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BIOACTIVE CONSTITUENTS FROM CHINESE NATURAL MEDICINES. XXV.[1] NEW FLAVONOL BISDESMOSIDES, SARMENOSIDES I, II, III, AND IV, WITH HEPATOPROTECTIVE ACTIVITY FROM *SEDUM SARMENTOSUM* (CRASSULACEAE)

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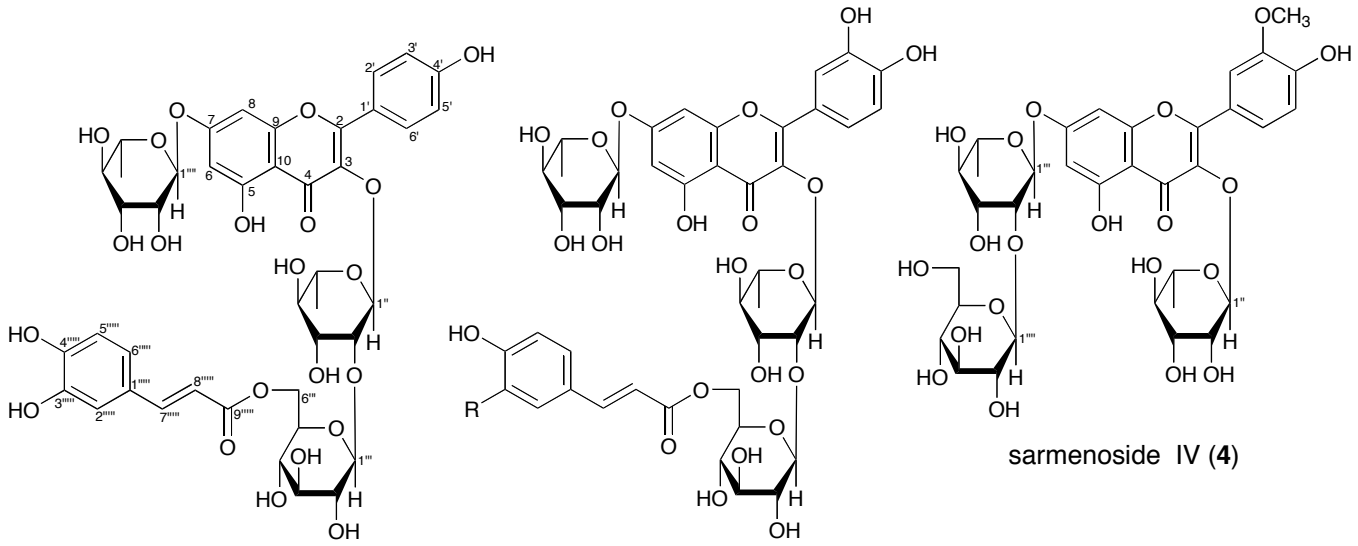
Abstract —Four new flavonol bisdesmosides, sarmenosides I, II, III, and IV, were isolated from the whole plant of *Sedum sarmentosum* (Crassulaceae). Their structures were elucidated on the basis of chemical and physicochemical evidence. Among them, sarmenoside III was found to show potent hepatoprotective effect (IC_{50} = 4.4 μM) on D-galactosamine-induced cytotoxicity in primary cultured mouse hepatocytes.

During the course of our characterization studies on the bioactive constituents from Chinese natural medicines,[2-15] we have reported the isolation and structure elucidation of 27 megastigmane constituents, including sarmentoic acid, sarmentol A, sedumosides A_1–A_6, B, C, D, E_1—E_3, F_1, F_2, and G—I, from the whole plant of *Sedum sarmentosum* (Crassulaceae).[16–18] The extract of *S. sarmentosum* and several megastigmane constituents were found to show hepatoprotective effects on D-galactosamine (D-GalN)-induced cytotoxicity in primary cultured mouse hepatocytes.[18] As a continuing study on this herbal medicine, we have isolated four new flavonol bisdesmosides, sarmenosides I (**1**), II (**2**), III (**3**), and IV (**4**). This paper deals with the isolation and structure elucidation of the new flavonol bisdesmosides (**1**—**4**) and hepatoprotective effects of flavonoid and lignan constituents from this herbal medicine on D-GalN-induced cytotoxicity in primary cultured mouse hepatocytes.

Structures of Sarmenosides I (1), II (2), III (3), and IV (4)

The hot water extract from the whole plant of *S. sarmentosum* was treated with methanol to give the methanol-soluble part (0.57% from the fresh plant). The methanol-soluble part was subjected to Diaion

HP-20 column chromatography (H_2O → MeOH) to give the water- and methanol-eluted fractions (0.44 and 0.13%, respectively) as previously reported.[16] The methanol-eluted fraction was subjected to normal- and reversed-phase silica gel column chromatographies, and finally HPLC to give **1** (0.00005%), **2** (0.00064%), **3** (0.00001%), and **4** (0.00002%) together with apigenin 7-*O*-*β*-D-glucopyranoside (**5**, 0.00005%), luteolin 7-*O*-*β*-D-glucopyranoside (**6**, 0.00006%), tricin 7-*O*-*β*-D-glucopyranoside (**7**, 0.00002%), kaempferol 7-*O*-*β*-D-glucopyranoside (**8**, 0.00004%), kaempferol 3-*O*-*β*-D-glucopyranosyl(1→2)-*α*-L-rhamnopyranosyl-7-*O*-*α*-L-rhamnopyranoside (**9**, 0.00015%), grosvenorine (**10**, 0.00010%), quercetin 3,7-di-*O*-*α*-L-rhamnopyranoside (**11**, 0.00007%), quercetin 3-*O*-*β*-D-glucopyranosyl(1→2)-*α*-L-rhamnopyranosyl-7-*O*-*α*-L-rhamnopyranoside (**12**, 0.00004%), isorhamnetin 7-*O*-*β*-D-glucopyranoside (**13**, 0.00009%), isorhamnetin 3-*O*-*β*-D-glucopyranosyl-7-*O*-*α*-L-rhamnopyranoside (**14**, 0.00005%), isorhamnetin 3,7-di-*O*-*β*-D-glucopyranoside (**15**, 0.00014%), isorhamnetin 3-*O*-*β*-D-glucopyranosyl(1→2)-*α*-L-rhamnopyranosyl-7-*O*-*α*-L-rhamnopyranoside (**16**, 0.00005%), herbacetin 8-methyl ether 3,7-di-*O*-*β*-D-glucopyranoside (**17**, 0.00003%), limocitrin 3-*O*-*β*-D-glucopyranoside (**18**, 0.00008%), limocitrin 3,7-di-*O*-*β*-D-glucopyranoside (**19**, 0.00057%), (–)-pinoresinol 4,4'-di-*O*-*β*-D-glucopyranoside (**20**, 0.00005%), (+)-isolariciresinol (**21**, 0.00012%), woorenoside XI (**22**, 0.00015%), (+)-isolariciresinol 3a-*O*-*β*-D-glucopyranoside (**23**, 0.00003%), **24** (0.00005%), (+)-lariciresinol 4-*O*-*β*-D-glucopyranoside (**25**, 0.00007%), (+)-lariciresinol 4,4'-bis-*O*-*β*-D-glucopyranoside (**26**, 0.00031%), 2-phenylethyl *β*-D-glucopyranoside (**27**, 0.00001%), 2-phenylethyl D-rutinoside (**28**, 0.00003%), eugenyl *β*-D-glucopyranoside (**29**, 0.00007%), 4*R*-*p*-menth-1-ene-7,8-diol 7-*O*-*β*-D-glucopyranoside (**30**, 0.00006%), 4*R*-*p*-menth-1-ene-7,8-diol 8-*O*-*β*-D-glucopyranoside (**31**, 0.00004%), octa-1-en-3-yl *α*-L-rhamnopyranosyl (1→6)-*β*-D-glucopyranoside (**32**, 0.00043%), 1-acetyl *β*-carboline (**33**, 0.00001%), **34** (0.00003%), and **35** (0.00005%).[17]

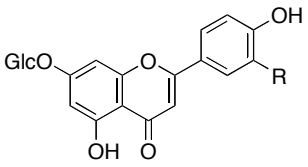


sarmenoside I (**1**)

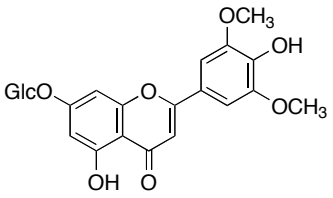
sarmenoside II (**2**) : R = H
sarmenoside III (**3**) : R = OH

sarmenoside IV (**4**)

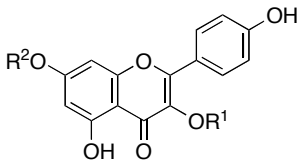
Chart 1



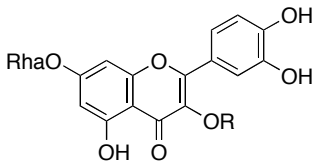
apigenin 7-*O*-β-D-glucopyranoside (**5**): R = H
luteolin 7-*O*-β-D-glucopyranoside (**6**): R = OH



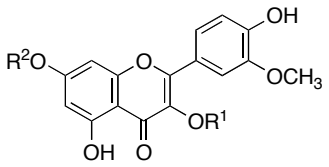
tricin 7-*O*-β-D-glucopyranoside (**7**)



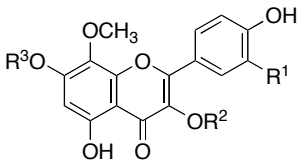
kaempferol 7-*O*-β-D-glucopyranoside (**8**): R^1 = H, R^2 =Glc
9: R^1 = Rha^{2}—^{1}Glc, R^2 = Rha
grosvenorine (**10**): R^1 = Rha, R^2 = Rha^{2}—^{1}Glc



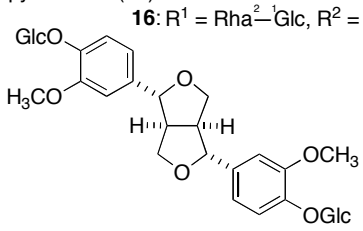
quercetin 3,7-di-*O*-α-L-rhamnopyranoside (**11**): R = Rha
12: R = Rha^{2}—^{1}Glc



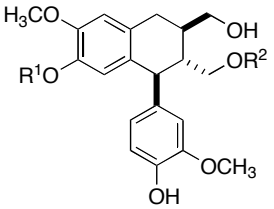
isorhamnetin 7-*O*-β-D-glucopyranoside (**13**): R^1 = H, R^2 = Glc
14: R^1 = Glc, R^2 = Rha
isorhamnetin 3,7-di-*O*-β-D-glucopyranoside (**15**): R^1 = R^2 = Glc
16: R^1 = Rha^{2}—^{1}Glc, R^2 = Rha



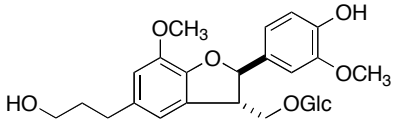
herbacetin 8-methyl ether 3,7-di-*O*-β-D-glucopyranoside (**17**): R^1 = H, R^2 = R^3 = Glc
limocitrin 3-*O*-β-D-glucopyranoside (**18**): R^1 = OCH_3, R^2 = Glc, R^3 = H
limocitrin 3,7-di-*O*-β-D-glucopyranoside (**19**): R^1 = OCH_3, R^2 = R^3 = Glc



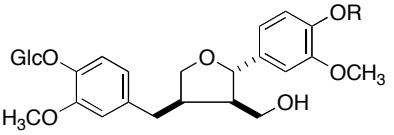
(–)-pinoresinol 4,4'-di-*O*-β-D-glucopyranoside (**20**)



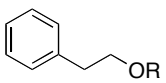
(+)-isolariciresinol (**21**): R^1 = R^2 = H
woorenoside XI (**22**): R^1 = Glc, R^2 = H
(+)-isolariciresinol 3a-*O*-β-D-glucopyranoside (**23**): R^1 = H, R^2 = Glc



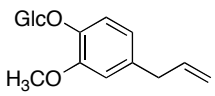
24



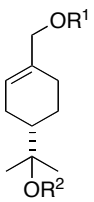
(+)-lariciresinol 4-*O*-β-D-glucopyranoside (**25**): R = H
(+)-lariciresinol 4,4'-bis-*O*-β-D-glucopyranoside (**26**): R = Glc



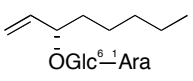
2-phenylethyl β-D-glucopyranoside (**27**): R = Glc
2-phenylethyl D-rutinoside (**28**): R = Glc^{2}—^{1}Rha



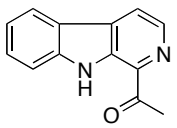
eugenyl β-D-glucopyranoside (**29**)



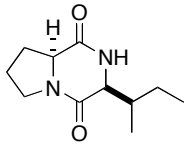
4*R*-*p*-menth-1-ene-7,8-diol 7-*O*-β-D-glucopyranoside (**30**): R^1 = Glc, R^2 = H
4*R*-*p*-menth-1-ene-7,8-diol 8-*O*-β-D-glucopyranoside (**31**): R^1 = H, R^2 = Glc



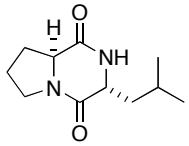
octa-1-en-3-yl α-L-arabinopyranosyl (1→6)-β-D-glucopyranoside (**32**)



1-acetyl β-carboline (**33**)



34



35

Glc: β-D-glucopyranosyl; Rha: α-L-rhamnopyranosyl; Ara: α-L-arabinopyranosyl

Chart 2

Sarmenoside I (**1**) was isolated as an amorphous powder with negative optical rotation ($[\alpha]_D^{24}$ –80.6° in MeOH). The IR spectrum of **1** showed absorption bands ascribable to hydroxyl (3431 cm^{-1}), ester carbonyl (1655 cm^{-1}), and ether (1024 cm^{-1}) functions and aromatic ring (1603, 1541, 1458 cm^{-1}). In the positive- and negative-ion fast atom bombardment (FAB)-MS of **1**, quasimolecular ion peaks were observed at *m/z* 925 $(M+Na)^+$ and *m/z* 901 $(M–H)^–$, respectively. High-resolution MS analysis of the quasimolecular ion peak $(M+Na)^+$ in the positive-ion FAB-MS revealed the molecular formula of **1** to be $C_{42}H_{46}O_{22}$. On alkaline hydrolysis of **1** with 10% aqueous potassium hydroxide (KOH)–50% aqueous 1,4-dioxane (1:1, v/v), kaempferol 3-*O*-β-D-glucopyranosyl(1→2)-α-L-rhamnopyranosyl-7-*O*-α-L-rhamnopyranoside (**9**)[17,19] was obtained together with caffeic acid, which was identified by HPLC analysis. The ^{1}H- (DMSO-d_6) and ^{13}C-NMR (Table 1) spectra of **1**, which were assigned by various NMR experiments,[20] showed signals assignable to a kaempferol part [δ 6.43, 6.70 (1H each, both d, *J* = 2.2 Hz, 6, 8-H), 6.93, 7.78 (2H each, both d, *J* = 8.9 Hz, 3',5', 2',6'-H)], and a β-D-glucopyranosyl and two α-L-rhamnopyranosyl moieties [δ 0.92, 1.13 (3H each, both d, *J* = 6.1 Hz, 6'', 6''''-H_3), 4.33 (1H, d, *J* = 8.0 Hz, 1'''-H), 5.61 (1H, br s, 1''-H), 5.53 (1H, d, *J* = 1.2 Hz, 1''''-H)] together with a caffeoyl group [δ 6.12, 7.38 (1H each, both d, *J* = 15.9 Hz, 8'''', 7''''-H), 6.68 (1H, d, *J* = 8.3 Hz, 5''''-H), 6.86 (1H, dd, *J* = 1.8, 8.3 Hz, 6''''-H), 6.93 (1H, d, *J* = 1.8 Hz, 2''''-H)]. Comparison of the ^{13}C-NMR data for **1** with those for **9** revealed an acylation shift around the 6'''-position of the glucopyranosyl moiety [**9**: δ_C 76.6 (5'''-C), 60.5 (6'''-C); **1**: δ_C 73.6 (5'''-C), 62.8 (6'''-C)]. Furthermore, in the heteronuclear multiple-bond correlations (HMBC) experiment of **1**, long-range correlation was observed between the 6'''-protons [δ 4.16, 4.21 (1H each, both m)] and the ester carbonyl carbon (δ_C 166.2). On the basis of the above-mentioned evidence, the structure of sarmenoside I was determined to be kaempferol 3-*O*-(6-*O*-caffeoyl)-β-D-glucopyranosyl(1→2)-α-L-rhamnopyranosyl-7-*O*-α-L-rhamnopyranoside (**1**).

Table 1. ^{13}C-NMR Data for Sarmenosides I (**1**), II (**2**), and III (**3**)

	1	**2**	**3**		**1**	**2**	**3**
2	157.1	157.0	157.1	3-*O*-Rha-1''	100.5	100.6	100.6
3	134.5	134.6	134.5	2''	81.4	81.7	81.5
4	177.8	177.9	177.9	3''	70.0	70.2	70.1
5	160.9	160.9	160.9	4''	71.6	71.7	71.6
6	99.3	99.3	99.3	5''	70.4	70.4	70.3
7	161.6	161.6	161.6	6''	17.3	17.4	17.3
8	94.5	94.3	94.3	2''-*O*-Glc-1'''	105.9	106.1	106.1
9	155.9	155.9	155.8	2'''	73.6	73.5	73.6
10	105.6	105.6	105.6	3'''	75.9	75.9	75.8
1'	120.1	120.4	120.3	4'''	69.5	69.5	69.1
2'	130.6	115.5	115.4	5'''	73.6	73.6	73.5
3'	115.3	145.2	145.1	6'''	62.8	62.7	62.5
4'	160.2	148.7	148.7	7-*O*-Rha-1''''	98.4	98.4	98.4
5'	115.3	115.5	115.5	2''''	69.7	69.8	69.7
6'	130.6	120.9	120.9	3''''	70.2	70.1	70.1
acyl-1'''''	125.3	124.9	125.3	4''''	71.5	71.6	71.5
2'''''	114.8	130.0	114.8	5''''	70.0	70.0	70.0
3'''''	145.4	115.5	145.4	6''''	17.8	17.8	17.8
4'''''	148.2	159.6	148.2				
5'''''	115.5	115.5	115.5				
6'''''	120.9	130.0	121.0				
7'''''	145.0	144.5	145.0				
8'''''	113.6	113.9	113.9				
9'''''	166.2	166.3	166.3				

Measured at 125 MHz in DMSO-d_6.

Rha: α-L-rhamnopyranosyl; Glc: β-D-glucopyranosyl

Sarmenoside II (**2**) was obtained as an amorphous powder with negative optical rotation ($[\alpha]_D^{24}$ –111.2° in MeOH). The molecular formula, $C_{42}H_{46}O_{22}$, of **2** was determined from the positive-ion FAB-MS [*m/z* 925 $(M+Na)^+$] and by high resolution positive-ion FAB-MS measurement. Sarmenoside III (**3**), $[\alpha]_D^{26}$ –

87.6° (MeOH), was also obtained as an amorphous powder and the molecular formula, $C_{42}H_{46}O_{23}$, was determined from the positive-ion FAB-MS data and by high resolution positive-ion FAB-MS measurement. Treatment of **2** and **3** with 10% KOH–50% aqueous 1,4-dioxane (1:1, v/v), gave quercetin 3-*O*-*β*-D-glucopyranosyl(1→2)-*α*-L-rhamnopyranosyl-7-*O*-*α*-L-rhamnopyranoside (**12**)[17,21] together with *p*-coumaric acid (from **2**) and caffeic acid (from **3**), which were identified by HPLC analysis. The ^{1}H-(DMSO-d_6) and ^{13}C-NMR (Table 1) spectra[20] of **2** showed signals assignable to a quercetin part [*δ* 6.42, 6.68 (1H each, both d, *J* = 2.2 Hz, 6, 8-H), 6.92 (1H, d, *J* = 8.2 Hz, 5'-H), 7.31 (1H, dd, *J* = 2.2, 8.2 Hz, 6'-H), 7.43 (1H, d, *J* = 2.1 Hz, 2'-H)], and a *β*-D-glucopyranosyl and two *α*-L-rhamnopyranosyl moieties [*δ* 0.97 (3H, d, *J* = 6.1 Hz, 6''-H_3), 1.15 (3H, d, *J* = 6.1 Hz, 6''''-H_3), 4.31 (1H, d, *J* = 7.9 Hz, 1'''-H), 5.58 (1H, br s, 1''-H), 5.54 (1H, d, *J* = 0.7 Hz, 1''''-H)] together with a *p*-coumaroyl group [*δ* 6.25, 7.45 (1H each, both d, *J* = 15.9 Hz, 8'''', 7''''-H), 6.71, 7.41 (2H each, both d, *J* = 8.9 Hz, 3'''',5'''', 2'''',6''''-H)]. The proton and carbon signals in ^{1}H- (DMSO-d_6) and ^{13}C-NMR (Table 1) spectra[20] of **3** were superimposable on those of **2**, except for the signals due to an acyl group [*δ* 6.15, 7.37 (1H each, both d, *J* = 15.9 Hz, 8'''', 7''''-H), 6.67 (1H, d, *J* = 8.3 Hz, 5''''-H), 6.88 (1H, dd, *J* = 2.5, 8.3 Hz, 6''''-H)]. The HMBC experiments on **2** and **3** showed long-range correlations between the 6'''-protons [**2**: *δ* 4.11 (1H, br d, *J* = *ca.* 11 Hz), 4.17 (1H, dd, *J* = 4.3, 11.3 Hz); **3**: *δ* 4.01 (1H, br d, *J* = *ca.* 11 Hz), 4.17 (1H, dd, *J* = 2.8, 11.3 Hz)] and the ester carbonyl carbon (**2**: δ_C 166.3; **3**: δ_C 166.3), respectively. Consequently, the structures of sarmenosides II and III were determined to be quercetin 3-*O*-(6-*O*-*p*-coumaroyl)-*β*-D-glucopyranosyl(1→2)-*α*-L-rhamnopyranosyl-7-*O*-*α*-L-rhamnopyranoside (**2**) and quercetin 3-*O*-(6-*O*-caffeoyl)-*β*-D-glucopyranosyl(1→2)-*α*-L-rhamnopyranosyl-7-*O*-*α*-L-rhamnopyranoside (**3**).

Sarmenoside IV (**4**) with negative optical rotation ($[\alpha]_D^{22}$ –77.5° in MeOH) was also isolated as an amorphous powder. The molecular formula $C_{34}H_{42}O_{20}$ of **4** was also determined from the positive- and negative-ion FAB-MS [*m/z* 793 $(M+Na)^+$, *m/z* 769 $(M-H)^-$] and by high-resolution positive-ion FAB-MS measurement. Acid hydrolysis of **4** with 1.0 M hydrochloric acid (HCl) liberated L-rhamnose and D-glucose, which were identified by HPLC analysis using an optical rotation detector.[2,4–6,9–14,16,17] The ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (Table 2) spectra[20] of **4** indicated the presence of *meta*-coupled and *ortho*-coupled A_2B_2-type aromatic protons [*δ* 6.48, 6.85 (1H each, both d, *J* = 2.2 Hz, 6, 8-H), 6.94 (1H, d, *J* = 8.6 Hz, 5'-H), 7.44 (1H, dd, *J* = 2.2, 8.6 Hz, 6'-H), 7.48 (1H, d, *J* = 2.2 Hz, 2'-H)], and a *β*-D-glucopyranosyl and two *α*-L-rhamnopyranosyl moieties [*δ* 0.81 (3H, d, *J* = 6.1 Hz, 6''-H), 1.15 (3H, d, *J* = 6.1 Hz, 6''''-H_3), 4.38 (1H, d, *J* = 7.9 Hz, 1''''-H), 5.28 (1H, d, *J* = 1.6 Hz, 1''-H), 5.94 (1H, d, *J* = 1.5 Hz, 1'''- H)] together with a methoxyl protons [*δ* 3.86 (3H, s, 3'-OCH_3)]. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra of **4** were very similar to those of grosvenorine

Table 2. ^{13}C-NMR Data for Sarmenoside IV (**4**)

	4		**4**
2	157.7	3-*O*-Rha-1''	101.8
3	134.6	2''	70.0
4	177.9	3''	70.4
5	160.9	4''	71.1
6	99.6	5''	70.7
7	161.4	6''	17.3
8	94.7	7-*O*-Rha-1'''	97.1
9	156.1	2'''	79.9
10	105.8	3'''	70.0
1'	120.5	4'''	72.1
2'	112.7	5'''	69.9
3'	147.2	6'''	17.8
4'	149.7	2'''-*O*-Glc-1''''	105.6
5'	115.4	2''''	73.9
6'	122.8	3''''	76.2
3'-OCH_3	55.7	4''''	69.9
		5''''	76.8
		6''''	61.0

Measured at 125 MHz in DMSO-d_6.
Rha: *α*-L-rhamnopyranosyl;
Glc: *β*-D-glucopyranosyl

(**10**),[17,22] except for the signals due to the B ring in the aglycon part. The connectivities of oligoglycoside moieties to the aglycon part were characterized by a HMBC experiment on **4**. Thus, the HMBC experiment of **4** showed long-range correlations between the following proton and carbon pairs (1''-H and C-3; 1'''-H and C-7; 1''''-H and C-2'''). Finally, the position of a methoxyl group in **4** was clarified by nuclear Overhauser enhancement spectroscopy (NOESY) experiment, which showed NOE correlation between the methoxyl protons and the 2'-proton. Consequently, the structure of sarmenoside IV was determined to be isorhamnetin 3-*O*-α-L-rhamnopyranosyl-7-*O*-β-D-glucopyranosyl(1→2)-α-L-rhamnopyranoside (**4**).

Protective Effects on D-GalN-induced Cytotoxicity in Primary Cultured Mouse Hepatocytes

Table 3. Inhibitory Effects of Constituents from *S. sarmentosum* on D-GalN-induced Cytotoxicity in Primary Cultured Mouse Hepatocytes

	Inhibition (%)					IC_{50}
	0 μM	3 μM	10 μM	30 μM	100 μM	μM
sarmenoside I (**1**)	0.0 ± 6.6	5.0 ± 7.2	12.5 ± 7.2	35.9 ± 7.0	73.3 ± 10.6**	46
sarmenoside II (**2**)	0.0 ± 6.2	9.4 ± 7.2	23.4 ± 9.5	26.8 ± 7.0	54.2 ± 8.0**	94
sarmenoside III (**3**)	0.0 ± 1.8	42.8 ± 2.2**	65.4 ± 1.6**	86.4 ± 6.0**	99.2 ± 2.5**	4.4
sarmenoside IV (**4**)	0.0 ± 6.3	5.6 ± 3.9	5.6 ± 4.7	13.3 ± 5.8	38.7 ± 7.5**	
apigenin 7-*O*-Glc (**5**)	0.0 ± 3.4	1.5 ± 5.8	13.2 ± 7.0	21.7 ± 4.1*	24.0 ± 2.3*	
luteolin 7-*O*-Glc (**6**)	0.0 ± 2.1	2.5 ± 2.3	0.7 ± 10.2	36.8 ± 2.6**	62.3 ± 2.6**	57
tricin 7-*O*-Glc (**7**)	0.0 ± 3.8	0.6 ± 2.3	6.2 ± 2.5	10.8 ± 2.9	22.0 ± 3.8**	
kaempferol 7-*O*-Glc (**8**)	0.0 ± 1.5	4.2 ± 1.5	14.0 ± 1.0**	46.4 ± 2.2**	83.5 ± 0.7**	32
9	0.0 ± 2.6	1.0 ± 1.4	9.8 ± 4.5	28.9 ± 5.9**	61.8 ± 6.6**	66
grosvenorine (**10**)	0.0 ± 2.7	1.8 ± 3.1	16.1 ± 1.3	25.6 ± 4.2**	51.6 ± 2.4**	
quercetin 3,7-di-*O*-Rha (**11**)	0.0 ± 1.4	-3.4 ± 0.8	9.2 ± 2.4*	24.7 ± 3.1**	28.5 ± 2.5**	
12	0.0 ± 2.3	3.9 ± 2.8	6.3 ± 3.5	9.8 ± 2.2	49.1 ± 3.9**	
isorhamnetin 7-*O*-Glc (**13**)	0.0 ± 2.2	-7.3 ± 1.4	-13.2 ± 0.6	-12.2 ± 0.9	12.9 ± 1.5**	
14	0.0 ± 1.6	6.6 ± 3.0*	8.7 ± 1.3**	10.5 ± 1.1**	11.5 ± 0.9**	
isorhamnetin 3,7-di-*O*-Glc (**15**)	0.0 ± 1.8	1.1 ± 2.5	-2.5 ± 1.4	-0.7 ± 2.5	20.9 ± 4.4**	
16	0.0 ± 2.4	7.3 ± 1.8	8.9 ± 2.0	17.8 ± 2.1	27.0 ± 3.8*	
17	0.0 ± 2.4	2.0 ± 4.4	10.3 ± 3.9	16.6 ± 3.7*	28.3 ± 2.3**	
limocitrin 3-*O*-Glc (**18**)	0.0 ± 3.0	7.6 ± 3.1	10.6 ± 2.2*	25.3 ± 1.8**	56.7 ± 2.7**	96
limocitrin 3,7-di-*O*-Glc (**19**)	0.0 ± 1.4	0.0 ± 1.6	-3.0 ± 0.9	-3.0 ± 1.8	8.3 ± 8.0	
20	0.0 ± 3.6	4.5 ± 2.0	7.1 ± 2.0	29.9 ± 3.1**	69.8 ± 4.3**	59
(+)-isolariciresinol (**21**)	0.0 ± 4.7	6.7 ± 3.3	20.7 ± 2.0*	42.8 ± 4.6**	79.1 ± 6.0**	33
woorenoside XI (**22**)	0.0 ± 7.5	2.2 ± 6.2	7.2 ± 3.5	11.4 ± 4.8	29.7 ± 7.7**	
23	0.0 ± 2.8	7.3 ± 2.2	19.9 ± 1.3**	44.9 ± 3.0**	92.5 ± 3.5**	24
24	0.0 ± 2.5	5.5 ± 2.8	6.6 ± 3.9	23.2 ± 4.1*	47.9 ± 6.4**	
(+)-lariciresinol 4-*O*-Glc (**25**)	0.0 ± 0.6	7.7 ± 0.5	26.5 ± 1.5**	68.8 ± 2.3**	108.2 ± 3.5**	18
26	0.0 ± 1.0	2.9 ± 1.8	4.2 ± 3.2	19.9 ± 0.5**	16.8 ± 0.8**	
27	0.0 ± 2.2	1.2 ± 2.5	11.1 ± 1.1**	6.1 ± 1.2	12.0 ± 2.0**	
28	0.0 ± 0.9	1.9 ± 2.2	3.3 ± 0.9	17.4 ± 3.0**	19.7 ± 1.0**	
29	0.0 ± 2.7	4.2 ± 1.1	4.2 ± 0.8	33.2 ± 1.4**	93.2 ± 0.9**	37
30	0.0 ± 2.0	5.4 ± 0.7	9.4 ± 1.1**	7.4 ± 1.9*	15.2 ± 1.5**	
31	0.0 ± 0.9	1.9 ± 2.6	2.7 ± 2.8	8.4 ± 2.2	12.1 ± 0.9**	
32	0.0 ± 1.7	2.0 ± 2.0	7.5 ± 1.6	10.0 ± 3.7**	7.5 ± 2.0	
33	0.0 ± 1.4	12.8 ± 1.0**	18.2 ± 0.4**	32.3 ± 2.5**	4.6 ± 1.6	
34	0.0 ± 1.1	4.0 ± 0.8	7.8 ± 2.1*	8.7 ± 0.9**	18.6 ± 1.3**	
35	0.0 ± 3.3	-1.4 ± 2.1	3.4 ± 1.8	5.6 ± 3.6	17.9 ± 2.3**	
silybin[a]	0.0±0.3	4.8 ± 1.1	7.7 ± 0.7	45.2 ± 8.8**	77.0 ± 5.5**	41

Each value represents the mean±S.E.M. (N=4). Significantly different from the control, *p<0.05, **p<0.01.

[a]Commercial silybin was purchased from Funakoshi Co., Ltd. (Tokyo, Japan).

Previously, we have reported the isolation and structure elucidation of several constituents with hepatoprotective effects from *Hovenia dulcis*,[23] *Bupleurum scorzonerifolium*,[24,25] *Curcuma zedoaria*,[26–28] *Angelica furcijuga*,[29,30] *Betula platyphylla* var. *japonica*,[31] *Pisum sativum*,[32] *Salacia reticulata*,[33] *Tilia argentea*,[34] *Anastatica hierochuntica*,[35] *Panax notoginseng*,[36] *Cyperus longus*,[37] *Erycibe* expansa,[38] and *Camellia sinensis*.[39] Since the extract of this herbal medicine and several megastigmane constituents showed hepatoprotective effect,[18] the inhibitory effects of flavonoid and lingan constituents from the same extract including sarmenosides I—IV (**1**—**4**), on D-GalN-induced cytotoxicity in primary cultured mouse hepatocytes were also examined. As shown in Table 3, sarmenosides I (**1**, IC_{50} = 46 μM), II (**2**, 94 μM), and III (**3**, 4.4 μM), luteolin 7-*O*-β-D-glucopyranoside (**6**, 57 μM), kaempferol 7-*O*-β-D-glucopyranoside (**8**, 32 μM), kaempferol 3-*O*-β-D-glucopyranosyl(1→2)-α-L-rhamnopyranosyl-7-*O*-α-L-rhamnopyranoside (**9**, 66 μM), grosvenorine (**10**, *ca*. 100 μM), limocitrin 3-*O*-β-D-glucopyranoside (**18**, 96 μM), (–)-pinoresinol 4,4'-di-*O*-β-D-glucopyranoside (**20**, 59 μM), (+)-isolariciresinol (**21**, 33 μM), (+)-isolariciresinol 3a-*O*-β-D-glucopyranoside (**23**, 24 μM), (+)-lariciresinol 4-*O*-β-D-glucopyranoside (**25**, 18 μM), and eugenyl β-D-glucopyranoside (**29**, 37 μM), were found to show inhibitory activity. Especially, the hepatoprotective activity of sarmenoside III (**3**) was stronger than that of commercial silybin (41 μM), which is well known to show potent hepatoprotective activity.[38,39]

EXPERIMENTAL

The following instruments were used to obtain physical data : specific rotations, Horiba SEPA-300 digital polarimeter (l = 5 cm); UV spectra, Shimadzu UV-1600; IR spectra, Shimadzu FTIR-8100 spectrophotometer; FAB-MS and high-resolution FAB-MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL EX-270 (270 MHz) and JNM-LA500 (500 MHz) spectrometers; ^{13}C-NMR spectra, JEOL EX-270 (68 MHz) and JNM-LA500 (125 MHz) spectrometers with tetramethylsilane as an internal standard; HPLC detector, Shimadzu RID-6A refractive index and SPD-10A*vp* UV-VIS detectors; and HPLC column, Cosmosil 5C_{18}-MS-II (250 × 4.6 mm i.d.) and (250 × 20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

The following experimental conditions were used for chromatography: normal-phase column chromatography; Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150–350 mesh), reversed-phase column chromatography; Diaion HP-20 (Nippon Rensui): TLC, pre-coated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (normal-phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed-phase); HPTLC, pre-coated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm) (reversed-phase) and detection was achieved by spraying with 1% $Ce(SO_4)_2$-10% aqueous H_2SO_4, followed by heating.

Plant Material

S. sarmentosum was cultivated at Huangshan, Anhui province, China and plant material was identified by one of authors (M. Y.). A voucher specimen (2005.01. Eishin-02) of this plant is on file in our laboratory.[16–18]

Isolation of Sarmenosides I (1), II (2), III (3), and IV (4)

Fraction 5-10 (1818 mg), which was obtained from the methanol-eluted fraction of hot water extract from the fresh whole plant of *S. sarmentosum* as reported previously,[17] was purified by Sephadex LH-20 column chromatography [150 g, $CHCl_3$–MeOH (1:1, v/v)] and finally HPLC [MeOH–H_2O (35:65, 40:60 v/v) and CH_3CN–MeOH–H_2O (15:8:77, v/v/v)] to furnish sarmenosides I (**1**, 28.1 mg, 0.00005%), II (**2**, 343.9 mg, 0.00064%), III (**3**, 6.3 mg, 0.00001%), and IV (**4**, 10.6 mg, 0.00002%) together with kaempferol 3-*O*-β-D-glucopyranosyl(1→2)-α-L-rhamnopyranosyl-7-*O*-α-L-rhamnopyranoside (**9**, 79.9 mg, 0.00015%), grosvenorine (**10**, 53.6 mg, 0.00010%), quercetin 3,7-di-*O*-α-L-rhamnopyranoside (**11**, 37.6 mg, 0.00007%), quercetin 3-*O*-β-D-glucopyranosyl(1→2)-α-L-rhamnopyranosyl-7-*O*-α-L-rhamnopyranoside (**12**, 23.9 mg, 0.00004%), isorhamnetin 3,7-di-*O*-α-L-rhamnopyranoside (**15**, 32.8 mg, 0.00006%), and isorhamnetin 3-*O*-β-D-glucopyranosyl(1→2)-α-L-rhamnopyranosyl-7-*O*-α-L-rhamnopyranoside (**16**, 26.8 mg, 0.00005%).

Sarmenoside I (**1**): an amorphous powder, $[\alpha]_D^{24}$ –80.6° (*c* = 1.00, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{42}H_{46}O_{22}Na$ $(M+Na)^+$: 925.2378. Found: 925.2383. UV [MeOH, nm (log ε)]: 266 (4.38), 329 (4.08). IR (KBr): 3431, 2932, 1655, 1603, 1541, 1509, 1491, 1458, 1270, 1208, 1175, 1024, 961, 816 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 0.92, 1.13 (3H each, both d, *J* = 6.1 Hz, 6", 6""-H_3), 3.06 (1H, dd, *J* = 8.0, 8.9 Hz, 2"'-H), 3.14 (1H, dd, *J* = 9.5, 9.5 Hz, 4"-H), 3.20 (1H, m, 3"'-H), 3.21 (1H, m, 4"'-H), 3.31 (1H, m, 4""-H), 3.32 (1H, m, 5"'-H), 3.38 (1H, m, 5"-H), 3.44 (1H, m, 5""-H), 3.56 (1H, dd, *J* = 3.7, 9.5 Hz, 3"-H), 3.64 (1H, dd, *J* = 3.4, 9.5 Hz, 3""-H), 3.86 (1H, m, 2""-H), 4.14 (1H, br d, *J* = *ca.* 2 Hz, 2"-H), 4.16, 4.21 (1H each, both m, 6"'-H_2), 4.33 (1H, d, *J* = 8.0 Hz, 1"'-H), 5.61 (1H, br s, 1"-H), 5.53 (1H, d, *J* = 1.2 Hz, 1""-H), 6.12, 7.38 (1H each, both d, *J* = 15.9 Hz, 8"", 7""-H), 6.43, 6.70 (1H each, both d, *J* = 2.2 Hz, 6, 8-H), 6.68 (1H, d, *J* = 8.3 Hz, 5""-H), 6.86 (1H, dd, *J* = 1.8, 8.3 Hz, 6""-H), 6.93 (1H, d, *J* = 1.8 Hz, 2""-H), 6.93, 7.78 (2H each, both d, *J* = 8.9 Hz, 3',5', 2',6'-H), 12.55 (1H, br s, 5-OH). ^{13}C-NMR (DMSO-d_6, 125 MHz) δc: given in Table 1. Positive-ion FAB-MS: *m/z* 925 $(M+Na)^+$. Negative-ion FAB-MS: *m/z* 901 $(M-H)^-$.

Sarmenoside II (**2**): an amorphous powder, $[\alpha]_D^{24}$ –111.2° (*c* = 1.06, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{42}H_{46}O_{22}Na$ $(M+Na)^+$: 925.2378. Found: 925.2374. UV [MeOH, nm (log ε)]: 257 (4.42), 317 (4.45). IR (KBr): 3389, 2934, 1655, 1605, 1516, 1491, 1449, 1348, 1271, 1206, 1169, 1022, 963, 814 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 0.97 (3H, d, *J* = 6.1 Hz, 6"-H_3), 1.15 (3H, d, *J* = 6.1 Hz, 6""-H_3), 3.08 (1H, dd, *J* = 7.9, 8.5 Hz, 2"'-H), 3.17 (1H, dd, *J* = 9.5, 9.5 Hz, 4"-H), 3.22 (1H, m, 4"'-H), 3.24 (1H, m, 3"'-H), 3.30 (1H, m, 5"'-H), 3.33 (1H, dd, *J* = 9.5, 9.5 Hz, 4""-H), 3.46 (1H, m, 5""-H), 3.61 (1H, m, 5"-H), 3.64 (1H, dd, *J* = 3.4, 9.5 Hz, 3"-H), 3.66 (1H, dd, *J* = 3.4, 9.5 Hz, 3""-H), 3.88 (1H, m, 2""-H), [4.11 (1H, br d, *J* = *ca.* 11 Hz), 4.17 (1H, dd, *J* = 4.3, 11.3 Hz), 6"'-H_2], 4.20 (1H, br d, *J* = *ca.* 3 Hz, 2"-H), 4.31 (1H, d, *J* = 7.9 Hz, 1"'-H), 5.58 (1H, br s, 1"-H), 5.54 (1H, d, *J* = 0.7 Hz, 1""-H), 6.25, 7.45 (1H each, both d, *J* = 15.9 Hz, 8"", 7""-H), 6.42, 6.68 (1H each, both d, *J* = 2.2 Hz, 6, 8-H), 6.71, 7.41 (2H each, both d, *J* = 8.9 Hz, 3"",5"", 2"",6""-H), 6.92 (1H, d, *J* = 8.2 Hz, 5'-H), 7.31 (1H, dd, *J* = 2.2, 8.2 Hz, 6'-H), 7.43 (1H, d, *J* = 2.1 Hz, 2'-H), 12.61 (1H, br s, 5-OH). ^{13}C-NMR (DMSO-d_6, 125 MHz) δc: given in Table 1. Positive-ion FAB-MS: *m/z* 925 $(M+Na)^+$. Negative-ion FAB-MS: *m/z* 901 $(M-H)^-$.

Sarmenoside III (**3**): an amorphous powder, $[\alpha]_D^{26}$ –87.6° (*c* = 0.11, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{42}H_{46}O_{23}Na$ $(M+Na)^+$: 941.2328. Found: 941.2336. UV [MeOH, nm (log *ε*)]: 255 (4.44), 336 (4.37). IR (KBr): 3431, 2940, 1651, 1605, 1509, 1500, 1458, 1348, 1273, 1175, 1052, 966, 820 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 500 MHz) *δ*: 0.93 (3H, d, *J* = 6.1 Hz, 6''-H), 1.12 (3H, d, *J* = 6.4 Hz, 6''''-H_3), 3.04 (1H, dd, *J* = 7.7, 8.3 Hz, 2'''-H), 3.13 (1H, dd, *J* = 9.5, 9.5 Hz, 4''-H), 3.17 (1H, m, 3'''-H), 3.19 (1H, m, 4'''-H), 3.25 (1H, m, 5'''-H), 3.29 (1H, m, 4''''-H), 3.40 (1H, m, 5''''-H), 3.59 (1H, dd, *J* = 3.4, 9.5 Hz, 3''-H), 3.61 (1H, m, 5''-H), 3.63 (1H, dd, *J* = 3.4, 9.5 Hz, 3''''-H), 3.84 (1H, m, 2''''-H), [4.01 (1H, br d, *J* = *ca*. 11 Hz), 4.17 (1H, dd, *J* = 2.8, 11.3 Hz), 6'''-H_2], 4.16 (1H, br s, 2''-H), 4.28 (1H, d, *J* = 7.7 Hz, 1'''-H), 5.50 (1H, br s, 1''-H), 5.52 (1H, br s, 1''''-H), 6.15, 7.37 (1H each, both d, *J* = 15.9 Hz, 8''''', 7''''-H), 6.41, 6.70 (1H each, both d, *J* = 2.2 Hz, 6, 8-H), 6.67 (1H, d, *J* = 8.3 Hz, 5''''-H), 6.88 (1H, dd, *J* = 2.5, 8.3 Hz, 6''''-H), 6.95 (1H, d, *J* = 2.5 Hz, 2''''-H), 6.89 (1H, d, *J* = 8.5 Hz, 5'-H), 7.30 (1H, dd, *J* = 2.1, 8.5 Hz, 6'-H), 7.43 (1H, d, *J* = 2.1 Hz, 2'-H), 12.61 (1H, br s, 5-OH). ^{13}C-NMR (DMSO-d_6, 125 MHz) *δ*c: given in Table 1. Positive-ion FAB-MS: *m/z* 941 $(M+Na)^+$. Negative-ion FAB-MS: *m/z* 917 $(M-H)^-$.

Sarmenoside IV (**4**): an amorphous powder, $[\alpha]_D^{22}$ –77.5° (*c* = 0.62, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{34}H_{42}O_{20}Na$ $(M+Na)^+$: 793.2176. Found: 793.2161. UV [MeOH, nm (log *ε*)]: 255 (4.27), 349 (4.12). IR (KBr): 3389, 2918, 1653, 1647, 1605, 1559, 1541, 1509, 1489, 1474, 1458, 1341, 1210, 1169, 1025, 970, 814 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 500 MHz) *δ*: 0.81 (3H, d, *J* = 6.1 Hz, 6''-H), 1.15 (3H, d, *J* = 6.1 Hz, 6''''-H_3), 3.06 (1H, dd, *J* = 7.9, 8.3 Hz, 2''''-H), 3.07 (1H, m, 4''''-H), 3.14 (1H, m, 4''-H), 3.15 (1H, m, 5''''-H), 3.17 (1H, m, 5''-H), 3.17 (1H, m, 3''''-H), 3.29 (1H, m, 4'''-H), 3.42, 3.66 (1H each, both m, 6''''-H_2), 3.46 (1H, m, 5'''-H), 3.51 (1H, m, 3''-H), 3.68 (1H, m, 3'''-H), 3.86 (3H, s, 3'-OCH_3), 3.93 (1H, dd, *J* = 1.6, 3.7 Hz, 2'''-H), 3.98 (1H, br s, 2''-H), 4.38 (1H, d, *J* = 7.9 Hz, 1''''-H), 5.28 (1H, d, *J* = 1.6 Hz, 1''-H), 5.94 (1H, d, *J* = 1.5 Hz, 1'''-H), 6.48, 6.85 (1H each, both d, *J* = 2.2 Hz, 6, 8-H), 6.94 (1H, d, *J* = 8.6 Hz, 5'-H), 7.44 (1H, dd, *J* = 2.2, 8.6 Hz, 6'-H), 7.48 (1H, d, *J* = 2.2 Hz, 2'-H), 12.60 (1H, br s, 5-OH). ^{13}C-NMR (DMSO-d_6, 125 MHz) *δ*c: given in Table 2. Positive-ion FAB-MS: *m/z* 793 $(M+Na)^+$. Negative-ion FAB-MS: *m/z* 769 $(M-H)^-$.

Alkaline Hydrolysis of 1—3

A solution of sarmenosides I—III (**1**—**3**, each 3.0 mg) in 50% aqueous 1,4-dioxane (0.5 mL) was treated with 10% aqueous KOH (0.5 mL) and the whole mixture was stirred at 37 °C for 1 h. A part of the reaction mixture was subjected to HPLC analysis [column: Cosmosil C_{18}-PAQ, 250 × 4.6 mm i.d.; mobile phase: MeOH–H_2O (45:55, v/v); detection: UV (254 nm); flow rate: 0.7 mL/min] to identify caffeic acid (**a**, t_R 10.9 min) from **1** and **3**, and *p*-coumaric acid (**b**, t_R 18.0 min) from **2**. The rest of each reaction mixture was neutralized with Dowex HCR W2 (H^+ form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure yielded a product, which was subjected to HPLC [MeOH–H_2O (40:60, v/v)] to give **5** (1.3 mg from **1**) and **6** (0.9 mg from **2**, 1.4 mg from **3**).

Acid Hydrolysis of 4

A solution of sarmenoside IV (**4**, 3.0 mg) in 1 M HCl (1.0 mL) was heated under reflux for 3 h. After cooling, the reaction mixture was extracted with EtOAc. The aqueous layer was subjected to HPLC

analysis under the following conditions, respectively: HPLC column, Kaseisorb LC NH_2-60-5, 4.6 mm i.d. × 250 mm (Tokyo Kasei Co., Ltd., Tokyo, Japan); detection, optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan); mobile phase, CH_3CN–H_2O (85:15, v/v); flow rate 0.8 mL/min]. Identification of L-rhamnose and D-glucose present in the aqueous layer was carried out by comparison of its retention time and optical rotation with those of authentic samples, t_R: (i) 7.8 min (L-rhamnose, negative optical rotation) and (ii) 13.9 min (D-glucose, positive optical rotation), respectively.

Bioassay Method

Protective Effect on Cytotoxicity Induced by D-GalN in Primary Cultured Mouse Hepatocytes

The hepatoprotective effects of the constituents were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay using primary cultured mouse hepatocytes.[39] Hepatocytes were isolated from male ddY mice (30—35 g) by collagenase perfusion method. The cell suspension at 4×10^4 cells in 100 μL William's E medium containing fetal calf serum (10%), penicillin G (100 units/mL), and streptomycin (100 μg/ml) was inoculated in a 96-well microplate, and precultured for 4 h at 37°C under a 5% CO_2 atmosphere. The fresh medium (100 μL) containing D-GalN (2 mM) and a test sample were added and the hepatocytes were cultured for 44 h. The medium was exchanged with 100 μL of the fresh medium, and 10 μL of MTT (5 mg/mL in phosphate buffered saline) solution was added to the medium. After 4 h culture, the medium was removed, 100 μL of isopropanol containing 0.04 M HCl was then added to dissolve the formazan produced in the cells. The optical density (O.D.) of the formazan solution was measured by microplate reader at 570 nm (reference: 655 nm). Inhibition (%) was obtained by following formula.

$$\text{Inhibition (\%)} = [(\text{O.D.(sample)} - \text{O.D.(control)})/(\text{O.D.(normal)} - \text{O.D.(control)})] \times 100$$

Cytotoxic effects of the constituents were assessed by MTT colorimetric assay. Briefly, after 44 h incubation with a test sample in the absence of D-GalN, MTT assay was performed as described above.

Statistics

Values are expressed as means±S.E.M. One-way analysis of variance followed by Dunnett's test was used for statistical analysis.

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19 Kaempferol 3-*O*-β-D-glucopyranosyl(1→2)-α-L-rhamnopyranosyl-7-*O*-α-L-rhamnopyranoside (**9**): ^{13}C-NMR (DMSO-d_6, 125 MHz) δc: 157.5 (C-2), 134.8 (C-3), 177.8 (C-4), 160.8 (C-5), 99.4 (C-6), 161.7 (C-7), 94.6 (C-8), 156.0 (C-9), 105.7 (C-10), 120.2 (C-1'), 130.6 (C-2',6'), 115.4 (C-3',5'), 160.2 (C-4'), 100.9 (C-1''), 81.2 (C-2''), 70.2 (C-3''), 71.7 (C-4''), 70.2 (C-5''), 17.4 (C-6''), 106.0 (C-1'''), 73.9 (C-2'''), 76.3 (C-3'''), 69.3 (C-4'''), 76.6 (C-5'''), 60.5 (C-6'''), 98.3 (C-1''''), 69.8 (C-2''''), 70.4 (C-3''''), 71.6 (C-4''''), 70.1 (C-5''''), 17.9 (C-6'''').

20 The ^{1}H- and ^{13}C-NMR spectra of **1**—**4** were assigned with the aid of distortionless enhancement by polarization transfer (DEPT), homocorrelation spectroscopy (^{1}H-^{1}H COSY), heteronuclear multiple quantum coherence (HMQC), and HMBC experiments.

21 Quercetin 3-*O*-β-D-glucopyranosyl(1→2)-α-L-rhamnopyranosyl-7-*O*-α-L-rhamnopyranoside (**12**): ^{13}C-NMR (DMSO-d_6, 125 MHz) δc: 157.4 (2-C), 134.7 (3-C), 177.8 (4-C), 160.8 (5-C), 99.3 (6-C), 161.6 (7-C), 94.4 (8-C), 155.9 (9-C), 105.6 (10-C), 120.1 (1'-C), 115.4 (2'-C), 145.2 (3'-C), 148.9 (4'-C), 115.4 (5'-C), 121.0 (6'-C), 100.9 (1''-C), 81.3 (2''-C), 70.3 (3''-C), 71.6 (4''-C), 70.3 (5''-C), 17.3

(6''-C), 106.1 (1'''-C), 73.8 (2'''-C), 76.1 (3'''-C), 68.9 (4'''-C), 76.4 (5'''-C), 60.1 (6'''-C), 98.4 (1''''-C), 69.7 (2''''-C), 70.3 (3''''-C), 71.5 (4''''-C), 70.1 (5''''-C), 17.8 (6''''-C).

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