Hyaluronidase Inhibitors from "Cimicifugae Rhizoma" (a Mixture of the Rhizomes of *Cimicifuga dahurica* and *C. heracleifolia*)

Atsufumi Iwanaga,[†,‡] Genjiro Kusano,[‡] Tsutomu Warashina,[§] and Toshio Miyase*[,†]

School of Pharmaceutical Sciences, University of Shizuoka, Yada 52-1, Suruga-ku, Shizuoka 422-8526, Japan, Shinnihon Pharmaceutical Co., Ltd., Akasaka 1-14-22, Chuo-ku, Fukuoka 810-0042, Japan, and Institute of Environmental Sciences, University of Shizuoka, Yada 52-1, Suruga-ku, Shizuoka 422-8526, Japan

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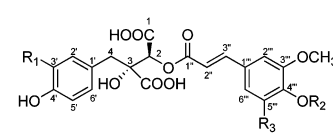
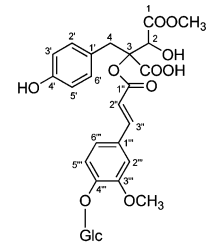
From the 80% acetone extract of "Cimicifugae Rhizoma" (a mixture of *Cimicifuga dahurica* and *C. heracleifolia* used medicinally), seven new fukiic acid derivatives (**1**−**7**) and a new phenylethanoid derivative (**8**) were isolated along with eight known compounds (**9**−**16**). Fukinolic acid (**9**) and cimicifugic acids A−J (**10**−**16**, **5**−**7**) showed stronger hyaluronidase inhibitory activities than the positive control, rosmarinic acid.

"Cimicifugae Rhizoma" has been used as an antipyretic, analgesic, and wound-healing agent along with other crude drugs in traditional Chinese-Japanese prescriptions.[1,2] The Japanese pharmacopoeia indicates that "Cimicifugae Rizoma" is the rhizomes of *Cimicifuga simplex* Wormskjord, *Cimicifuga dahurica* (Turcz.) Maximowicz, *Cimicifuga heracleifolia* Komarov, and *Cimicifuga foetida* L. (Ranunculaceae). Several constituents have been identified from "Cimicifugae Rhizoma". These include fukiic acid derivatives such as fukinolic acid (**9**) and cimicifugic acids A−G (**10**−**16**),[3−5] which show biological effects such as relaxant action on smooth muscle,[6] inhibitory activities on germination,[7] and α-amylase,[7] carboxypeptidase A,[7] estrogenic,[8] and neutrophil elastase activities.[9] The structures of the fukiic acid derivatives are similar to that of rosmarinic acid, which has shown strong hyaluronidase inhibitory activity.[10] In the course of studies on the water-soluble constituents of a sample of "Cimicifugae Rhizoma" [a prepared mixture of the rhizomes of *C. dahurica* and *C. heracleifolia* (ratio about 1:9)], we obtained seven new fukiic acid derivatives (**1**−**7**) and a new phenylethanoid (**8**), along with the known fukinolic acid (**9**) and cimicifugic acids A−G (**10**−**16**). The present report deals with the isolation of these constituents, the structure elucidation of the new compounds, and their hyaluronidase inhibitory activities. As a result, compounds **6**, **7**, fukinolic acid (**9**), and cimicifugic acids A−G (**10**−**16**) were found to show stronger inhibitory activities than rosmarinic acid.



	R_1	R_2	R_3
1	OH	Gal	H
2	H	Gal	H
3	H	Glc	H
6	OH	H	OCH_3
7	H	H	OCH_3



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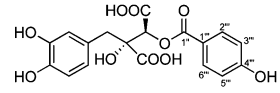

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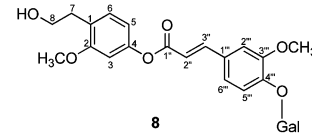

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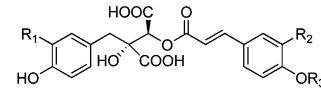

	R_1	R_2	R_3
9	OH	OH	H
10	OH	OMe	H
11	OH	OH	Me
12	OH	H	H
13	H	OH	H
14	H	OMe	H
15	H	OH	Me
16	H	OMe	Me

Results and Discussion

The MeOH eluate obtained from the Diaion HP-20 column chromatography of the 80% acetone fraction of the plant material extracted gave seven new fukiic acid derivatives (**1**−**7**) and a new phenylethanoid (**8**), together with eight known compounds (**9**−**12**,[3] **13**−**15**,[4] **16**[5]). The known compounds were identified by comparison of NMR data with reported values.

Shomaside A (**1**) was isolated as a pale brown powder. The molecular formula was established as $C_{27}H_{30}O_{16}$ on the basis of the HRFABMS data ([M − H]$^-$ ion at *m*/*z* 609.1451). The UV absorption λ_{max} (MeOH) at 320 nm (log ε 4.14) was indicative of an oxycinnamoyl residue. Acid hydrolysis of **1** afforded D-galactose as the component sugar. The ^{1}H NMR spectrum of **1** indicated the presence of a fukiic acid moiety showing typical AB-type proton signals assignable to isolated methylene protons at δ 2.93 and 3.05 (each 1H, d, J = 14 Hz), an oxymethine proton signal at δ 5.66 (1H, s), and a 1,3,4-trisubstituted benzene moiety at δ 6.75 (1H, d, J = 2 Hz, H-2′), 6.65 (1H, d, J = 8 Hz, H-5′), and 6.60 (1H, dd, J = 8, 2 Hz, H-6′). The ^{13}C NMR spectrum of **1** showed a methylene carbon signal (δ 42.3), an oxymethine carbon signal (δ 77.6), a quaternary carbon signal (δ 80.0), and two carboxyl carbon signals (δ 170.2 and 174.6), suggesting that **1** has a fukiic acid moiety. In addition, a feruloyl moiety was shown at δ 6.59 (1H, d, J = 16 Hz, H-2″), 7.81 (1H, d, J = 16 Hz, H-3″), 3.91 (3H, s, OCH_3), 7.31 (1H, d, J = 2 Hz, H-2‴), 7.21 (1H, d, J = 8 Hz, H-5‴), and 7.23 (1H, dd, J = 8, 2 Hz, H-6‴). The presence of a β-D-galactopyranosyl moiety was shown from resonances at δ 5.33 (1H, d, J = 8 Hz, Gal-1), 3.66 (1H, dd, J = 8, 3 Hz, Gal-2), 4.16 (1H, dd, J = 3, 3 Hz, Gal-3), 3.61 (1H, dd, J = 9, 3 Hz, Gal-4), 3.84 (1H, overlapped, Gal-5), 3.68 (1H, dd, J = 13, 6 Hz, Gal-6), and 3.86 (1H, dd, J = 13, 3 Hz, Gal-6). The ^{13}C NMR data also showed six carbon signals for galactose at δ 100.3 (Gal-1), 72.0 (Gal-2), 72.9 (Gal-3), 68.7 (Gal-4), 76.0 (Gal-5), and 62.9 (Gal-6). The methoxy group was located at C-3‴ by the analysis of the

* To whom correspondence should be addressed. Tel: +81-54-264-5625. E-mail: miyase@ys7.u-shizuoka-ken.ac.jp.
† School of Pharmaceutical Sciences, University of Shizuoka.
‡ Shinnihon Pharmaceutical Co., Ltd.
§ Institute of Environmental Sciences, University of Shizuoka.

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ROE between the methoxy group and H-2‴. The location of the galactopyranoside linkage was confirmed to be C-4‴ by the HMBC spectrum. The anomeric proton (δ 5.33) was long-range coupled with the carbon at δ 150.7, which in turn was long-range coupled with the proton at δ 7.23 due to H-6‴ because of its lower chemical shift. The ^{1}H and ^{13}C NMR data were very similar to those of cimicifugic acid A (**10**)[3] except for additional signals for a β-D-galactopyranosyl unit. The CD spectrum of **1** gave similar Cotton effects to those of fukinolic acid, for which the relative configuration was elucidated by synthesis and analysis.[3,11] The absolute stereochemistry was deduced as 2*S*, 3*R* from the positive Cotton effects at 280 nm (+2800), 226 nm (+8400), and 209 nm (+12 200) in the CD spectrum. Thus, the structure of shomaside A was established as shown.

Shomaside B (**2**) was isolated as a pale brown powder. The molecular formula was established as $C_{27}H_{30}O_{15}$ on the basis of the HRFABMS data ([M − H] $^-$ ion at *m*/*z* 593.1525). The difference of the molecular formula of compound **2** from that of compound **1** was due to a lack of an oxygen atom. The UV and NMR data of **2** were found to be similar to those of **1**. However, the ^{1}H NMR spectrum of **2** indicated the presence of a *p*-hydroxybenzyl moiety at δ 7.09 (2H, d, *J* = 8 Hz, H-2′, H-6′) and 6.67 (2H, d, *J* = 8 Hz, H-3′, H-5′), showing typical AB-type proton signals at δ 2.99 and 3.10 (each 1H, d, *J* = 14 Hz) and an oxymethine proton signal at δ 5.67 (1H, s). On the basis of the above evidence, compound **2** was deduced as a glycoside of the known cimicifugic acid E (**14**),[4] which has a piscidic acid moiety. The ^{13}C NMR spectrum of **2** showed a methylene carbon signal (δ 42.0), an oxymethine carbon signal (δ 77.6), a quaternary oxygenated carbon signal (δ 80.1), and two carboxyl carbon signals (δ 170.3 and 174.6), suggesting that **2** also has a piscidic acid moiety. In addition, a feruloyl moiety occurred at δ 6.59 (1H, d, *J* = 16 Hz, H-2″), 7.81 (1H, d, *J* = 16 Hz, H-3″), 3.91 (3H, s, OCH_3), 7.30 (1H, d, *J* = 2 Hz, H-2‴), 7.21 (1H, d, *J* = 8 Hz, H-5‴), and 7.23 (1H, dd, *J* = 8, 2 Hz, H-6‴) and a β-D-galactopyranosyl moiety at δ 5.33 (1H, d, *J* = 8 Hz, Gal-1), 3.66 (1H, dd, *J* = 8, 3 Hz, Gal-2), 4.16 (1H, dd, *J* = 3, 3 Hz, Gal-3), 3.62 (1H, dd, *J* = 9, 3 Hz, Gal-4), 3.87 (1H, overlapped, Gal-5), 3.68 (1H, dd, *J* = 13, 6 Hz, Gal-6), and 3.87 (1H, dd, *J* = 13, 3 Hz, Gal-6). The ^{13}C NMR data also showed six carbon signals for galactose at δ 100.3 (Gal-1), 72.0 (Gal-2), 72.8 (Gal-3), 68.6 (Gal-4), 76.0 (Gal-5), and 62.9 (Gal-6). The methoxy group was located at C-3‴ by the analysis of the ROE between the methoxy group and H-2‴. The location of the galactopyranoside linkage was confirmed to be C-4‴ by the HMBC spectrum. The CD spectrum of **2** also showed positive Cotton effects at 279 nm (+3300) and 228 nm (+15 200). The absolute configulation of **2** was deduced to be 2*S*, 3*R*.

Shomaside C (**3**) was isolated as a pale brown powder. The MS and NMR data were very similar to those of **2** except for the sugar moiety. Acid hydrolysis of **3** gave D-glucose. In the HMBC spectrum, the anomeric proton signal at δ 4.98 (1H, d, *J* = 7.5 Hz) was long-range coupled to the carbon signal at δ 150.3, which was assigned to C-4‴. Therefore, these observations confirmed that compound **3** is cimicifugic acid E 4‴-glucopyranoside. The absolute configulation of **3** was also deduced to be 2*S*, 3*R* from the CD spectrum, showing positive Cotton effects at 279 nm (+2400) and 227 nm (+6800).

Shomaside D (**4**) was isolated as a pale brown powder. In the HRFABMS, a fragment ion peak [M − $COOCH_3$ − 2H]$^-$ appeared at *m*/*z* 547.1449, in agreement with the molecular formula, $C_{26}H_{27}O_{13}$, for a piscidic acid methyl ester derivative. This fragmentation appears to be related to the rule of mass shift.[12] The ^{1}H and ^{13}C NMR spectroscopic data of **4** were analogous to those of **3**. However, the ^{1}H NMR spectrum of **4** showed an additional methoxy group signal (δ 3.70), suggesting the presence of a carbomethoxy group, instead of two carboxyl groups in **2**. In the HMBC experiment, a ^{1}H, ^{13}C long-range correlation was observed between a carbomethoxy proton (δ 3.70) and C-1 (δ 173.4). Thus, it was confirmed that the esterification site of **4** is at C-1 and not C-5 (δ 175.7). Differences between **4** and **3** were observed in the chemical shift change in the ^{1}H NMR spectrum for H-2 from δ 5.65 to δ 4.55 and then in the ^{13}C NMR spectrum for C-2 from δ 77.9 to δ 76.6 and C-3 from δ 80.1 to δ 81.5. A correlation between H-2 and C-1″ in the HMBC spectrum of **3** was observed, whereas this was not observed for **4**. The anomeric proton signal at δ 4.97 (1H, d, *J* = 8 Hz) was long-range coupled to the carbon signal at δ 150.2, which was assigned to C-4‴. These observations confirmed that a glucosyl feruloyl moiety is linked to C-3 and a secondary hydroxy group is linked to C-2 in **4**. The absolute configurations of two asymmetric carbon atoms at C-2 and C-3 were not decided because of the low yield of the compound.

Cimicifugic acid H (**5**) was isolated as a pale brown powder. The molecular formula was established as $C_{18}H_{16}O_{10}$ on the basis of the HRFABMS data ([M − H]$^-$ ion at *m*/*z* 391.0069). Compound **5** showed typical absorptions due to a *p*-hydroxybenzoyl group at 324 nm (log ε 4.20) and 290 nm (log ε 4.15). Signals for vinylic protons were not observed in the ^{1}H NMR spectrum, whereas the ^{1}H NMR spectrum of **5** showed signals assignable to an isolated methylene at δ 2.95 and 3.06 (each 1H, d, *J* = 14 Hz, H-4), an oxymethine at δ 5.72 (1H, s, H-2), and a 1,2,4-trisubstituted benzene moiety at δ 6.74 (1H, d, *J* = 2 Hz, H-2′), 6.64 (1H, d, *J* = 8 Hz, H-5′), and 6.59 (1H, dd, *J* = 8, 2 Hz, H-6′). In the ^{13}C NMR spectrum, a methylene carbon signal (δ 42.3), an oxymethine carbon signal (δ 77.8), a quaternary oxygenated carbon signal (δ 80.5), and two carboxyl carbon signals (δ 171.0, 174.5) were observed, similar to compound **1**, suggesting the presence of a fukiic acid moiety. On the other hand, compound **5** was found to contain a *p*-hydroxybenzoyl unit, since the ^{1}H and ^{13}C NMR spectra gave resonances for four aromatic protons at δ 8.40 (2H, d, *J* = 9 Hz, H-2‴, H-6‴) and 6.87 (2H, d, *J* = 9 Hz, H-3‴, H-5‴) and a resonance for a carbonyl carbon signal at δ 167.3 (C-1″). In addition, in the HMBC spectrum, ^{1}H−^{13}C long-range correlations were found from H-2, H-2‴, and H-6‴ to C-1″, suggesting the *p*-hydroxybenzoyl group is linked to the fukiic acid moiety. The absolute configuration of **5** was deduced to be 2*S*, 3*R* from the CD spectrum, showing positive Cotton effects at 232 nm (+6500) and 208 nm (+19 100).

Cimicifugic acid I (**6**) was isolated as a pale brown powder. The molecular formula was established as $C_{22}H_{22}O_{12}$ on the basis of the HRFABMS data ([M − H]$^-$ ion at *m*/*z* 477.1023). Compound **6** showed typical UV oxycinnamoyl absorptions (MeOH) at 331 nm (log ε 4.10) and 293 nm (log ε 3.78). The presence of a fukiic acid moiety was suggested because the values of chemical shifts of **6** were consistent with those of compound **1** in the ^{1}H and ^{13}C NMR spectra. On the other hand, the presence of a sinapoyl moiety was shown at δ 6.53 (1H, d, *J* = 16 Hz, H-2″), 7.79 (1H, d, *J* = 16 Hz, H-3″), 3.90 (6H, s, OCH_3), and 6.97 (2H, s, H-2‴, H-6‴) in the ^{1}H NMR spectrum. This was also confirmed by the HMBC spectrum, which showed correlations from H-2‴ (δ 6.97) to C-1‴, C-3″, C-3‴, and C-4‴; H-6 (δ 6.97) to C-1‴, C-2‴, C-3″, and C-5‴; OCH_3 (δ 3.90) to C-3‴; and OCH_3 (δ 3.90) to C-5‴. The long-range correlation between H-2 and C-1 in the HMBC spectrum supported the linkage of the sinapoyl moiety with C-2 of the fukiic acid unit. The CD spectrum of **6** showed positive Cotton effects at 326 nm (+2700), 285 nm (+2800), and 232 nm (+17 800), similar to those of fukinolic acid. Therefore, the absolute configulation of **6** was also deduced as 2*S*, 3*R*.

Cimicifugic acid J (**7**) was isolated as a pale brown powder. The molecular formula was established as $C_{22}H_{22}O_{11}$ on the basis of the HRFABMS data ([M − H]$^-$ ion at *m*/*z* 461.1084). The ^{1}H and ^{13}C NMR spectra of **7** were nearly identical with those of **6**, but showed resonances for a piscidic acid unit as in the case of compounds **2** and **3**. The sinapoyl moiety of **7** was observed from a ROE between the methoxy group signal and those of H-2‴ and

Table 1. NMR Spectrpscopic Data (400 MHz) of Compounds **1**−**3**

	1				**2**				**3**			
position	δ_H (J in Hz)	δ_C	HMBC (H to C)	ROE	δ_H (J in Hz)	δ_C	HMBC (H to C)	ROE	δ_H (J in Hz)	δ_C	HMBC (H to C)	ROE
1		170.2				170.3				170.5		
2	5.66 (s)	77.6	1, 1″, 3, 4, 5		5.67 (s)	77.6	1, 1″, 3		5.65 (s)	77.9	1″, 3	
3		80.0				80.1				80.1		
4	2.93 (d, 14)	42.3	1′, 2, 2′, 3, 5, 6′		2.99 (d, 14)	42.0	1′, 2, 2′, 3, 6′		2.99 (d, 14)	42.0	3, 1′, 2′, 6′	
	3.05 (d, 14)		1′, 2, 2′, 3, 5, 6′		3.10 (d, 14)		1′, 2, 2′, 3, 5, 6′		3.08 (d, 14)		3, 1′, 2′, 6′	
5		174.6				174.6				174.7		
1′		128.0				127.3				127.4		
2′	6.75 (d, 2)	118.9	3′, 4, 4′, 6′		7.09 (d, 8)	132.5	3′, 4, 4′, 6′		7.09 (d, 8)	132.6	4, 4′, 6′	
3′		145.8			6.67 (d, 8)	115.8	1′, 4′, 5′		6.67 (d, 8)	115.8		
4′		145.3				157.4				157.4		
5′	6.65 (d, 8)	116.0	1′, 3′		6.67 (d, 8)	115.8	1′, 3′, 4′		6.67 (d, 8)	115.8	1′, 3′, 4′	
6′	6.60 (dd, 8, 2)	123.1	2′, 4, 4′		7.09 (d, 8)	132.5	2′, 4, 4′, 5′		7.09 (d, 8)	132.6	4, 2′, 4′	
1″		167.9				167.9				167.9		
2″	6.59 (d, 16)	116.4	1″, 1‴		6.59 (d, 16)	116.3	1″, 1‴		6.59 (d, 16)	116.6	1″, 1‴	
3″	7.81 (d, 16)	147.5	1″, 1‴, 2″, 2‴, 6‴		7.81 (d, 16)	147.5	1″, 1‴, 2″, 2‴, 6‴		7.81 (d, 16)	147.3	1″, 2‴, 6‴	
1‴		130.3				130.3				130.6		
2‴	7.31 (d, 2)	112.7	1‴, 3″, 4‴, 6‴		7.30 (d, 2)	112.8	1‴, 3″, 4‴, 6‴		7.32 (d, 2)	112.8	3″, 4‴, 6‴	
3‴		151.1				151.1				151.2		
4‴		150.7				150.6				150.3		
5‴	7.21 (d, 8)	117.4	1‴, 3″, 3‴, 4‴		7.21 (d, 8)	117.4	1‴, 3″, 3‴, 4‴		7.20 (d, 8)	117.6	4‴	
6‴	7.23 (dd, 8, 2)	123.8	1‴, 2‴, 3″, 4‴		7.23 (dd, 8, 2)	123.8	1‴, 2‴, 3″, 4‴		7.23 (dd, 8, 2)	123.8	4‴	
OMe-3‴	3.91 (s)	56.9	3‴	2‴	3.91 (s)	56.9	3‴	2‴	3.92 (s)	56.8	3‴	2‴
Gal-1 (Glc-1)	5.33 (d, 8)	100.3	4‴		5.33 (d, 8)	100.3	4‴		4.98 (d, 7.5)	102.3	4‴	
2	3.66 (dd, 8, 3)	72.0	Gal-1		3.66 (dd, 8, 3)	72.0	Gal-1		3.51 (dd, 7.5, 7.5)	74.9	Glc-1, Glc-3	
3	4.16 (dd, 3, 3)	72.9	Gal-1, Gal-2, Gal-4, Gal-5		4.16 (dd, 3, 3)	72.8	Gal-1, Gal-2, Gal-4, Gal-5		3.51 (overlapped)	77.9	Glc-2, Glc-4	
4	3.61 (dd, 9, 3)	68.7	Gal-5, Gal-6		3.62 (dd, 9, 3)	68.6	Gal-5, Gal-6		3.39 (overlapped)	71.3		
5	3.84 (overlapped)	76.0	Gal-4, Gal-6		3.87 (overlapped)	76.0	Gal-4, Gal-6		3.40 (overlapped)	78.3		
6	3.68 (dd, 13, 6)	62.9	Gal-4		3.68 (dd, 13, 6)	62.9	Gal-4		3.70 (dd, 12, 5)	62.5		
	3.86 (dd, 13, 3)		Gal-4, Gal-5		3.87 (dd, 13, 3)		Gal-4, Gal-5		3.88 (dd, 12, 1.5)			

Table 2. NMR Spectrpscopic Data (400 MHz) of Compounds **4**−**6**

	4				**5**			**6**		
position	δ_H (J in Hz)	δ_C	HMBC (H to C)	ROE	δ_H (J in Hz)	δ_C	HMBC (H to C)	δ_H (J in Hz)	δ_C	HMBC (H to C)
1		173.4				171.0			170.4	
2	4.55 (s)	76.6	1, 3		5.72 (s)	77.8	1, 1″, 3, 4	5.67 (s)	77.5	1, 1″
3		81.5				80.5			80.5	
4	2.99 (d, 14)	41.8	1′, 2, 3, 5		2.95 (d, 14)	42.3	1′, 2′, 3, 6′	2.93 (d, 14)	42.2	1′, 2′, 6′
	3.13 (d, 14)		1′, 2, 3, 5		3.06 (d, 14)		1′, 2, 2′, 3, 6′	3.07 (d, 14)		1′, 2′, 3, 5, 6′
5		175.7				174.5			174.6	
COOMe-2	3.70 (s)	52.3	1							
1′		128.1				128.0			127.9	
2′	7.07 (d, 8)	132.5	3′, 4, 4′, 6′		6.74 (d, 2)	118.9	3′, 4, 6′	6.75 (brs)	118.8	3′, 4, 4′, 6′
3′	6.65 (d, 8)	116.0	1′, 4′, 5′			145.8			145.7	
4′		157.3				145.4			145.3	
5′	6.65 (d, 8)	116.0	1′, 3′, 4′		6.64 (d, 8)	116.0	1′, 3′	6.65 (d, 8)	115.9	1′, 3′
6′	7.07 (d, 8)	132.5	2′, 4, 4′, 5′		6.59 (dd, 8, 2)	123.1	2′, 4, 4′	6.60 (brd, 8)	123.0	2′, 4, 4′
1″		170.3				167.3			168.1	
2″	6.38 (d, 16)	118.2	1″					6.53 (d, 16)	115.0	1″, 1‴, 3″
3″	7.62 (d, 16)	145.9	1″, 1‴, 2‴, 5‴, 6‴					7.79 (d, 16)	148.3	1″, 1‴, 2″, 2‴, 6‴
1‴		131.3				121.6			126.6	
2‴	7.24 (d, 2)	112.6	1‴, 3″, 6‴		8.40 (d, 9)	133.3	1″, 4‴, 6‴	6.97 (s)	107.2	1‴, 3″, 3‴, 4‴
3‴		151.6			6.87 (d, 9)	116.3	1‴, 4‴		150.0	
4‴		150.2				164.0			140.0	
5‴	7.18 (d, 8)	118.6	1‴		6.87 (d, 9)	116.3	1‴, 4‴		150.0	
6‴	7.15 (dd, 8, 2)	123.3	1‴, 3″, 2‴		8.40 (d, 9)	133.3	1″, 2‴, 4‴	6.97 (s)	107.2	1‴, 2‴, 3″, 5‴
OMe-3‴	3.90 (s)	57.3	3‴	2‴				3.90(s)	56.9	3‴
OMe-5‴								3.90(s)	56.9	5‴
Glc-1	4.97 (d, 8)	102.7	4‴							
2	3.51 (overlapped)	75.1	Glc-1, Glc-3							
3	3.42 (overlapped)	78.1	Glc-2							
4	3.42 (overlapped)	71.7	Glc-3							
5	3.49 (overlapped)	78.4	Glc-3, Glc-4							
6	3.70 (m)	62.9	Glc-5							
	3.88 (dd, 11, 2)									

H-6‴. The CD spectrum of **7** showed positive Cotton effects at 324 nm (+1400), 275 nm (+1600), and 229 nm (+9400) similar to those of compound **6**. Thus, the structure of **7** was established as 2-sinapoylpiscidic acid.

Shomaside E (**8**) was isolated as a pale brown powder. The HRFABMS exhibited a molecular ion peak at *m*/*z* 506.1819, corresponding to $C_{25}H_{30}O_{11}$. The ^{1}H NMR spectrum of **8** showed a feruloyl moiety at δ 6.47 (1H, d, J = 16 Hz, H-2″), 7.45 (1H, d, J = 16 Hz, H-3″), 3.88 (3H, s, OCH_3), 7.16 (1H, d, J = 2 Hz, H-2‴), 7.17 (1H, d, J = 8 Hz, H-5‴), and 7.11 (1H, dd, J = 8, 2 Hz, H-6‴), similar to that of compound **1**. Also observed was a β-D-glactopyranosyl unit, from the anomeric proton signal at δ 5.30 (1H, d, J = 8 Hz, Gal-1) and signals at δ 3.65 (1H, dd, J = 8, 3 Hz, Gal-2), 4.16 (1H, d, J = 3, 3 Hz, Gal-3), 3.61(1H, dd, J = 9.5, 3 Hz, Gal-4), 3.85 (1H, overlapped, Gal-5), 3.69 (1H, dd, J = 13, 5.5 Hz, Gal-6), and 3.85 (1H, overlapped, Gal-6) and from the carbon signals at δ 100.4 (Gal-1), 72.1 (Gal-2), 72.9 (Gal-3), 68.7 (Gal-4), 76.0 (Gal-5), and 62.9 (Gal-6). In the ^{1}H NMR spectrum, aromatic proton signals at δ 6.82 (1H, d, J = 2 Hz, H-3), 6.67 (1H, dd, J = 8, 2 Hz, H-5), and 6.73 (1H, d, J = 8 Hz, H-6), a methoxy proton signal at δ 3.83 (3H, s, OCH_3), and two triplet proton signals in the aliphatic proton region due to H_2-7 (δ 2.77, t, J = 7.5 Hz) and H_2-8 (δ 3.49, t, J = 7.5 Hz) were observed. In the HMBC spectrum, the H_2-7 protons and the methoxy protons at δ 3.83 were long-range coupled to C-1, C-5, C-8, and C-2, respectively. These data suggested the presence of a 1,2,4-trisubstituted benzene moiety. Unlike compounds **1**−**7**, oxymethine proton and carbon signals and a quaternary oxygenated carbon signal were not observed. Consequently, the structure of shomaside E (**8**) was established as shown.

In this study, in order to investigate the biological activities of isolated compounds **1**−**16**, their effects on hyaluronidase inhibitory activities were measured. Table 4 shows the 50% inhibition concentration (IC_{50}) values. As a result, strong inhibition (IC_{50} <200 μM) was observed for compounds **6** (cimicifugic acid I) and **7** (cimicifugic acid J) and the known compounds **9**−**11**, **13**−**16**. All these compounds showed stronger inhibitory activities than rosmarinic acid, used as a positive control. However, the hyaluronidase inhibitory activities of glycosides, such as compounds **1**, **3**, **4** and **8**, were less potent than that of rosmarinic acid.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P-2200 digital polarimeter. UV spectra were measured in methanol on a JASCO V-630 spectrometer. Circular dichroism spectra were measured on AVIV model 215S spectrometer. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on an α-400 FT-NMR spectrometer, and chemical shifts are given as δ values with TMS as internal standard at 35 °C in methanol-d_4. Inverse-detected heteronuclear correlations were measured using HMQC (optimized for $^1J_{C-H}$ = 145 Hz) and HMBC (optimized for $^nJ_{C-H}$ = 8 Hz) pulse sequences with a pulse field gradient. HRFABS 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. GC was run on a Hitachi G-3000 gas chromatograph.

Plant Material. The standardized "Cimicifugae Rhizoma" (*Cimicifuga dahurica* (Turcz.) Maximowicz and *Cimicifuga heracleifolia* Komarov, in a ratio of about 1:9) (lot number: P0106120072) was supplied by Tochimoto Tenkaido Co., Ltd., Suehiro-cho 3-21, Kita-ku, Osaka, Osaka, Japan, in October 2008. The plants were identified by Dr. Yutaka Yamamoto, Tochimoto Tenkaido Co., Ltd.[13] A voucher specimen (No. 20081021) was deposited at the herbarium of University of Shizuoka.

Extraction and Isolation. The dried mixture of the rhizomes of *C. dahurica* and *C. heracleifolia* (cut crude drug 154 g) was extracted with water−acetone (20:80) (1 L) under reflux for 1.5 h twice. The extract was concentrated under reduced pressure to give a dark brown residue (25 g). The residue was suspended in hot water (1 L) and extracted with ethyl ether continuously for 5 h. After the water layer (2 L) was acidified with concentrated HCl, it was fractionated over Mitsubishi Diaion HP-20 (400 g), eluting with water (5 L) and MeOH (2 L). The fraction eluted with MeOH was concentrated under reduced pressure to give a brown residue (4.87 g). The MeOH eluate was subjected to preparative HPLC [column, Tosoh TSKgel ODS-80Ts 5.5 × 180 cm; solvent, 0.1% trifluoroacetic acid−CH_3CN (90:10−67:33)

Table 3. NMR Spectrpscopic Data (400 MHz) of Compounds **7** and **8**

	7				**8**				
position	δ_H (J in Hz)	δ_C	HMBC (H to C)	ROE	position	δ_H (J in Hz)	δ_C	HMBC (H to C)	ROE
1		170.6			1		132.1		
2	5.66 (s)	77.7	1, 1″, 3, 4, 5′		2		146.1		
3		80.1			3	6.82 (d, 2)	113.6	2, 4, 5	
4	2.99 (d, 14)	42.0	1′, 2, 2′, 3, 5, 6′		4		142.0		
	3.09 (d, 14)		1′, 2, 2′, 3, 5, 6′		5	6.67 (dd, 8, 2)	122.3	3, 4, 7	
5		174.9			6	6.73 (d, 8)	116.3	1, 2, 4, 7	
					7	2.77 (t, 7.5)	36.2	1, 5, 8	
1′		127.4			8	3.49 (t, 7.5)	42.4	1, 7	
2′	7.09 (d, 8)	132.6	3′, 4, 6′		OMe-2	3.83 (s)	56.5	2	
3′	6.67 (d, 8)	115.8	1′, 4′, 5′		1″		168.9		
4′		157.4			2″	6.47 (d, 16)	120.6	1″, 1‴	
5′	6.67 (d, 8)	115.8	1′, 3′, 4′		3″	7.45 (d, 16)	141.4	1″, 1‴, 2″, 2‴, 6‴	
6′	7.09 (d, 8)	132.6	2′, 4, 5′		1‴		131.1		
1″		168.1			2‴	7.16 (d, 2)	112.6	3″, 3‴, 4‴	
2″	6.54 (d, 16)	115.1	1″, 1‴		3‴		151.1		
3″	7.78 (d, 16)	148.3	1″, 1‴, 2″, 2‴, 6‴		4‴		149.0		
1‴		126.7			5‴	7.17 (d, 8)	117.6	1‴, 3‴, 4‴, 6‴	
2‴	6.97 (s)	107.3	1‴, 3″, 3‴, 4‴, 6‴		6‴	7.11 (dd, 8, 2)	122.7	2‴, 3″, 4‴	
3‴		150.0			OMe-3‴	3.88 (s)	56.8	3‴	2‴
4‴		140.0							
5‴		150.0			Gal-1	5.30 (d, 8)	100.4	4‴	
6‴	6.97 (s)	107.3	1‴, 2‴, 3″, 4‴, 5‴		2	3.65 (dd, 8, 3)	72.1	Gal-1	
OMe-3‴	3.90 (s)	56.9	3‴	2‴	3	4.16 (dd, 3, 3)	72.9	Gal-1, Gal-2, Gal-5	
OMe-5‴	3.90 (s)	56.9	5‴	6‴	4	3.61 (dd, 9.5, 3)	68.7	Gal-5, Gal-6	
					5	3.85 (overlapped)	76.0	Gal-4, Gal-6	
					6	3.69 (dd, 13, 5.5)	62.9	Gal-4	
						3.85 (overlapped)		Gal-4, Gal-5	

Table 4. Hyaluronidase Inhibitory Activities (IC_{50}) of Isolated Fukiic Acid Derivatives

compound	IC_{50}, μM
1	573
2	430
3	663
4	>658
5	525
6	143
7	193
8	>790
9	144
10	112
11	82
12	251
13	153
14	120
15	92
16	138
rosmarinic acid	545

linear gradient, UV 320 nm] to give 38 fractions (Frs. A−Z and a−l). The 87:13 solvent gave Fr. D [shomaside D (**4**) (13.5 mg)], the 77:23 solvent gave Fr. W [fukinolic acid (**9**)[3] (85 mg)], the 75:25 solvent gave Fr. a [cimicifugic acid D (**13**)[4] (136 mg)], the 74:26 solvent gave Fr. c [cimicifugic acid C (**12**)[3] (7.3 mg)] and Fr. e [cimicifugic acid A (**10**)[3] (88 mg)], the 73:27 solvent gave Fr. f [cimicifugic acid B (**11**)[3] (302 mg)] and Fr. h (**7**) (23.4 mg), the 71:29 solvent gave Fr. i [cimicifugic acid E (**14**)[4] (170 mg)] and Fr. j [cimicifugic acid F (**15**)[4] (655 mg)], and the 70:30 solvent gave Fr. k [cimicifugic acid G (**16**)[5] (19.7 mg)]. Then, Fr. N (35 mg) was subjected to semipreparative HPLC [column, Cosmosil 5PE-MS, 2 × 25 cm; solvent, 0.05% trifluoroacetic acid−CH_3CN (85:15), UV 320 nm] to give **1** (9.1 mg). Fr. S (74.9 mg) was subjected to semipreparative HPLC [column, Develosil C30-UG-5, 2 × 25 cm; solvent, 0.05% trifluoroacetic acid−CH_3CN (82.5:17.5), UV 320 nm] to give **2** (25.8 mg). Fr. R (17.8 mg) was subjected to semipreparative HPLC [column, Cosmosil 5PE-MS, 2 × 25 cm; solvent, 0.1% trifluoroacetic acid−CH_3CN (85:15), UV 320 nm] to give **3** (3.4 mg). Fr. U (41.6 mg) was subjected to semipreparative HPLC [column, Develosil C30-UG-5, 2 × 25 cm; solvent, 0.05% trifluoroacetic acid−CH_3CN (82.5:17.5), UV 320 nm] to give **5** (12.0 mg). Fr. d (16.3 mg) was subjected to semipreparative HPLC [column, Cosmosil 5PE-MS, 2 × 25 cm; solvent, 0.05% trifluoroacetic acid−CH_3CN (77.5:22.5), UV 320 nm] to give **6** (5.4 mg). Fr. V (35.5 mg) was subjected to semipreparative HPLC [column, Cosmosil 5C_{18}-AR-II 2 × 25 cm; solvent, 0.05% trifluoroacetic acid−CH_3CN (82.5:17.5), UV 280 nm] to give **8** (6.8 mg).

Shomaside A (1): pale brown powder, $[\alpha]^{24}_D$ −5.1 (*c* 0.79, MeOH); UV (MeOH) λ_{max} (log ε) 291 (4.15), 320 (4.14) nm; CD (MeOH) [θ] (nm) +2800 (280), +8400 (226), +12 200 (209); ^{1}H NMR and ^{13}C NMR, Table 1; HRFABMS *m*/*z* 609.1451 (calcd for $C_{27}H_{30}O_{16}$ − H, 609.1456).

Shomaside B (2): pale brown powder, $[\alpha]^{24}_D$ −5.0 (*c* 1.39, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.30), 296 (4.16), 321 (4.19) nm; CD (MeOH) [θ] (nm) +3300 (279), +15 200 (228); ^{1}H NMR and ^{13}C NMR, Table 1; HRFABMS *m*/*z* 593.1525 (calcd for $C_{27}H_{30}O_{15}$ − H, 593.1507).

Shomaside C (3): pale brown powder, $[\alpha]^{24}_D$ −8.3 (*c* 0.42, MeOH); UV (MeOH) λ_{max} (log ε) 219sh (4.26), 293 (4.09), 319 (4.10) nm; CD (MeOH) [θ] (nm) +2400 (279), +6800 (227); ^{1}H NMR and ^{13}C NMR, Table 1; HRFABMS *m*/*z* 593.1517 (calcd for $C_{27}H_{30}O_{15}$ − H, 593.1507).

Shomaside D (4): pale brown powder, $[\alpha]^{24}_D$ −11.8 (*c* 0.55, MeOH); UV (MeOH) λ_{max} (log ε) 286 (4.03), 314 (3.97) nm; CD (MeOH) [θ] (nm) +1200 (276), +4800 (224); ^{1}H NMR and ^{13}C NMR, Table 2; HRFABMS *m*/*z* 547.1449 (calcd for $C_{28}H_{32}O_{15}$ − $COOCH_3$ − 2H, 547.1452).

Cimicifugic acid H (5): pale brown powder, $[\alpha]^{22}_D$ +25.5 (*c* 0.55, MeOH); UV (MeOH) λ_{max} (log ε) 290 (4.15), 324 (4.20) nm; CD (MeOH) [θ] (nm) +6500 (232), +19 100 (208); ^{1}H NMR and ^{13}C NMR, Table 2; HRFABMS *m*/*z* 391.0669 (calcd for $C_{18}H_{16}O_{10}$ − H, 391.0665).

Cimicifugic acid I (6): pale brown powder, $[\alpha]^{24}_D$ +45.3 (*c* 0.96, MeOH); UV (MeOH) λ_{max} (log ε) 293sh (3.78), 331 (4.10) nm; CD (MeOH) [θ] (nm) +2700 (326), +2800 (285), +17 800 (232); ^{1}H NMR and ^{13}C NMR, Table 3; HRFABMS *m*/*z* 477.1023 (calcd for $C_{22}H_{22}O_{12}$ − H, 477.1033).

Cimicifugic acid J (7): pale brown powder, $[\alpha]^{24}_D$ +19.4 (*c* 0.49, MeOH); UV (MeOH) λ_{max} (log ε) 225 (4.22), 329 (4.04) nm; CD (MeOH) [θ] (nm) +1400 (324), +1600 (275), +9400 (229); ^{1}H NMR and ^{13}C NMR, Table 3; HRFABMS *m*/*z* 461.1084 (calcd for $C_{22}H_{22}O_{11}$ − H, 461.1084).

Shomaside E (8): pale brown powder, $[\alpha]^{24}_D$ −42.9 (*c* 1.12, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.28), 288 (4.25), 314 (4.19) nm; ^{1}H NMR and ^{13}C NMR, Table 3; HRFABMS *m*/*z* 506.1819 (calcd for $C_{25}H_{30}O_{11}$ − H, 506.1788).

Fukinolic acid (9): CD (MeOH) [θ] (nm) +2300 (322), +3400 (283), 12 600 (228), +17 400 (207).

Acid Hydrolysis of Compounds 1, 2, 3, 4, and 8.[14] **C**ompounds **1**, **2**, **3**, **4**, and **8** (ca. 1 mg) were dissolved in dioxane (80 μL) and 2 M HCl (20 μL), respectively. The solution was heated at 60° for 1 h. After hydrolysis, this reaction mixture was diluted with H_2O and extracted with EtOAc. The H_2O layer was neutralized with an Amberlite IRA-60E column, and the eluate was concentrated to dryness. The residue was stirred with D-cysteine methyl ester hydrochloride (3 mg) in pyridine (25 μL) at 60° for 1.5 h. After warming, the trimethylsilylation reagent (hexamethyldisilazane and trimethylsilyl chloride) was added, and the warming at 60° was continued for another 30 min. The precipitates were centrifuged, and the supernatant was subjected to GC analysis. GC conditions: column, GL Sciences capillary column TC-1 0.25 mm × 30 m; carrier gas, N_2; column temperature 230 °C; t_R 21.2 min (D-glucose), 20.2 min (L-glucose), 22.9 min (D-galactose), 21.7 min (L-galactose). D-Glucose was detected from the mixture of compounds **3** and **4**. D-Galactose was detected from the mixture of compounds **1**, **2**, and **8**.

Assay of Inhibitory Effects on Hyaluronidase. Inhibitory effects on hyaluronidase were determined by the Morgan−Elson method[15] as modified by Kakegawa et al.[16] Thes, 0.1 mL of a hyaluronidase solution (Type IV-S, derived from bovine testis, Sigma 400 NF units/mL) was added to 0.2 mL of a 0.1 mol/L acetate buffer (pH 3.5) containing a study sample, and the mixture was allowed to react for 20 min at 37 °C. Next, 0.2 mL of 2.5 mmol/L calcium chloride was added as an activator, and the mixture was allowed to react for 20 min at 37 °C. A 0.5 mL aliquot of a 0.4 mg/mL potassium hyaluronate solution (derived from a rooster comb) was added to the mixture and was allowed to react for 40 min at 37 °C. Subsequently, 0.2 mL of 0.4 mol/L sodium hydroxide was added to stop the reaction, and the mixture was cooled. Boric acid solution (0.2 mL) was added to each solution, and the mixture was boiled for 3 min. The mixture was cooled using ice, 6 mL of a *p*-dimethylaminobenzaldehyde reagent was added, and the mixture was allowed to react for 20 min at 37 °C. After the reaction, the absorbance at λ 585 nm was measured. A blank test was performed in the same fashion as a negative control. The following is the formula used for inhibitory effects on hyaluronidase activity: Rate of inhibiting hyaluronidase activity (%) = [1 − (St − Sb)/(Ct − Cb)] × 100, where St is absorbance of the solution containing a study sample at λ 585 nm, Sb is absorbance of the blank solution without a study sample at λ 585 nm, Ct is absorbance of the control solution at λ 585 nm, and Cb is absorbance of the blank control solution at λ 585 nm. The IC_{50} value of inhibitory effects on hyaluronidase was determined within the range of the concentration.

Note Added after ASAP Publication: This paper was published on the Web on Mar 1, 2010, with errors in the compound references in the paragraphs immediately preceding the Experimental Section. The corrected version was reposted on Mar 5, 2010.

Supporting Information Available: ^1H and ^{13}C NMR spectra of new compounds and HPLC chart of the "Cimicifugae Rhizoma" used in this study are available free of charge via the Internet at http://pubs.acs.org.

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