

Bioactive Constituents from Chinese Natural Medicines. XXIII.¹⁾ Absolute Structures of New Megastigmane Glycosides, Sedumosides A₄, A₅, A₆, H, and I, and Hepatoprotective Megastigmanes from *Sedum sarmentosum*

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The methanol-eluted fraction of the hot water extract from the whole plant of *Sedum sarmentosum* (Crassulaceae) was found to show hepatoprotective effect on D-galactosamine-induced cytotoxicity in primary cultured mouse hepatocytes. From the active fraction, five new megastigmane glycosides, sedumosides A₄, A₅, A₆, H, and I, were isolated together with 22 megastigmane constituents. Their absolute stereostructures were elucidated on the basis of chemical and physicochemical evidence. Among them, sedumside F₁ (IC₅₀=47 μM), (3*S*,5*R*,6*S*,9*R*)-megastigmane-3,9-diol (61 μM), and myrsiniosides A (52 μM) and D (62 μM) were found to show the strong hepatoprotective activity.

Key words *Sedum sarmentosum*; sedumside; megastigmane; hepatoprotective activity; Chinese natural medicine; Crassulaceae

During the course of our characterization studies on the bioactive constituents from Chinese natural medicines,^{1–16)} we reported the isolation and structure elucidation of sarmentoic acid (1), sarmentol A (2), and sedumosides A₁–A₃ (3–5), B (6), C (7), D (8), E₁–E₃ (9–11), F₁ (12), F₂ (13), and G (14) from the whole plant of *Sedum sarmentosum* (Crassulaceae).^{1,16)} As a continuing study on this herbal medicine, the methanolic extract of the hot water extract from *S. sarmentosum* was found to show hepatoprotective effect on D-galactosamine (D-GalN)-induced cytotoxicity in primary cultured mouse hepatocytes. Through bioassay-guided

separation, we isolated five new megastigmane glycosides, sedumosides A₄ (15), A₅ (16), A₆ (17), H (18), and I (19), from the active methanol-eluted fraction together with other megastigmane constituents (1–14, 20–27). This paper deals with the absolute stereostructure elucidation of these new constituents (15–19) and the hepatoprotective effects of megastigmane constituents (1–5, 9–27) and related megastigmanes (1a, 19a) on D-GalN-induced cytotoxicity in primary cultured mouse hepatocytes.

The hot water extract of the whole plant of *S. sarmentosum* was treated with methanol to give the methanol-soluble

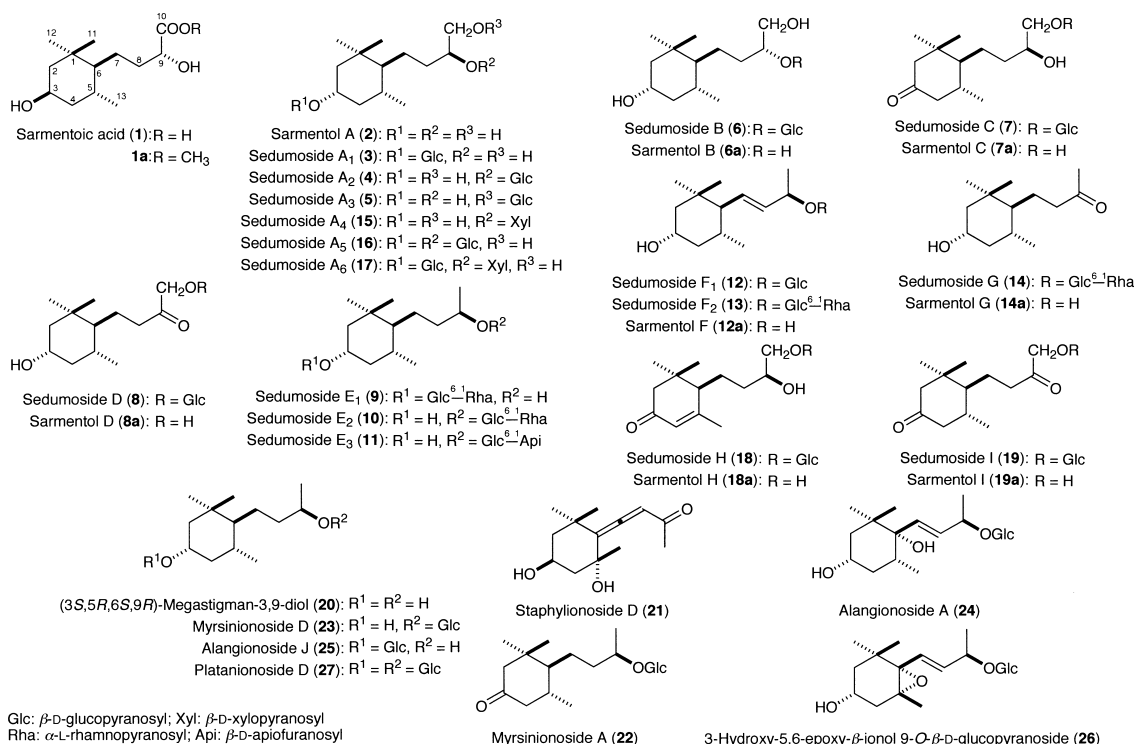


Chart 1

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Table 1. Inhibitory Effects of Methanol-Soluble Part and Its Fractions from the Hot Water Extract of *S. sarmentosum* on D-GalN-Induced Cytotoxicity in Primary Cultured Mouse Hepatocytes

	Inhibition (%)				
	0 $\mu\text{g/ml}$	10 $\mu\text{g/ml}$	30 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$	300 $\mu\text{g/ml}$
MeOH-soluble part	0.0 $\pm$ 2.9	24.5 $\pm$ 1.1**	26.0 $\pm$ 1.2**	36.1 $\pm$ 2.7**	46.0 $\pm$ 1.3**
MeOH-eluted fraction	0.0 $\pm$ 3.0	30.0 $\pm$ 1.3**	46.0 $\pm$ 2.5**	64.1 $\pm$ 2.9**	113.5 $\pm$ 1.4**
H ₂ O-eluted fraction	0.0 $\pm$ 2.8	9.4 $\pm$ 5.8	—	—	—

Each value represents the mean $\pm$ S.E.M. ($n=4$). Significantly different from the control, ** $p<0.01$. (—): Cytotoxicity.

Table 2. ¹³C-NMR Data for **15**—**19**, **18a**, **18b**, and **19a**

Position	15 ^{a)}	16 ^{a)}	17 ^{b)}	17 ^{a)}	18 ^{a)}	18a ^{a)}	18b ^{a)}	19 ^{a)}	19a ^{a)}
1	36.8	36.8	35.9	36.8	37.3	37.4	40.1	40.2	40.3
2	51.9	48.5	48.1	48.5	47.9	48.1	51.8	57.0	57.0
3	67.4	75.8	74.2	75.7	202.3	202.3	215.2	214.0	214.0
4	46.5	44.8	44.3	44.8	125.4	125.5	46.2	50.7	50.7
5	34.8	35.0	33.7	34.9	169.6	169.7	33.8	37.5	37.5
6	54.3	54.4	53.2	54.4	52.2	52.4	50.4	52.6	52.8
7	26.0	26.1	25.3	26.1	26.8	27.2	21.9	23.2	23.6
8	35.2	35.2	35.1	35.3	33.9	34.1	36.7	41.3	40.7
9	82.8	82.5	82.9	82.9	71.5	73.2	73.6	210.6	212.1
10	65.1	64.9	65.5	65.1	75.2	67.3	67.4	74.7	68.8
11	21.4	21.4	21.0	21.4	27.4	27.5	29.5	21.1	21.0
12	31.3	31.4	30.9	31.3	29.0	29.1	28.0	30.3	30.3
13	21.4	21.6	21.1	21.5	24.9	24.8	19.0	21.4	21.3
1'	105.1	102.7	103.0	102.7	104.7			104.2	
2'	75.5	75.1	75.4	75.1	75.1			74.9	
3'	78.0	78.1	78.7	78.1	77.8			77.8	
4'	71.2	71.7	71.7	71.7	71.6			71.6	
5'	67.1	77.9	78.5	77.9	77.9			78.1	
6'		62.7	62.9	62.9	62.7			62.8	
1''		104.0	105.6	105.1					
2''		75.6	75.6	75.5					
3''		78.1	78.7	78.0					
4''		71.7	71.1	71.3					
5''		77.9	67.5	67.1					
6''		62.7							

Measured in a) CD₃OD or b) pyridine-*d*₅ at 125 MHz.

part (0.57% from the fresh plant). The methanol-soluble part was subjected to Diaion HP-20 column chromatography (H₂O→MeOH) to give the water- and methanol-eluted fractions (0.44% and 0.13%, respectively) as previously reported.¹⁶⁾ As shown in Table 1, the methanol-soluble part and methanol-eluted fraction were found to show hepatoprotective activity. From the methanol-eluted fraction, **15** (0.00001%), **16** (0.00003%), **17** (0.00002%), **18** (0.00022%), and **19** (0.00026%) were isolated using normal- and reverse-phase silica gel column chromatography, and finally HPLC together with 22 megastigmane constituents (**1**—**14**, **20**—**27**), which were reported previously.^{1,16)}

Absolute Stereostructures of Sedumosides A₄ (15), A₅ (16), A₆ (17), H (18), and I (19) Sedumoside A₄ (**15**) was isolated as an amorphous powder with negative optical rotation ($[\alpha]_D^{26}$ -11.1° in MeOH). The IR spectrum of **15** showed absorption bands at 3405, 1072, and 1039 cm⁻¹ ascribable to hydroxyl and ether functions. In the positive-ion fast atom bombardment (FAB)-MS of **15**, a quasimolecular ion peak was observed at m/z 385 (M+Na)⁺. The molecular formula C₁₈H₃₄O₇ of **15** was determined based on high-resolution positive-ion FAB-MS measurement. Acid hydrolysis of **15** with 1.0 M hydrochloric acid (HCl) liberated the agly-

cone, sarmentol A¹⁶⁾ (**2**), together with D-xylose, which was identified by HPLC analysis using an optical rotation detector.^{1,2,4-6,9-12,14,16)} The ¹H- (CD₃OD) and ¹³C-NMR (Table 2) spectra¹⁷⁾ of **15** showed signals assignable to a sarmentol A part { δ 0.84, 0.95 (3H each, both s, 11, 12-H₃), 0.98 (3H, d, $J=6.8$ Hz, 13-H₃), [3.53 (1H, dd, $J=6.7$, 12.5 Hz), 3.62 (1H, m), 10-H₂], 3.62 (1H, m, 9-H), 3.70 (1H, m, 3-H)} and a β -D-xylopyranosyl moiety [δ 4.34 (1H, d, $J=7.7$ Hz, 1'-H)]. In the heteronuclear multiple-bond correlation (HMBC) experiment of **15**, a long-range correlation was observed between the 1'-proton and the 9-carbon (δ_C 82.8). On the basis of the above-mentioned evidence, the absolute stereostructure of sedumoside A₄ was elucidated to be sarmentol A 9-O- β -D-xylopyranoside (**15**).

Sedumosides A₅ (**16**) and A₆ (**17**) were also isolated as amorphous powders with negative optical rotations (**16**: $[\alpha]_D^{19}$ -16.7° ; **17**: $[\alpha]_D^{17}$ -26.8° both in MeOH), respectively. The molecular formula C₂₅H₄₆O₁₃ of **16** was determined from the positive-ion FAB-MS [m/z 577 (M+Na)⁺] and by high-resolution positive-ion FAB-MS. On the other hand, the molecular formula of **17**, C₂₄H₄₄O₁₂, was also determined based on high-resolution positive-ion FAB-MS. The acid hydrolysis of **16** and **17** liberated **2** (from **16** and **17**), together with D-glu-

cose (from **16** and **17**) and D-xylose (from **17**), which were identified by HPLC analysis using an optical rotation detector.^{1,2,4–6,9–12,14,16} The ¹H- (CD₃OD) and ¹³C-NMR (Table 2) spectra¹⁷ of **16** showed signals assignable to a sarmentol A part together with two β-D-glucopyranosyl moieties [δ 4.33 (1H, d, $J=7.7$ Hz, 1'-H), 4.42 (1H, d, $J=7.7$ Hz, 1''-H)]. The long-range correlations in the HMBC experiment on **16** were observed between the 1'-proton and the 3-carbon (δ_C 75.8) and between the 1''-proton and the 9-carbon (δ_C 82.5). On the other hand, the proton and carbon signals in the ¹H- (pyridine-*d*₅) and ¹³C-NMR (Table 2) spectra¹⁷ of **17** were superimposable on those of **16**, except for the signals due to sugar parts {a β-D-glucopyranosyl [δ 5.02 (1H, d, $J=8.0$ Hz, 1'-H)] and a β-D-xylopyranosyl moiety [δ 5.04 (1H, d, $J=8.2$ Hz, 1''-H)]}. The connectivities of the sugar parts in **17** were also characterized in HMBC experiments, which showed long-range correlations between the 1'-proton and the 3-carbon (δ_C 74.2) and between the 1''-proton and the 9-carbon (δ_C 82.9). On the basis of the above-mentioned evidence, the absolute stereostructures of sedumosides A₅ and A₆ were elucidated to be sarmentol A 3,9-di-O-β-D-glucopyranoside (**16**) and sarmentol A 3-O-β-D-glucopyranosyl-9-O-β-D-xylopyranoside (**17**).

Sedumoside H (**18**) was obtained as an amorphous powder and exhibited a positive optical rotation ($[\alpha]_D^{27} +71.4^\circ$ in MeOH). In the UV spectrum of **18**, an absorption maximum was observed at 240 (log ϵ 4.08) nm. The IR spectrum of **18** showed absorption bands at 3389, 1669, 1076, and 1038 cm⁻¹ assignable to hydroxyl, α,β-unsaturated carbonyl, and ether functions. In the positive-ion FAB-MS of **18**, a quasimolecular ion peak was observed at m/z 411 (M+Na)⁺ and high-resolution FAB-MS analysis revealed the molecular formula to be C₁₉H₃₂O₈. Acid hydrolysis of **18** with 1.0 M HCl liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.^{1,2,4–6,9–12,14,16} Enzymatic hydrolysis of **18** with β-glucosidase gave a new megastigmane, sarmentol H (**18a**), as the aglycon. The proton and carbon signals in the ¹H- (CD₃OD) and ¹³C-NMR (Table 2) spectra¹⁷ of **18a** indicated the presence of three methyls [δ 1.02, 1.09, 2.04 (3H each, all s, 12, 11, 13-H₃)], a methylene and a methine bearing an oxygen function [δ 3.45 (2H, d, $J=5.5$ Hz, 10-H₂), 3.54 (1H, m, 9-H)], and an olefin [δ 5.81 (1H, s, 4-H)]. The ¹H–¹H correlation spectroscopy (¹H–¹H COSY) experiment on **18a** indicated the presence of a partial structure shown by the bold line in Fig. 1. In the HMBC experiment on **18a**, long-range correlations were observed between the following proton and carbon pairs: 2-H₂ and 3-C; 4-H and 2, 3, 6, 13-C; 6-H and 1, 5, 7-C; 7-H₂ and 6, 8-C; 8-H₂ and 6, 7, 9-C; 9-H and 7, 8, 10-C; 10-H₂ and 9-C; 11-H₃ and 1, 2, 6, 12-C; 12-H₃ and 1, 2, 6, 11-C; and 13-H₃ and 4–6-C. The relative stereostructure of **18a** was characterized in a NOESY experiment, which showed NOE correlations between the following proton pairs: 2α-H and 12-H₃; 2β-H and 11-H₃; 4-H and 13-H₃; 6-H and 12-H₃; and 7-H₂ and 11-H₃ (Fig. 1). To clarify the absolute stereostructure of **18a**, we carried out the conversion of **18a** into sarmentol C (**7a**), of which the absolute stereostructure was elucidated previously.¹⁶ Thus hydrogenation of **18a** with 10% palladium carbon (Pd-C) under an H₂ atmosphere gave **7a** and **18b** in an approximate 1:3 ratio. The reductant (**18b**) was produced by hydrogenation of **18a** via intermediates (i and

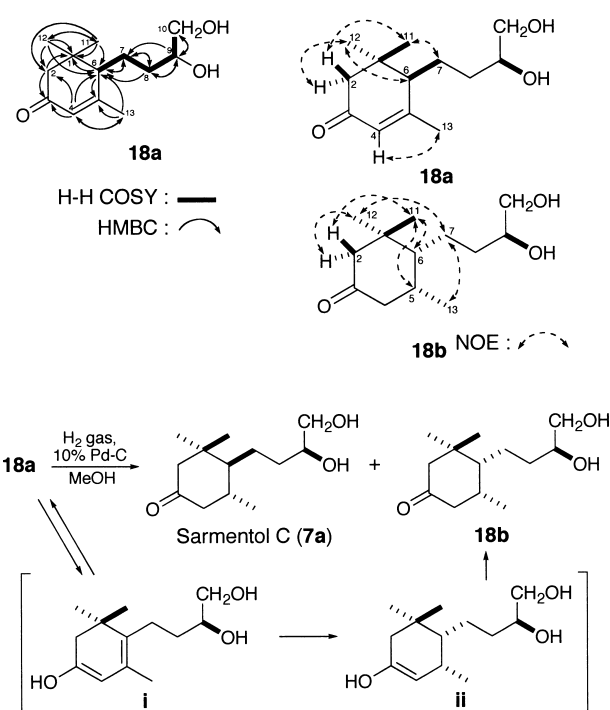


Fig. 1

ii), as shown in Fig. 1. The proton and carbon signals in the ¹H- (CD₃OD) and ¹³C-NMR (Table 2) spectra¹⁷ of **18b** were superimposable on those of **7a** { δ 0.97, 1.05 (3H each, both s, 11, 12-H₃), 1.01 (3H, d, $J=7.0$ Hz, 13-H₃), [3.46 (1H, dd, $J=6.4, 11.0$ Hz), 3.49 (1H, dd, $J=4.9, 11.0$ Hz), 10-H₂], 3.60 (1H, m, 9-H)}, except for the signals around the 6-position. The relative stereostructure of **18b** was clarified in a NOESY experiment, which showed NOE correlations between the following proton pairs: 2α-H and 12-H₃; 2β-H and 11-H₃; 5-H and 11-H₃; 6-H and 11-H₃; and 7-H₂ and 12, 13-H₃. The absolute stereostructure of **18b** was confirmed by the application of the octant rule.¹⁸ The circular dichroic (CD) spectrum of **18b** showed a positive Cotton effect at 291 nm ($\Delta\epsilon +0.17$ in MeOH), so that the absolute configuration of the 5-position in **18b** was confirmed to be the *R* orientation. Consequently, **18b** was clarified to be the 6-diastereoisomer of **7a** and sarmentol H was characterized to be (6*R*,9*S*)-4-megastigmen-9,10-dihydroxy-3-one (**18a**). Next, the ¹H- (CD₃OD) and ¹³C-NMR (Table 2) spectra¹⁷ of **18** showed signals assignable to a sarmentol H part together with a β-D-glucopyranosyl moiety [δ 4.28 (1H, d, $J=7.7$ Hz, 1'-H)]. In the HMBC experiment on **18**, a long-range correlation was observed between the 1'-proton and the 10-carbon (δ_C 75.2), so that the connectivity of a β-D-glucopyranosyl part in **18** was clarified to be the 10-position of **18a**. On the basis of those findings, the absolute stereostructure of sedumoside H was elucidated to be sarmentol H 10-O-β-D-glucopyranoside (**18**).

Sedumoside I (**19**), $[\alpha]_D^{24} -0.2^\circ$ (MeOH), was also obtained as an amorphous powder. The molecular formula C₁₉H₃₂O₈, of **19** was determined from the positive-ion FAB-MS and high-resolution positive-ion FAB-MS. The acid hydrolysis of **19** liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.^{1,2,4–6,9–12,14,16} The ¹H- (CD₃OD) and ¹³C-NMR (Table 2) spectra¹⁷ of **19** showed signals assignable to three methyls [δ 0.77, 1.07

(3H each, both s, 11, 12-H₃), 1.08 (3H, d, $J=6.7$ Hz, 13-H₃) and a methylene bearing an oxygen function [δ 4.33, 4.52 (1H each, both d, $J=17.4$ Hz, 10-H₂)] together with a β -glucopyranosyl moiety [δ 4.31 (1H, d, $J=7.7$ Hz, 1'-H)]. The planar structure of **19** was confirmed by ¹H-¹H COSY and HMBC experiments, as shown in Fig. 2. Thus the ¹H-¹H COSY experiment on **19** indicated the presence of two partial structures (4-C—8-C; 1'-C—6'-C), while long-range correlations in the HMBC experiment on **19** were observed between the following proton and carbon pairs: 2-H and 1, 3-C; 4-H₂ and 3, 6-C; 5-H and 3-C; 6-H and 1-C; 7-H₂ and 9-C; 8-H₂ and 9-C; 10-H₂ and 9-C; 11-H₃ and 1, 2, 6, 12-C; 12-H₃ and 1, 2, 6, 11-C; 13-H₃ and 4—6-C; and 1'-H and 10-C. In the NOESY experiment on **19**, NOE correlations were observed between the following proton pairs: 2 α -H and 6-H, 12-H₃; 2 β -H and 11-H₃; 4 α -H and 6-H, 13-H₃; 6-H and 12-H₃; and 7-H₂ and 11-H₃ (Fig. 2). Next, enzymatic hydrolysis of **19** with β -glucosidase gave a new megastigmane, sarmentol I (**19a**), as the aglycon. The CD spectra of **19** and **19a** both showed a positive Cotton effect [**19**, 285 nm ($\Delta\epsilon$ +0.09); **19a**, 288 nm ($\Delta\epsilon$ +0.12), both in MeOH]. By the application of the octant rule to **19** and **19a**, the absolute configuration of the 5-positions were confirmed to be *R* orientations.¹⁸⁾ Thus the absolute stereostructures of **19a** and **19** were determined to be (5*R*,6*S*)-megastigman-10-hydroxy-3,9-dione and its 10-*O*- β -D-glucopyranoside, respectively.

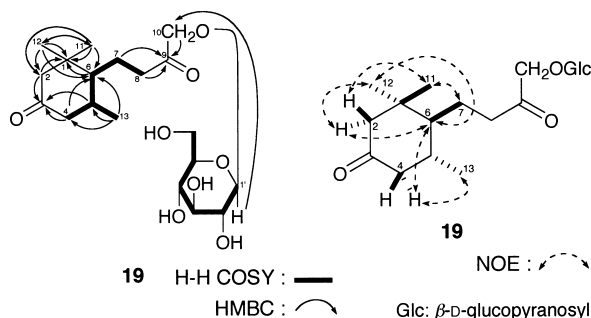


Fig. 2

Protective Effects on D-GalN-Induced Cytotoxicity in Primary Cultured Mouse Hepatocytes Previously, we reported the isolation and structure elucidation of several constituents with hepatoprotective effects from *Hovenia dulcis*,¹⁹⁾ *Bupleurum scorzoniferifolium*,^{20,21)} *Curcuma zedoaria*,^{22–24)} *Angelica furcijuga*,^{25,26)} *Betula platyphylla* var. *japonica*,²⁷⁾ *Pisum sativum*,²⁸⁾ *Salacia reticulata*,²⁹⁾ *Tilia argentea*,³⁰⁾ *Anastatica hierochuntica*,³¹⁾ *Panax notoginseng*,³²⁾ *Cyperus longus*,³³⁾ *Erycibe expansa*,³⁴⁾ and *Camellia sinensis*.³⁵⁾ Since the methanol-soluble part and methanol-eluted fraction were found to show hepatoprotective activities (*vide ante*), the inhibitory effects of megastigmane constituents (**1–5**, **9–27**) and related compounds (**1a**, **19a**) were also examined. As shown in Table 3, sedumoside F₁ (**12**, IC₅₀=47 μ M), (3*S*,5*R*,6*S*,9*R*)-megastigmane-3,9-diol (**20**, 61 μ M), myrsiniosides A (**22**, 52 μ M) and D (**23**, 62 μ M), and sarmentol I (**19a**, 32 μ M) were found to show hepatoprotective activity, which were equivalent to that of the hepatoprotective agent silybin (41 μ M).

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter ($l=5$ cm); CD spectra, JASCO J-720WI spectrometer; UV spectra, Shimadzu UV-1600 spectrometer; IR

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

	Inhibition (%)				
	0 μ M	3 μ M	10 μ M	30 μ M	100 μ M
Sarmentol acid (1)	0.0 $\pm$ 1.5	16.2 $\pm$ 3.3**	19.6 $\pm$ 3.6**	21.6 $\pm$ 3.4**	29.5 $\pm$ 2.3**
1a	0.0 $\pm$ 0.8	18.7 $\pm$ 1.9**	23.5 $\pm$ 2.3**	25.2 $\pm$ 2.5**	33.6 $\pm$ 2.3**
Sarmentol A (2)	0.0 $\pm$ 9.8	2.5 $\pm$ 2.9	15.4 $\pm$ 1.2	20.4 $\pm$ 3.2*	38.8 $\pm$ 1.3**
Sedumoside A ₁ (3)	0.0 $\pm$ 3.6	16.9 $\pm$ 2.1**	19.3 $\pm$ 1.2**	22.3 $\pm$ 1.5**	31.4 $\pm$ 0.8**
Sedumoside A ₂ (4)	0.0 $\pm$ 2.3	19.3 $\pm$ 1.7**	23.5 $\pm$ 2.9**	27.0 $\pm$ 1.2**	43.8 $\pm$ 4.3**
Sedumoside A ₃ (5)	0.0 $\pm$ 2.8	17.7 $\pm$ 0.8**	18.6 $\pm$ 2.0**	23.8 $\pm$ 2.3**	32.8 $\pm$ 2.2**
Sedumoside A ₄ (15)	0.0 $\pm$ 3.3	4.1 $\pm$ 2.3	8.8 $\pm$ 2.8	19.2 $\pm$ 3.4*	41.8 $\pm$ 2.7**
Sedumoside A ₅ (16)	0.0 $\pm$ 1.8	23.2 $\pm$ 1.9**	26.3 $\pm$ 1.1**	27.9 $\pm$ 1.8**	47.6 $\pm$ 1.7**
Sedumoside A ₆ (17)	0.0 $\pm$ 1.3	3.2 $\pm$ 0.6	5.2 $\pm$ 3.1	9.9 $\pm$ 2.4*	24.3 $\pm$ 1.6**
Sedumoside E ₁ (9)	0.0 $\pm$ 1.6	5.0 $\pm$ 0.7	5.8 $\pm$ 1.7	8.9 $\pm$ 1.0*	18.3 $\pm$ 1.7**
Sedumoside E ₂ (10)	0.0 $\pm$ 1.6	20.6 $\pm$ 1.9**	21.4 $\pm$ 2.4**	27.5 $\pm$ 2.0**	30.2 $\pm$ 3.3**
Sedumoside E ₃ (11)	0.0 $\pm$ 1.0	2.8 $\pm$ 1.9	6.2 $\pm$ 1.9	20.3 $\pm$ 0.2**	48.5 $\pm$ 0.6**
Sedumoside F ₁ (12)	0.0 $\pm$ 3.4	17.7 $\pm$ 1.1	25.7 $\pm$ 2.4**	42.7 $\pm$ 1.0**	62.6 $\pm$ 2.7**
Sedumoside F ₂ (13)	0.0 $\pm$ 0.6	15.9 $\pm$ 0.8**	16.2 $\pm$ 1.5**	19.4 $\pm$ 1.9**	25.1 $\pm$ 2.1**
Sedumoside H (18)	0.0 $\pm$ 1.2	−2.3 $\pm$ 1.6	1.0 $\pm$ 1.2	1.8 $\pm$ 2.1	5.4 $\pm$ 1.5
Sedumoside I (19)	0.0 $\pm$ 1.2	20.9 $\pm$ 1.7**	22.4 $\pm$ 1.5**	29.9 $\pm$ 1.2**	45.1 $\pm$ 2.5**
Sarmentol I (19a)	0.0 $\pm$ 2.8	3.8 $\pm$ 2.8	12.8 $\pm$ 1.7**	41.3 $\pm$ 2.4**	86.5 $\pm$ 2.7**
(3 <i>S</i> ,5 <i>R</i> ,6 <i>S</i> ,9 <i>R</i>)-Megastigmane-3,9-diol (20)	0.0 $\pm$ 2.3	18.8 $\pm$ 1.4**	25.5 $\pm$ 0.7**	41.6 $\pm$ 2.5**	57.2 $\pm$ 1.5**
Staphylionoside D (21)	0.0 $\pm$ 3.1	2.2 $\pm$ 2.0	2.0 $\pm$ 2.5	6.3 $\pm$ 0.9	12.2 $\pm$ 1.6**
Myrsinioside A (22)	0.0 $\pm$ 2.2	17.7 $\pm$ 3.0*	27.4 $\pm$ 5.1**	45.0 $\pm$ 4.8**	57.9 $\pm$ 2.5**
Myrsinioside D (23)	0.0 $\pm$ 1.4	14.9 $\pm$ 1.8**	21.4 $\pm$ 1.5**	39.1 $\pm$ 1.5**	58.8 $\pm$ 0.9**
Alangionoside A (24)	0.0 $\pm$ 0.9	16.5 $\pm$ 4.6*	19.6 $\pm$ 3.8*	24.3 $\pm$ 2.6**	31.9 $\pm$ 4.0**
Alangionoside J (25)	0.0 $\pm$ 2.5	17.9 $\pm$ 3.0*	20.1 $\pm$ 3.1**	25.3 $\pm$ 1.3**	34.0 $\pm$ 1.8**
26	0.0 $\pm$ 1.4	−1.5 $\pm$ 3.5	4.3 $\pm$ 3.1	6.9 $\pm$ 3.4	26.9 $\pm$ 5.0**
Platanionoside D (27)	0.0 $\pm$ 1.4	13.9 $\pm$ 4.6	21.0 $\pm$ 4.7**	27.4 $\pm$ 2.1**	46.2 $\pm$ 4.8**
Silybin ^{a)}	0.0 $\pm$ 0.3	4.8 $\pm$ 1.1	7.7 $\pm$ 0.7	45.2 $\pm$ 8.8**	77.0 $\pm$ 5.5**

Each value represents the mean $\pm$ 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).

spectra, Shimadzu FTIR-8100 spectrometer; EI-MS and high-resolution MS, JEOL JMS-GCMATE mass spectrometer; FAB-MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; ^1H -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; and HPLC detector, Shimadzu RID-6A refractive index and SPD-10Avp UV-VIS detectors. HPLC column, Cosmosil 5C₁₈-MS-II (Nacalai Tesque Inc., 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: ordinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., Aichi, Japan, 150–350 mesh); reverse-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., Aichi, Japan, 100–200 mesh); TLC, precoated TLC plates with Silica gel 60F₂₅₄ (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reverse phase); reverse-phase HPTLC, precoated TLC plates with Silica gel RP-18 F_{254S} (Merck, 0.25 mm); and detection was achieved by spraying with 1% Ce(SO₄)₂–10% aqueous H₂SO₄, followed by heating.

Plant Material *S. sarmentosum* was cultivated in 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.^{1,16)}

Extraction and Isolation Fractions 1-5 (1510 mg), 2-5 (3300 mg), 2-8 (1800 mg), 2-10 (1360 mg), 3-7 (230 mg), and 5-6 (665 mg) were obtained from the methanol-eluted fraction of the hot water extract from the fresh whole plant of *S. sarmentosum* as reported previously.^{1,16)} Fraction 1-5 (1510 mg) was purified on Sephadex LH-20 column chromatography [150 g, CHCl₃–MeOH (1:1, v/v)] and finally HPLC [MeOH–H₂O (35:65, v/v)] to furnish sedumside I (**19**, 107.2 mg, 0.00020%). Fraction 2-5 (3300 mg) was further separated by HPLC [CH₃CN–H₂O (15:85, v/v)] to furnish sedumside H (**18**, 83.7 mg, 0.00016%) and **19** (34.1 mg, 0.00006%). Fraction 2-8 (1800 mg) was purified on Sephadex LH-20 column chromatography [150 g, CHCl₃–MeOH (1:1, v/v)] and finally HPLC [CH₃CN–MeOH–H₂O (20:8:72, v/v/v) and MeOH–H₂O (40:60, v/v)] to furnish sedumside A₄ (**15**, 4.9 mg, 0.00001%). Fraction 2-10 (1360 mg) was further separated by HPLC [CH₃CN–MeOH–H₂O (20:8:72, v/v/v) and MeOH–H₂O (40:60, v/v)] to furnish **18** (37.5 mg, 0.00007%). Fraction 3-7 (230 mg) was purified by HPLC [MeOH–H₂O (29:71, v/v)] to furnish sedumside A₆ (**17**, 12.0 mg, 0.00002%). Fraction 5-6 (665 mg) was purified by HPLC [CH₃CN–MeOH–H₂O (10:8:82, v/v/v)] to give sedumside A₅ (**16**, 18.4 mg, 0.00003%).

Sedumside A₄ (**15**): An amorphous powder, $[\alpha]_D^{26} -11.1^\circ$ ($c=0.25$, MeOH). High-resolution positive-ion FAB-MS: Calcd for C₁₈H₃₄O₇Na (M+Na)⁺ 385.2202; Found 385.2209. IR (KBr, cm⁻¹): 3405, 2924, 2870, 1473, 1072, 1039. ^1H -NMR (500 MHz, CD₃OD) δ : 0.53 (1H, ddd, $J=2.0$, 4.6, 10.7 Hz, 6-H), 0.84, 0.95 (3H each, both s, 11, 12-H₃), 0.98 (3H, d, $J=6.8$ Hz, 13-H₃), 0.90 (1H, ddd, $J=11.9$, 11.9, 11.9 Hz, 4 α -H), 1.07, 1.66 (1H each, both m, 7-H₂), 1.09 (1H, dd, $J=11.9$, 11.9 Hz, 2 α -H), 1.45 (1H, m, 5-H), 1.49, 1.65 (1H each, both m, 8-H₂), 1.64 (1H, ddd, $J=2.4$, 4.0, 11.9 Hz, 2 β -H), 1.89 (1H, m, 4 β -H), [3.18 (1H, dd, $J=10.4$, 11.3 Hz), 3.84 (1H, dd, $J=5.2$, 11.3 Hz), 5'-H₂], 3.20 (1H, dd, $J=7.7$, 9.2 Hz, 2'-H), 3.31 (1H, m, 3'-H), 3.47 (1H, ddd, $J=5.2$, 8.9, 10.4 Hz, 4'-H), [3.53 (1H, dd, $J=6.7$, 12.5 Hz), 3.62 (1H, m, 10-H₂), 3.62 (1H, m, 9-H), 3.70 (1H, m, 3-H), 4.34 (1H, d, $J=7.7$ Hz, 1'-H)]. ^{13}C -NMR (125 MHz, CD₃OD) δ : see Table 2. Positive-ion FAB-MS m/z : 385 (M+Na)⁺.

Sedumside A₅ (**16**): An amorphous powder, $[\alpha]_D^{19} -16.7^\circ$ ($c=0.93$, MeOH). High-resolution positive-ion FAB-MS: Calcd for C₂₅H₄₆O₁₃Na (M+Na)⁺ 577.2836; Found 577.2831. IR (KBr, cm⁻¹): 3410, 2941, 2898, 1474, 1171, 1076, 1030. ^1H -NMR (500 MHz, CD₃OD) δ : 0.55 (1H, ddd, $J=1.9$, 5.2, 11.3 Hz, 6-H), 0.83, 0.97 (3H each, both s, 11, 12-H₃), 0.98 (3H, d, $J=6.5$ Hz, 13-H₃), 1.02 (1H, ddd, $J=12.2$, 12.2, 12.2 Hz, 4 α -H), 1.08, 1.65 (1H each, both m, 7-H₂), 1.13 (1H, dd, $J=12.2$, 12.2 Hz, 2 α -H), 1.45 (1H, m, 5-H), 1.57, 1.64 (1H each, both m, 8-H₂), 1.79 (1H, ddd, $J=2.2$, 3.7, 12.2 Hz, 2 β -H), 2.01 (1H, m, 4 β -H), 3.17 (1H, dd, $J=7.7$, 9.2 Hz, 2'-H), 3.20 (1H, dd, $J=7.7$, 9.2 Hz, 2'-H), 3.25–3.29 (4H, m, 4', 5', 4'', 5''-H), 3.33 (1H, dd, $J=9.2$, 9.2 Hz, 3'-H), 3.35 (1H, dd, $J=9.2$, 9.2 Hz, 3'-H), [3.52 (1H, dd, $J=5.8$, 11.9 Hz), 3.65 (1H, dd, $J=3.4$, 11.9 Hz, 10-H₂), [3.64 (2H, m), 3.85 (2H, dd, $J=2.0$, 12.0 Hz), 6', 6''-H₂], 3.69 (1H, m, 9-H), 3.84 (1H, m, 3-H), 4.33 (1H, d, $J=7.7$ Hz, 1'-H), 4.42 (1H, d, $J=7.7$ Hz, 1'-H)]. ^{13}C -NMR (125 MHz, CD₃OD) δ : see Table 2. Positive-ion FAB-MS m/z : 577 (M+Na)⁺.

Sedumside A₆ (**17**): An amorphous powder, $[\alpha]_D^{17} -26.8^\circ$ ($c=0.60$,

MeOH). High-resolution positive-ion FAB-MS: Calcd for C₂₄H₄₄O₁₂Na (M+Na)⁺: 547.2730; Found: 547.2728. IR (KBr, cm⁻¹): 3410, 2940, 2918, 1541, 1474, 1171, 1081, 1047. ^1H -NMR (500 MHz, pyridine-*d*₅) δ : 0.51 (1H, ddd, $J=2.8$, 4.6, 10.7 Hz, 6-H), 0.77, 0.92 (3H each, both s, 11, 12-H₃), 0.90 (3H, d, $J=6.1$ Hz, 13-H₃), 1.16, 1.92 (1H each, both m, 7-H₂), 1.19 (1H, ddd, $J=11.3$, 11.3, 11.3 Hz, 4 α -H), 1.27 (1H, dd, $J=11.9$, 11.9 Hz, 2 α -H), 1.27 (1H, m, 5-H), 1.66, 1.80 (1H each, both m, 8-H₂), 2.02 (1H, ddd, $J=2.0$, 3.1, 11.9 Hz, 2 β -H), 2.21 (1H, m, 4 β -H), [3.70 (1H, dd, $J=10.4$, 11.3 Hz), 4.35 (1H, d, $J=5.2$, 11.3 Hz), 5''-H₂], 3.96 (2H, m, 10-H₂), 3.98 (1H, m, 5'-H), 4.04 (2H, m, 9, 2''-H), 4.05 (1H, m, 2'-H), 4.12 (1H, m, 3-H), 4.15 (1H, dd, $J=8.8$, 8.8 Hz, 3'-H), 4.21 (1H, m, 4''-H), 4.29 (2H, m, 3', 4'-H), [4.43 (1H, dd, $J=5.2$, 11.9 Hz), 4.58 (1H, dd, $J=2.5$, 11.9 Hz), 6'-H₂], 5.02 (1H, d, $J=8.0$ Hz, 1'-H), 5.04 (1H, d, $J=8.2$ Hz, 1'-H)]. ^1H -NMR (500 MHz, CD₃OD) δ : 0.53 (1H, ddd, $J=2.2$, 4.6, 10.7 Hz, 6-H), 0.82, 0.95 (3H each, both s, 11, 12-H₃), 0.97 (3H, d, $J=6.5$ Hz, 13-H₃), 1.02 (1H, ddd, $J=11.9$, 11.9, 11.9 Hz, 4 α -H), 1.06, 1.65 (1H each, both m, 7-H₂), 1.13 (1H, dd, $J=12.2$, 12.2 Hz, 2 α -H), 1.44 (1H, m, 5-H), 1.49, 1.65 (1H each, both m, 8-H₂), 1.79 (1H, ddd, $J=2.2$, 4.3, 12.2 Hz, 2 β -H), 2.01 (1H, m, 4 β -H), 3.11 (1H, dd, $J=7.6$, 9.2 Hz, 2'-H), [3.17 (1H, dd, $J=10.4$, 11.0 Hz), 3.83 (1H, m), 5'-H₂], 3.18 (1H, dd, $J=7.6$, 9.4 Hz, 2'-H), 3.25 (2H, m, 4', 5'-H), 3.31 (1H, m, 3''-H), 3.33 (1H, m, 3'-H), 3.47 (1H, m, 4''-H), [3.51 (1H, dd, $J=7.0$, 12.8 Hz), 3.60 (1H, m), 10-H₂], 3.61 (1H, m, 9-H), [3.64 (1H, dd, $J=5.2$, 11.9 Hz), 3.85 (1H, m), 6'-H₂], 3.84 (1H, m, 3-H), 4.33 (1H, d, $J=7.7$ Hz, 1'-H), 4.33 (1H, d, $J=7.7$ Hz, 1'-H)]. ^{13}C -NMR (125 MHz, pyridine-*d*₅ and CD₃OD) δ : see Table 2. Positive-ion FAB-MS: m/z 547 (M+Na)⁺.

Sedumside H (**18**): An amorphous powder, $[\alpha]_D^{27} +71.4^\circ$ ($c=0.21$, MeOH). High-resolution positive-ion FAB-MS: Calcd for C₁₉H₃₂O₈Na (M+Na)⁺: 411.1995; Found: 411.1989. CD [MeOH, nm ($\Delta\epsilon$)]: 211 (+4.40), 237 (+3.42), 335 (+0.75). UV [MeOH, nm ($\log \epsilon$)]: 240 (4.08). IR (KBr, cm⁻¹): 3389, 3011, 2961, 2876, 1669, 1471, 1076, 1038, 752. ^1H -NMR (500 MHz, CD₃OD) δ : 1.01, 1.09, 2.04 (3H each, all s, 12, 11, 13-H₃), 1.49, 1.98 (1H each, both m, 7-H₂), 1.51, 1.61 (1H each, both m, 8-H₂), 1.96 (1H, m, 6-H), 2.00 (1H, d, $J=17.1$ Hz, 2 β -H), 2.46 (1H, d, $J=17.1$ Hz, 2 α -H), 3.21 (1H, dd, $J=7.7$, 8.6 Hz, 2'-H), 3.28 (1H, m, 5'-H), 3.30 (1H, m, 4'-H), 3.36 (1H, m, 3'-H), [3.43 (1H, dd, $J=6.4$, 10.1 Hz), 4.74 (1H, dd, $J=3.4$, 10.1 Hz), 10-H₂], [3.64 (1H, dd, $J=4.9$, 11.6 Hz), 3.84 (1H, brd, $J=ca$. 12 Hz), 6'-H₂], 3.75 (1H, m, 9-H), 4.28 (1H, d, $J=7.7$ Hz, 1'-H), 5.81 (1H, s, 4-H). ^{13}C -NMR (125 MHz, CD₃OD) δ : see Table 2. Positive-ion FAB-MS: m/z 411 (M+Na)⁺.

Sedumside I (**19**): An amorphous powder, $[\alpha]_D^{27} -0.2^\circ$ ($c=1.41$, MeOH). High-resolution EI-MS: Calcd for C₁₉H₃₂O₈ (M⁺): 388.2097; Found: 388.2095. CD [MeOH, nm ($\Delta\epsilon$)]: 285 (+0.09). IR (KBr, cm⁻¹): 3431, 2961, 1723, 1715, 1559, 1472, 1076, 1044, 753. ^1H -NMR (500 MHz, CD₃OD) δ : 0.77, 1.07 (3H each, both s, 11, 12-H₃), 1.08 (3H, d, $J=6.7$ Hz, 13-H₃), 1.21 (1H, ddd, $J=3.1$, 6.1, 10.7 Hz, 6-H), 1.46, 1.81 (1H each, both m, 7-H₂), 1.81 (1H, m, 5-H), 1.96 (1H, dd, $J=2.2$, 13.2 Hz, 2 β -H), 2.22 (1H, ddd, $J=2.2$, 4.6, 14.1 Hz, 4 β -H), 2.16 (1H, dd, $J=14.1$, 14.1 Hz, 4 α -H), 2.39 (1H, d, $J=13.2$ Hz, 2 α -H), 2.69 (2H, m, 8-H₂), 3.25 (1H, m, 2'-H), 3.26 (1H, m, 4'-H), 3.27 (1H, m, 5'-H), 3.36 (1H, m, 3'-H), 3.64, 3.87 (1H each, both m, 6'-H₂), 4.31 (1H, d, $J=7.7$ Hz, 1'-H), 4.33, 4.52 (1H each, both d, $J=17.4$ Hz, 10-H₂). ^{13}C -NMR (125 MHz, CD₃OD) δ : see Table 2. EI-MS (%): m/z 388 (M⁺, 1), 370 (M⁺–H₂O, 1), 255 (100), 227 (66), 208 (42).

Acid Hydrolysis of 15–19 A solution of sedumside A₄ (**15**, 3.1 mg), A₅ (**16**, 2.0 mg), or A₆ (**17**, 2.5 mg) in 1 M HCl (1.0 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was extracted with EtOAc. The EtOAc extract was neutralized with Amberlite IRA-400 (OH⁻ form), and the resin was removed by filtration. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by HPLC [MeOH–H₂O (35:65, v/v)] to give sarmentol A⁽¹⁶⁾ (**2**, 1.2 mg, 91% from **15**; 1.3 mg, 79% from **16**; or 0.8 mg, 73% from **17**). Through the similar procedure, a solution of sedumside H (**18**) or I (**19**) (each 1.0 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 layers of **15–18** were subjected to HPLC analysis under the following conditions, respectively: HPLC column, Kaseisorb LC NH₂-60-5, 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, CH₃CN–H₂O (85:15, v/v); flow rate 0.8 ml/min. Identification of D-xylose (i) from **15** and **17** and D-glucose (ii) from **15–19** present in the aqueous layer was carried out by comparison of the retention time and optical rotation with those of authentic sample, t_R : (i) 9.5 min (D-xylose, positive optical rotation), and (ii) 13.9 min (D-glucose, positive optical rotation), respectively.

Enzymatic Hydrolysis of 18 and 19 with β -Glucosidase A solution of

sedumosides H (**18**, 13.2 mg) or I (**19**, 14.1 mg) in H₂O (2.0 ml) was treated with β -glucosidase (each 15.0 mg, from Almond, Oriental Yeast Co., Ltd., Tokyo, Japan) and the solution was stirred at 37 °C for 16 h. After EtOH was added to the reaction mixture, the solvent was removed under reduced pressure and the residue was purified by HPLC [MeOH–H₂O (40:60, v/v)] to furnish sarmentols H (**18a**, 7.1 mg, 91% from **18**) and I (**19a**, 7.5 mg, 91% from **19**), respectively.

Sarmentol H (18a): Colorless oil, $[\alpha]_D^{25} + 64.1^\circ$ ($c=0.40$, MeOH). High-resolution EI-MS: Calcd for C₁₃H₂₂O₃ (M⁺): 226.1569; Found: 226.1572. CD [MeOH, nm ($\Delta\epsilon$)]: 211 (+1.69), 232 (+1.35), 334 (+0.47). UV [MeOH, nm (log ϵ)]: 240 (4.00). IR (film, cm⁻¹): 3423, 2957, 2874, 1657, 1045. ¹H-NMR (500 MHz, CD₃OD) δ : 1.02, 1.09, 2.04 (3H each, all s, 12, 11, 13-H₃), 1.44, 1.66 (1H each, both m, 8-H₂), 1.49, 1.98 (1H each, both m, 7-H₂), 1.96 (1H, m, 6-H), 2.00 (1H, d, $J=17.1$ Hz, 2 β -H), 2.46 (1H, d, $J=17.1$ Hz, 2 α -H), 3.45 (2H, d, $J=5.5$ Hz, 10-H₂), 3.54 (1H, m, 9-H), 5.81 (1H, s, 4-H). ¹³C-NMR (125 MHz, CD₃OD) δ_C : see Table 2. EI-MS (%): m/z 226 (M⁺, 22), 208 (M⁺–H₂O, 19), 193 (26), 175 (34), 151 (94), 95 (100).

Sarmentol I (19a): Colorless oil, $[\alpha]_D^{24} + 19.9^\circ$ ($c=0.31$, MeOH). High-resolution EI-MS: Calcd for C₁₃H₂₂O₃ (M⁺): 226.1569; Found: 226.1563. IR (film, cm⁻¹): 3432, 2961, 2895, 1792, 1723, 1716, 1558, 1541, 1472, 1418, 1374, 1283, 1073, 1028, 860. ¹H-NMR (500 MHz, CD₃OD) δ : 0.77, 1.06 (3H each, both s, 11, 12-H₃), 1.07 (3H, d, $J=6.8$ Hz, 13-H₃), 1.21 (1H, ddd, $J=2.9, 5.1, 11.0$ Hz, 6-H), 1.46, 1.81 (1H each, both m, 7-H₂), 1.81 (1H, m, 5-H), 1.97 (1H, dd, $J=2.2, 13.2$ Hz, 2 β -H), 2.16 (1H, dd, $J=14.1, 14.1$ Hz, 4 α -H), 2.22 (1H, ddd, $J=2.2, 4.6, 14.1$ Hz, 4 β -H), 2.39 (1H, d, $J=13.2$ Hz, 2 α -H), 2.58 (2H, m, 8-H₂), 4.20 (2H, s, 10-H₂). ¹³C-NMR (125 MHz, CD₃OD) δ_C : see Table 2. EI-MS (%): m/z 226 (M⁺, 7), 208 (M⁺–H₂O, 9), 195 (45), 177 (9), 167 (29), 69 (100).

Hydrogenation of 18a A solution of sarmentol H (**18a**, 7.0 mg) in MeOH (4.0 ml) was treated with 10% palladium carbon (Pd–C, 13.0 mg) and the whole mixture was stirred at room temperature under an H₂ atmosphere for 1 h. The catalyst was filtered off, and the solvent from the filtrate was evaporated under reduced pressure to give a residue, which was purified by HPLC [MeOH–H₂O (40:60, v/v)] to give sarmentol C⁽¹⁶⁾ (**7a**, 1.0 mg, 14%) and **18b** (3.0 mg, 43%).

18b: Colorless oil, $[\alpha]_D^{25} - 2.9^\circ$ ($c=0.16$, MeOH). High-resolution positive-ion FAB-MS: Calcd for C₁₃H₂₄O₃Na (M+Na)⁺: 251.1616; Found: 251.1623. CD [MeOH, nm ($\Delta\epsilon$)]: 291 (+0.17). IR (film, cm⁻¹): 3421, 2957, 2874, 1717, 1653, 1474, 1397, 1049. ¹H-NMR (500 MHz, CD₃OD) δ : 0.97, 1.05 (3H each, both s, 11, 12-H₃), 1.01 (3H, d, $J=7.0$ Hz, 13-H₃), 1.30 (1H, m, 6-H), 1.46, 1.62 (1H each, both m, 8-H₂), 1.48, 1.71 (1H each, both m, 7-H₂), 1.93 (1H, d, $J=14.1$ Hz, 2 β -H), 2.14 (2H, d, $J=8.6$ Hz, 4-H₂), 2.35 (1H, d, $J=14.1$ Hz, 2 α -H), [3.46 (1H, dd, $J=6.4, 11.0$ Hz), 3.49 (1H, dd, $J=4.9, 11.0$ Hz), 10-H₂], 3.60 (1H, m, 9-H). ¹³C-NMR (125 MHz, CD₃OD) δ_C : see Table 2. Positive-ion FAB-MS: m/z 251 (M+Na)⁺.

Bioassay Method. Protective Effects on Cytotoxicity Induced by D-GalN in Primary Cultured Mouse Hepatocytes The hepatoprotective effects of the constituents were determined in the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay using primary cultured mouse hepatocytes.³⁵ Hepatocytes were isolated from male ddY mice (30–35 g) using the collagenase perfusion method. The cell suspension at 4×10^4 cells in 100 μ l William's E medium containing fetal calf serum (10%, v/v), penicillin G (100 units/ml), and streptomycin (100 μ g/ml) was inoculated in a 96-well microplate, and precultured for 4 h at 37 °C under a 5% CO₂ atmosphere. Fresh medium (100 μ l) 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 μ l of fresh medium, and 10 μ l of MTT (5 mg/ml in phosphate buffered saline) solution was added to the medium. After 4 h culture, the medium was removed, and 100 μ l 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 using the following formula:

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

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

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

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