Bioactive Constituents from Chinese Natural Medicines. XXIII.[1] Absolute Structures of New Megastigmane Glycosides, Sedumosides A_4, A_5, A_6, 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 A_4, A_5, A_6, 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, sedumoside F_1 (IC_{50}=47 μM), (3*S*,5*R*,6*S*,9*R*)-megastigmane-3,9-diol (61 μM), and myrsinionosides A (52 μM) and D (62 μM) were found to show the strong hepatoprotective activity.

Key words *Sedum sarmentosum*; sedumoside; 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 A_1—A_3 (**3**—**5**), B (**6**), C (**7**), D (**8**), E_1—E_3 (**9**—**11**), F_1 (**12**), F_2 (**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 A_4 (**15**), A_5 (**16**), A_6 (**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

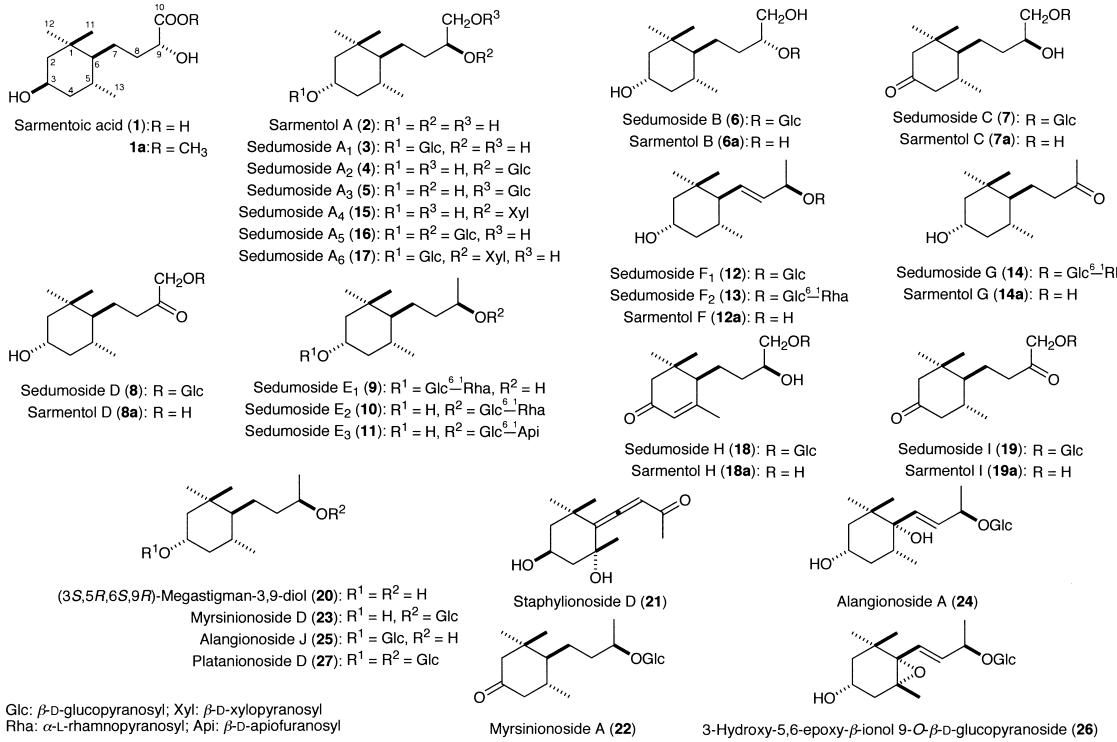

Chart 1

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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 μg/ml	10 μg/ml	30 μg/ml	100 μg/ml	300 μg/ml
MeOH-solube part	0.0±2.9	24.5±1.1**	26.0±1.2**	36.1±2.7**	46.0±1.3**
MeOH-eluted fraction	0.0±3.0	30.0±1.3**	46.0±2.5**	64.1±2.9**	113.5±1.4**
H_2O-eluted fraction	0.0±2.8	9.4±5.8	—	—	—

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

Table 2. ^{13}C-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*) CD_3OD 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 ($H_2O \rightarrow$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 A_4 (15), A_5 (16), A_6 (17), H (18), and I (19) Sedumoside A_4 (**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 $C_{18}H_{34}O_7$ 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 aglycone, sarmentol A[16] (**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 ^{1}H- (CD_3OD) and ^{13}C-NMR (Table 2) spectra[17] of **15** showed signals assignable to a sarmentol A part {δ 0.84, 0.95 (3H each, both s, 11, 12-H_3), 0.98 (3H, d, J=6.8 Hz, 13-H_3), [3.53 (1H, dd, J=6.7, 12.5 Hz), 3.62 (1H, m), 10-H_2], 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 A_4 was elucidated to be sarmentol A 9-*O*-β-D-xylopyranoside (**15**).

Sedumosides A_5 (**16**) and A_6 (**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 $C_{25}H_{46}O_{13}$, 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**, $C_{24}H_{44}O_{12}$, 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 ^{1}H- (CD_3OD) and ^{13}C-NMR (Table 2) spectra[17] 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 ^{1}H- (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[17] 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 A_5 and A_6 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° 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 $C_{19}H_{32}O_8$. 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 ^{1}H- (CD_3OD) and ^{13}C-NMR (Table 2) spectra[17] of **18a** indicated the presence of three methyls [δ 1.02, 1.09, 2.04 (3H each, all s, 12, 11, 13-H_3)], a methylene and a methine bearing an oxygen function [δ 3.45 (2H, d, J=5.5 Hz, 10-H_2), 3.54 (1H, m, 9-H)], and an olefin [δ 5.81 (1H, s, 4-H)]. The ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H 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-H_2 and 3-C; 4-H and 2, 3, 6, 13-C; 6-H and 1, 5, 7-C; 7-H_2 and 6, 8-C; 8-H_2 and 6, 7, 9-C; 9-H and 7, 8, 10-C; 10-H_2 and 9-C; 11-H_3 and 1, 2, 6, 12-C; 12-H_3 and 1, 2, 6, 11-C; and 13-H_3 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-H_3; 2β-H and 11-H_3; 4-H and 13-H_3; 6-H and 12-H_3; and 7-H_2 and 11-H_3 (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 H_2 atmosphere gave **7a** and **18b** in an approximate 1 : 3 ratio. The reductant (**18b**) was produced by hydrogenation of **18a** *via* intermediates (**i** and **ii**), as shown in Fig. 1. The proton and carbon signals in the ^{1}H- (CD_3OD) and ^{13}C-NMR (Table 2) spectra[17] of **18b** were superimposable on those of **7a** {δ 0.97, 1.05 (3H each, both s, 11, 12-H_3), 1.01 (3H, d, J=7.0 Hz, 13-H_3), [3.46 (1H, dd, J=6.4, 11.0 Hz), 3.49 (1H, dd, J=4.9, 11.0 Hz), 10-H_2], 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-H_3; 2β-H and 11-H_3; 5-H and 11-H_3; 6-H and 11-H_3; and 7-H_2 and 12, 13-H_3. 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 ($\Delta\varepsilon$ +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 ^{1}H- (CD_3OD) and ^{13}C-NMR (Table 2) spectra[17] 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**).

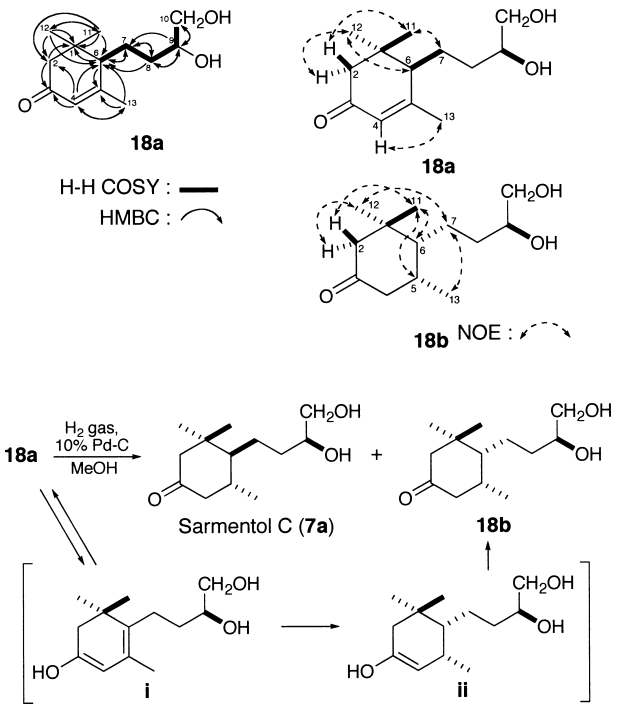

Fig. 1

Sedumoside I (**19**), $[\alpha]_D^{24}$ −0.2° (MeOH), was also obtained as an amorphous powder. The molecular formula $C_{19}H_{32}O_8$, 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 ^{1}H- (CD_3OD) and ^{13}C-NMR (Table 2) spectra[17] of **19** showed signals assignable to three methyls [δ 0.77, 1.07

(3H each, both s, 11, 12-H_3), 1.08 (3H, d, J=6.7 Hz, 13-H_3)] and a methylene bearing an oxygen function [δ 4.33, 4.52 (1H each, both d, J=17.4 Hz, 10-H_2)] 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 ^{1}H–^{1}H COSY and HMBC experiments, as shown in Fig. 2. Thus the ^{1}H–^{1}H 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-H_2 and 3, 6-C; 5-H and 3-C; 6-H and 1-C; 7-H_2 and 9-C; 8-H_2 and 9-C; 10-H_2 and 9-C; 11-H_3 and 1, 2, 6, 12-C; 12-H_3 and 1, 2, 6, 11-C; 13-H_3 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: 2α-H and 6-H, 12-H_3; 2β-H and 11-H_3; 4α-H and 6-H, 13-H_3; 6-H and 12-H_3; and 7-H_2 and 11-H_3 (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 ($\Delta\varepsilon$ +0.09); **19a**, 288 nm ($\Delta\varepsilon$ +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.

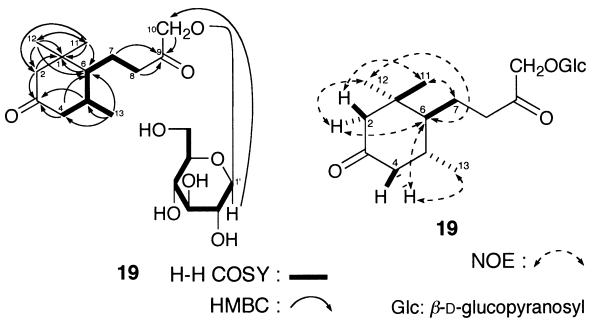

Fig. 2

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 scorzonerifolium*,[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 F_1 (**12**, IC_{50}=47 μM), (3*S*,5*R*,6*S*,9*R*)-megastigmane-3,9-diol (**20**, 61 μM), myrsinionosides A (**22**, 52 μM) and D (**23**, 62 μM), and sarmentol I (**19a**, 32 μM) were found to show hepatoprotective activity, which were equivalent to that of the hepatoprotective agent silybin (41 μM).

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 μM	3 μM	10 μM	30 μM	100 μM
Sarmentoic acid (**1**)	0.0±1.5	16.2±3.3**	19.6±3.6**	21.6±3.4**	29.5±2.3**
1a	0.0±0.8	18.7±1.9**	23.5±2.3**	25.2±2.5**	33.6±2.3**
Sarmentol A (**2**)	0.0±9.8	2.5±2.9	15.4±1.2	20.4±3.2*	38.8±1.3**
Sedumoside A_1 (**3**)	0.0±3.6	16.9±2.1**	19.3±1.2**	22.3±1.5**	31.4±0.8**
Sedumoside A_2 (**4**)	0.0±2.3	19.3±1.7**	23.5±2.9**	27.0±1.2**	43.8±4.3**
Sedumoside A_3 (**5**)	0.0±2.8	17.7±0.8**	18.6±2.0**	23.8±2.3**	32.8±2.2**
Sedumoside A_4 (**15**)	0.0±3.3	4.1±2.3	8.8±2.8	19.2±3.4*	41.8±2.7**
Sedumoside A_5 (**16**)	0.0±1.8	23.2±1.9**	26.3±1.1**	27.9±1.8**	47.6±1.7**
Sedumoside A_6 (**17**)	0.0±1.3	3.2±0.6	5.2±3.1	9.9±2.4*	24.3±1.6**
Sedumoside E_1 (**9**)	0.0±1.6	5.0±0.7	5.8±1.7	8.9±1.0*	18.3±1.7**
Sedumoside E_2 (**10**)	0.0±1.6	20.6±1.9**	21.4±2.4**	27.5±2.0**	30.2±3.3**
Sedumoside E_3 (**11**)	0.0±1.0	2.8±1.9	6.2±1.9	20.3±0.2**	48.5±0.6**
Sedumoside F_1 (**12**)	0.0±3.4	17.7±1.1	25.7±2.4**	42.7±1.0**	62.6±2.7**
Sedumoside F_2 (**13**)	0.0±0.6	15.9±0.8**	16.2±1.5**	19.4±1.9**	25.1±2.1**
Sedumoside H (**18**)	0.0±1.2	−2.3±1.6	1.0±1.2	1.8±2.1	5.4±1.5
Sedumoside I (**19**)	0.0±1.2	20.9±1.7**	22.4±1.5**	29.9±1.2**	45.1±2.5**
Sarmentol I (**19a**)	0.0±2.8	3.8±2.8	12.8±1.7**	41.3±2.4**	86.5±2.7**
(3*S*,5*R*,6*S*,9*R*)-Megastigmane-3,9-diol (**20**)	0.0±2.3	18.8±1.4**	25.5±0.7**	41.6±2.5**	57.2±1.5**
Staphylionoside D (**21**)	0.0±3.1	2.2±2.0	2.0±2.5	6.3±0.9	12.2±1.6**
Myrsinionoside A (**22**)	0.0±2.2	17.7±3.0*	27.4±5.1**	45.0±4.8**	57.9±2.5**
Myrsinionoside D (**23**)	0.0±1.4	14.9±1.8**	21.4±1.5**	39.1±1.5**	58.8±0.9**
Alangionoside A (**24**)	0.0±0.9	16.5±4.6*	19.6±3.8*	24.3±2.6**	31.9±4.0**
Alangionoside J (**25**)	0.0±2.5	17.9±3.0*	20.1±3.1**	25.3±1.3**	34.0±1.8**
26	0.0±1.4	−1.5±3.5	4.3±3.1	6.9±3.4	26.9±5.0**
Platanionoside D (**27**)	0.0±1.4	13.9±4.6	21.0±4.7**	27.4±2.1**	46.2±4.8**
Silybin[a)]	0.0±0.3	4.8±1.1	7.7±0.7	45.2±8.8**	77.0±5.5**

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).

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; ^{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; and HPLC detector, Shimadzu RID-6A refractive index and SPD-10A*vp* UV–VIS detectors. HPLC column, Cosmosil 5C_{18}-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 60F_{254} (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 WF_{254S} (Merck, 0.25 mm); 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 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, $CHCl_3$–MeOH (1 : 1, v/v)] and finally HPLC [MeOH–H_2O (35 : 65, v/v)] to furnish sedumoside I (**19**, 107.2 mg, 0.00020%). Fraction 2-5 (3300 mg) was further separated by HPLC [CH_3CN–H_2O (15 : 85, v/v)] to furnish sedumoside 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, $CHCl_3$–MeOH (1 : 1, v/v)] and finally HPLC [CH_3CN–MeOH–H_2O (20 : 8 : 72, v/v/v) and MeOH–H_2O (40 : 60, v/v)] to furnish sedumoside A_4 (**15**, 4.9 mg, 0.00001%). Fraction 2-10 (1360 mg) was further separated by HPLC [CH_3CN–MeOH–H_2O (20 : 8 : 72, v/v/v) and MeOH–H_2O (40 : 60, v/v)] to furnish **18** (37.5 mg, 0.00007%). Fraction 3-7 (230 mg) was purified by HPLC [MeOH–H_2O (29 : 71, v/v)] to furnish sedumoside A_6 (**17**, 12.0 mg, 0.00002%). Fraction 5-6 (665 mg) was purified by HPLC [CH_3CN–MeOH–H_2O (10 : 8 : 82, v/v/v)] to give sedumoside A_5 (**16**, 18.4 mg, 0.00003%).

Sedumoside A_4 (**15**): An amorphous powder, $[\alpha]_D^{26}$ −11.1° (*c*=0.25, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{18}H_{34}O_7Na$ $(M+Na)^+$ 385.2202; Found 385.2209. IR (KBr, cm^{-1}): 3405, 2924, 2870, 1473, 1072, 1039. ^{1}H-NMR (500 MHz, CD_3OD) 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-H_3), 0.98 (3H, d, *J*=6.8 Hz, 13-H_3), 0.90 (1H, ddd, *J*=11.9, 11.9, 11.9 Hz, 4α-H), 1.07, 1.66 (1H each, both m, 7-H_2), 1.09 (1H, dd, *J*=11.9, 11.9 Hz, 2α-H), 1.45 (1H, m, 5-H), 1.49, 1.65 (1H each, both m, 8-H_2), 1.64 (1H, ddd, *J*=2.4, 4.0, 11.9 Hz, 2β-H), 1.89 (1H, m, 4β-H), [3.18 (1H, dd, *J*=10.4, 11.3 Hz), 3.84 (1H, dd, *J*=5.2, 11.3 Hz), 5′-H_2], 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-H_2], 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, CD_3OD) δ_C: see Table 2. Positive-ion FAB-MS *m/z*: 385 $(M+Na)^+$.

Sedumoside A_5 (**16**): An amorphous powder, $[\alpha]_D^{19}$ −16.7° (*c*=0.93, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{25}H_{46}O_{13}Na$ $(M+Na)^+$ 577.2836; Found 577.2831. IR (KBr, cm^{-1}): 3410, 2941, 2898, 1474, 1171, 1076, 1030. ^{1}H-NMR (500 MHz, CD_3OD) 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-H_3), 0.98 (3H, d, *J*=6.5 Hz, 13-H_3), 1.02 (1H, ddd, *J*=12.2, 12.2, 12.2 Hz, 4α-H), 1.08, 1.65 (1H each, both m, 7-H_2), 1.13 (1H, dd, *J*=12.2, 12.2 Hz, 2α-H), 1.45 (1H, m, 5-H), 1.57, 1.64 (1H each, both m, 8-H_2), 1.79 (1H, ddd, *J*=2.2, 3.7, 12.2 Hz, 2β-H), 2.01 (1H, m, 4β-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-H_2], [3.64 (2H, m), 3.85 (2H, dd, *J*=2.0, 12.0 Hz), 6′, 6″-H_2], 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, CD_3OD) δ_C: see Table 2. Positive-ion FAB-MS *m/z*: 577 $(M+Na)^+$.

Sedumoside A_6 (**17**): An amorphous powder, $[\alpha]_D^{17}$ −26.8° (*c*=0.60, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{24}H_{44}O_{12}Na$ $(M+Na)^+$: 547.2730; Found: 547.2728. IR (KBr, cm^{-1}): 3410, 2940, 2918, 1541, 1474, 1171, 1081, 1047. ^{1}H-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-H_3), 0.90 (3H, d, *J*=6.1 Hz, 13-H_3), 1.16, 1.92 (1H each, both m, 7-H_2), 1.19 (1H, ddd, *J*=11.3, 11.3, 11.3 Hz, 4α-H), 1.27 (1H, dd, *J*=11.9, 11.9 Hz, 2α-H), 1.27 (1H, m, 5-H), 1.66, 1.80 (1H each, both m, 8-H_2), 2.02 (1H, ddd, *J*=2.0, 3.1, 11.9 Hz, 2β-H), 2.21 (1H, m, 4β-H), [3.70 (1H, dd, *J*=10.4, 11.3 Hz), 4.35 (1H, d, *J*=5.2, 11.3 Hz), 5″-H_2], 3.96 (2H, m, 10-H_2), 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′-H_2], 5.02 (1H, d, *J*=8.0 Hz, 1′-H), 5.04 (1H, d, *J*=8.2 Hz, 1″-H). ^{1}H-NMR (500 MHz, CD_3OD) 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-H_3), 0.97 (3H, d, *J*=6.5 Hz, 13-H_3), 1.02 (1H, ddd, *J*=11.9, 11.9, 11.9 Hz, 4α-H), 1.06, 1.65 (1H each, both m, 7-H_2), 1.13 (1H, dd, *J*=12.2, 12.2 Hz, 2α-H), 1.44 (1H, m, 5-H), 1.49, 1.65 (1H each, both m, 8-H_2), 1.79 (1H, ddd, *J*=2.2, 4.3, 12.2 Hz, 2β-H), 2.01 (1H, m, 4β-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″-H_2], 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-H_2], 3.61 (1H, m, 9-H), [3.64 (1H, dd, *J*=5.2, 11.9 Hz), 3.85 (1H, m), 6′-H_2], 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 CD_3OD) δ_C: see Table 2. Positive-ion FAB-MS: *m/z* 547 $(M+Na)^+$.

Sedumoside H (**18**): An amorphous powder, $[\alpha]_D^{27}$ +71.4° (*c*=0.21, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{19}H_{32}O_8Na$ $(M+Na)^+$: 411.1995; Found: 411.1989. CD [MeOH, nm ($\Delta\varepsilon$)]: 211 (+4.40), 237 (+3.42), 335 (+0.75). UV [MeOH, nm (log ε)]: 240 (4.08). IR (KBr, cm^{-1}): 3389, 3011, 2961, 2876, 1669, 1471, 1076, 1038, 752. ^{1}H-NMR (500 MHz, CD_3OD) δ: 1.01, 1.09, 2.04 (3H each, all s, 12, 11, 13-H_3), 1.49, 1.98 (1H each, both m, 7-H_2), 1.51, 1.61 (1H each, both m, 8-H_2), 1.96 (1H, m, 6-H), 2.00 (1H, d, *J*=17.1 Hz, 2β-H), 2.46 (1H, d, *J*=17.1 Hz, 2α-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-H_2], [3.64 (1H, dd, *J*=4.9, 11.6 Hz), 3.84 (1H, br d, *J*=*ca.* 12 Hz), 6′-H_2], 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, CD_3OD) δ_C: see Table 2. Positive-ion FAB-MS: *m/z* 411 $(M+Na)^+$.

Sedumoside I (**19**): An amorphous powder, $[\alpha]_D^{27}$ −0.2° (*c*=1.41, MeOH). High-resolution EI-MS: Calcd for $C_{19}H_{32}O_8$ (M^+): 388.2097; Found: 388.2095. CD [MeOH, nm ($\Delta\varepsilon$)]: 285 (+0.09). IR (KBr, cm^{-1}): 3431, 2961, 1723, 1715, 1559, 1472, 1076, 1044, 753. ^{1}H-NMR (500 MHz, CD_3OD) δ: 0.77, 1.07 (3H each, both s, 11, 12-H_3), 1.08 (3H, d, *J*=6.7 Hz, 13-H_3), 1.21 (1H, ddd, *J*=3.1, 6.1, 10.7 Hz, 6-H), 1.46, 1.81 (1H each, both m, 7-H_2), 1.81 (1H, m, 5-H), 1.96 (1H, dd, *J*=2.2, 13.2 Hz, 2β-H), 2.22 (1H, ddd, *J*=2.2, 4.6, 14.1 Hz, 4β-H), 2.16 (1H, dd, *J*=14.1, 14.1 Hz, 4α-H), 2.39 (1H, d, *J*=13.2 Hz, 2α-H), 2.69 (2H, m, 8-H_2), 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′-H_2), 4.31 (1H, d, *J*=7.7 Hz, 1′-H), 4.33, 4.52 (1H each, both d, *J*=17.4 Hz, 10-H_2). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: see Table 2. EI-MS (%): *m/z* 388 (M^+, 1), 370 (M^+-H_2O, 1), 255 (100), 227 (66), 208 (42).

Acid Hydrolysis of 15—19 A solution of sedumosides A_4 (**15**, 3.1 mg), A_5 (**16**, 2.0 mg), or A_6 (**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–H_2O (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 sedumosides 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 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 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 H_2O (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–H_2O (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° (c=0.40, MeOH). High-resolution EI-MS: Calcd for $C_{13}H_{22}O_3$ (M^+): 226.1569; Found: 226.1572. CD [MeOH, nm ($\Delta\varepsilon$)]: 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. ^{1}H-NMR (500 MHz, CD_3OD) δ: 1.02, 1.09, 2.04 (3H each, all s, 12, 11, 13-H_3), 1.44, 1.66 (1H each, both m, 8-H_2), 1.49, 1.98 (1H each, both m, 7-H_2), 1.96 (1H, m, 6-H), 2.00 (1H, d, J=17.1 Hz, 2β-H), 2.46 (1H, d, J=17.1 Hz, 2α-H), 3.45 (2H, d, J=5.5 Hz, 10-H_2), 3.54 (1H, m, 9-H), 5.81 (1H, s, 4-H). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: see Table 2. EI-MS (%): m/z 226 (M^+, 22), 208 (M^+–H_2O, 19), 193 (26), 175 (34), 151 (94), 95 (100).

Sarmentol I (**19a**): Colorless oil, $[\alpha]_D^{24}$ +19.9° (c=0.31, MeOH). High-resolution EI-MS: Calcd for $C_{13}H_{22}O_3$ (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. ^{1}H-NMR (500 MHz, CD_3OD) δ: 0.77, 1.06 (3H each, both s, 11, 12-H_3), 1.07 (3H, d, J=6.8 Hz, 13-H_3), 1.21 (1H, ddd, J=2.9, 5.1, 11.0 Hz, 6-H), 1.46, 1.81 (1H each, both m, 7-H_2), 1.81 (1H, m, 5-H), 1.97 (1H, dd, J=2.2, 13.2 Hz, 2β-H), 2.16 (1H, dd, J=14.1, 14.1 Hz, 4α-H), 2.22 (1H, ddd, J=2.2, 4.6, 14.1 Hz, 4β-H), 2.39 (1H, d, J=13.2 Hz, 2α-H), 2.58 (2H, m, 8-H_2), 4.20 (2H, s, 10-H_2). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: see Table 2. EI-MS (%): m/z 226 (M^+, 7), 208 (M^+–H_2O, 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 H_2 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–H_2O (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° (c=0.16, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{13}H_{24}O_3Na$ $(M+Na)^+$: 251.1616; Found: 251.1623. CD [MeOH, nm ($\Delta\varepsilon$)]: 291 (+0.17). IR (film, cm^{-1}): 3421, 2957, 2874, 1717, 1653, 1474, 1397, 1049. ^{1}H-NMR (500 MHz, CD_3OD) δ: 0.97, 1.05 (3H each, both s, 11, 12-H_3), 1.01 (3H, d, J=7.0 Hz, 13-H_3), 1.30 (1H, m, 6-H), 1.46, 1.62 (1H each, both m, 8-H_2), 1.48, 1.71 (1H each, both m, 7-H_2), 1.93 (1H, d, J=14.1 Hz, 2β-H), 2.14 (2H, d, J=8.6 Hz, 4-H_2), 2.35 (1H, d, J=14.1 Hz, 2α-H), [3.46 (1H, dd, J=6.4, 11.0 Hz), 3.49 (1H, dd, J=4.9, 11.0 Hz), 10-H_2], 3.60 (1H, m, 9-H). ^{13}C-NMR (125 MHz, CD_3OD) 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 μl William's E medium containing fetal calf serum (10%, v/v), 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. 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 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, and 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 using the 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 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±S.E.M. One-way analysis of variance followed by Dunnett's test was used for statistical analysis.

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References and Notes

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