxylic acids in the structure and structural features around the acids.

Experimental

General Procedures Optical rotations were recorded on a Jasco P-2300 polarimeter. Circular dichroism (CD) spectra were recorded on a Jasco J-700 spectropolarimeter; and UV, on a Shimadzu MPS-2450. ^{1}H-NMR (400 MHz), ^{13}C-NMR (100 MHz), ^{1}H–^{1}H COSY, heteronuclear multiple quantum correlation (HMQC) (optimized for $^1J_{C-H}$=145 Hz) and HMBC (optimized for $^nJ_{C-H}$=8 Hz) spectra were recorded on a Jeol JNM-AL400 FT-NMR spectrometer, and chemical shifts were given as δ values with TMS as an internal standard. HR-FAB- and HR-electron ionization (EI)-MS data were obtained on a Jeol JMS700 mass spectrometer, using a m-nitrobenzyl alcohol or a glycerol matrix. A porous polymer gel (Mitsubishi Chemical, Diaion HP-20, 60×300 mm) and octadecyl silica (ODS) (Cosmosil 140 C_{18}-OPN, Nacalai Tesque, 150 g) were used for column chromatography. Preparative HPLC was performed on a Jasco 2089 and detected with UV at 320 or 210 nm (columns, TSKgel ODS-80Ts, 55×600 mm×3; Cosmosil AR-II, Nacalai tesque, 20×250 mm; Cosmosil 5PE-MS, Nacalai Tesque, 20×250 mm; Mightisil RP-18 GP, Kanto Chemical, 10×250 mm).

Plant Material *K. japonica* was collected in July 2009 in Shizuoka, Japan. The plant was identified by Prof. Akira Ueno, School of Pharmaceutical Sciences, University of Shizuoka. A voucher specimen has been deposited in the herbarium of Tohoku Pharmaceutical University, No.20090701.

Extraction and Isolation Powdered aerial parts of *K. japonica* (300 g) were extracted with acetone–water (8 : 2) at room temperature for two weeks (6 L). The extract was concentrated at reduced pressure (22.0 g), suspended in water (1.5 L) and subjected to extraction with diethyl ether (1.0 L) three times. The aqueous layer extract (11.8 g) was dissolved in water and passed through a porous polymer gel (Mitsubishi Diaion HP-20, 70×180 mm) eluted with EtOH–water (95 : 5) after being washed with water (10 L). The 95% EtOH fraction (4.5 g) was subjected to HPLC [TSKgel ODS-80Ts; mobile phase, acetonitrile–0.1% trifluoroacetic acid (TFA) (15 : 85)→(40 : 60)], to give 44 fractions. Each fraction was subjected to HPLC [columns; AR-II, mobile phases 15%, 17.5%, 20%, 25%, 27.5%, and 30% acetonitrile in 0.2% trifluoroacetic acid (TFA); 5PE-MS, mobile phases 12.5%, 22.5%, 25%, and 30% acetonitrile in 0.2% TFA; AR-II, mobile phases 12.5%, 17.5%, 27.5%, and 30% acetonitrile in 0.2% TFA and 30% MeOH in 0.2% TFA] to yields compounds **1** (1.7 mg), **2** (2.4 mg), **3** (9.6 mg), **4** (1.3 mg), **5** (1.1 mg), **6** (3.1 mg), **7** (0.8 mg), **8** (4.2 mg), **9** (39.8 mg), **10** (3.4 mg), **11** (43.4 mg), **12** (3.2 mg), **13** (639.1 mg), **14** (27.1 mg), **15** (57.5 mg), **16** (195.2 mg), **17** (287.8 mg), **18** (125.7 mg), **19** (2.9 mg), **20** (49.7 mg), **21** (6.5 mg), **22** (3.6 mg), **23** (4.6 mg), **24** (2.1 mg), **25** (6.0 mg), **26** (2.3 mg), **27** (1.3 mg), **28** (6.3 mg), **29** (6.9 mg).

Shimobashiric acid A (**1**): Colorless amorphous solid, $[\alpha]_D^{22}$ +205.0° (c=0.16, MeOH), UV (MeOH) λ_{max} (logε): 201 (5.22), 293 (4.21), 333 (4.37). CD (c=0.016, MeOH) nm ([θ]): 205 (−15600), 234 (−6500), 250 (5200), 301 (8300), 332 (9700). HR-FAB-MS (positive): m/z 373.0928 $[M+H]^+$ (Calcd for $C_{19}H_{17}O_8$: 373.0923). ^{1}H-NMR: (CD_3OD, 400 MHz), δ: 6.77 (1H, d, J=2.0 Hz, H-2), 6.77 (1H, d, J=8.5 Hz, H-5), 6.67 (1H, dd, J=8.5, 2.0 Hz, H-6), 4.55 (1H, brs, H-7), 4.78 (overlapped, H-8), 6.89 (1H, d, J=2.0 Hz, H-2′), 6.75 (1H, d, J=8.5 Hz, H-5′), 6.99 (1H, dd, J=8.5, 2.0 Hz, H-6′), 7.68 (1H, d, J=2.0 Hz, H-7′), 3.56 (3H, s, H-OMe). ^{13}C-NMR: (CD_3OD, 100 MHz), δ: 133.7 (C-1), 115.1 (C-2), 147.4 (C-3), 146.3 (C-4), 116.4 (C-5), 119.4 (C-6), 49.5 (C-7), 83.6 (C-8), 173.7 (C-9), 126.9 (C-1′), 114.3 (C-2′), 149.1 (C-3′), 150.7 (C-4′), 117.2 (C-5′), 128.0 (C-6′), 141.7 (C-7′), 122.5 (C-8′), 174.5 (C-9′), 56.4 (C-OMe).

Shimobashiric acid B (**2**): Colorless amorphous solid, $[\alpha]_D^{21}$ +47.0° (c=0.2, MeOH), UV (MeOH) λ_{max} (logε): 204 (4.60), 288 (4.13), 324 (4.09). CD (c=0.020, MeOH) nm ([θ]): 247 (9600), 297 (6400). HR-FAB-MS (positive): m/z 375.1082 $[M+H]^+$ (Calcd for $C_{19}H_{19}O_8$: 375.1080). ^{1}H-NMR: (acetone-d_6, 400 MHz), δ: 6.86 (1H, d, J=2.0 Hz, H-2), 6.75 (1H, d, J=8.0 Hz, H-5), 6.69 (1H, dd, J=8.0, 2.0 Hz, H-6), 3.04 (1H, dd, J=14.5, 8.5 Hz, H-7), 3.13 (1H, dd, J=14.5, 4.5 Hz, H-7), 5.23 (1H, dd, J=8.5, 4.5 Hz, H-8), 7.18 (1H, d, J=2.0 Hz, H-2′), 7.00 (1H, d, J=8.5 Hz, H-5′), 7.13 (1H, dd, J=8.5, 2.0 Hz, H-6′), 7.59 (1H, d, J=16.0 Hz, H-7′), 6.37 (1H, d, J=16.0 Hz, H-8′), 3.90 (3H, s, H-OMe). ^{13}C-NMR: (acetone-d_6, 100 MHz), δ: 129.2 (C-1), 117.3 (C-2), 145.6 (C-3), 144.7 (C-4), 115.9 (C-5), 121.7 (C-6), 37.5 (C-7), 73.7 (C-8), 171.0 (C-9), 128.5 (C-1′), 114.6 (C-2′), 147.7 (C-3′), 150.8 (C-4′), 112.4 (C-5′), 122.5 (C-6′), 146.3 (C-7′), 115.8 (C-8′), 166.7 (C-9′), 56.3 (C-OMe).

Shimobashiric acid C (**3**): Colorless amorphous solid, $[\alpha]_D^{22}$ −4.1° (c=1.21, MeOH), UV (MeOH) λ_{max} (logε): 204 (4.92),

284 (4.05). CD (c=0.012, MeOH) nm ([θ]): 252 (11900). HR-FAB-MS (positive): m/z 743.1597 $[M+Na]^+$ (Calcd for $C_{36}H_{32}O_{17}Na$: 743.1587). ^{1}H-NMR: (DMSO-d_6, 400 MHz), δ: 6.54 (1H, d, J=2.0 Hz, H-2), 6.62 (1H, d, J=8.0 Hz, H-5), 6.34 (1H, dd, J=8.0, 2.0 Hz, H-6), 2.50 (overlapped, H-7), 2.58 (1H, dd, J=14.0, 6.0 Hz, H-7), 4.43 (1H, t, J=6.0 Hz, H-8), 6.64 (1H, d, J=2.0 Hz, H-2′), 6.57 (1H, d, J=8.0 Hz, H-5′), 6.31 (1H, dd, J=8.0, 2.0 Hz, H-6′), 4.05 (1H, dd, J=10.5, 7.0 Hz, H-7′), 3.76 (1H, dd, J=10.5, 7.0 Hz, H-8′), 6.60 (1H, d, J=2.0 Hz, H-2″), 6.65 (1H, d, J=8.0 Hz, H-5″), 6.43 (1H, dd, J=8.0, 2.0 Hz, H-6″), 2.71 (1H, dd, J=14.0, 7.0 Hz, H-7″), 2.76 (1H, dd, J=14.0, 6.0 Hz, H-7″), 4.58 (1H, dd, J=7.0, 6.0 Hz, H-8″), 6.72 (1H, d, J=2.0 Hz, H-2‴), 6.63 (1H, d, J=8.0 Hz, H-5‴), 6.41 (1H, dd, J=8.0, 2.0 Hz, H-6‴), 4.13 (1H, dd, J=10.5, 7.0 Hz, H-7‴), 3.67 (1H, dd, J=10.5, 7.0 Hz, H-8‴). ^{13}C-NMR: (DMSO-d_6, 100 MHz), δ: 116.4 (C-1), 119.9 (C-2), 145.0[b] (C-3), 144.2[a] (C-4), 115.2 (C-5), 120.3 (C-6), 35.9 (C-7), 73.1 (C-8), 170.2 (C-9), 129.2 (C-1′), 115.4 (C-2′), 144.7[b] (C-3′), 144.0[a] (C-4′), 115.4[c] (C-5′), 117.8[c] (C-6′), 39.7 (C-7′), 46.2 (C-8′), 171.1 (C-9′), 126.8 (C-1″), 119.6 (C-2″), 145.0[b] (C-3″), 144.1[a] (C-4″), 115.4[c] (C-5″), 120.3 (C-6″), 36.2 (C-7″), 73.2 (C-8″), 170.0 (C-9″), 129.3 (C-1‴), 114.7 (C-2‴), 144.8[b] (C-3‴), 144.0 [a] (C-4‴), 115.4 (C-5‴), 118.5 (C-6‴), 40.8 (C-7‴), 46.5 (C-8‴), 170.2 (C-9‴). [a,b,c]: Assignments are interchangeable. ^{1}H-NMR: (CD_3OD, 400 MHz), δ: 6.78 (1H, d, J=2.0 Hz), 6.70 (1H, d, J=8.0 Hz), 6.68 (1H, d, J=8.0 Hz), 6.67 (1H, d, J=8.0 Hz), 6.66 (1H, d, J=2.0 Hz), 6.66 (1H, d, J=2.0 Hz), 6.64 (1H, d, J=8.0 Hz), 6.63 (1H, d, J=2.0 Hz), 6.53 (1H, dd, J=8.0, 2.0 Hz), 6.48 (1H, dd, J=8.0, 2.0 Hz), 6.45 (1H, dd, J=8.0, 2.0 Hz), 6.35 (1H, dd, J=8.0, 2.0 Hz), 4.73 (1H, dd, J=7.5, 5.0 Hz), 4.59 (1H, dd, J=6.0, 6.0 Hz), 4.21 (2H, br dd, J=10.5, 7.0 Hz), 3.91 (1H, dd, J=10.5, 7.0 Hz), 3.75 (1H, dd, J=10.5, 7.0 Hz). 2.86 (1H, dd, J=14.0, 5.0 Hz), 2.81 (1H, dd, J=14.0, 7.5 Hz), 2.71 (1H, dd, J=14.0, 6.0 Hz), 2.61 (1H, dd, J=14.0, 6.0 Hz), ^{13}C-NMR: (CD_3OD, 100 MHz), δ: 173.4, 173.2, 172.8, 172.7, 146.2, 146.1, 146.0, 145.9, 145.5, 145.3, 145.2, 145.2, 131.7, 131.6, 128.9, 128.6, 122.2, 122.2, 120.5, 119.5, 117.8, 117.6, 116.5, 116.4, 116.4, 116.3, 116.0, 115.6, 74.9, 74.9, 48.7, 48.0, 42.8, 42.1, 37.9, 37.6. ^{1}H-NMR: (D_2O, 400 MHz), δ: 6.68 (1H, d, J=2.0 Hz), 6.66 (1H, d, J=8.0 Hz), 6.64 (1H, d, J=8.0 Hz), 6.63 (1H, d, J=8.0 Hz), 6.62 (1H, d, J=2.0 Hz), 6.59 (1H, d, J=8.0 Hz), 6.56 (1H, d, J=2.0 Hz), 6.55 (1H, d, J=2.0 Hz), 6.47 (1H, dd, J=8.0, 2.0 Hz), 6.37 (1H, dd, J=8.0, 2.0 Hz), 6.35 (1H, dd, J=8.0, 2.0 Hz), 6.09 (1H, dd, J=8.0, 2.0 Hz), 4.59 (1H, dd, J=8.5, 4.5 Hz), 4.52 (1H, dd, J=7.0, 5.0 Hz), 4.05 (2H, m), 3.74 (1H, dd, J=10.5, 7.5 Hz), 3.65 (1H, dd, J=10.5, 6.0 Hz). 2.80 (1H, dd, J=14.5, 4.5 Hz), 2.68 (1H, dd, J=14.5, 8.5 Hz), 2.62 (1H, dd, J=14.5, 7.5 Hz), 2.57 (1H, dd, J=14.5, 5.5 Hz), ^{13}C-NMR: (D_2O, 100 MHz), δ: 174.0, 173.9, 173.7, 173.5, 144.6, 144.5, 144.4, 144.4, 143.8, 143.7, 143.6, 143.6, 131.4, 131.2, 129.1, 128.8, 122.7, 122.5, 120.6, 119.4, 117.7, 117.5, 116.8, 116.7, 116.7, 116.7, 116.2, 115.7, 74.8, 74.7, 47.8, 46.8, 41.7, 41.2, 36.7, 36.4.

Shimobashiric acid D (**4**): Colorless amorphous solid, $[\alpha]_D^{21}$ −7.7° (c=0.13, MeOH), UV (MeOH) λ_{max} (logε): 204 (4.95), 284 (4.19). CD (c=0.013, MeOH) nm ([θ]): 250 (11600). HR-FAB-MS (positive): m/z 771.1899 $[M+Na]^+$ (Calcd for $C_{38}H_{36}O_{16}Na$: 771.1900). ^{1}H-NMR: (CD_3OD, 400 MHz), δ: 6.70 (1H, d, J=2.0 Hz, H-2), 6.73 (1H, d, J=8.0 Hz, H-5), 6.50 (1H, dd, J=8.0, 2.0 Hz, H-6), 2.65 (1H, dd, J=14.0, 5.0 Hz, H-7), 2.74 (1H, dd, J=14.0, 7.0 Hz, H-7), 4.66 (1H, dd, J=7.0, 5.0 Hz, H-8), 6.80 (1H, d, J=2.0 Hz, H-2′), 6.72 (1H, d, J=8.0 Hz, H-5′), 6.54 (1H, dd, J=8.0, 2.0 Hz, H-6′), 4.29 (1H, dd, J=11.0, 7.0 Hz, H-7′), 3.96 (1H, dd, J=11.0, 7.0 Hz, H-8′), 3.83 (3H, s, H-3′-OMe), 6.73 (1H, d, J=2.0 Hz, H-2″), 6.75 (1H, d, J=8.0 Hz, H-5″), 6.57 (1H, dd, J=8.0, 2.0 Hz, H-6″), 2.82 (1H, dd, J=14.0, 8.0 Hz, H-7″), 2.89 (1H, dd, J=14.0, 4.5 Hz, H-7″), 4.79 (1H, dd, J=8.0, 4.5 Hz, H-8″), 6.91 (1H, d, J=2.0 Hz, H-2‴), 6.74 (1H, d, J=8.0 Hz, H-5‴), 6.63 (1H, dd, J=8.0, 2.0 Hz, H-6‴), 4.34 (1H, dd, J=11.0, 7.0 Hz, H-7‴), 3.90 (1H, dd, J=10.5, 7.0 Hz, H-8‴), 3.84 (3H, s, H-3‴-OMe). ^{13}C-NMR: (CD_3OD, 100 MHz), δ: 129.1 (C-1), 117.6 (C-2), 146.1[a] (C-3), 145.4[a] (C-4), 116.4[b] (C-5), 122.1 (C-6), 37.7 (C-7), 75.2 (C-8), 172.9 (C-9), 131.7 (C-1′), 112.8 (C-2′), 148.8 (C-3′), 146.3 (C-4′), 116.4[b] (C-5′), 120.5 (C-6′), 42.6 (C-7′), 48.1 (C-8′), 173.6 (C-9′), 56.5 (C-3′-OMe), 128.9 (C-1″), 117.8 (C-2″), 145.5[a] (C-3″), 145.3[a] (C-4″), 116.5[b] (C-5″), 122.1 (C-6″), 38.0 (C-7″), 75.2 (C-8″), 173.6 (C-9″), 131.8 (C-1‴), 112.4 (C-2‴), 148.9 (C-3‴), 146.4 (C-4‴), 116.7[b] (C-5‴), 121.3 (C-6‴), 43.2 (C-7‴), 49.0 (C-8‴), 172.9 (C-9‴), 56.6 (C-3‴-OMe). [a,b]: Assignments are interchangeable.

Shimobashiraside A (**5**): Colorless amorphous solid, $[\alpha]_D^{21}$ −70.0° (c=0.1, MeOH), UV (MeOH) λ_{max} (logε): 202 (4.52), 251 (4.20), 331 (4.15). HR-FAB-MS (positive): m/z 583.1646 $[M+H]^+$ (Calcd for $C_{26}H_{31}O_{15}$: 583.1662). ^{1}H-NMR: (CD_3OD, 400 MHz), δ: 6.42 (1H, d, J=5.5 Hz, H-5), 7.94 (1H, d, J=5.5 Hz, H-6), 2.44 (3H, s, H-7), 4.76 (overlapped, H-Glc-1), 3.3—3.45 (overlapped, H-Glc-2,3,4,5), 3.62 (1H, dd, J=11.5, 6.5 Hz, H-Glc-6), 3.96 (overlapped, H-Glc-6), 4.98 (1H, d, J=1.5 Hz, H-Api-1), 3.89 (1H, br s, H-Api-2), 3.82 (1H, d, J=9.5 Hz, H-Api-4), 3.96 (1H, d, J=9.5 Hz, H-Api-4), 4.21 (1H, d, J=11.5 Hz, H-Api-5), 4.25 (1H, d, J=11.5 Hz, H-Api-5), 7.04 (1H, d, J=1.5 Hz, H-2′), 6.78 (1H, d, J=8.0 Hz, H-5′), 6.95 (1H, dd, J=8.0, 1.5 Hz, H-6′), 7.58 (1H, d, J=16.0 Hz, H-7′), 6.28 (1H, d, J=16.0 Hz, H-8′). ^{13}C-NMR: (CD_3OD, 100 MHz), δ: 164.7 (C-2), 143.6 (C-3), 177.2 (C-4), 117.3 (C-5), 157.2 (C-6), 15.9 (C-7), 105.4 (C-Glc-1), 75.0 (C-Glc-2), 78.5 (C-Glc-3), 71.4 (C-Glc-4), 77.5 (C-Glc-5), 68.6 (C-Glc-6), 110.6 (C-Api-1), 78.0 (C-Api-2), 79.0 (C-Api-3), 75.4 (C-Api-4), 67.4 (C-Api-5), 127.8 (C-1′), 115.3 (C-2′), 146.9 (C-3′), 149.8 (C-4′), 116.6 (C-5′), 123.1 (C-6′), 147.5 (C-7′), 114.8 (C-8′), 168.9 (C-9′).

Shimobashiraside B (**6**): Colorless amorphous solid, $[\alpha]_D^{22}$ −60.0° (c=0.28, MeOH), UV (MeOH) λ_{max} (logε): 202 (4.34), 244 (4.07), 327 (4.11). HR-FAB-MS (positive): m/z 597.1804 $[M+H]^+$ (Calcd for $C_{27}H_{33}O_{15}$: 597.1819). ^{1}H-NMR: (CD_3OD, 400 MHz), δ: 6.42 (1H, d, J=5.5 Hz, H-5), 7.94 (1H, d, J=5.5 Hz, H-6), 2.44 (3H, s, H-7), 4.77 (1H, d, J=7.5 Hz, H-Glc-1), 3.3—3.4 (overlapped, H-Glc-2,3,4,5), 3.62 (1H, dd, J=11.5, 6.5 Hz, H-Glc-6), 3.96 (1H, J=11.5, 2.0 Hz, H-Glc-6), 4.99 (1H, d, J=2.0 Hz, H-Api-1), 3.89 (1H, d, J=2.0 Hz, H-Api-2), 3.82 (1H, d, J=9.5 Hz, H-Api-4), 3.97 (1H, d, J=9.5 Hz, H-Api-4), 4.22 (1H, d, J=11.5 Hz, H-Api-5), 4.25 (1H, d, J=11.5 Hz, H-Api-5), 7.20 (1H, d, J=2.0 Hz, H-2′), 6.82 (1H, d, J=8.0 Hz, H-5′), 7.08 (1H, dd, J=8.0, 2.0 Hz, H-6′), 7.64 (1H, d, J=16.0 Hz, H-7′), 6.38 (1H, d, J=16.0 Hz, H-8′), 3.90 (3H, s, H-OMe). ^{13}C-NMR: (CD_3OD, 100 MHz), δ: 164.7 (C-2), 143.5 (C-3), 177.2 (C-4), 117.3 (C-5), 157.2 (C-6), 15.9 (C-7), 105.4 (C-Glc-1), 75.0 (C-Glc-2), 78.5 (C-Glc-3), 71.4 (C-Glc-4), 77.5 (C-Glc-5), 68.6 (C-Glc-6), 110.6 (C-Api-1), 78.0 (C-Api-2), 79.0 (C-Api-3), 75.4 (C-Api-4), 67.5 (C-Api-5), 127.7 (C-1′), 115.1 (C-2′), 149.5 (C-3′), 150.8 (C-4′), 116.6 (C-5′), 124.3 (C-6′), 147.3 (C-7′), 111.8 (C-8′), 168.9 (C-9′), 56.5

(C-OMe).

Shimobashiraside C (**7**): Colorless amorphous solid, $[\alpha]_D^{23}$ −65.0° (*c*=0.08, MeOH), UV (MeOH) λ_{max} (logε): 205 (5.08), 310 (4.02). HR-FAB-MS (positive): *m/z* 437.1090 $[M+H]^+$ (Calcd for $C_{20}H_{21}O_{11}$: 437.1083). ^{1}H-NMR: (DMSO-d_6, 400 MHz), *δ*: 6.72 (1H, d, *J*=9.0 Hz, H-3), 7.19 (1H, dd, *J*=9.0, 3.0 Hz, H-4), 7.41 (1H, d, *J*=3.0 Hz), 4.84 (1H, d, *J*=7.5 Hz, H-Glc-1), 3.1-3.5 (3H, overlapped, H-Glc-2-4), 3.74 (1H, m, H-Glc-5), 4.33 (1H, dd, *J*=11.5, 7.5 Hz, H-Glc-6), 4.61 (1H, dd, *J*=11.5, 2.0 Hz, H-Glc-6), 6.95 (overlapped, H-3′), 7.54 (1H, m, H-4′), 6.97 (overlapped, H-5′), 7.77 (1H, dd, *J*=7.5, 2.0 Hz, H-6′). ^{13}C-NMR: (DMSO-d_6, 100 MHz), *δ*: 113.5 (C-1), 156.4 (C-2), 117.3 (C-3), 124.7 (C-4), 149.1 (C-5), 117.2 (C-6), 171.3 (C-7), 101.3 (C-Glc-1), 73.1 (C-Glc-2), 76.1 (C-Glc-3), 70.1 (C-Glc-4), 73.4 (C-Glc-5), 64.6 (C-Glc-6), 112.8 (C-1′), 160.2 (C-2′), 119.3 (C-3′), 135.8 (C-4′), 117.3 (C-5′), 130.0 (C-6′), 168.6 (C-7′).

Shimobashiraside D (**8**): Colorless amorphous solid, $[\alpha]_D^{22}$ +58.5° (*c*=0.39, MeOH), UV (MeOH) λ_{max} (logε): 202 (4.40), 231 (4.12), 287 (3.97), 326 (3.71). HR-FAB-MS (positive): *m/z* 583.2032 $[M+H]^+$ (Calcd for $C_{27}H_{35}O_{14}$: 583.2027). ^{1}H-NMR: (CD_3OD, 400 MHz), *δ*: 2.96 (1H, m, H-2), 3.39 (1H, dd, *J*=7.5, 3.0 Hz, H-3), 3.42 (overlapped, H-3), 7.23 (4H, m, H-2′,3′,5′,6′), 7.14 (1H, m, H-4′), 4.94 (1H, d, *J* =10.0 Hz, H-1″), 3.61 (1H, dd, *J*=10.0, 9.0 Hz, H-2″), 3.51 (1H, dd, *J*=9.0, 9.0 Hz, H-3″), 3.51 (1H, dd, *J*=9.0, 9.0 Hz, H-4″), 3.42 (overlapped, H-5″), 3.80 (1H, dd, *J*=12.0, 4.0 Hz, H-6″), 3.85 (1H, dd, *J*=12.0, 2.0 Hz, H-6″), 4.94 (1H, d, *J*=10.0 Hz, H-1‴), 3.61 (1H, dd, *J*=10.0, 9.0 Hz, H-2‴), 3.51 (1H, dd, *J*=9.0, 9.0 Hz, H-3‴), 3.51 (1H, dd, *J*=9.0, 9.0 Hz, H-4‴), 3.42 (overlapped, H-5‴), 3.80 (1H, dd, *J*=12.0, 4.0 Hz, H-6‴), 3.85 (1H, dd, *J*=12.0, 2.0 Hz, H-6‴). ^{13}C-NMR: (CD_3OD, 100 MHz), *δ*: 31.7 (C-2), 47.4 (C-3), 206.8 (C-4), 162.2 (C-5), 104.3 (C-6), 163.1 (C-7), 104.3 (C-8), 162.2 (C-9), 106.1 (C-10), 143.1 (C-1′), 129.5 (C-2′,6′), 129.3 (C-3′,5′), 126.8 (C-4′), 76.6 (C-1″), 74.1 (C-2″), 79.0 (C-3″), 71.0 (C-4″), 82.7 (C-5″), 61.8 (C-6″), 76.7 (C-1‴), 74.1 (C-2‴), 79.0 (C-3‴), 71.0 (C-4‴), 82.7 (C-5‴), 61.8 (C-6‴).

Shimobashiraside E (**9**): Colorless amorphous solid, $[\alpha]_D^{23}$ −18.7° (*c*=3.12, MeOH). HR-FAB-MS (positive): *m/z* 345.1548 $[M+H]^+$ (Calcd for $C_{16}H_{25}O_8$: 345.1550). ^{1}H-NMR: (CD_3OD, 400 MHz), *δ*: 8.36 (1H, brs, H-2), 6.77 (1H, d, *J*=1.0 Hz, H-4), 7.57 (1H, dd, *J*=1.5, 1.0 Hz, H-5), 2.87 (2H, m, H-7), 1.57 (1H, m, H-8), 1.84 (overlapped, H-8), 1.84 (overlapped, H-9), 3.39 (1H, m, H-10), 3.80 (1H, dd, *J*=9.5, 6.5 Hz, H-10), 0.97 (3H, d, *J*=7.0 Hz, H-11), 4.25 (1H, d, *J*=8.0 Hz, H-Glc-1), 3.18 (1H, dd, *J*=9.0, 8.0 Hz, H-Glc-2), 3.35 (1H, m, H-Glc-3), 3.29 (1H, m, H-Glc-4), 3.29 (1H, m, H-Glc-5), 3.67 (1H, dd, *J*=12.0, 5.0 Hz, H-Glc-6), 3.87 (1H, dd, *J*=12.0, 2.0 Hz, H-Glc-6). ^{13}C-NMR: (CD_3OD, 100 MHz), *δ*: 150.0 (C-2), 128.8 (C-3), 109.3 (C-4), 145.8 (C-5), 198.3 (C-6), 38.7 (C-7), 29.6 (C-8), 34.2 (C-9), 75.5 (C-10), 17.3 (C-11), 104.5 (C-Glc-1), 75.1 (C-Glc-2), 78.1 (C-Glc-3), 71.7 (C-Glc-4), 77.9 (C-Glc-5), 62.8 (C-Glc-6).

Shimobashiraside F (**10**): Colorless amorphous solid, $[\alpha]_D^{22}$ −7.9° (*c*=0.28, MeOH). HR-FAB-MS (positive): *m/z* 361.1504 $[M+H]^+$ (Calcd for $C_{16}H_{25}O_9$: 361.1499). ^{1}H-NMR: (CD_3OD, 400 MHz), *δ*: 8.37 (1H, brs, H-2), 6.79 (1H, d, *J*=1.0 Hz, H-4), 7.58 (1H, dd, *J*=1.5, 1.0 Hz, H-5), 2.99 (1H, dd, *J*=15.5, 9.0 Hz, H-7), 3.05 (1H, dd, *J*=15.5, 3.0 Hz, H-7), 4.16 (1H, dd, *J*=9.0, 3.0 Hz, H-8), 1.29 (3H, s, H-10), 1.31 (3H, s, H-11), 4.54 (1H, d, *J*=8.0 Hz, H-Glc-1), 3.16 (1H, dd, *J*=9.0, 8.0 Hz, H-Glc-2), 3.34 (1H, m, H-Glc-3), 3.26 (1H, m, H-Glc-4), 3.26 (1H, m, H-Glc-5), 3.62 (1H, dd, *J*=12.0, 5.0 Hz, H-Glc-6), 3.82 (1H, dd, *J*=12.0, 2.0 Hz, H-Glc-6). ^{13}C-NMR: (CD_3OD, 100 MHz), *δ*: 150.6 (C-2), 129.7 (C-3), 109.3 (C-4), 145.9 (C-5), 197.1 (C-6), 43.6 (C-7), 74.7 (C-8), 80.8 (C-9), 24.0 (C-10), 22.1 (C-11), 98.6 (C-Glc-1), 75.4 (C-Glc-2), 78.2 (C-Glc-3), 71.6 (C-Glc-4), 77.9 (C-Glc-5), 62.9 (C-Glc-6).

(*S*)-PGME and (*R*)-PGME Amides of 1 for Determining the Stereochemistry of C-8 To **1** (each 0.8 mg) in *N*,*N*-dimethylformamide (DMF) (0.5 mL) was added (*S*)- or (*R*)-PGME (5 mg), and then benzotriazol-1-yl-oxy-tris-pyrrolidinophonium hexafluorophosphate (PyBOP) (10 mg), 1-hydroxybenzotriazole (HOBT) (5 mg), and *N*-methylmorpholine (20 μL) were added and the mixture was stirred for 10 h at room temperature. The reactions gave (*S*)-amide: (**1a**) and (*R*)-amide (**1b**).[16]

(*S*)-PGME Amide of **1** (**1a**): Colorless amorphous solid, FAB-MS (positive): *m/z* 520 $[M+H]^+$, 542 $[M+Na]^+$, ^{1}H-NMR: (CD_3OD, 400 MHz), *δ*: 6.70 (1H, d, *J*=2.0 Hz, H-2), 6.72 (1H, d, *J*=8.5 Hz, H-5), 6.59 (1H, dd, *J*=8.5, 2.0 Hz, H-6), 4.54 (1H, brs, H-7), 4.86 (overlapped, H-8), 6.86 (1H, d, *J*=2.0 Hz, H-2′), 6.74 (1H, d, *J*=8.5 Hz, H-5′), 6.98 (1H, dd, *J*=8.5, 2.0 Hz, H-6′), 7.69 (1H, d, *J*=2.0 Hz, H-7′), 3.54 (3H, s, H-OMe).

(*R*)-PGME Amide of **1** (**1b**): Colorless amorphous solid, FAB-MS (positive): *m/z* 520 $[M+H]^+$, 542 $[M+Na]^+$, ^{1}H-NMR: (CD_3OD, 400 MHz), *δ*: 6.79 (1H, d, *J*=2.0 Hz, H-2), 6.77 (1H, d, *J*=8.5 Hz, H-5), 6.71 (1H, dd, *J*=8.5, 2.0 Hz, H-6), 4.59 (1H, brs, H-7), 4.86 (overlapped, H-8), 6.90 (1H, d, *J*=2.0 Hz, H-2′), 6.75 (1H, d, *J*=8.5 Hz, H-5′), 6.99 (1H, dd, *J*=8.5, 2.0 Hz, H-6′), 7.68 (1H, d, *J*=2.0 Hz, H-7′), 3.58 (3H, s, H-OMe).

(*S*)-PGME and (*R*)-PGME Amides of 3-(3,4-Dihydroxyphenyl)-2-hydroxypropanoic Acid To 3-(3,4-dihydroxyphenyl)-2-hydroxypropanoic acid (5 mg, each) obtained from rosmarinic acid[16] in DMF (1.0 mL) was added (*S*)- or (*R*)-PGME (10 mg), and then benzotriazol-1-yl-oxy-tris-pyrrolidinophonium hexafluorophosphate (PyBOP) (15 mg), 1-hydroxybenzotriazole (HOBT) (5 mg), and *N*-methylmorpholine (20 μL) were added and the mixture was stirred for 10 h at room temperature. The reactions gave (*S*)-amide and (*R*)-amide.[16] The retention time of (*S*)-amide was 19.4 min and that of (*R*)-amide was 20.1 min. The analytical HPLC was performed on a Shiseido Capcell Pak C18 column (4.6×250 mm) using acetonitrile–0.2% TFA in water (22.5 : 77.5) as the mobile phase (flow rate, 1 mL/min; detector, UV 210 nm).

Acidic Hydrolysis of Compounds 2—4 and Their (*S*)-PGME Amides Each compound (**2**, **3**, **4**: each 1.0 mg) was dissolved in 7% HCl (1 mL) and stirred for 2 h at 90°C. After concentration, the residues were dissolved in DMF and (*S*)-PGME (5 mg), PyBOP (7 mg), HOBT (3 mg), and *N*-methylmorpholine (15 μL) were added. The mixtures were then stirred for 10 h at room temperature to give (*S*)-amide; t_R=19.4 min in the HPLC analysis [column, Shiseido Capcell Pak C18 (4.6×250 mm); mobile phase, acetonitrile–0.2% TFA in water (22.5 : 77.5); flow rate, 1 mL/min; detector, UV 210 nm].

Acid Hydrosis and Sugar Identification Compounds **5**—**7**, **9**, **10** and **21** (each 0.5—1.0 mg) were hydrolyzed with 7% HCl (1 mL) at 60°C for 2 h. The reaction mixture was neutralized with an Amberlite IRA400 column, and the eluate was concentrated. The residues were stirred with L-cysteine

Table 1. Hyaluronidase Inhibitory Activity of Compounds **3**, **9**—**18**, **20**—**29**, and DSCG

Compound	Hyaluronidase inhibition (%)[a]	IC_{50} (μM)
3	88.7	594
9	5.93	N.D.[b]
10	−2.50	N.D.
11	0.57	N.D.
12	65.8	814
13	86.5	309[c]
14	78.7	737
15	2.01	N.D.
16	3.46	N.D.
17	93.8	548[c]
18	86.5	267
20	−2.97	N.D.
21	5.54	N.D.
22	1.43	N.D.
23	−1.78	N.D.
24	28.6	N.D.
25	8.10	N.D.
26	7.41	N.D.
27	7.45	N.D.
28	3.81	N.D.
29	0.79	N.D.
DSCG	83.9	297[c]

a) Final concentration: 1.0 mM. *b*) N.D., not determined. *c*) Previously reported value.[3]

methyl ester (5 mg) and *o*-tolyl isothiocyanate (10 μL) in pyridine (0.5 mL), by using the procedure reported by Tanaka *et al.*[21] The reaction mixtures were analyzed by HPLC (column, Cosmosil $5C_{18}$–AR II column, 4.6×250 mm; mobile phase, CH_3CN–0.2% TFA in H_2O (25 : 75), 1.0 mL/min; detector, UV at 210 nm) at 20°C. D-Glucose (t_R 15.7 min) was identified as the sugar moieties of **5**—**7**, **9**, and **10** based on comparisons with authentic samples of D-glucose (t_R 15.7 min) and L-glucose (t_R 14.3 min). Compound **21** has an D-apiofuranose,[8] and sugar analyses by HPLC (column, Cosmosil $5C_{18}$–AR II column, 4.6×250 mm; mobile phase, CH_3CN–0.2% TFA in H_2O (25 : 75), 0.8 mL/min; detector, UV at 250 nm) were conducted. Chromatograms of **5**, **6**, and **21** showed peaks at 17.7 min (D-glucose) and 30.6 min (suggesting D-apiose).[8]

Assay of Hyaluronidase Inhibition The assay was carried out according to the Morgan–Elson method, which was modified by Davidson and Aronson.[25—27] Each compound (final concentration: 1, 0.3, 0.1, 0.03 mM) was dissolved in 0.1 M acetate buffer as the sample solution. Hyaluronidase activity was measured as described previously.[2,3] Disodium cromoglycate (DSCG) was used as a positive control. The final concentration of hyaluronidase was 400 unit/mL.

Acknowledgments We thank Mr. S. Sato and Mr. T. Matsuki of Tohoku Pharmaceutical University for assisting with the MS measurements.

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