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Novel megastigmanes with lipid accumulation inhibitory and lipid metabolism-promoting activities in HepG2 cells from *Sedum sarmentosum*☆

Osamu Muraoka [a], Toshio Morikawa [a], Yi Zhang [b], Kiyofumi Ninomiya [a], Seikou Nakamura [b], Hisashi Matsuda [b], Masayuki Yoshikawa [a,b,*]

[a] *Pharmaceutical Research and Technology Institute, Kinki University, 3-4-1 Kowakae, Higashi-osaka, Osaka 577-8502, Japan*
[b] *Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607-8412, Japan*

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ABSTRACT

Four novel megastigmanes, neosedumosides I (**1**), II (**2**), III (**3**), and IV (**4**) were isolated from the whole plant of *Sedum sarmentosum* (Crassulaceae). Absolute stereostructures of these constituents were determined on the basis of chemical and physicochemical evidence. Among them, **1**–**3** were found to show lipid accumulation inhibitory activity in HepG2 cells. Furthermore, **2** and **3** were found to also show lipid metabolism-promoting activity.

1. Introduction

During the course of our characterization studies on bioactive constituents from Chinese natural medicines,[1–5] we have reported the isolation and structural elucidation of 27 megastigmane constituents, including sarmentoic acid, sarmentol A, sedumosides A_1–A_6, B, C, D, E_1–E_3, F_1, F_2, and G–I, and four flavonol glycosides, sarmenosides I–IV from the whole plant of *Sedum sarmentosum* (Crassulaceae).[2–5] As a continuing study on this herbal medicine, we have isolated four novel bicyclic megastigmane glycosides, neosedumosides I (**1**), II (**2**), III (**3**), and IV (**4**). This paper deals with the isolation and structural elucidation of these new megastigmanes (**1**–**4**) and their lipid accumulation inhibitory and lipid metabolism-promoting activities.

2. Results and discussion

The MeOH-eluted fraction (72.0 g) from the whole plant of *S. sarmentosum*[3] was subjected to SiO_2 and ODS column chromatographies and finally HPLC (ODS column, eluted with CH_3CN–MeOH–H_2O solvent system) to furnish five novel megastigmane glycosides, neosedumosides I (**1**, 25.4 mg), II (**2**, 12.3 mg), III (**3**, 54.2 mg), and IV (**4**, 9.2 mg) (Chart 1).

Neosedumoside I (**1**), $[\alpha]_D^{25}$ +40.0 (MeOH), was obtained as an amorphous powder. Its IR spectrum showed absorption bands at 3389, 1653, and 1036 cm^{-1} ascribable to hydroxyl, α,β-unsaturated olefin, and ether functions, respectively. In the UV spectrum, an absorption maximum was observed at 241 nm (log ε 4.06 in MeOH) ascribable to the enone moiety. The EIMS of **1** showed a molecular ion peak at *m*/*z* 386 (M^+), and the molecular formula was determined as $C_{19}H_{30}O_8$ by high-resolution EIMS measurement. The ^1H and ^{13}C NMR spectra of **1** (CD_3OD, Tables 1 and 2) showed

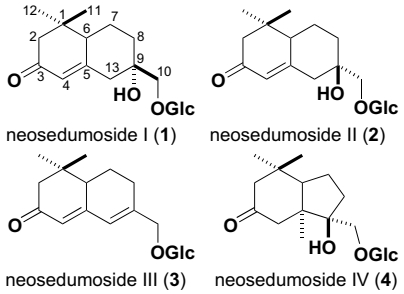

Chart 1. Structures of neosedumosides I–IV (**1**–**4**).

☆ See Ref. 1.

* Corresponding author. Tel.: +81 75 595 4633; fax: +81 75 595 4768.
E-mail address: myoshika@mb.kyoto-phu.ac.jp (M. Yoshikawa).

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Table 1
^{1}H NMR data for neosedumosides I (**1**), II (**2**), III (**3**), and IV (**4**) (at 500 MHz in CD_3OD, *J* values in Hz)

	1	2	3	4
2α	2.19 (d, 15.6)	2.19 (d, 15.6)	2.44 (d, 16.2)	1.88 (d, 17.4)
2β	2.25 (d, 15.6)	2.26 (d, 15.6)	2.17 (dd, 0.9, 16.2)	2.43 (d, 17.4)
3				
4(α)	5.83 (br s)	5.83 (br s)	5.79 (br s)	2.09 (d, 17.7)
4β				2.83 (d, 17.7)
6	2.23 (m)	2.19 (m)	2.50 (m)	1.80 (m)
7α	1.95 (m)	1.89 (m)	2.05 (m)	1.77 (2H, m)
7β	1.43 (m)	1.78 (m)	1.45 (m)	
8α	1.61 (ddd, 4.0, 13.1, 13.4)	1.74 (m)	2.40 (m)	1.64 (m)
8β	2.15 (m)	1.81 (m)	2.30 (m)	1.71 (m)
10	3.39, 3.78 (both d, 10.7)	3.40, 3.83 (both d, 10.1)	4.22, 4.43 (both d, 14.7)	3.48, 4.04 (both d, 10.5)
11	0.95 (s)	0.98 (s)	0.88 (s)	0.97 (s)
12	1.06 (s)	1.07 (s)	1.15 (s)	1.05 (s)
13	2.37 (d, 13.9) 2.70 (dd, 2.1, 13.9)	2.48 (d, 14.6) 2.54 (dd, 1.9, 14.6)	6.45 (s)	1.10 (s)
1′	4.25 (d, 7.6)	4.29 (d, 7.7)	4.29 (d, 7.7)	4.22 (d, 7.7)
2′	3.22 (dd, 7.7, 9.2)	3.23 (dd, 7.7, 9.2)	3.23 (m)	3.22 (dd, 7.7, 9.2)
3′	3.36 (m)	3.36 (m)	3.36 (dd, 8.5, 8.5)	3.35 (dd, 9.2, 9.2)
4′	3.26 (dd, 8.6, 8.6)	3.28 (m)	3.28 (m)	3.27 (m)
5′	3.25 (m)	3.28 (m)	3.26 (m)	3.26 (m)
6′	3.65 (dd, 5.5, 11.4) 3.83 (dd, 1.8, 11.4)	3.65 (dd, 5.5, 11.9) 3.87 (dd, 1.8, 11.9)	3.66 (dd, 5.5, 11.9) 3.86 (dd, 2.1, 11.9)	3.65 (dd, 5.8, 11.9) 3.86 (dd, 1.5, 11.9)

signals assignable to two methyls [δ 0.95, 1.06 (3H each, both s, H_3-11, 12)], four methylenes, a methine, a methylene bearing an oxygen function [δ 3.39, 3.78 (1H each, both d, *J*=10.7 Hz, H_2-10)], a quaternary carbon bearing an oxygen function (δ_C 74.5), a trisubstituted olefin [δ 5.83 (1H, br s, H-4), δ_C 125.5 (C-4), 165.7 (C-5)], and a conjugated carbonyl carbon (δ_C 202.5) together with those of a glucopyranosyl moiety [δ 4.25 (1H, d, *J*=7.6 Hz, H-1′)]. Acid hydrolysis of **1** with 1 M HCl liberated the D-glucose, which was identified by HPLC analysis using an optical rotation detector.[2–5] The bicyclic neomegastigmane skeleton of **1** was constructed on the basis of various NMR experiments.[6] Namely, the ^{1}H–^{1}H COSY experiments on **1** indicated the presence of two partials written in bold lines, while in the HMBC experiments, long range correlations were observed between the following proton and carbon pairs: H_2-2 and C-1, 3, 4; H_2-4 and C-2, 5, 6, 13; H-6 and C-1, 4, 5; H_2-7 and C-5; H_2-8 and C-6; H_2-10 and C-8, 9, 13; H_3-11 and C-1, 2, 6, 12; H_3-12 and C-1, 2, 6, 11; H-1′ and C-10 (Fig. 1). Next, the relative stereostructure of **1** was clarified by the NOESY experiment, in which

Table 2
^{13}C NMR data for **1**–**4** (at 125 MHz)

	1[a]	2[a]	3[a]	4[a]	4[b]
1	35.8	35.7	37.3	34.7	34.2
2	51.6	51.3	54.7	48.8	48.7
3	202.5	202.5	202.8	217.3	212.1
4	125.5	125.6	123.8	46.8	46.1
5	165.7	167.1	159.7	48.8	47.9
6	49.3	50.0	46.9	56.9	55.6
7	24.5	23.7	23.4	26.7	25.8
8	35.2	34.1	28.2	34.7	34.2
9	74.5	74.6	151.8	84.8	83.7
10	74.5	79.0	72.7	75.3	75.2
11	24.3	24.6	20.3	29.3	28.0
12	28.9	28.9	29.0	30.4	30.0
13	46.1	46.1	126.5	29.5	28.7
1′	104.9	105.0	104.0	105.0	106.0
2′	75.2	75.2	75.1	75.1	75.2
3′	78.0	77.9	78.1	78.0	78.8
4′	71.6	71.6	71.6	71.7	71.6
5′	77.9	78.0	78.0	78.1	78.7
6′	62.7	62.7	62.8	62.8	62.7

[a] In CD_3OD.
[b] In pyridine-d_5.

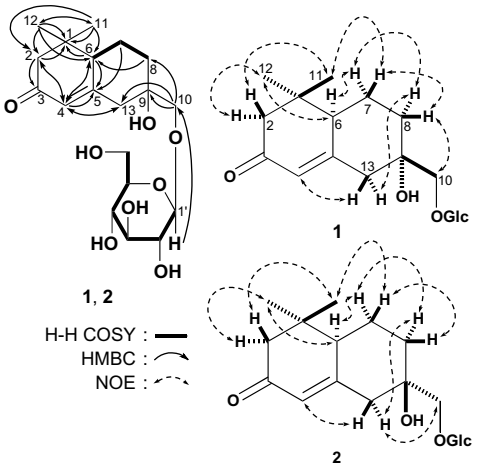


Figure 1. ^{1}H–^{1}H COSY, HMBC, and NOE correlations of **1** and **2**.

correlations were observed between the following proton pairs: H-2α and H_3-12; H-2β and H_3-11; H-4 and H-13β; H-6 and H-7α, H_3-12; H-7α and H-8α; H-7β and H-8β, H_3-11; H-8α and H-13α; H-8β and H_2-10 (Fig. 1). On the basis of above-mentioned evidence, the relative stereostructure of **1** was elucidated as shown in Figure 1.

Neosedumoside II (**2**) was isolated as an amorphous powder with positive optical rotation ($[\alpha]_D^{25}$ +39.6 (MeOH)). The EIMS of **2** showed a molecular ion peak at *m*/*z* 386 (M^+), and the molecular formula, $C_{19}H_{30}O_8$, was found to be the same as that of **1** by high-resolution EIMS measurement. The ^{1}H and ^{13}C NMR spectroscopic properties of **2** (CD_3OD, Tables 1 and 2) were quite similar to those of **1**. That is, **2** showed signals due to two methyls [δ 0.98, 1.07 (3H each, both s, H_3-11, 12)], four methylenes, a methine, a methylene bearing an oxygen function [δ 3.40, 3.83 (1H each, both d, *J*=10.1 Hz, H_2-10)], a quaternary carbon bearing an oxygen function (δ_C 79.0), a trisubstituted olefin [δ 5.83 (1H, br s, H-4), δ_C 125.6 (C-4), 167.1 (C-5)], and a conjugated carbonyl carbon (δ_C 202.5) together with those of a glucopyranosyl moiety. On acid hydrolysis with 1 M HCl, **2** liberated the D-glucose. The planar structure of **2** was characterized to be the same as that of **1** by means of ^{1}H–^{1}H COSY and HMBC experiments as shown in Figure 1. The NOESY spectrum of **2** showed distinct correlation between H-13α and H_2-10 (Fig. 1). Thus, **2** was clarified to be the stereoisomer of **1** at the 10-position.

Neosedumoside III (**3**) was isolated as a white powder with negative optical rotation ($[\alpha]_D^{27}$ −63.7 (MeOH)). The positive-ion FABMS showed a quasimolecular ion peak at *m*/*z* 391 $(M+Na)^+$ and its molecular formula, $C_{19}H_{28}O_7$, was determined by high-resolution FABMS measurement. In the UV spectrum, an absorption maximum was observed at 290 nm (log ε 4.12 in MeOH), which was suggestive of a hetero-annular diene chromophore. The IR spectrum showed absorption bands due to hydroxyl, olefin, and ether groups at 3649, 1653, and 1076 cm^{-1}, respectively. ^{1}H and ^{13}C NMR spectra of **3** (CD_3OD, Tables 1 and 2) showed signals assignable to two methyls [δ 0.88, 1.15 (3H each, both s, H_3-11, 12)], three methylenes, a methine, a methylene bearing an oxygen function [δ 4.22, 4.43 (1H each, both d, *J*=14.7 Hz, H_2-10)], two trisubstituted olefins [δ 5.79, 6.45 (1H each, both br s, H-4, 13), δ_C 123.8 (C-4), 126.5 (C-13), 151.8 (C-9), 159.7 (C-5)], and a conjugated carbonyl carbon (δ_C 202.8) together with those of a glucopyranosyl moiety. The acid hydrolysis of **3** liberated the D-glucose. Connectivities of the quaternary carbons and the β-D-glucopyranosyl part were elucidated on the basis of various NMR measurements, and the relative stereostructure of **3** was unambiguously clarified as shown in Figure 2 by the NOESY experiment.

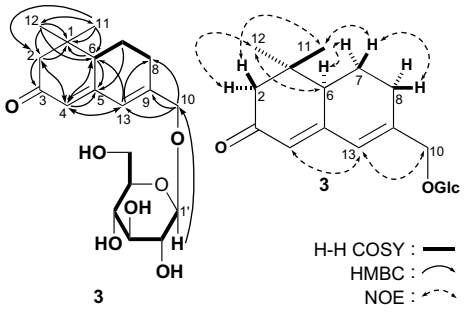
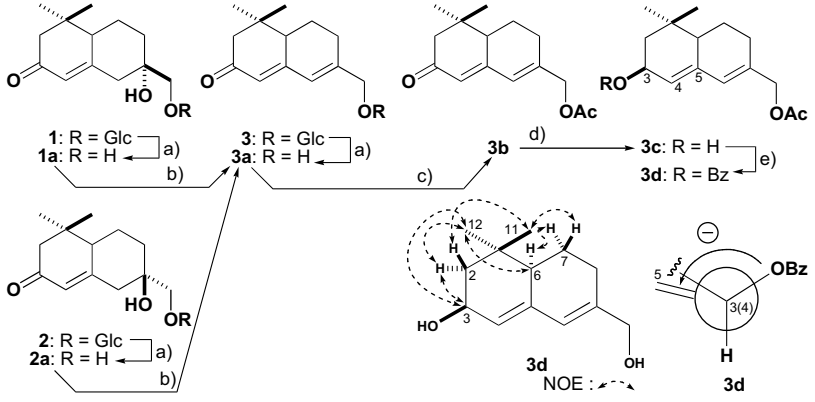


Figure 2. ^{1}H–^{1}H COSY, HMBC, and NOE correlations of **3**.

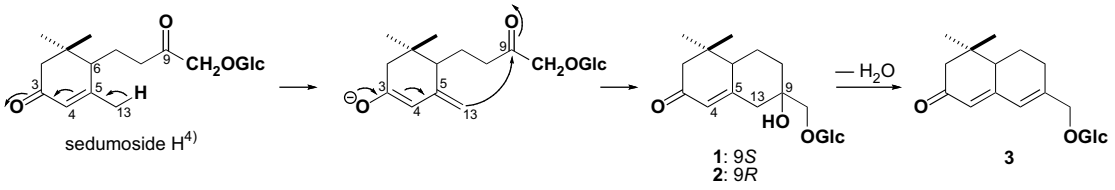
Finally, absolute stereostructures of **1**–**3** were determined by the CD excitation chirality method for allylic benzoates.[7–9] As shown in Scheme 1, enzymatic hydrolysis of **1**–**3** with a β-glucosidase gave the corresponding aglycones named neosarmentols I–III (**1a**–**3a**), respectively. Dehydration of **1a** and **2a** with $SOCl_2$/pyridine gave **3a**, thus, the stereochemistry of **1a**–**3a** was found to be the same except for the 9-position in **1a** and **2a**. Next, acetylation of **3a** using Ac_2O/pyridine yielded 10-monoacetate (**3b**), which was then treated with $NaBH_4$ in the presence of $CeCl_3$ to give the corresponding 3β-hydroxy derivative (**3c**) as the main product [δ 4.30 (1H, br dd, J=ca. 7, 11 Hz, H-3β)]. The orientation of the 3β-hydroxyl in **3c** was also clarified by NOE correlations observed between the following proton pairs: H-3 and H_3-12, H-2α. Treatment of **3c** with benzoyl chloride in the presence of 4-DMAP yielded 3-benzoate (**3d**), which showed a negative Cotton effect [225 nm ($\Delta\varepsilon$ −3.05) in MeOH]. The above-mentioned evidence led us to conclude that the stereochemistry at the 3-position in **3d** was *S* orientation. The plausible biogenetic pathways of **1**–**3** are shown in Scheme 2.

Neosedumoside IV (**4**) was isolated as an amorphous powder with negative optical rotation ($[\alpha]_D^{27}$ −27.6 (MeOH)). The EIMS showed a molecular ion peak at m/z 388 (M^+), and the molecular formula, $C_{19}H_{32}O_8$, was determined by high-resolution EIMS measurement. Its IR spectrum showed absorption bands at 3389, 1708, 1076, and 1035 cm^{-1}due to hydroxyl, carbonyl, and ether functions. The ^{1}H and ^{13}C NMR spectra (CD_3OD, Tables 1 and 2) showed signals assignable to three methyls [δ 0.97, 1.05, 1.10 (3H each, all s, H_3-11, 12, 13)], four methylenes, a methine [δ 1.80 (1H, m, H-6)], a methylene bearing an oxygen function [δ 3.48, 4.04 (1H each, both d, J=10.5 Hz, H_2-10)], and a carbonyl carbon (δ_C 217.3) together with those of a β-D-glucopyranosyl part. The acid hydrolysis of **4** liberated the D-glucose. The relative stereostructure was clarified by various NMR experiments as shown in Figure 3. In order to determine the absolute structure, following chemical modifications were carried out. As shown in Scheme 3, treatment of the aglycone, neosarmentol IV (**4a**), which was obtained by enzymatic hydrolysis of **4**, with $NaBH_4$ gave the reductant (**4b**). Then, **4b** was treated with 1,1′-thiocarbonyldiimidazole in the presence of 4-DMAP to give 9,10-thiocarbonate (**4c**). Compound **4c** showed a negative Cotton effect [305 nm ($\Delta\varepsilon$ −0.85) in MeOH] associated by the $n\rightarrow\pi^*$ transition of cyclic thiocarbonate moiety.[10,11] On the basis of these evidences, the absolute configuration at the 9-position was determined as *R* orientation. A variety of megastigmane constituents have been isolated from many kinds of plant materials, however, as far as we know, this is the first report on isolation and structural elucidation as well as bioactivities of bicyclic megastigmanes having a bicyclo[4.4.0]decane or bicyclo[4.3.0]nonane skeleton from natural resources.

The whole plant of *S. sarmentosum* has traditionally been used for the treatment of liver disease in China and South Korea.[12] It is recognized that fatty liver is a significant risk factor for serious liver disease.[13,14] There is a strong association between fatty liver disease and hyperinsulinemic insulin-resistance.[15,16] Thus, fatty liver is often associated with obesity and type 2 diabetes.[15] As a part of our characterization studies of antidiabetic constituents from natural medicines, effects of the neosedumosides I–IV (**1**–**4**) on triglyceride contents in HepG2 cells were examined in this study using two methods; 1. triglyceride (TG) accumulation inhibitory activity in HepG2 cells and 2. TG metabolism-promoting activity in high glucose pre-treated HepG2 cells. Firstly, effects of **1**–**4** on oleic acid–albumin-induced TG accumulation inhibitory activity in HepG2 cells were examined.[17] As shown in Table 3, **1** (% of control at 100 μM: 75.1±1.8), **2** (87.8±1.5), and **3** (81.6±4.0) were found to



Scheme 1. (a) β-glucosidase (Sigma, from almond)/H_2O, 37 °C, 16 h, **1a** (21% from **1**), **2a** (64% from **2**), **3a** (48% from **3**); (b) $SOCl_2$/pyridine, rt, 1 h, **3a** (48% from **1a**, 62% from **2a**); (c) Ac_2O/pyridine, rt, 18 h, 90%; (d) $NaBH_4$, $CeCl_3$/EtOH, rt, 20 min, 72%; (e) BzCl, 4-DMAP/pyridine, rt, 6 h, 64%.



Scheme 2. Plausible biogenetic pathways of **1**–**3**.

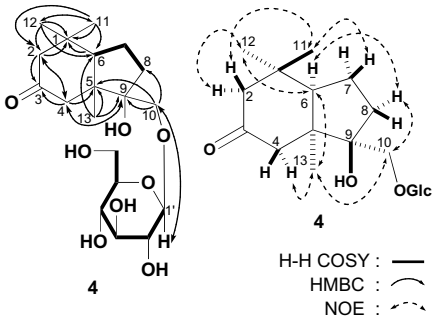


Figure 3. ^{1}H–^{1}H COSY, HMBC, and NOE correlations of **4**.

inhibit TG accumulation in HepG2 cells. Lipid metabolism-promoting activity[8,9] of **1**–**4** on stored TG contents in high glucose pretreated HepG2 cells was also examined. As the result, **2** (% of control at 1 μM: 92.6±1.8) and **3** (92.2±2.3) were found to show reduction of triglyceride levels in HepG2 cells (Table 4). Thus, these megastigmane constituents (**2** and **3**) were found to show not only TG accumulation inhibitory activity but also TG metabolism-promoting activity in the hepatocyte.

3. Experimental

3.1. General

The following instruments were used to obtain spectral and 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 spectra, Shimadzu FTIR-8100 spectrometer; ^{1}H and ^{13}C NMR spectra, JEOL JNM-LA500 (500 and 125 MHz) spectrometer with tetramethylsilane as an internal standard; EIMS, CIMS, high-resolution EIMS, and high-resolution CIMS, JEOL JMS-GCMATE mass spectrometer; 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 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: 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); 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.

Table 3
Effects of neosedumosides I–IV (**1**–**4**) on oleic acid–albumin-induced triglyceride accumulation in HepG2 cells

Conc. (μM)	TG/protein (% of control)				
	0	0.1	1	10	100
Neosedumoside I (**1**)	100.0±3.5	80.4±1.5**	79.6±2.9**	78.3±2.0**	75.1±1.8**
Neosedumoside II (**2**)	100.0±2.9	97.8±0.9	91.6±4.8	91.2±2.8	87.8±1.5*
Neosedumoside III (**3**)	100.0±3.1	94.3±4.2	98.3±2.5	96.6±2.7	81.6±4.0**
Neosedumoside IV (**4**)	100.0±1.8	91.8±2.8	98.8±1.1	100.5±3.1	107.6±3.3
Bezafibrate	100.0±1.1	90.5±1.3*	93.4±1.4	86.7±1.2**	72.7±0.6**

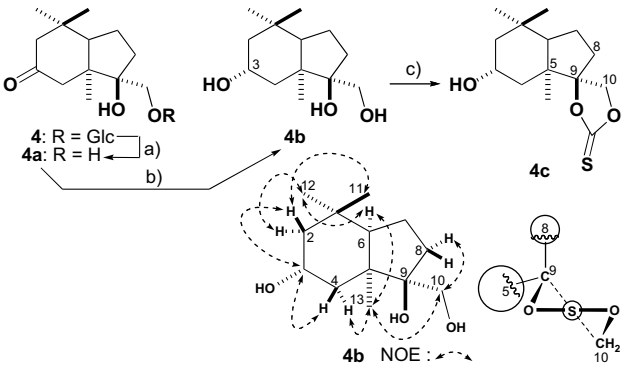
Significantly different from the control, $^{*}p < 0.05$, $^{**}p < 0.01$.

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.[2]

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 this herbal medicine) 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 1 (12.1 g) was subjected to reversed-phase silica gel CC [300 g, MeOH–H_2O (5:95→10:90→20:80→30:70→50:50→70:30, v/v)→MeOH] to afford 13 fractions [Fr. 1-1 (550 mg), Fr. 1-2 (980 mg), Fr. 1-3 (1460 mg), Fr. 1-4 (1230 mg), Fr. 1-5 (1510 mg), Fr. 1-6 (1800 mg), Fr. 1-7 (540 mg), Fr. 1-8 (600 mg), Fr. 1-9 (710 mg), Fr. 1-10 (220 mg), Fr. 1-11 (1170 mg), Fr. 1-12 (1030 mg), and Fr. 1-13 (150 mg)] as reported previously.[2] The fraction 1-7 (540 mg) was subjected to Sephadex LH-20 CC [150 g, $CHCl_3$–MeOH (1:1, v/v)], and then by HPLC [MeOH–H_2O (42:58, v/v)] to furnish neosedumoside III (**3**, 21.3 mg, 0.00004%) together with myrsinionoside A[2] (48.5 mg, 0.00009%), secoisolariciresinol[3] (56.3 mg, 0.00010%). The fraction 2 (19.2 g) was subjected to reversed-phase silica gel CC [600 g, MeOH–H_2O (20:80→30:70→40:60→70:30, v/v)→MeOH] to afford 12 fractions [Fr. 2-1 (200 mg), Fr. 2-2 (4630 mg), Fr. 2-3 (1160 mg), Fr. 2-4



Scheme 3. (a) β-Glucosidase/H_2O, 37 °C, 16 h, 93%; (b) $NaBH_4$/MeOH, rt, 2 h, 60%; (c) 1,1′-thiocarbonyldiimidazole, 4-DMAP/toluene, 60 °C, 3 h, 19%.

Table 4
Effects of neosedumosides I–IV (**1**–**4**) on triglyceride contents in high glucose pre-treated HepG2 cells

Conc. (μM)	TG/protein (% of control)				
	0	0.01	0.1	1	10
Neosedumoside I (**1**)	100.0±1.4		96.6±1.0	99.3±1.6	97.2±1.2
Neosedumoside II (**2**)	100.0±0.8		95.3±1.8	92.6±1.8**	89.7±2.2**
Neosedumoside III (**3**)	100.0±1.0		95.3±0.3*	92.2±2.3*	94.2±3.0
Neosedumoside IV (**4**)	100.0±2.0		92.0±3.1	93.4±2.3	92.8±2.7
Metformin	100.0±2.0	94.0±2.1	90.0±2.7*	89.3±3.1*	

Significantly different from the control, $^{*}p < 0.05$, $^{**}p < 0.01$.

(1950 mg), Fr. 2-5 (3300 mg), Fr. 2-6 (650 mg), Fr. 2-7 (700 mg), Fr. 2-8 (1800 mg), Fr. 2-9 (810 mg), Fr. 2-10 (1360 mg), Fr. 2-11 (2270 mg), and Fr. 2-12 (770 mg)] as reported previously.[2] The fraction 2-4 (1950 mg) was subjected to normal-phase silica gel CC [100 g, $CHCl_3 \rightarrow CHCl_3$–MeOH (50:1 → 20:1 → 10:1, v/v) → $CHCl_3$–MeOH–H_2O (20:3:1, v/v/v, lower layer) → MeOH] to give seven fractions [Fr. 2-4-1 (90.5 mg), Fr. 2-4-2 (50.1 mg), Fr. 2-4-3 (284.0 mg), Fr. 2-4-4 (153.8 mg), Fr. 2-4-5 (348.2 mg), Fr. 2-4-6 (721.1 mg), and Fr. 2-4-7 (300.0 mg)]. The fraction 2-4-5 (348.2 mg) was further purified by HPLC [CH_3CN–MeOH–H_2O (10:8:82, v/v/v) and MeOH–H_2O (30:70 or 32:68, v/v)] to furnish neosedumosides I (**1**, 25.4 mg, 0.00005%) and II (**2**, 12.3 mg, 0.00002%) together with sedumoside D[2] (43.0 mg, 0.00008%), staphylionoside D[2] (3.2 mg, 0.00001%), 3-hydroxy-5,6-epoxy-β-ionol 9-*O*-β-D-glucopyranoside[2] (22.0 mg, 0.00004%), 4*R*-*p*-menth-1-ene-7,8-diol 7-*O*-β-D-glucopyranoside[3] (31.3 mg, 0.00006%), and 4*R*-*p*-menth-1-ene-7,8-diol 8-*O*-β-D-glucopyranoside[3] (22.9 mg, 0.00004%). The fraction 2-8 (1800 mg) was purified by Sephadex LH-20 CC [150 g, MeOH–H_2O (30:70, v/v)] and then by HPLC [CH_3CN–MeOH–H_2O (20:8:72, v/v/v) and MeOH–H_2O (40:60, v/v)] to furnish neosedumosides III (**3**, 32.9 mg, 0.00006%) and IV (**4**, 9.2 mg, 0.00002%) together with sarmentoic acid[2] (429.8 mg, 0.00080%), sarmentoic acid methyl ester[2] (24.5 mg, 0.00005%), alangioside J[2] (80.9 mg, 0.00015%), sedumosides A_4[4] (4.9 mg, 0.00001%), F_1[3] (82.5 mg, 0.00015%), F_2[3] (22.6 mg, 0.00004%), and G[3] (2.5 mg, 0.00001%).

3.3.1. Neosedumoside I (*1*)

An amorphous powder, $[\alpha]_D^{25}$ +40.0 (*c* 1.35, MeOH). High-resolution EIMS: calcd for $C_{19}H_{30}O_8$ $(M)^+$: 386.1940. Found: 386.1936. UV [MeOH, nm (log ε)]: 241 (4.06). CD (MeOH, nm, Δε): 242 (+2.92), 283 (−0.02), 319 (+0.37). IR (KBr): 3389, 1653, 1036 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) δ: given in Table 1. ^{13}C NMR (125 MHz, CD_3OD) δ_C: given in Table 2. EIMS *m*/*z*: 386 (M^+, 3), 206 (86), 190 (92), 178 (13), 148 (100).

3.3.2. Neosedumoside II (*2*)

An amorphous powder, $[\alpha]_D^{25}$ +39.6 (*c* 1.90, MeOH). High-resolution EIMS: calcd for $C_{19}H_{30}O_8$ $(M)^+$: 386.1940. Found: 386.1934. UV [MeOH, nm (log ε)]: 241 (4.10). CD (MeOH, nm, Δε): 239 (+2.35), 278 (−0.05), 321 (+0.42). IR (KBr): 3389, 1653, 1037 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) δ: given in Table 1. ^{13}C NMR (125 MHz, CD_3OD) δ_C: given in Table 2. EIMS *m*/*z*: 386 (M^+, 1), 206 (85), 190 (100), 178 (12), 148 (4).

3.3.3. Neosedumoside III (*3*)

An amorphous powder, $[\alpha]_D^{27}$ −63.7 (*c* 1.00, MeOH). High-resolution positive-ion FABMS: calcd for $C_{19}H_{28}O_7Na$ $(M+Na)^+$: 391.1733. Found: 391.1738. UV [MeOH, nm (log ε)]: 290 (4.12). CD (MeOH, nm, Δε): 283 (−2.63). IR (KBr): 3649, 1653, 1576, 1076 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) δ: given in Table 1. ^{13}C NMR (125 MHz, CD_3OD) δ_C: given in Table 2. Positive-ion FABMS *m*/*z*: 391 $(M+Na)^+$.

3.3.4. Neosedumoside IV (*4*)

An amorphous powder, $[\alpha]_D^{27}$ −27.6 (*c* 0.70, MeOH). High-resolution EIMS: calcd for $C_{19}H_{32}O_8$ $(M)^+$: 388.2097. Found: 388.2099. CD (MeOH, nm, Δε): 282 (−0.73), 317 (+0.02). IR (KBr): 3389, 1708, 1076, 1035 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) δ: given in Table 1. ^1H NMR (500 MHz, pyridine-d_5) δ: 0.88, 0.97, 1.06 (3H each, all s, H_3-11, 12, 13), 1.65 (1H, m, H-6), 1.65 (1H, m, H-7α), 1.84 (1H, m, H-8α), 1.88 (1H, m, H-7α), 1.94 (1H, m, H-8β), 2.01 (1H, d, *J*=16.8 Hz, H-2α), 2.28 (1H, d, *J*=17.4 Hz, H-4α), 2.60 (1H, d, *J*=16.8 Hz, H-2β), 3.16 (1H, d, *J*=17.4 Hz, H-4β), 3.80, 4.28 (1H each, both d, *J*=10.4 Hz, H_2-10), 3.96 (1H, m, H-5′), 4.04 (1H, dd, *J*=7.6, 8.0 Hz, H-2′), 4.22 (1H, m, H-3′), 4.23 (1H, m, H-4′), [4.38 (1H, dd, *J*=4.9, 11.3 Hz), 4.55 (1H, br d, *J*=ca. 11 Hz), H_2-6′], 4.90 (1H, d, *J*=7.6 Hz, H-1′). ^{13}C NMR (125 MHz, CD_3OD and pyridine-d_5) δ_C: given in Table 2. EIMS *m*/*z*: 388 (M^+, 4), 226 (45), 208 (96), 195 (60), 151 (100).

3.4. Acid hydrolysis of 1–4

A solution of **1**–**4** (each 1.0 mg) in 1 M HCl (1.0 mL) was heated under reflux for 3 h. After being cooled, the reaction mixture was washed with EtOAc. The aqueous layer was subjected to HPLC [column, Kaseisorb LC NH_2-60-5, 4.6 mm i.d.×250 mm (Tokyo Kasei Co., Ltd., Tokyo, Japan); detector, Shodex OR-2 optical rotator (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-glucose present in the aqueous layer was carried out by comparison of its retention time and optical rotation with those of an authentic sample, t_R: 13.9 min (D-glucose, positive optical rotation).

3.5. Enzymatic hydrolysis of 1–4 with β-glucosidase

A solution of **1**–**4** (19.6, 11.8, 9.6, and 6.3 mg, respectively) in H_2O (2.0 mL) was treated with a β-glucosidase (12.1, 7.8,10.2, 5.0, and 4.5 mg, respectively, from almond, Oriental yeast Co., Ltd., Tokyo, Japan), and the solution was stirred at 37 °C for 16 h. EtOH was added to the reaction mixture, and the solvent was removed under reduced pressure. The residue obtained starting from **1** and **2** was purified by HPLC [MeOH–H_2O (50:50, v/v)] to furnish neosarmentols I (**1a**, 2.4 mg, 21% from **1**) and II (**2a**, 4.2 mg, 64% from **2**), respectively. The residue obtained starting from **3** was purified by HPLC [MeOH–H_2O (60:40, v/v)] to furnish neosarmentol III (**3a**, 2.6 mg, 48%). The residue obtained starting from **4** was purified by HPLC [MeOH–H_2O (55:45, v/v)] to furnish neosarmentol IV (**4a**, 3.4 mg, 93% from **4**).

3.5.1. Neosarmentol I (*1a*)

Colorless oil, $[\alpha]_D^{23}$ +126.8 (*c* 0.06, MeOH). High-resolution EIMS: calcd for $C_{13}H_{20}O_3$ $(M)^+$: 224.1412. Found: 224.1415. UV [MeOH, nm (log ε)]: 243 (4.02). CD (MeOH, nm, Δε): 241 (+2.10), 283 (−0.11), 321 (+0.47). IR (film): 3397, 1653, 1287, 1082 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.94, 1.07 (3H each, both s, H_3-11, 12), 1.30 (1H, m, H-7β), 1.64 (1H, ddd, *J*=4.0, 12.5, 13.4 Hz, H-8α), 1.97 (1H, m, H-7α), 2.08 (1H, ddd, *J*=2.8, 5.5, 12.5 Hz, H-8β), 2.14 (1H, dd, *J*=4.9, 13.4 Hz, H-6), 2.21 (2H, s, H_2-2), 2.37 (1H, d, *J*=13.8 Hz, H-13α), 2.65 (1H, dd, *J*=2.2, 13.8 Hz, H-13β), 3.44, 3.47 (1H each, both d, *J*=11.0 Hz, H_2-10), 5.83 (1H, br s, H-4). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 34.8 (C-1), 50.7 (C-2), 199.4 (C-3), 125.2 (C-4), 161.4 (C-5), 48.1 (C-6), 23.5 (C-7), 34.3 (C-8), 73.7 (C-9), 66.1 (C-10), 24.1 (C-11), 28.7 (C-12), 45.0 (C-13). EIMS *m*/*z*: 224 (M^+, 18), 206 (53), 193 (37), 151 (81), 123 (100).

3.5.2. Neosarmentol II (*2a*)

Colorless oil, $[\alpha]_D^{22}$ +116.0 (*c* 0.22, MeOH). High-resolution EIMS: calcd for $C_{13}H_{20}O_3$ $(M)^+$: 224.1412. Found: 224.1419. UV [MeOH, nm (log ε)]: 242 (4.10). CD (MeOH, nm, Δε): 239 (+1.96), 283 (−0.01), 314 (+0.42). IR (film): 3391, 1659, 1258, 1042 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 1.00, 1.07 (3H each, both s, H_3-11, 12), 1.70 (1H, m, H-7β), 1.53 (1H, ddd, *J*=4.0, 12.8, 13.5 Hz, H-8α), 1.89 (1H, ddd, *J*=3.1, 6.1, 13.5 Hz, H-8β), 1.93 (1H, m, H-7α), 2.05 (1H, dd, *J*=4.6,

12.8 Hz, H-6), 2.18 (1H, d, J=15.6 Hz, H-2α), 2.28 (1H, d, J=15.6 Hz, H-2β), 2.30 (1H, d, J=13.7 Hz, H-13α), 2.50 (1H, dd, J=2.5, 13.7 Hz, H-13β), 3.51, 3.54 (1H each, both d, J=11.0 Hz, H_2-10), 5.89 (1H, br s, H-4). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 34.8 (C-1), 50.1 (C-2), 199.5 (C-3), 125.5 (C-4), 162.2 (C-5), 49.2 (C-6), 23.2 (C-7), 33.3 (C-8), 74.1 (C-9), 70.8 (C-10), 24.8 (C-11), 28.6 (C-12), 44.5 (C-13). EIMS m/z: 224 (M^+, 18), 206 (44), 193 (39), 151 (83), 123 (100).

*3.5.3. Neosarmentol III (**3a**)*

Colorless oil, $[\alpha]_D^{24}$ −125.3 (c 0.13, MeOH). High-resolution EIMS: calcd for $C_{13}H_{18}O_2$ $(M)^+$: 206.1307. Found: 206.1308. UV [MeOH, nm (log ε)]: 291 (4.32). CD (MeOH, nm, Δε): 285 (−3.80). IR (film): 3422, 1659, 1586, 1370, 1312, 1289, 1138 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.90, 1.13 (3H each, both s, H_3-11, 12), 1.44 (1H, m, H-7β), 2.03 (1H, m, H-7α), 2.20 (1H, m, H-8β), 2.25 (1H, d, J=16.2 Hz, H-2β), 2.31 (1H, m, H-8α), 2.38 (1H, d, J=16.2 Hz, H-2α), 2.38 (1H, m, H-6), 4.22 (2H, s, H_2-10), 5.83 (1H, s, H-4), 6.30 (1H, s, H-13). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 36.3 (C-1), 54.0 (C-2), 199.9 (C-3), 123.7 (C-4), 155.8 (C-5), 45.8 (C-6), 22.2 (C-7), 27.0 (C-8), 151.1 (C-9), 65.8 (C-10), 20.1 (C-11), 28.9 (C-12), 123.5 (C-13). EIMS m/z: 206 (M^+, 84), 191 (40), 173 (12), 163 (9), 150 (100), 122 (56).

*3.5.4. Neosarmentol IV (**4a**)*

Colorless oil, $[\alpha]_D^{23}$ −49.4 (c 0.17, $CHCl_3$). High-resolution EIMS: calcd for $C_{13}H_{22}O_3$ $(M)^+$: 226.1569. Found: 226.1577. CD (MeOH, nm, Δε): 281 (−0.88), 317 (+0.03). IR (film): 3397, 1712, 1287, 1082 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.96, 1.05, 1.06 (3H each, all s, H_3-11, 13, 12), 1.65 (2H, m, H_2-8), 1.76 (1H, m, H-6), 1.78 (2H, m, H_2-7), 1.93 (1H, d, J=17.7 Hz, H-2α), 2.10 (1H, d, J=17.4 Hz, H-4α), 2.17 (1H, t, J=4.0 Hz, O*H*), 2.42 (1H, d, J=17.7 Hz, H-2β), 2.72 (1H, d, J=17.4 Hz, H-4β), 3.55, 3.65 (1H each, both dd, J=4.0, 11.0 Hz, H_2-10). ^{1}H NMR (500 MHz, C_6D_6) δ: 0.69, 0.73, 0.76 (3H each, all s, H_3-11, 13, 12), 1.28 (1H, m, H-8α), 1.31 (1H, m, H-6), 1.40 (1H, m, H-7α), 1.49 (1H, m, H-8β), 1.59 (1H, m, H-7β), 1.85 (1H, d, J=17.4 Hz, H-2α), 1.99 (1H, d, J=17.4 Hz, H-4α), 2.39 (1H, d, J=17.4 Hz, H-2β), 2.85 (1H, d, J=17.4 Hz, H-4β), 3.25, 3.35 (1H each, both d, J=10.7 Hz, H_2-10). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 33.7 (C-1), 47.7 (C-2), 213.9 (C-3), 45.5 (C-4), 47.0 (C-5), 55.6 (C-6), 25.6 (C-7), 34.0 (C-8), 84.2 (C-9), 65.9 (C-10), 28.1 (C-11), 30.1 (C-12), 28.7 (C-13). ^{13}C NMR (125 MHz, C_6D_6) δ_C: 33.5 (C-1), 47.8 (C-2), 212.1 (C-3), 45.8 (C-4), 47.1 (C-5), 55.6 (C-6), 25.9 (C-7), 34.0 (C-8), 84.2 (C-9), 65.9 (C-10), 28.1 (C-11), 30.0 (C-12), 28.7 (C-13). EIMS m/z: 226 (M^+, 16), 208 (41), 195 (44), 151 (100).

3.6. Dehydration of 1a and 2a

A solution of **1a** (1.0 mg) in dry pyridine (0.5 mL) was treated with thionyl chloride ($SOCl_2$, 20 μL). The mixture was stirred at room temperature for 1 h and was poured into ice-water, then extracted with EtOAc. The extract was successively washed with saturated aqueous $NaHCO_3$ and brine, and filtrated. After removal of the solvent under reduced pressure, the resulting residue was purified by HPLC [MeOH–H_2O (60:40, v/v)] to give **3a** (0.4 mg, 48%). Through the similar procedure, the same product **3a** (0.6 mg, 62%) was obtained from **2a** (1.0 mg).

3.7. Acetylation of 3a

To a solution of **3a** in dry pyridine (1.0 mL) was added acetic anhydride (0.8 mL), and the mixture was stirred at room temperature for 18 h. The reaction mixture was poured into ice-water and extracted with EtOAc. The extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, and filtrated. After removal of the solvent under reduced pressure, the residue was purified by normal-phase silica gel CC (500 mg, $CHCl_3$) to give **3b** (2.8 mg, 90%).

*3.7.1. Compound **3b***

Colorless oil, $[\alpha]_D^{24}$ −72.9 (c 0.14, $CHCl_3$). High-resolution EIMS: calcd for $C_{15}H_{20}O_3$ $(M)^+$: 248.1412. Found: 248.1409. UV [MeOH, nm (log ε)]: 287 (4.16). CD (MeOH, nm, Δε): 283 (−3.13). IR (film): 1748, 1663, 1592, 1372, 1310, 1289, 1225, 1138, 1032 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.90, 1.13 (3H each, both s, H_3-11, 12), 1.44 (1H, m, H-7β), 2.02 (1H, m, H-7α), 2.13 (3H, s, Ac), 2.25 (1H, d, J=16.2 Hz, H-2β), 2.25 (1H, m, H-8β), 2.31 (1H, m, H-8α), 2.37 (1H, d, J=16.2 Hz, H-2α), 2.37 (1H, m, H-6), 4.62 (2H, s, H_2-10), 5.83 (1H, s, H-4), 6.23 (1H, s, H-13). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 36.2 (C-1), 53.9 (C-2), 199.8 (C-3), 124.4 (C-4), 155.1 (C-5), 45.5 (C-6), 22.1 (C-7), 27.0 (C-8), 145.6 (C-9), 66.5 (C-10), 20.1 (C-11), 28.8 (C-12), 125.8 (C-13), 20.9, 170.6 (C–Ac). EIMS m/z: 248 (M^+, 3), 188 (47), 160 (77), 145 (66), 132 (100), 104 (48).

3.8. $NaBH_4$–$CeCl_3$ reduction of 3b

To a solution of **3b** (2.8 mg) in EtOH (2.0 mL) was added sodium borohydride ($NaBH_4$, 4.0 mg) in the presence of cerium chloride ($CeCl_3$, 11.0 mg), and the mixture was stirred at room temperature for 20 min. The reaction was quenched by acetone, and the solvent was removed under reduced pressure. To the residue was added ice-water and the mixture was extracted with EtOAc. The extract was washed with brine, and filtered. Removal of the solvent under reduced pressure gave a residue, which was purified by normal-phase silica gel CC (500 mg, $CHCl_3$) to furnish **3c** (2.0 mg, 72%).

*3.8.1. Compound **3c***

Colorless oil, $[\alpha]_D^{22}$ −40.0 (c 0.10, $CHCl_3$). High-resolution EIMS: calcd for $C_{15}H_{22}O_3$ $(M)^+$: 250.1569. Found: 250.1565. UV [MeOH, nm (log ε)]: 241 (3.98). CD (MeOH, nm, Δε): 241 (−3.26). IR (film): 3406, 1742, 1372, 1368, 1238, 1028 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.81, 1.03 (3H each, both s, H_3-11, 12), 1.22 (1H, m, H-7β), 1.33 (1H, dd, J=11.3, 11.3 Hz, H-2β), 1.89 (1H, dd, J=6.9, 11.3 Hz, H-2α), 1.91 (1H, m, H-7α), 1.95 (1H, m, H-6), 2.09 (3H, s, Ac), 2.17 (2H, m, H_2-8), 4.30 (1H, br dd, J=ca. 7, 11 Hz, H-3), 4.54 (2H, s, H_2-10), 5.54 (1H, br s, H-4), 6.06 (1H s, H-13). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 33.9 (C-1), 48.1 (C-2), 66.5 (C-3), 126.9 (C-4), 136.7 (C-5), 44.7 (C-6), 22.6 (C-7), 27.3 (C-8), 135.9 (C-9), 67.6 (C-10), 20.1 (C-11), 29.2 (C-12), 127.7 (C-13), 21.0, 170.9 (C–Ac). EIMS m/z: 250 (M^+, 3), 232 (49), 190 (100), 175 (10), 172 (49).

3.9. Benzoylation of 3c

To a solution of **3c** (2.0 mg) in dry pyridine (1.0 mL) was added benzoyl chloride (10 μL) in the presence of 4-DMAP (2.0 mg), and the mixture was stirred at room temperature for 6 h. The reaction mixture was poured into ice-water and extracted with EtOAc. The extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, brine, and filtrated. Removal of the solvent under reduced pressure yielded a residue, which was purified by normal-phase silica gel CC [500 mg, hexane–EtOAc (3:1, v/v)] to give **3d** (1.8 mg, 64%).

*3.9.1. Compound **3d***

Colorless oil, $[\alpha]_D^{25}$ +3.8 (c 0.10, $CHCl_3$). High-resolution EIMS: calcd for $C_{22}H_{26}O_4$ $(M)^+$: 354.1829. Found: 354.1831. UV [MeOH, nm (log ε)]: 237 (4.08). CD (MeOH, nm, Δε): 225 (−3.05). IR (film): 1742, 1717, 1603, 1451, 1368, 1269, 1113, 1026 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.92, 1.08 (3H each, both s, H_3-11, 12), 1.30 (1H, m, H-7β), 1.63 (1H, dd, J=11.0, 11.0 Hz, H-2β), 1.95 (1H, m, H-7α), 2.01 (1H, m, H-2α), 2.02 (1H, m, H-6), 2.09 (3H, s, Ac), 2.18 (2H, m, H_2-8), 4.55 (2H, s, H_2-10), 5.60 (1H, br s, H-4), 5.68 (1H, m, H-3), 6.09 (1H, s, H-13), 7.43 (2H, dd, J=7.7, 7.7 Hz, H-3′,5′), 7.55 (1H, m, H-5′), 8.04 (2H, dd, J=1.3, 7.7 Hz, H-2′,6′). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 33.9 (C-1), 43.4 (C-2), 70.5 (C-3), 122.6 (C-4), 138.3 (C-5), 44.7 (C-6), 22.5 (C-7), 27.2 (C-8), 136.4 (C-9), 67.4 (C-10), 20.1 (C-11), 29.1 (C-12), 127.3

(C-13), 20.9, 170.9 (C–Ac), 128.3, 128.3×2, 129.6×2, 132.8, 166.4 (C–Bz). EIMS *m*/*z*: 294 (M^+, 59), 232 (57), 172 (63), 157 (100).

3.10. $NaBH_4$ reduction of 4a

A mixture of **4a** (1.5 mg) and $NaBH_4$ (1.0 mg) in MeOH (1.0 mL) was stirred at room temperature for 2 h. The reaction was quenched by acetone. After removal of the solvent under reduced pressure, the residue was purified by HPLC [Shiseido Chiral CD-Ph, 4.6×250 mm i.d. (Shiseido Co., Ltd., Tokyo, Japan), MeOH–H_2O (50:50, v/v)] to give **4b** (0.9 mg, 60%).

*3.10.1. Compound **4b***

Colorless oil, $[\alpha]_D^{23}$ +28.0 (*c* 0.04, MeOH). High-resolution positive-ion FABMS: calcd for $C_{13}H_{24}O_3Na$ $(M+Na)^+$: 251.1623. Found: 251.1628. IR (film): 3405, 1045 cm^{-1}. ^1H NMR (500 MHz, C_6D_6) δ: 0.87, 1.01, 1.10 (3H each, all s, H_3-11, 13, 12), 1.13 (1H, dd, *J*=5.5, 13.7 Hz, H-2α), 1.27 (1H, dd, *J*=5.5, 14.4 Hz, H-4α), 1.32 (1H, dd, *J*=7.6, 8.2 Hz, H-6), 1.37 (1H, m, H-8β), 1.43 (2H, m, H_2-7), 1.52 (1H, dd, *J*=4.8, 13.7 Hz, H-2β), 1.68 (1H, ddd, *J*=5.5, 8.9, 15.1 Hz, H-8α), 1.76 (1H, dd, *J*=5.5, 14.4 Hz, H-4β), 3.09, 3.31 (1H each, both d, *J*=10.3 Hz, H_2-10), 4.01 (1H, m, H-3). ^{13}C NMR (125 MHz, C_6D_6) δ_C: 32.7 (C-1), 43.5 (C-2), 67.2 (C-3), 37.8 (C-4), 46.1 (C-5), 55.3 (C-6), 23.4 (C-7), 33.8 (C-8), 84.5 (C-9), 66.7 (C-10), 28.0 (C-11), 32.4 (C-12), 26.7 (C-13). Positive-ion FABMS *m*/*z*: 251 $(M+Na)^+$.

3.11. Thiocarbonylation of 4b

To a solution of **4b** (0.9 mg) in dry toluene (1.0 mL) was added 1,1′-thiocarbonyldiimidazole (3.5 mg) in the presence of 4-dimethylaminopyridine (4-DMAP, 1.0 mg), and the mixture was stirred at 60 °C for 3 h. The reaction mixture was poured into ice-water and extracted with EtOAc. The extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, brine, and filtrated. Removal of the solvent under reduced pressure gave a residue, which was purified by normal-phase silica gel CC [500 mg, hexane–EtOAc (1:2→1:3, v/v)] to give **4c** (0.2 mg, 18.8%).

3.12. 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 calf 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 days with replacement with the fresh medium every 2 days. Then the medium was removed, and the cells were homogenized in distilled water (105 μL/well) with sonication. The triglyceride (TG) and protein contents in the homogenate were determined using commercial kits (Triglyceride E-test Wako and BCA protein assay kit, 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.[18,19]

3.13. Lipid metabolism-promoting activity of stored triglyceride in HepG2 cells induced by high glucose

HepG2 cells were inoculated in a 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 DMEM containing high glucose (4500 mg/L) and cultured for 6 days with replacement with the fresh medium every 2 days. After accumulation of the lipid, the medium was exchanged to 200 μL/well of DMEM containing low-glucose (1000 mg/L) and a test sample, and the cells were cultured. After 20 h, the TG and protein contents in the cells were determined by the same manner as described above. An antidiabetic agent, metformin was used as a reference compound.[14,15]

3.14. 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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