Cyclic Spermidine Alkaloids and Flavone Glycosides from *Meehania fargesii*

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The dried whole plant of *Meehania fargesii* (H. LÉV.) C. Y. WU is utilized as an antipyretic and antidote for poison in China. Water soluble fractions, obtained from an 80% acetone extract of the whole plant, showed hyaluronidase inhibitory activity. From these fractions, hyaluronidase inhibitors were isolated along with two new spermidine alkaloids (meefarnines A and B), four new flavone glycosides (fargenins A—D), and 10 known compounds. The structure of each was elucidated by spectroscopic methods.

Key words spermidine alkaloid; polyamine; flavone glycoside; *Meehania fargesii*; Lamiaceae; hyaluronidase inhibitor

Meehania fargesii (H. LÉV.) C. Y. WU is a climbing herbaceous plant (Lamiaceae) known as Chinese herb. The dried or fresh whole plant and the dried root have been used to treat colds, pain and poisoning.[1)] Two caffeic acid esters in this plant were reported.[2)] However, studies on other constituents and the biological activities of this plant are not sufficient. The water soluble fractions obtained from an 80% acetone extract of *M. fargesii* were found to have an inhibitory effect on hyaluronidase. Hyaluronidase degrades hyaluronic acid and its inhibitors are known to have anti-allergic activity.[3,4)] Moreover, they are potent anti-inflammatory, anti-aging, and anti-cancer agents.[5)] In the present study, we investigated constituents of fractions which showed strong hyaluronidase inhibitory activity from whole plants of *M. fargesi*. Rosmarinic acid, lithospermic acid B, diosmetin-7-*O*-*β*-D-glucuronide, and apigenin-7-*O*-*β*-D-glucuronide were identified as hyaluronidase inhibitors. Two new cyclic spermidine alkaloids, meefarnines A and B (**1**, **2**), four new flavone glycosides, fargenins A—D (**3**—**6**), and the methyl ester (**4a**) of **4** have also been isolated together with 10 known compounds and we describe the elucidation of their structure.

Dried whole plants of *M. fargesii* were extracted with acetone–water (8 : 2). The concentrated extract was partitioned between water and diethyl ether. The water layer was fractionated using the inhibitory activity against hyaluronidase as a marker during column chromatography (a porous polymer gel column and octadecyl silica (ODS) columns). From the active fractions (Fr. 1A—D, Table 3), 21 compounds were isolated. Known compounds were identified from spectroscopic data as apigenin-7-*O*-*β*-D-glucuronopyranoside,[6)] apigenin-7-*O*-*β*-D-glucuronopyranoside methyl ester,[6,7)] acacetin-7-*O*-*β*-D-glucuronopyranoside,[6,8)] luteolin-7-*O*-*β*-D-glucuronopyranoside,[9)] hyperin,[10)] diosmetin-7-*O*-*β*-D-glucuronopyranoside methyl ester,[7)] acacetin,[11)] pectolarigenin,[12)] ladanein,[12)] 4-{[6-*O*-(4-hydroxy-3,5-dimethoxybenzoyl)-*β*-D-glucopyranosyl]oxy}-3-methoxybenzoic acid,[13)] rosmarinic acid,[14)] 7-epiblechnic acid,[15)] and lithospermic acid B.[16)] Four principal components of Fr. 1A and Fr. 1B, rosmarinic acid, lithospermic acid B, diosmetin-7-*O*-*β*-D-glucuronopyranoside, and apigenin-7-*O*-*β*-D-glucuronopyranoside, were assayed for hyaluronidase inhibitory activity. Rosmarinic acid is already known as a hyaluronidase inhibitor.[17)] Lithospermic acid B showed strong inhibitory activity, and diosmetin-7-*O*-*β*-D-glucuronopyranoside and apigenin-7-*O*-*β*-D-glucuronopyranoside had moderate inhibitory activity as shown in Table 4.

Meefarnines A (**1**) and B (**2**) were revealed to have the molecular formula $C_{25}H_{31}N_3O_4$ based on high resolution (HR)-FAB-MS [*m*/*z* 438.2411 (**1**) and 438.2394 (**2**), Calcd for $C_{25}H_{32}N_3O_4$, 438.2395]. However, the ^{13}C-NMR spectrum acquired in methanol-d_4 at 30 °C showed more than 25 signals and the ^{1}H-NMR spectrum showed the presence of two or more sets of closely spaced resonances (Table 1). These spectra were attributed to the restricted rotation of the amide bonds.[18—21)] ^{1}H- and ^{13}C-NMR spectra of **1** were acquired in dimethyl sulfoxide (DMSO)-d_6 at temperatures between 30 °C and 100 °C (Fig. 2). The ^{1}H- and ^{13}C-NMR spectra at 100 °C of **1** and **2** showed one set of resonances (Table 1), which were similar to those of a cyclic spermidine alkaloid: (*S*)-dihydroperiphylline[22,23)] except for the aromatic and olefinic signals. For **1**, analyses of the ^{1}H-NMR and ^{1}H–^{1}H correlation spectroscopy (COSY) spectra in methanol-d_4 showed the presence of a N–C(=O)–CH_2–CH–N spin system [δ 3.72 (overlapped, H-4), 3.79 (dd, J=7.5, 7.0 Hz, H-4), and 2.36 (br d, J=7.0 Hz, H-3)], a N–$(CH_2)_3$–N spin system [δ 2.13 (m, H-6), 2.43 (ddd, J=12.5, 6.5, 2.0 Hz, H-6), 2.53 (ddd, J=12.5, 5.5, 2.0 Hz, H-6), 1.37 (m, H-7), 1.60 (overlapped, H-7), 1.90 (overlapped, H-7), 3.40—3.52 (overlapped, H-8), and 3.65 (m, H-8)], and a N–$(CH_2)_4$–N spin system [δ 3.36—3.55 (overlapped, H-10), 3.69—3.77 (overlapped, H-10), 1.80 (overlapped, H-11), 1.40—1.53 (overlapped, H-12), 1.61—1.70 (overlapped, H-12), 3.21 (m, H-13), and 3.36—3.46 (overlapped, H-13)]. The proton signals of H-6 and H-7 were divided due to the rotamer of the amide

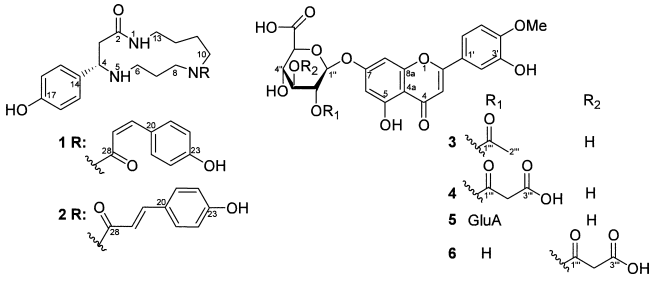

Fig. 1. Structures of **1**—**6**

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Table 1. ^{1}H- and ^{13}C-NMR Spectroscopic Data for Compounds **1** and **2**

Position	**1**						**2**					
	δ_H (J in Hz)[a]	δ_C[b]	HMBC (H to C)	δ_H (J in Hz)[c]	δ_C[d]	NOE (H to H)	δ_H (J in Hz)[a]	δ_C[b]	HMBC (H to C)	δ_H (J in Hz)[c]	δ_C[d]	NOE (H to H)
1				7.68 (m)						7.75 (m)		
2		175.0			170.5			175.0 175.1			170.5	
3	2.36 (br d, 7.0)	46.9 47.0	4	2.19 (dd, 13.0, 3.5) 2.25 (dd, 13.0, 11.0)	45.6		2.40 (br d, 6.5)	47.1 47.2	2	2.22 (dd, 13.0, 3.0) 2.78 (dd, 13.0, 11.0)	45.6	
4	3.72[e] 3.79 (dd, 7.5, 7.0)	61.2	2, 3, 6, 14, 15, 19	3.77 (dd, 11.0, 3.5)	58.6	15, 19	3.86 (m)	61.1 61.2	2, 6, 14, 15, 19	3.82 (dd, 11.0, 3.0)	58.6	15, 19
6	2.13 (m) 2.43 (ddd, 12.5, 6.5, 2.0) 2.53 (ddd, 12.5, 5.5, 2.0)	43.8[f] 43.9[f]	4, 8	2.11 (m) 2.45[e]	42.1		2.25 (ddd, 12.5, 6.5, 3.0) 2.59 (m)	43.7 44.0		2.17 (m) 2.49[e]	42.2	
7	1.37 (m) 1.60[e] 1.90[e]	28.8 30.4		1.50[e] 1.65[e]	26.0[g]		1.58 (m) 1.78—1.94[e]	29.5 31.7		1.25 (m) 1.71[e]	26.2[g]	
8	3.40—3.52[e] 3.65 (m)	43.3[f] 46.4[f]	7, 10, 28	3.12[e] 3.30—3.50[e]	48.2[g]		3.62—3.80[e]	45.4 45.7	7, 28	3.20[e] 3.40[e]	47.8[g]	
10	3.36—3.55[e] 3.69—3.77[e]	47.2[f] 51.0	28	3.25—3.55[e]	43.2[g]		3.49—3.60[e] 3.65—3.78[e]	49.5 50.2	12, 28	3.35—3.60[e]	43.1	
11	1.80[e]	25.5 26.4	10, 12, 13	1.35—1.95[e]	24.4[h]		1.75—1.88[e]	26.4 27.9		1.45—2.00[e]	24.6	
12	1.40—1.53[e] 1.61—1.70[e]	26.0 26.6	10, 13	1.35—1.95[e]	24.6[h]		1.60—1.72[e]	26.0 26.2		1.45—2.00[e]	24.6	
13	3.21 (m) 3.36—3.46[e]	40.0 40.2	2, 12	3.14[e] 3.30[e]	37.4		3.25—3.38[e] 3.43—3.57[e]	40.0	2, 12	3.20[e] 3.33[e]	37.4	
14		135.5			134.0			135.5			134.1	
15	7.09 (br d, 8.5)	128.4 128.5	16, 17	7.07 (br d, 8.5)	126.7		7.13 (br d, 8.5)	128.5	14, 16, 17, 19	7.09 (br d, 8.5)	126.7	
16	6.74 (br d, 8.5) 6.76 (br d, 8.5)	116.5	14, 17	6.70 (br d, 8.5)	115.5		6.75 (br d, 8.5)	116.5	15, 17, 18	6.70 (br d, 8.5)	114.7	
17		157.8			155.7			157.9			155.8	
18	6.74 (br d, 8.5) 6.76 (br d, 8.5)	116.5	14, 17	6.70 (br d, 8.5)	115.5		6.75 (br d, 8.5)	116.5	16, 17, 19	6.70 (br d, 8.5)	114.7	
19	7.09 (br d, 8.5)	128.4 128.5	17, 18	7.07 (br d, 8.5)	126.7		7.13 (br d, 8.5)	128.5	14, 15, 17, 18	7.09 (br d, 8.5)	126.7	
20		128.0 128.1			126.7			126.9			125.3	
21	7.21 (br d, 8.5) 7.22 (br d, 8.5)	131.1 131.2	20, 22, 23, 26	7.21 (br d, 8.5) 6.65 (br d, 8.5)	129.4	26	7.40 (br d, 8.5) 7.42 (br d, 8.5)	130.9	22, 23, 25, 26	7.34 (br d, 8.5)	128.5	
22	6.70 (br d, 8.5) 6.71 (br d, 8.5)	116.5	23		114.7		6.75 (br d, 8.5)	117.5	20, 24	6.64 (br d, 8.5)	115.7	
23		160.0			157.6			163.0			159.5[g]	
24	6.70 (br d, 8.5) 6.71 (br d, 8.5)	116.5	23	6.65 (br d, 8.5)	114.7		6.75 (br d, 8.5)	117.5	20, 22	6.64 (br d, 8.5)	115.7	
25	7.21 (br d, 8.5) 7.22 (br d, 8.5)	131.1 131.2	20, 23, 24, 26	7.21 (br d, 8.5)	129.4	26	7.40 (br d, 8.5) 7.42 (br d, 8.5)	130.9	21, 23, 24, 26	7.34 (br d, 8.5)	128.5	
26	6.51 (d, 12.5) 6.56 (d, 12.5)	134.3	28	6.39 (d, 13.0)	131.3	21, 25, 27	7.50 (d, 15.5)	144.4	21, 25, 28	7.33 (d, 16.0)	138.4	
27	5.90 (d, 12.5)	121.0 121.1	28	5.87 (d, 13.0)	120.7		6.76[e]	114.2 114.4	28	6.66 (d, 16.0)	113.2	
28		172.0 172.1			167.6			169.4			165.3[g]	

a) Recorded in CD_3OD at 30 °C, 400 MHz. *b*) Recorded in CD_3OD at 30 °C, 100 MHz. *c*) Recorded in DMSO-d_6 at 100 °C, 400 MHz. *d*) Recorded in DMSO-d_6 at 100 °C, 100 MHz. *e*) Unclear signal pattern due to overlapping. *f*,*h*) Assignments are interchangeable. *g*) Data were obtained from the HMQC and HMBC spectra.

Table 2. ^{1}H- and ^{13}C-NMR Spectroscopic Data (400 MHz, DMSO-d_6) for Compounds **3**—**6**

Position	**3**		**4**		**4a**		**5**		**6**		Diosmetin-7-*O*-glucuronide	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
2		164.2		164.2		164.2		164.1		164.2		164.1
3	6.83, s	103.8	6.84, s	103.8	6.85, s	103.8	6.82, s	103.8	6.84, s	103.9	6.83, s	103.8
4		181.9		181.9		181.9		182.0		181.9		181.9
4a		105.8		105.8		105.8		105.5		105.6		105.5
5		161.3		161.2		161.2		161.1		161.2		161.2
6	6.42, d (2.5)	99.1	6.43, d (2.0)	99.4	6.42, d (2.0)	99.2	6.42, d (2.0)	99.2	6.50, d (2.5)	99.4	6.48, d (2.0)	99.2
7		161.8		161.8		161.7		162.2		162.2		162.5
8	6.78, d (2.5)	94.7	6.76, d (2.0)	95.0	6.78, d (2.0)	94.8	6.76, d (2.0)	94.6	6.86, d (2.5)	94.7	6.85, d (2.0)	94.6
8a		156.9		156.8		156.8		156.9		156.9		157.0
5-OH	12.96, s		12.95, s		12.97, s		12.93, s		12.97, s		12.95, s	
1′		122.8		122.8		122.8		122.9		122.8		122.9
2′	7.44, d (2.0)	113.1	7.45, d (2.0)	113.1	7.45, d (2.0)	113.1	7.45, d (2.0)	113.1	7.46, d (2.5)	113.1	7.46, d (2.5)	113.1
3′		146.8		146.8		146.8		146.8		146.8		146.8
4′		151.3		151.3		151.3		151.3		151.3		151.3
5′	7.10, d (9.0)	112.1	7.12, d (8.5)	112.1	7.11, d (8.5)	112.1	7.12, d (8.5)	112.1	7.12, d (8.5)	112.1	7.10, d (8.5)	112.1
6′	7.57, dd (9.0, 2.0)	118.9	7.57, dd (8.5, 2.0)	118.9	7.57, dd, (8.5, 2.0)	118.9	7.57, dd (8.5, 2.0)	118.8	7.57, dd (8.5, 2.5)	118.9	7.57, dd (8.5, 2.5)	118.9
3′-OH	9.42, s		9.46, s		9.45, s		9.45, s		9.45, s		9.45, s	
4′-OMe	3.87, s	55.8	3.88, s	55.8	3.88, s	55.8	3.88, s	55.8	3.88, s	55.8	3.88, s	55.8
1″	5.57, d (8.5)	96.7	5.56, d (8.0)	96.7	5.62, d (8.5)	96.5	5.45, d (7.0)	97.5	5.48, d (8.0)	98.7	5.29, d (7.0)	99.4
2″	4.82, dd (8.5, 9.0)	73.0	4.86, dd (8.0, 8.5)	73.6	4.86, dd (8.5, 9.0)	73.8	3.56, overlapped	82.3	3.51, overlapped	70.5	3.30—3.50, overlapped	72.8
3″	3.54, overlapped	72.9	3.61, overlapped	72.9	3.50—3.60, overlapped	72.6	3.45—3.52, overlapped	74.8	4.99, t (9.5)	77.3	3.30—3.50, overlapped	75.4
4″	3.51, overlapped	71.2	3.55, overlapped	71.2	3.50—3.60, overlapped	71.2	3.45—3.52, overlapped	70.7	3.63, dd (10.0, 9.5)	69.0	3.30—3.50, overlapped	71.2
5″	4.14, d (9.0)	75.3	4.16, d (9.0)	75.2	4.32, d (9.0)	75.0	4.11, d (9.5)	75.0	4.22, d, (10.0)	74.9	4.06, d (9.0)	75.6
6″		169.5		169.6		169.7		170.1		169.3		170.0
6″-OMe					3.70, s	52.0						
1‴		169.1		165.8		166.4	4.55, d (7.5)	104.3		166.3		
2‴	2.03, s	20.7	3.40, overlapped	41.5	3.53, s	41.3	3.03, dd (8.5, 7.5)	74.1	3.42, s	41.4		
3‴				167.5		167.4	3.23, overlapped	75.5		167.7		
4‴					3.52, s	51.9	3.26, overlapped	71.6				
5‴							3.66, d (9.0)	75.8				
6‴								169.7				

Table 3. Hyaluronidase Inhibitory Activity of Fractions of *M. fargesii* Obtained with a Porous Polymer Gel and an ODS Column

Fraction	Hyaluronidase inhibition (%)
Water eluate	−6.38±3.94
30% MeOH eluate	26.8±4.62
MeOH eluate	82.8±1.52
Fr. 1A	86.9±3.97
Fr. 1B	92.8±2.16
Fr. 1C	65.5±4.07
Fr. 1D	65.1±6.92
Fr. 1E	31.9±2.55

All values represent the mean±S.E. (n=3).

Table 4. Hyaluronidase Inhibitory Activity of Compounds from *M. fargesii*

Compound	Content (%)[a)]	IC_{50} (μM)
Rosmarinic acid	1.36	309
Lithospermic acid B	1.63	164
Apigenin-7-β-D-glucuronide	0.96	548
Diosmetin-7-β-D-glucuronide	1.94	644
DSCG		297

a) g/g of the methanol eluate fraction.

bond. These spin system protons and corresponding carbons displayed the presence of a cyclic spermidine 13-membered ring moiety, which was either a periphylline-type[22)] skeleton (1,4,9-triazacyclotridecan-4-one) or a celacinnine-type[24)] skeleton (1,4,9-triazacyclotridecan-2-one). Both skeletons were possible *via* some biosynthetic pathway of naturally occurring macrocyclic spermidine alkaloids.[25)] In the heteronuclear multiple bond correlation (HMBC) spectrum, the H-6 signals were long-range coupled with the carbon of C-4 (δ 61.2). Other long-range couplings included the H-8 signals with the carbon of C-10 (δ 51.0), and H-13 signals with the carbon of C-2 (δ 175.0). These data demonstrared that the 13-membered ring spermidine moiety was the periphylline-type skeleton as shown in Fig. 3. The absolute configuration of C-4 was assumed to be *S* since all other known macrocyclic spermidine alkaloids have the (*S*)-configuration. The circular dichroism (CD) spectrum of **1** revealed a weak cotton effect around 210—240 nm, which was comparable to that reported for the (*S*)-configuration of cyclic spermidines.[25—28)]

The ^{1}H-NMR spectrum of the AB-type benzene ring signals at δ 6.70 (br d, J=8.5 Hz, H-22, 24), 6.71 (br d, J=8.5 Hz, H-22, 24), 7.21 (br d, J=8.5 Hz, H-21, 25), and 7.22 (br d, J=8.5 Hz, H-21, 25) and the olefinic signals at δ 6.51 (d, J=13.0 Hz, H-26), 6.56 (d, J=13.0 Hz, H-26), and 5.90 (d, J=13 Hz, H-27) suggested the presence of a (*Z*)-*p*-coumaroyl unit. In the HMBC spectrum, the H-8 and -10 signals were long-range coupled with the carbon of C-28 (δ 172.0, 172.1). The other AB type benzene ring signals at δ 6.74 (br d, J=8.5 Hz, H-16, 18), 6.76 (br d, J=8.5 Hz, H-16, 18), and 7.09 (br d, J=8.5 Hz, H-15, 19) showed the presence of a *p*-hydroxybenzene moiety. The proton signals of H-16, 18, 21, 22, 24, 25 and 26 were divided due to the rotamer of the amide bond. In the HMBC spectrum, the H-4 methine signals were long-range coupled with the carbons of C-14 (δ 135.5) and C-15 and 19 (δ 128.4, 128.5). Hence, **1** was identified as (*S*)-4-(4-hydroxyphenyl)-9-[(2*Z*)-1-oxo-3-(4-hydroxyphenyl)-2-propen-1-yl]-1,5,9-triazacyclotridecan-2-one.

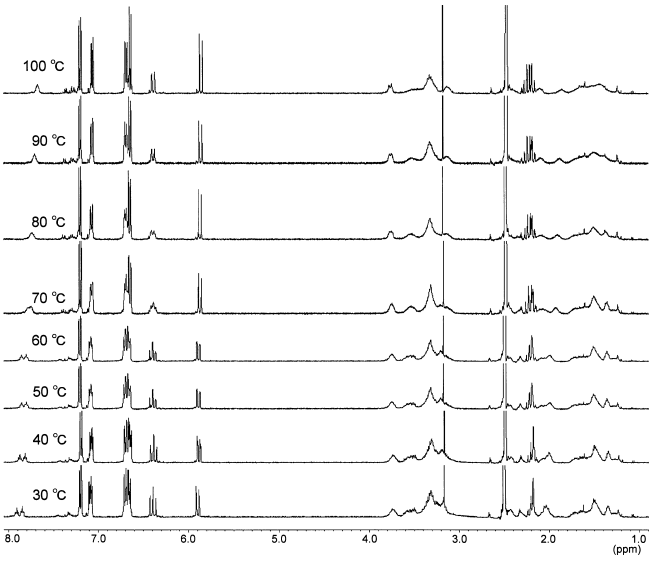

Fig. 2. ^{1}H-NMR Spectra of **1** in DMSO-d_6 at Temperatures from 30 to 100 °C

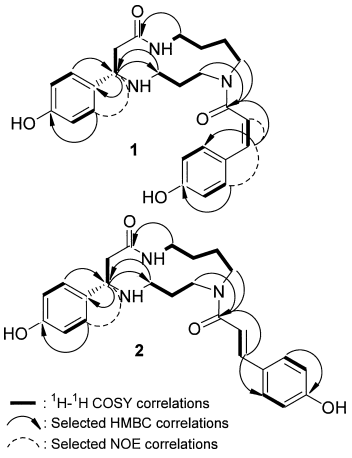

Fig. 3. ^{1}H–^{1}H COSY, Key HMBC and Key NOE Correlations for Compounds **1** and **2**

The ^{1}H- and ^{13}C-NMR spectra (in methanol-d_4) of **2** were similar to those of **1** except for the olefinic signals. The ^{1}H-NMR spectrum of the olefinic signals at δ 6.76 (overlapped, H-27) and 7.50 (d, J=15.5 Hz, H-26) suggested the presence of a (*E*)-*p*-coumaroyl unit. Hence, **2** was identified as (*S*)-4-(4-hydroxyphenyl)-9-[(2*E*)-1-oxo-3-(4-hydroxyphenyl)-2-propen-1-yl]-1,5,9-triazacyclotridecan-2-one.

Diosmetin-7-*O*-glucuronide was obtained as a flavone glycoside. The full assignments of the ^{1}H- and ^{13}C-NMR spectra of this compound in DMSO-d_6 are shown in Table 2. A nuclear Overhauser effect (NOE) correlation between H-OMe (δ 3.88, 3H, s) and H-5′ (δ 7.10, d, J=8.5 Hz) supported that aglycone had a diosmetin skeleton. This compound seemed to be a main flavone glycoside in *M. fargesii*.

Five novel flavone glucuronides were obtained as amorphous powders. The ^{1}H- and ^{13}C-NMR data are shown in Table 2. These spectra and the UV spectra[29)] of compounds **3**—**6** suggested that they were diosmetin-7-glycoside derivatives. For fargenin A (**3**), the molecular formula $C_{24}H_{22}O_{13}$

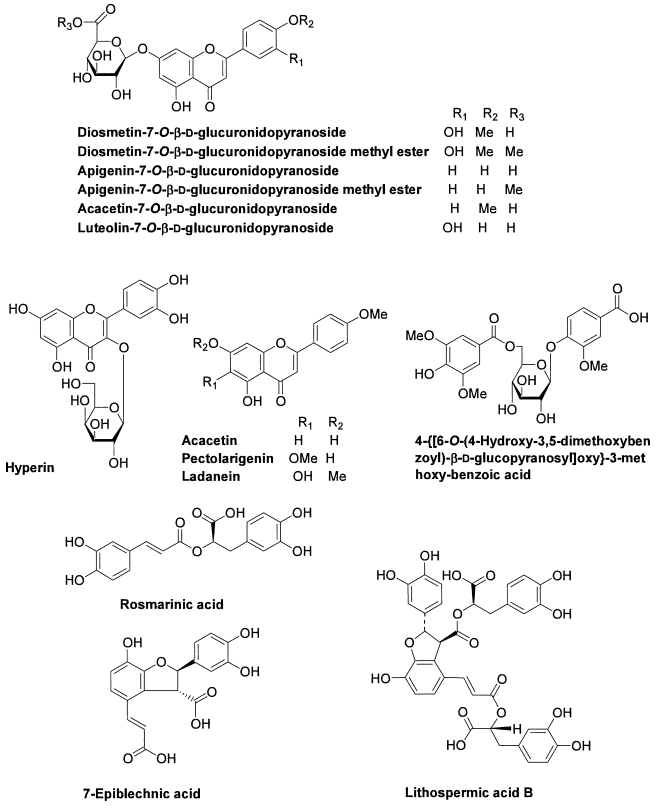

Fig. 4. Structure of Known Compounds

was deduced by HR-FAB-MS (*m/z* 517.0997, Calcd for $C_{24}H_{21}O_{13}$, 517.0981). The ^{1}H- and ^{13}C-NMR spectra of **3**, with an anomeric proton signal at δ 5.57 (1H, d, J=8.5 Hz, H-1″), suggested the presence of a monosaccharide. The corresponding anomeric carbon (δ 96.7, C-1″), four oxymethine carbons [δ 73.0 (C-2″), 72.9 (C-3″), 71.2 (C-4″), 75.3 (C-5″)] and a carbonyl carbon (δ 169.5, C-6″) indicate the 2-acylated glucuronic acid moiety. The H-2″ resonance at the δ 4.82 (1H, dd, J=8.5, 9.0 Hz) proton signal of **3** was shifted downfield relative to the H-2″ signal of diosmetin-7-*O*-glucuronide. Singlet methyl protons at δ 2.03 (3H) suggested the presence of an acetyl group. In the HMBC spectrum, the H-2″ signal was long-range coupled with the acetyl group carbon of C- 1‴ (δ 169.1). From these data, the structure of **3** was identified as shown in Fig. 1.

The ^{1}H- and ^{13}C-NMR spectra of fargenin B (**4**) were similar to those of **3**. The ^{1}H-NMR signal at δ 3.40 (overlapped) and the ^{13}C-NMR signals at δ 41.5, 165.8, and 167.5 suggested that **4** had a malonyl group instead of the acetyl group of **3**. In the HMBC spectrum, the H-2″ signal (δ 4.86, dd, J= 8.5, 8.0 Hz) was long-range coupled with the carbonyl carbon of C-1‴ (δ 165.8). The compound **4** showed an $[M+H]^+$ ion peak in HR-FAB-MS at 563.1043 corresponding to a molecular formula of $C_{25}H_{22}O_{15}$ (Calcd for $C_{25}H_{23}O_{15}$, *m/z* 563.1036). Hence, the structure of **4** was determined as shown in Fig. 1. The compound **4a** was obtained as the 6″,3‴-dimethyl ester of **4**. In the HMBC spectra, two methoxy singlet proton signals at δ 3.52 (3H, s) and 3.70 (3H, s) were correlated with the carbonyl carbon signals at δ 167.4 (C-3‴) and 169.7 (C-6″), respectively.

^{1}H- and ^{13}C-NMR spectra showed that fargenin C (**5**) was a 2″-substituted diosmetin glucuronic acid similar to **3**. On the basis of HR-FAB-MS, the molecular formula $C_{28}H_{28}O_{18}$ was deduced (*m/z* 653.1373, Calcd for $C_{28}H_{28}O_{18}$, 653.1353). The anomeric carbon at δ 104.3 suggested the presence of another monosaccharide moiety. The corresponding anomeric proton at δ 4.55 (1H, d, J=7.5 Hz, H-1‴), and the ^{1}H–^{1}H COSY and ^{13}C-NMR signals at δ 74.1 (C-2‴), 75.5 (C-3‴), 71.6 (C-4‴), 75.8 (C-5‴), and 169.7 (C-6‴) showed the presence of a β-glucuronopyranosyl moiety. Sugar analysis showed D-glucuronic acid.[30] These data suggested that **5** had a β-D-glucuronic acid moiety instead of the acetyl group of **3**. In the HMBC spectrum, the H-1‴ signal was long-range coupled with the carbon of C-2″ (δ 82.3). Hence, the structure of **5** was identified as shown in Fig. 1.

For fargenin D (**6**), the ^{1}H- and ^{13}C-NMR data suggested the presence of a malonyl moiety on an acylated glucuronic acid similar to **4**. The H-3″ resonance at the δ 4.99 (1H, t, J= 9.5 Hz) proton signal of **6** was shifted downfield relative to the H-3″ signal of diosmetin-7-*O*-glucuronide. In the HMBC spectrum, the H-3″ signal was long-range coupled with the carbon of C-1‴ (δ 166.3). The ^{1}H-NMR signal at δ 3.42 (2H, s) and the ^{13}C-NMR signals at δ 41.4, 166.3, and 167.7 suggested that **6** had a malonyl group on C-3″. The molecular formula $C_{25}H_{22}O_{15}$ was confirmed on the basis of HR-FAB-MS (*m/z* 563.1031, Calcd for $C_{25}H_{23}O_{15}$, 563.1036). Thus, the structure of **6** was established as shown in Fig. 1.

From the whole plant of *Meehania fargesii*, hyaluronidase inhibitors, two new spermidine alkaloids and four new flavone glycosides were isolated. Some phenylpropanoid oligomers are potent hyaluronidase inhibitors.[17,31] Lithospermic acid B is a phenylpropanoid tetramer with inhibitory activity comparable to that of the oligomers. Some flavonoids have been reported to inhibit of hyaluronidase,[32,33] and diosmetin-7-*O*-β-D-glucuronide and apigenin-7-*O*-β-D-glucuronide were also revealed as active compounds. Previously, in phytochemical studies of the *Meehania* genus, we found 23 spermidine alkaloidal glycosides in *M. urticifolia*.[27,28] Polyamine alkaloids have a rich diversity of skeletons and biological activities.[21,34,35] The 13-membered ring moieties of **1** and **2** were a periphylline-type skeleton, and different from that of cyclic spermidine alkaloidal glycosides from *M. urticifolia*, which have a celacinnine-type skeleton. Periphylline and celacinnine had been isolated from Celastraceae plants.[22,24] *Meehania* genus plants also have two types of skeletons. Compounds **3**, **4**, and **6** were esters of diosmetin-7-*O*-glucuronide which have hyaluronidase inhibitory activity, and **5** was diosmetin-diglucuronide.

Experimental

General Procedures Optical rotations were recorded on a Jasco P-2300 polarimeter. CD spectra were recorded on a Jasco J-700 spectropolarimeter; UV, on a Shimadzu MPS-2450; and IR on a Perkin Elmer Spectrum One FT-IR spectrometer. ^{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 tetramethylsilane (TMS) as an internal standard. HR-FAB-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 an ODS (Cosmosil 140 C_{18}-OPN, Nacalai Tesque, 150 g) were used for column chromatography. Preparative Yamazen Cartridge Column Chromatography (YCCC) was performed on a Jasco 2089 (column, Ultra Pack ODS-SM-50C-M, Yamazen, 37×100 mm; detector, UV at 210 nm). Preparative HPLC was performed on a Jasco 2089 and detected with UV at 210 nm (columns, ODS-100V, Tosoh,

20×250 mm; 5C_{18}-AR-II, Cosmosil, 20×250 mm; Capcell-Pak Ph, Shiseido, 20×250 mm; YMC-Pack ODS-AM, YMC, 10×300 mm; Mightysil RP-18 GP, Kanto Chemical, 10×250 mm).

Plant Material *Meehania fargesii* (H. Lév.) C. Y. Wu was collected in May 2008 in JiuJiang City Lushan of Jiangxi Province, China. The plant was identified by Ceming Tan of the Forestry Administration of Jiujiang county. A voucher specimen was deposited at the herbarium of Tohoku Pharmaceutical University, No. 20080601.

Extraction and Isolation Powdered whole plants (792 g) of *M. fargesii* were extracted with acetone–water (8 : 2), (7 l) twice at room temperature for a month. The extract was concentrated at reduced pressure, suspended in water (1.5 l), and extracted with ether (1.0 l) three times. The water layer extract (80.13 g) was dissolved in water and passed through a porous polymer gel column and eluted with water, 30% MeOH, and MeOH. The MeOH eluate extract (4.96 g) was chromatographed on a reversed-phase column using ODS and eluted with 30%, 40%, 50%, 60%, 80% MeOH, and MeOH (fractions 1A—F). Fraction 1A (2.10 g) was subjected to Yamazen Cartridge Column Chromatography (YCCC) [mobile phase, methanol–0.2% TFA (25 : 75), (35 : 65) and (45 : 55)] to give 15 fractions (Frs. 2A—O). Fractions 2D and 2E (394.1 mg) were subjected to HPLC [ODS-100V, mobile phase, acetonitrile–water (20 : 80), Capcell-Pak Ph, mobile phase, acetonitrile–water (15 : 85), and YMC-Pack ODS-AM, mobile phase, acetonitrile–0.2% TFA (20 : 80)] to yield 7-epiblechnic acid (1.1 mg). Fraction 2G (210.6 mg) was subjected to HPLC [ODS-100V, mobile phase, acetonitrile–water (25 : 75), and 5C_{18}-AR-II, mobile phase, acetonitrile–0.2% TFA (20 : 80)] to yield **5** (3.2 mg), luteolin-7-*O*-*β*-D-glucuronopyranoside (6.9 mg), and apigenin-7-*O*-*β*-D-glucuronopyranoside methyl ester (3.9 mg). Fraction 2H (132.9 mg) was subjected to HPLC [ODS-100V, mobile phase, acetonitrile–water (25 : 75), and 5C_{18}-AR-II, mobile phase, acetonitrile–0.2% TFA (20 : 80)] to yield hyperin (2.2 mg) and 4-{[6-*O*-(4-hydroxy-3,5-dimethoxybenzoyl)-*β*-D-glucopyranosyl]oxy}-3-methoxybenzoic acid (1.0 mg). Fractions 2I, 2J, and 2K (328.4 mg) were subjected to HPLC [ODS-100V, mobile phase, acetonitrile–water (25 : 75)] to yield rosmarinic acid (56.6 mg), diosmetin-7-*O*-*β*-D-glucuronopyranoside (2.7 mg) and apigenin-7-*O*-*β*-D-glucuronopyranoside (29.9 mg). Fractions 2L and 2M (206.8 mg) were subjected to HPLC [Capcell-Pak Ph, mobile phase, acetonitrile–0.2% TFA (25 : 75)] to yield lithospermic acid B (47.8 mg), apigenin-7-*O*-*β*-D-glucuronopyranoside methyl ester (6.3 mg), and diosmetin-7-*O*-*β*-D-glucuronopyranoside methyl ester (7.1 mg). Fraction 1B (1.26 g) was subjected to YCCC [mobile phase, methanol–0.2% TFA (35 : 65)→(50 : 50)], to give 15 fractions (Frs. 3A—O). Fractions 3B and 3C (96.5 mg) were subjected to HPLC [ODS-100V, mobile phase, acetonitrile–water (30 : 70), and Capcell-Pak Ph, mobile phase, acetonitrile–water (17.5 : 85.5)] to yield compounds **1** (5.7 mg) and **2** (2.5 mg). Fractions 3D and 3E (78.9 mg) were subjected to HPLC [ODS-100V, mobile phase, acetonitrile–0.2% TFA (35 : 65), and Capcell-Pak Ph, mobile phase, acetonitrile–0.2% TFA (35 : 65)] to yield apigenin-7-*O*-*β*-D-glucuronopyranoside (17.9 mg), rosmarinic acid (10.9 mg), and acacetin-7-*O*-*β*-D-glucuronopyranoside (3.0 mg). Fractions 3G, 3H, 3I, and 3J (336.1 mg) were subjected to HPLC [ODS-100V, mobile phase, acetonitrile–0.2% TFA (35 : 65)] to yield diosmetin-7-*O*-*β*-D-glucuronopyranoside (93.5 mg) and lithospermic acid B (33.1 mg). Fraction 3M (167.8 mg) was subjected to HPLC [ODS-100V, mobile phase, acetonitrile–0.2% TFA (35 : 65), and Capcell-Pak Ph, mobile phase, acetonitrile–0.2% TFA (27.5 : 72.5)] to yield **4** (4.8 mg), **4a** (0.8 mg), **6** (3.6 mg), and lithospermic acid B (21.0 mg). Fraction 1C (793.0 mg) was subjected to YCCC [mobile phase, methanol–0.2% TFA (45 : 55)] and HPLC [Capcell-Pak Ph, mobile phase, acetonitrile–0.2% TFA (30 : 70), and Mightysil RP-18 GP, mobile phase, acetonitrile–water (25 : 75)] to yield **3** (1.1 mg). Fraction 1D (350.6 mg) was subjected to YCCC [mobile phase, acetonitrile–0.2% TFA (45 : 55)] and HPLC [ODS-100V, mobile phase, acetonitrile–0.2% TFA (50 : 50)] to yield acacetin (1.1 mg), pectolarigenin (4.8 mg), and ladanein (1.7 mg).

Meefarnine A (**1**): Colorless amorphous powder. $[\alpha]_D^{24}$ +2.6 (c=0.53, MeOH). UV λ_{max} (MeOH) nm (log ε): 276 (4.60), 202 (4.92). CD (c=0.106, MeOH) $\lambda(\theta)$ 284 (1500), 228 (−1700), 207 (1300) nm. IR (KBr) cm^{-1}: 3398, 1644, 1610, 1514, 1030, 838. FAB-MS m/z: 438.2411 $[M+H]^+$ (Calcd for $C_{25}H_{32}N_3O_4$: 438.2395). ^{1}H- and ^{13}C-NMR, see Table 1.

Meefarnine B (**2**): Colorless amorphous powder. $[\alpha]_D^{24}$ −5.0 (c=0.28, MeOH). UV λ_{max} (MeOH) nm (log ε): 275 (4.57), 212 (4.71). IR (KBr) cm^{-1}: 3421, 1649, 1612, 1031, 838. FAB-MS m/z: 438.2394 $[M+H]^+$ (Calcd for $C_{25}H_{32}N_3O_4$: 438.2395). ^{1}H- and ^{13}C-NMR, see Table 1.

Fargenin A (**3**): Colorless amorphous powder. $[\alpha]_D^{24}$ −14.3 (c=0.14, DMSO). UV λ_{max} (MeOH) nm (log ε): 343 (4.79), 266 (4.73), 252 (4.77), 207 (5.04). IR (KBr) cm^{-1}: 3422, 1614, 1499, 1261, 1176, 1076. FAB-MS m/z: 517.0997 $[M-H]^-$ (Calcd for $C_{24}H_{21}O_{13}$: 517.0997). ^{1}H- and ^{13}C-NMR, see Table 2.

Fargenin B (**4**): Colorless amorphous powder. $[\alpha]_D^{23}$ −27.1 (c=0.48, DMSO). UV λ_{max} (MeOH) nm (log ε): 342 (4.52), 267 (4.47), 253 (4.49), 207 (4.77). IR (KBr) cm^{-1}: 3402, 1718, 1660, 1611, 1500, 1444, 1261, 1177, 1064. FAB-MS m/z: 563.1043 $[M+H]^+$ (Calcd for $C_{25}H_{23}O_{15}$: 563.1036). ^{1}H- and ^{13}C-NMR, see Table 2.

Compound **4a**: Colorless amorphous powder; $[\alpha]_D^{23}$ −30.0 (c=0.08, DMSO). UV λ_{max} (MeOH) nm (log ε): 342 (4.55), 266 (sh), 252 (4.55), 207 (4.81). FAB-MS m/z: 613.1188 $[M+Na]^+$ (Calcd for $C_{27}H_{26}O_{15}Na$: 613.1168). ^{1}H- and ^{13}C-NMR, see Table 2.

Fargenin C (**5**): Colorless amorphous powder. $[\alpha]_D^{24}$ −37.0 (c=0.40, DMSO). UV λ_{max} (MeOH) nm (log ε): 342 (4.64), 268 (4.61), 254 (4.62), 207 (4.90). IR (KBr) cm^{-1}: 3394, 1655, 1611, 1500, 1442, 1401, 1262, 1181, 1073. FAB-MS m/z: 653.1373 $[M+H]^+$ (Calcd for $C_{28}H_{29}O_{18}$: 653.1353). ^{1}H- and ^{13}C-NMR, see Table 2.

Fargenin D (**6**): Colorless amorphous powder. $[\alpha]_D^{23}$ −17.8 (c=0.36, DMSO). UV λ_{max} (MeOH) nm (log ε): 343 (4.50), 267 (4.44), 253 (4.47), 209 (4.68). IR (KBr) cm^{-1}: 3421, 1657, 1611, 1500, 1443, 1261, 1177, 1063. FAB-MS m/z: 563.1031 $[M+H]^+$ (Calcd for $C_{25}H_{23}O_{15}$: 563.1036). ^{1}H- and ^{13}C-NMR, see Table 2.

Acid Hydrolysis and Sugar Identification Compounds **3**—**6** (1 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 methyl ester (5 mg) and *o*-tolyl isothiocyanate (10 μl) in pyridine (0.5 ml), by using the procedure reported by Tanaka *et al.*[30] 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-Glucuronic acid (t_R 16.8 min) was identified as the sugar moieties of **3**—**6** based on comparisons with authentic samples of D-glucuronic acid (t_R 16.8 min) and L-glucuronic acid (using D-cysteine methyl ester and D-glucuronic acid, t_R 16.1 min).

Assay of Hyaluronidase Inhibition The assay was carried out according to the Morgan–Elson method, which was modified by Davidson and Aronson.[17,36,37] Each fraction (final concentration: 2.0 mg/ml) and each compound (final concentration: 1, 0.3, 0.1, 0.03, 0.01 mM) was dissolved in 0.1 M acetate buffer as the sample solution. Hyaluronidase activity was measured as described previously.[31] Disodium cromoglycate (DSCG) (Wako Pure Chemical Industries Ltd., Osaka, Japan) was regarded to be a positive control.

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