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Flavonol glycosides with lipid accumulation inhibitory activity from *Sedum sarmentosum*☆

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ABSTRACT

Three new flavonol glycosides, sarmenosides V–VII (**1**–**3**), were isolated from the whole plant of *Sedum sarmentosum* (Crassulaseae). The structures of **1**–**3** were determined on the basis of spectroscopic analysis. Among the flavonoid constituents from *S. sarmentosum*, **1** and **3** were found to show oleic acid–albumin-induced lipid accumulation inhibitory activity.

1. Introduction

The Crassulaceae plant *Sedum sarmentosum* is a perennial herb and widely distributed on the mountain slopes in China and Korea. The whole plant of *S. sarmentosum* has been used for the treatment of chronic viral hepatitis in Chinese and Korean traditional medicines (Kang et al., 2000). In the course of our search for bioactive constituents from Chinese natural medicines (Morikawa et al., 2010a,b; Pan et al., 2010), we have reported isolation and structure elucidation of megastigmene, flavonoid, lignin, and monoterpene constituents from the whole plant of *S. sarmentosum* (Morikawa et al., 2007; Muraoka et al., 2009; Ninomiya et al., 2007; Yoshikawa et al., 2007; Zhang et al., 2007). It is recognized that fatty liver is a significant risk factor for serious liver disfunction (Bellentani et al., 1994; El-Hassan et al., 1992). There is a strong association between fatty liver and hyperglicemia (Marceau et al., 1999; Marchesini et al., 1999). Thus, fatty liver is often associated with obesity and type 2 diabetes (Marchesini et al., 1999). In our previous studies on anti-fatty liver principles from natural medicines, we have reported that the megastigmane constituents from *S. sarmentosum* were found to show lipid accumulation inhibitory and lipid metabolism-promoting activities in HepG2 cells (Muraoka et al., 2009). As a continuing study on this natural medicine, we further isolated three new flavonol glycosides, sarmenosides V (**1**), VI (**2**), and VII (**3**). This paper deals with the isolation and structure elucidation of these new flavonol glycosides (**1**–**3**) and lipid accumulation inhibitory activities on flavonoid constituents from *S. sarmentosum*.

2. Results and discussion

The hot H_2O extract from the whole plant of *S. sarmentosum* was extracted with MeOH under reflux. The MeOH extract was subjected with Diaion HP-20 column chromatography to give H_2O- and MeOH-eluted fractions (Yoshikawa et al., 2007). The MeOH-eluted fraction was subjected to silica gel and ODS column chromatography and finally HPLC (ODS column, eluted with CH_3CN–MeOH–H_2O solvent system) to furnish three new flavonol glycosides, sarmenosides V (**1**), VI (**2**), and VII (**3**).

Sarmenoside V (**1**) was obtained as an amorphous powder with negative optical rotation ($[\alpha]_D^{24}$ −71.0 in MeOH). The IR spectrum of **1** showed absorption bands at 1718 cm^{-1} (ester carbonyl), 1653 cm^{-1} (carbonyl), and 1603, 1558, and 1456 cm^{-1} (aromatic ring), and broad bands at 3389, 1075, 1036 cm^{-1} suggestive of glycosyl moiety. In the positive-ion FABMS of **1**, a quasimolecular ion peak was observed at *m/z* 705 $[M+Na]^+$, and HRFABMS analysis revealed the molecular formula to be $C_{30}H_{34}O_{18}$. Treatment of 1 with 10% aqueous potassium hydroxide (KOH)–50% aqueous

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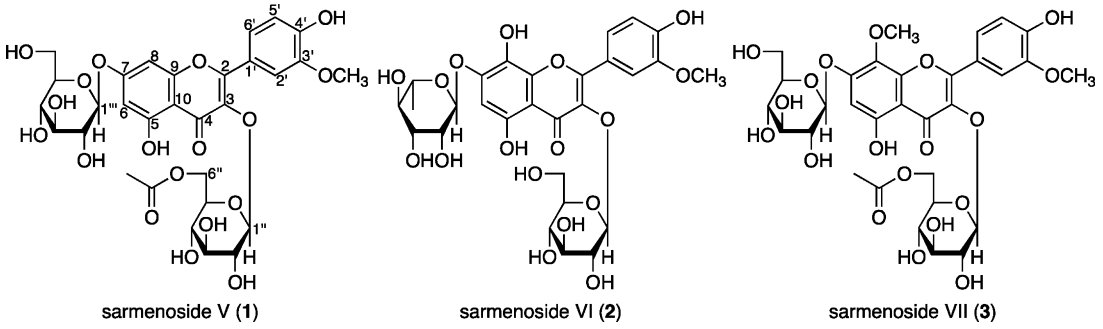

Plate 1.

1,4-dioxane (1:1, v/v) provided isorhamnetin 3,7-di-*O*-*β*-D-glucopyranoside (Morikawa et al., 2007). The ^{1}H (Table 1) and ^{13}C NMR (Table 2) spectra (DMSO-d_6) of **1**, which were assigned with the aid of DEPT, ^{1}H–^{1}H COSY, HMQC, and HMBC experiments, showed signals assignable to a isorhamnetin part [δ 3.87 (3H, s, 3′-OCH$_3$), 6.48, 6.84 (1H each, both br s, H-6, 8), 6.93 (1H, d, J = 8.4 Hz, H-5′), 7.59 (1H, dd, J = 2.4, 8.4 Hz, H-6′), 7.86 (1H, d, J = 2.4 Hz, H-2′), 12.57 (1H, br s, 5-OH)] and two glucopyranosyl units [δ 5.08 (1H, d, J = 7.5 Hz, 7-*O*-Glc-H-1‴), 5.45 (1H, d, J = 7.6 Hz, 3-*O*-Glc-H-1″)] together with an acetyl group [δ 1.74 (3H, s)]. The acylated flavonol structure of 1 was constructed on the basis of ^{1}H–^{1}H COSY and HMBC experiments as shown in Fig. 1. Thus, the ^{1}H–^{1}H COSY experiment of 1 indicated the presence of partial structures in bold lines. The connectivities of the glucopyranosyl parts and an acetyl group in **1** were characterized by the HMBC experiment, in which long-range correlations were observed between the 3-*O*-Glc-H-1″ and C-3 (δ_C 133.2), 7-*O*-Glc-H-1‴ and C-7 (δ_C 162.8), and 3-*O*-Glc-H$_2$-6″ [δ 4.02 (1H, dd, J = 6.1, 12.2 Hz), 4.09 (1H, dd, J = 2.5, 12.2 Hz)] and the acetyl carbonyl carbon (δ_C 169.8). On the basis of the above-mentioned evidence, the structure of sarmenoside V was determined as isorhamnetin 3-*O*-(6-*O*-acetyl-*β*-D-glucopyranosyl)-7-*O*-*β*-D-glucopyranoside (**1**).

Sarmenoside VI (**2**) was obtained as amorphous powder with negative optical rotation ($[\alpha]_D^{23}$ −46.9 in MeOH). The molecular formula, $C_{28}H_{32}O_{17}$, of **2** was determined from the positive-ion FABMS (m/z 633 $[M+Na]^+$) and by HRFABMS. Acid hydrolysis of **2** with 1 M HCl liberated L-rhamnose and D-glucose, which were identified by HPLC analysis (Zhang et al., 2007). The ^{1}H (Table 1) and ^{13}C NMR (Table 2) spectra (DMSO-d_6) of **2** indicated the presence of an aglycon part [δ 3.85 (3H, s, 3′-OCH$_3$), 6.62 (1H, s, H-6), 6.94 (1H, d, J = 8.3 Hz, H-5′), 7.64 (1H, dd, J = 2.1, 8.3 Hz, H-6′), 7.99 (1H, d, J = 2.1 Hz, H-2′), 12.03 (1H, br s, 5-OH)] (Wang et al., 1984) and a glucopyranosyl and a rhamnopyranosyl moieties [δ 1.13 (3H, d, J = 6.2 Hz, Rha-H$_3$-6‴), 5.51 (1H, d, J = 1.4 Hz, 7-*O*-Rha-H-1‴), 5.57 (1H, d, J = 7.6 Hz, 3-*O*-Glc-H-1″)]. In its HMBC experiment, long-range correlations were observed between the

Table 1
^{1}H NMR data (600 MHz, DMSO-d_6) for **1**–**3**.

Position	**1**	**2**	**3**
6	6.48 (br s)	6.62 (s)	6.68 (s)
8	6.84 (br s)		
2′	7.86 (d, 2.4)	7.99 (d, 2.1)	7.90 (d, 2.0)
5′	6.93 (d, 8.4)	6.94 (d, 8.3)	6.96 (d, 8.3)
6′	7.59 (dd, 2.4, 8.4)	7.64 (dd, 2.1, 8.3)	7.60 (dd, 2.0, 8.3)
5-O*H*	12.57 (br s)	12.03 (br s)	12.28 (br s)
8-*O*-C*H*$_3$			3.87 (3H, s)
3′-*O*-C*H*$_3$	3.87 (3H, s)	3.85 (3H, s)	3.87 (3H, s)
3-*O*-Glc			
1″	5.45 (d, 7.6)	5.57 (d, 7.6)	5.45 (d, 7.6)
2″	3.27 (m)	3.25 (m)	3.26 (m)
3″	3.31 (m)	3.25 (m)	3.30 (m)
4″	3.15 (m)	3.10 (m)	3.15 (m)
5″	3.35 (m)	3.12 (m)	3.33 (m)
6″	4.02 (dd, 6.1, 12.2)	3.38 (m)	4.03 (dd, 5.5, 11.7)
	4.09 (dd, 2.5, 12.2)	3.59 (br d, *ca.* 12)	4.07 (dd, 2.1, 11.7)
6″-OCOC*H*$_3$	1.74 (3H, s)		1.74 (3H, s)
7-*O*-Glc			
1‴	5.08 (d, 7.5)		5.08 (d, 7.6)
2‴	3.30 (m)		3.32 (m)
3‴	3.28 (m)		3.28 (m)
4‴	3.18 (m)		3.18 (m)
5‴	3.47 (m)		3.47 (m)
6‴	3.45 (m)		3.45 (m)
	3.71 (br d, *ca.* 12)		3.70 (br d, *ca.* 12)
7-*O*-Rha			
1‴		5.51 (d, 1.4)	
2‴		3.95 (br s)	
3‴		3.82 (dd, 3.4, 9.7)	
4‴		3.32 (dd, 9.7, 9.7)	
5‴		3.53 (qd, 6.2, 9.7)	
6‴		1.13 (3H, d, 6.2)	

Table 2
^{13}C NMR data (150 MHz, DMSO-d_6) for **1**–**3**.

Position	1	2	3
2	156.8	156.6	156.5
3	133.2	132.9	133.1
4	177.4	177.8	177.6
5	160.1	152.0	155.6
6	99.3	98.7	98.5
7	162.8	150.4	156.0
8	94.6	126.9	128.9
9	155.9	144.4	147.9
10	105.5	105.4	105.1
1′	120.7	121.1	120.8
2′	113.2	113.4	113.1
3′	146.8	146.8	146.8
4′	149.6	149.4	149.8
5′	115.1	115.1	115.3
6′	122.4	122.4	122.2
8-*O*-CH_3			61.2
3′-*O*-CH_3	55.6	55.5	55.4
3-*O*-Glc			
1″	100.9	100.6	101.1
2″	74.1	74.2	74.1
3″	76.3	76.3	76.5
4″	69.7	69.8	69.7
5″	73.8	77.3	73.8
6″	62.5	60.5	62.6
6″-*O*$COCH_3$	169.8		169.7
6″-*O*$COCH_3$	20.0		20.0
7-*O*-Glc			
1‴	99.8		100.2
2‴	73.0		73.0
3‴	76.0		76.0
4‴	69.5		69.5
5‴	77.1		77.1
6‴	60.5		60.5
7-*O*-Rha			
1‴		99.1	
2‴		69.7	
3‴		69.9	
4‴		71.6	
5‴		69.7	
6‴		17.8	

following proton and carbon pairs: 3-*O*-Glc-H-1″ and C-3 (δ_C 132.9), 7-*O*-Rha-H-1‴ and C-7 (δ_C 150.4). Consequently, the structure of sarmenoside VI was established to be capitatin 3-*O*-*β*-D-glucopyranosyl-7-*O*-*α*-L-rhamnopyranoside (**2**).

Sarmenoside VII (**3**) was obtained as an amorphous powder with a negative optical rotation ($[\alpha]_D^{26}$ −47.7 in MeOH). Its molecular formula, $C_{31}H_{36}O_{19}$, was determined by positive-FABMS and HRFABMS measurements. The proton and carbon signals in the ^{1}H (Table 1) and ^{13}C NMR (Table 2) spectra (DMSO-d_6) of **3** were similar to those of **1**, except for the presence of methoxyl group at C-8 in 3 in the aglycone part [δ 3.87 (6H, s, 8, 3′-OCH_3), 6.68 (1H, s, H-6), 6.96 (1H, d, J = 8.3 Hz, H-5′), 7.60 (1H, dd, J = 2.0, 8.3 Hz, H-6′), 7.90 (1H, d, J = 2.1 Hz, H-2′), 12.28 (1H, br s, 5-OH)]. The alkaline hydrolysis of **3** with 10% KOH–50% aqueous 1,4-dioxane (1:1, v/v) liberated limocitrin 3,7-di-*O*-*β*-D-glucopyranoside. Finally, the connectivity of an acetyl group in **3** was characterized by the HMBC experiment, in which long-range correlations were observed between the 3-*O*-Glc-H_2-6″ [δ 4.02 (1H, dd, J = 5.35, 11.7 Hz), 4.07 (1H, dd, J = 2.1, 11.7 Hz)] and the acetyl carbonyl carbon (δ_C 169.7). On the basis of above-mentioned evidence, the structure of sarmenoside VII was elucidated to be limocitrin 3-*O*-(6-*O*-acetyl-*β*-D-glucopyranosyl)-7-*O*-*β*-D-glucopyranoside (**3**).

Additionally, 21 flavonoid glycosides, apigenin 7-*O*-*β*-D-glucopyranoside, luteolin 7-*O*-*β*-D-glucopyranoside, tricin 7-*O*-*β*-D-glucopyranoside, kaempferol 7-*O*-*β*-D-glucopyranoside, quercetin 7-*O*-*β*-D-glucopyranoside, isorhamnetin 7-*O*-*β*-D-glucopyranoside, tamarixetin 7-*O*-*β*-D-glucopyranoside, kaempferol 3-*O*-*β*-D-glucopyranosyl-(**1** → **2**)-*α*-L-rhamnopyranosyl-7-*O*-*α*-L-rhamnopyranoside, sarmenoside I, grosvenorine, quercetin 3,7-di-*O*-*α*-L-rhamnopyranoside, quercetin 3-*O*-*β*-D-glucopyranosyl-(**1** → **2**)-*α*-L-rhamnopyranosyl-7-*O*-*α*-L-rhamnopyranoside, sarmenoside II, isorhamnetin 3-*O*-*β*-D-glucopyranosyl-(**1** → **2**)-*α*-L-rhamnopyranosyl-7-*O*-*α*-L-rhamnopyranoside, sarmenoside IV, isorhamnetin 3-*O*-*β*-D-glucopyranosyl-7-*O*-*α*-L-rhamnopyranoside, isorhamnetin 3,7-di-*O*-*β*-D-glucopyranoside, sinocrassoside C_1, herbacetin 8-methyl ether 3,7-di-*O*-*β*-D-glucopyranoside, limocitrin 3-*O*-*β*-D-glucopyranoside, and limocitrin 3,7-di-*O*-*β*-D-glucopyranoside, were isolated from this plant material (Plate 1) (Morikawa et al., 2007; Zhang et al., 2007). Continuing the studies on bioactive constituents from *S. sarmantosum*, inhibitory effects of flavonoid constituents from *S. sarmentosum* on oleic acid–albumin-induced triglyceride accumulation in HepG2 cells were examined. As shown in Table 3, sarmenosides V (**1**, % of control at 0.1 μM: 71.6 ± 2.0) and VII (**3**, 87.9 ± 1.8), quercetin 7-*O*-*β*-D-glucopyranoside (85.4 ± 1.1), isorhamnetin 7-*O*-*β*-D-glucopyranoside (87.5 ± 1.1), and sarmenoside II (81.5 ± 3.0) were found to show strong lipid accumulation inhibitory activity. Especially, sarmenosides II and V (**1**) were stronger than that of bezafibrate (90.5 ± 1.3). Bezafibrate is clinically used as a hypolipidemic agent that decreases liver triglyceride *via* peroxisome proliferator-activated receptor *α*-independent mechanism (Nakajima et al., 2009). Thus, these flavonoid constituents from *S. sarmentosum* may be useful for the preventions of fatty liver disease although other experiments to be studied further.

3. Experimental

3.1. General experimental procedures

The following instruments were used to obtain spectral and physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l = 5 cm); UV spectra, Shimadzu UV-1600 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; ^{1}H and ^{13}C NMR spectra, JEOL ECA-600 (600 and 150 MHz) spectrometer with tetramethylsilane as an internal standard; FABMS and high-resolution FABMS, JEOL JMS-SX 102A mass spectrometer; HPLC detector, Shimadzu RID-6A refractive index, Shimadzu SPD-10A UV-VIS, and Shodex OR-2 optical rotation detectors. HPLC column, Cosmosil 5C_{18}-MS-II (Nacalai Tesque Inc., 250 mm × 4.6 mm i.d.) and (250 mm × 20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

The following experimental conditions were used for chromatography: normal-phase silica gel column chromatography (CC), silica gel BW-200 (Fuji Silysia Chemical Ltd., 150–350 mesh); reversed-phase silica gel CC, Chromatorex ODS DM1020T (Fuji Silysia Chemical Ltd., 100–200 mesh); Sephadex LH-20 CC (Amersham Biosciences K. K.); normal-phase TLC, pre-coated TLC plates with silica gel 60F_{254} (Merck, 0.25 mm); reversed-phase TLC, pre-coated TLC plates with 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), detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4, followed by heating. All the organic extracts were dried over anhydrous $MgSO_4$ prior to evaporation.

3.2. Plant material

S. sarmentosum was cultivated at Huangshan, Anhui province, China and plant material was identified by one of the authors (M.Y.). A voucher specimen (2005.01. Eishin-02) of this plant is on file in our laboratory (Yoshikawa et al., 2007).

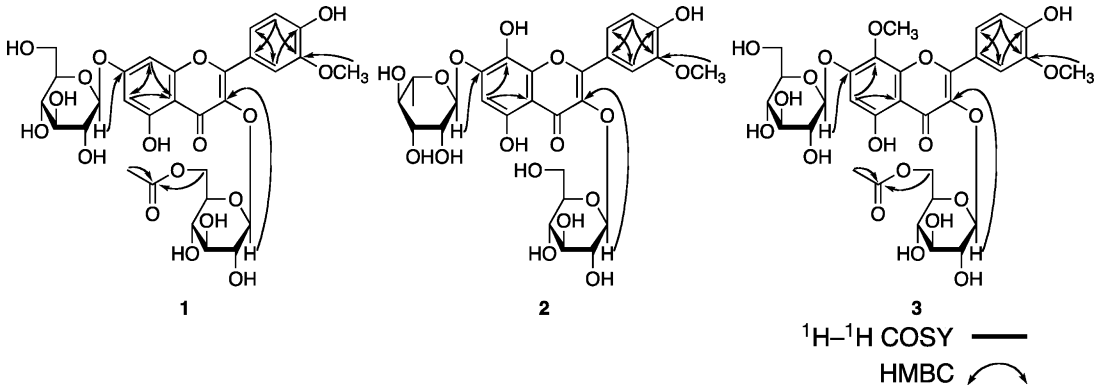

Fig. 1. Selected ^{1}H–^{1}H COSY and HMBC correlations of **1**–**3**.

3.3. Extraction and isolation

The hot H_2O extract (1950 g) from the fresh whole plant of *S. sarmentosum* (Huangshan, Anhui province, China, 1.25% from the fresh plant) was extracted three times with MeOH under reflux for 3 h. Evaporation of the solvent under reduced pressure provided a MeOH extract (887.5 g, 0.57%), and an aliquot (398.6 g) was subjected to Diaion HP-20 CC (4.0 kg, $H_2O \rightarrow$ MeOH, twice) to give H_2O- and MeOH-eluted fractions (305.0 and 93.6 g, respectively). The MeOH-eluted fraction (72.0 g) was subjected to normal-phase silica gel CC [2.0 kg, $CHCl_3$–MeOH–H_2O (10:3:0.5 → 7:3:1, v/v/v, lower layer) → MeOH] to give five fractions [Fr. 1 (12.1 g), Fr. 2 (19.2 g), Fr. 3 (10.4 g), Fr. 4 (8.7 g), and Fr. 5 (16.3 g)]. The fraction 3 (10.4 g) was subjected to reversed-phase silica gel CC [240 g, MeOH–H_2O (10:90 → 20:80 → 30:70 → 40:60, v/v) → MeOH] to afford 14 fractions [Fr. 3–1 (123.0 mg), Fr. 3–2 (675.1 mg), Fr. 3–3 (574.8 mg), Fr. 3–4 (1337 mg), Fr. 3–5 (797.8 mg), Fr. 3–6 (798.6 mg), Fr. 3–7 (230.3 mg), Fr. 3–8 (901.2 mg), Fr. 3–9 (645.6 mg), Fr. 3–10 (256.4 mg), Fr. 3–11 (511.7 mg), Fr. 3–12 (1238 mg), Fr. 3–13 (473.1 mg), and Fr. 3–14 (1320 mg)]. The fraction 3–11 (511.7 mg) was purified by Sephadex LH-20 column chromatography [150 g, $CHCl_3$–MeOH (1:1, v/v)] and finally HPLC [MeOH–H_2O (40:60, v/v)] to furnish sarmenosides V (**1**, 16.4 mg, 0.00003%), VI (**2**, 27.7 mg, 0.00005%), and VII (**3**, 12.8 mg, 0.00002%) together with quercetin 7-*O*-*β*-D-glucopyranoside (12.7 mg, 0.00002%) and sedumoside F_2 (72.2 mg, 0.00013%). The fraction 5 (8.7 g) was subjected to reversed-phase silica gel column chromatography [240 g, $H_2O \rightarrow$ MeOH–H_2O (10:90 → 20:80 → 30:70 → 40:60 → 50:50, v/v) → MeOH] to give 12 fractions [Fr. 5–1 (345.0 mg), Fr. 5–2 (408.3 mg), Fr. 5–3 (60.1 mg), Fr. 5–4 (318.3 mg), Fr. 5–5 (864.3 mg), Fr. 5–6 (664.5 mg), Fr. 5–7 (298.3 mg), Fr. 5–8 (672.4 mg), Fr. 5–9 (589.3 mg), Fr. 5–10 (1818 mg), Fr. 5–11 (388.1 mg), and Fr. 5–12 (1161 mg)]. The fraction 5–9 (589.3 mg) was separated by 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 give **2** (21.2 mg, 0.00004%) together with isorhamnetin 3,7-di-*O*-*β*-D-glucopyranoside (44.3 mg, 0.00008%), sinocrassoside C_1 (21.2 mg, 0.00004%) (Morikawa et al., 2008), and limocitrin 3,7-di-*O*-*β*-D-glucopyranoside (10.1 mg, 0.00002%).

3.3.1. Sarmenoside V (1 = isorhamnetin 3-O-(6-O-acetyl-β-D-glucopyranosyl)-7-O-β-D-glucopyranoside)

An amorphous powder $[\alpha]_D^{24}$ −71.0 (*c* 0.05, MeOH); UV [MeOH, nm (log *ε*)]: 255 (4.29), 355 (4.13); IR (KBr) ν_{max} cm^{-1}: 3389, 2941, 1718, 1653, 1603, 1558, 1456, 1075, 1036; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive-ion FABMS *m*/*z*: 705 [M+Na]$^+$; HRFABMS *m*/*z*: 705.1640 [M+Na]$^+$ (calcd for $C_{30}H_{34}O_{18}Na$, 705.1643).

3.3.2. Sarmenoside VI (2 = capitatin 3-O-β-D-glucopyranosyl-7-O-α-L-rhamnopyranoside)

An amorphous powder $[\alpha]_D^{23}$ −46.9 (*c* 0.14, MeOH); UV [MeOH, nm (log *ε*)]: 258 (4.08), 344 (3.94); IR (KBr) ν_{max} cm^{-1}: 3389, 2943, 1653, 1609, 1541, 1458, 1059; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive-ion FABMS *m*/*z*: 663 [M+Na]$^+$; HRFABMS *m*/*z*: 663.1542 [M+Na]$^+$ (calcd for $C_{28}H_{32}O_{17}Na$, 663.1537).

3.3.3. Sarmenoside VII (3 = limocitrin 3-O-(6-O-acetyl-β-D-glucopyranosyl)-7-O-β-D-glucopyranoside)

An amorphous powder $[\alpha]_D^{26}$ −47.7 (*c* 0.20, MeOH); UV [MeOH, nm (log *ε*)]: 257 (4.22), 358 (4.05); IR (KBr) ν_{max} cm^{-1}: 3420, 2934, 1720, 1653, 1601, 1559, 1456, 1071; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive-ion FABMS *m*/*z*: 735 [M+Na]$^+$; HRFABMS *m*/*z*: 735.1752 [M+Na]$^+$ (calcd for $C_{31}H_{36}O_{19}Na$, 735.1748).

3.4. Alkaline hydrolysis of 1 and 3

A solution of 1 (4.0 mg) in 10% aqueous KOH–50% aqueous 1,4-dioxane (1:1, v/v, 1.0 mL) was stirred at 40 °C for 1 h. The 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 gave a residue which was purified by HPLC [MeOH–H_2O (65:35, v/v)] to furnish isorhamnetin 3,7-di-*O*-*β*-D-glucopyranoside (2.5 mg, 67%). Through a similar procedure, limocitrin 3,7-di-*O*-*β*-D-glucopyranoside (2.2 mg, 59%) was obtained from **3** (4.0 mg).

3.5. Acid hydrolysis of 2

A solution of **2** (3.0 mg) in 1 M HCl (1 mL) were heated at 80 °C for 3 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and the resin was filtered. Removal of the solvent from the filtrate under reduced pressure, the residue was separated by Sep-Pak C18 cartridge column ($H_2O \rightarrow$ MeOH). The H_2O-eluted fraction was subjected to HPLC analysis under the following condition: 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 (i) and D-glucose (ii) present in the H_2O-eluted fraction was carried out by comparison of their retention times and optical rotations with those of authentic samples [t_R: (i) 7.8 min (negative optical rotation) and (ii) 13.9 min (positive optical rotation)]. The EtOAc-eluted fraction was purified by HPLC [MeOH–H_2O

Table 3
Effects of flavonoid constituents from *S. sarmentosum* on oleic acid–albumin-induced triglyceride accumulation in HepG2 cells.

	TG/protein (% of control)				
Conc. (μM)	0	0.1	1	10	100
Sarmenoside V (**1**)	100.0 ± 2.0	71.6 ± 2.0[b]	66.1 ± 2.0[b]	59.3 ± 0.6[b]	52.0 ± 1.0[b]
Sarmenoside VI (**2**)	100.0 ± 1.7	–	102.4 ± 1.6	99.9 ± 2.3	95.4 ± 0.7
Sarmenoside VII (**3**)	100.0 ± 2.6	87.4 ± 1.8[b]	84.9 ± 1.3[b]	88.0 ± 1.6[b]	74.2 ± 2.4[b]
Apigenin 7-*O*-Glc	100.0 ± 3.3	–	92.6 ± 1.6	92.9 ± 5.7	101.8 ± 2.4
Luteolin 7-*O*-Glc	100.0 ± 2.3	96.4 ± 2.4	87.2 ± 3.2[b]	87.0 ± 3.4[b]	85.5 ± 2.0[b]
Tricin 7-*O*-Glc	100.0 ± 2.1	91.3 ± 2.7	86.9 ± 1.3[b]	87.8 ± 2.3[b]	79.4 ± 1.4[b]
Kaempferol 7-*O*-Glc	100.0 ± 5.2	95.8 ± 3.4	92.7 ± 1.6	87.4 ± 1.8[b]	81.9 ± 1.8[b]
Quercetin 7-*O*-Glc	100.0 ± 2.0	85.4 ± 1.1[b]	85.8 ± 1.3[b]	81.3 ± 1.4[b]	72.0 ± 0.4[b]
Isorhamnetin 7-*O*-Glc	100.0 ± 4.1	87.5 ± 1.1[b]	86.8 ± 3.2[b]	86.8 ± 1.0[b]	77.8 ± 1.3[b]
Tamarixetin 7-*O*-Glc	100.0 ± 4.6	108.2 ± 1.4	93.1 ± 1.7	89.0 ± 1.4[b]	85.0 ± 2.0[b]
Kaempferol 3-*O*-Glc-(**1** → **2**)-Rha-7-*O*-Rha	100.0 ± 3.8	–	105.6 ± 2.8	107.4 ± 7.2	113.2 ± 1.0
Sarmenoside I	100.0 ± 4.9	104.9 ± 2.7	96.8 ± 3.6	90.4 ± 3.3	80.7 ± 4.6[b]
Grosvenorine	100.0 ± 2.0	98.9 ± 1.6	90.0 ± 2.7[b]	95.8 ± 0.9	90.5 ± 1.6[b]
Quercetin 3,7-di-*O*-Rha	100.0 ± 2.2	92.2 ± 2.4	87.5 ± 5.9[b]	96.3 ± 2.2	80.5 ± 4.6[b]
Quercetin 3-*O*-Glc-(**1** → **2**)-Rha-7-*O*-Rha	100.0 ± 1.4	–	111.0 ± 1.8	107.6 ± 6.9	92.2 ± 3.0
Sarmenoside II	100.0 ± 7.5	81.5 ± 3.0[b]	77.2 ± 2.8[b]	72.9 ± 3.3[b]	70.8 ± 0.7[b]
Isorhamnetin 3-*O*-Glc-(**1** → **2**)-Rha-7-*O*-Rha	100.0 ± 1.2	98.9 ± 1.8	92.1 ± 1.9[a]	92.0 ± 2.3[a]	85.0 ± 1.4[b]
Sarmenoside IV	100.0 ± 8.1	–	104.0 ± 4.0	99.6 ± 2.7	103.6 ± 2.5
Isorhamnetin 3-*O*-Glc-7-*O*-Rha	100.0 ± 3.3	102.2 ± 3.0	99.6 ± 2.7	92.2 ± 4.7	83.5 ± 3.4[b]
Isorhamnetin 3,7-di-*O*-Glc	100.0 ± 2.7	103.1 ± 2.1	87.1 ± 1.9[b]	83.3 ± 1.8[b]	79.3 ± 0.8[b]
Sinocrassoside C_1	100.0 ± 2.4	95.6 ± 5.4	98.2 ± 1.0	91.6 ± 3.6	87.3 ± 0.7[b]
Herbacetin 8-methyl ether 3,7-di-*O*-Glc	100.0 ± 3.2	95.1 ± 3.1	94.6 ± 2.3	93.6 ± 1.5	80.4 ± 0.3[b]
Limocitrin 3-*O*-Glc	100.0 ± 1.1	–	100.9 ± 2.1	99.4 ± 1.6	92.6 ± 1.0
Limocitrin 3,7-di-*O*-Glc	100.0 ± 0.6	–	93.2 ± 2.6	91.2 ± 1.0[b]	93.9 ± 3.2
Bezafibrate	100.0 ± 1.1	90.5 ± 1.3[a]	93.4 ± 1.4	86.7 ± 1.2[b]	72.7 ± 0.6[b]

Each value represents the mean ± S.E.M. ($n = 4$); Significantly different from the control, [a] $p < 0.05$, [b] $p < 0.01$.

(60:40, v/v)] to furnish capitatin (1.2 mg, 74%) (Wang et al., 1984).

3.6. Inhibitory effects on oleic acid–albumin-induced triglyceride accumulation in HepG2 cells

HepG2 cells (Dainippon Pharmaceuticals, Osaka, Japan) were maintained in Minimum Essential Medium Eagle (MEM, Sigma–Aldrich) containing 10% fetal bovine serum, 1% MEM non-essential amino acids (Invitrogen), penicillin G (100 units/mL), and streptomycin (100 μg/mL) at 37 °C under 5% CO_2 atmosphere. The cells were inoculated in 48-well tissue culture plate [10^5 cells/well in 200 μL/well in MEM]. After 20 h, the medium was replaced with 200 μL/well of Dulbecco's modified Eagle's medium (DMEM) containing low-glucose (1000 mg/L), 5% (v/v) oleic acid–albumin (Sigma–Aldrich), and a test sample. The cells were cultured for 4 d and replacement with the fresh medium every 2 d. Then the medium was removed, and the cells were homogenized in distilled water (105 μL/well) by sonication. The triglyceride (TG) and protein contents in the homogenate were determined using commercial kits [Triglyceride E-test Wako (Wako Pure Chemical Industries) and BCA Protein Assay Kit (Thermo Scientific), respectively]. Data were expressed as % of control of TG/protein (μg/mg). Each test compound was dissolved in DMSO, and the solution was added to the medium (final DMSO concentration was 0.5%). A PPAR-α agonist, bezafibrate was used as a reference compound (Lin et al., 2000; Hawley et al., 2002).

3.7. Statistics

Values were expressed as means ± S.E.M. One-way analysis of variance (ANOVA) followed by Dunnett's test was used for statistical analysis. Probability (p) values less than 0.05 were considered significant.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytol.2011.07.012.

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