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Lupane-Type Saponins from Leaves of *Acanthopanax sessiliflorus* and Their Inhibitory Activity on Pancreatic Lipase

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Three known saponins, chiisanoside, 11-deoxyisochiisanoside, and isochiisanoside, and one novel saponin, 3,4-*seco*-4(23),20(29)-lupadiene-3,28-dioic acid 28-*O*-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranosyl (1→6)-β-D-glucopyranoside, referred to as sessiloside, were isolated from a hot water extract of *Acanthopanax sessiliflorus* leaves. All of these saponins were lupane-type triterpene triglycosides, and their concentrations were 4.1, 1.0, 0.5, and 0.4% (w/w) of the total extract, respectively. Sessiloside and chiisanoside inhibited pancreatic lipase activity in vitro, and addition of the saponin-rich fraction to a high-fat diet suppressed the body weight gain of mice. The possibility of application of the lupane-type saponins from *A. sessiliflorus* leaves to the treatment of obesity is discussed.

KEYWORDS: Lipase inhibitor; *Acanthopanax sessiliflorus*; lupane-type saponin; chiisanoside; sessiloside; obesity

INTRODUCTION

Lipase (triacylglycerol hydrolase, EC 3.1.1.3), which catalyzes the hydrolysis of triacylglycerols in the gastrointestinal tract, is the key enzyme for dietary fat absorption, and it is generally thought that a potent and specific inhibitor of pancreatic lipase could be useful in the treatment of obesity (*1*). Orlistat, a hydrogenated derivative of lipstatin from *Streptomyces toxytricini*, binds covalently to the active site serine of pancreatic lipase (*2*). Orlistat, a potent inhibitor of lipases (*3*), has been proved useful in the treatment of obesity, but side effects such as fecal incontinence have also arisen due to its potency (*4*).

The hydrolysis of triacylglycerol by lipase occurs at an interface between water and oil; therefore, any substance decreasing the interface area of the lipid substrate may also act as an inhibitor of lipase. Natural products such as the proteins from soybean seed (*5*) and herring sperm (*6*) as well as low molecular weight substances such as condensed tannin from *Cassia mimosoides* (*7*), flavonoid from *Citrus unshiu* (*8*), an ethyl acetate extract, caulerpenyne, from *Caulerpa taxifolia* (*9*), and catechins from oolong tea (*10*) have been reported to inhibit lipase activity. Many of these inhibitors are amphoteric substances, which could influence the nature of the interface of the lipid substrate.

In the course of a search for a lipase inhibitor from various foodstuffs and natural products such as Chinese medicines and herbs, we found that a hot water extract from *Acanthopanax sessiliflorus* leaves inhibited porcine pancreatic lipase activity. Because saponins are amphoretic glycoconjugates and characteristic in Araliaceae plants (*11*−*16*), we expected that saponins would be active components in terms of the observed inhibitory effect associated with the extract from *A. sessiliflorus* leaves. In the present study, we isolated four saponins from *A. sessiliflorus* leaves and determined their inhibitory activities on pancreatic lipase in vitro and in vivo.

MATERIALS AND METHODS

Materials. The leaves of *A. sessiliflorus* were collected in Jilin, China, in August 2002 and purchased from Shinwa Bussan Co., Ltd. (Osaka, Japan). Porcine pancreatic lipase (type II) was purchased from Sigma (St. Louis, MO). Soybean oil and gum arabic were obtained from Nacalai Tesque Inc. (Kyoto, Japan). 3,5-Dinitrosalicylic acid reagent and *m*-galloylgallic acid were purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan). Soya saponin containing 69% soyasaponin I and 10% soyasaponin A1 (w/w) (*17*) was obtained from J-Oil Mills, Inc. (Iwata, Japan). Three-week-old male Sprague−Dawley

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rats and 4-week-old female ICR mice were obtained from Shimizu Experimental Animals (Kyoto, Japan). Silica gel 60 (0.063−0.2 mm), silica gel plates (kieselgel 60 F_{254}), and ODS plates (RP-18 WF_{254S}) for TLC were the products of Merck (Darmstadt, Germany). Chromatorex ODS (DM-1020T), Sephadex LH-20 (2−100 μm), Diaion HP 20, and IEC SP-825 were obtained from Fuji Silysia Co. (Tokyo, Japan), Pharmacia Fine Chemicals (Uppsala, Sweden), Mitsubishi Chemical Co. (Tokyo, Japan), and Showa Denko K.K. (Tokyo, Japan), respectively. The solvents used for column chromatography were obtained from Wako Pure Chemical Industry Co., Ltd.

Analytical Instruments. The infrared (IR) spectra were recorded in KBr disks on a Shimadzu FT-IR 8200 PC spectrometer (Shimadzu Corp., Kyoto, Japan). The nuclear magnetic resonance (NMR) spectra were recorded in pyridine-d_5 on a JNM-LA 500 spectrometer (^{1}H; 500 MHz, ^{13}C; 125 MHz, JEOL Ltd., Tokyo, Japan). Chemical shifts were assigned by means of DEPT and two-dimensional (2D) NMR methods (^{1}H−^{1}H COSY, ^{1}H−^{13}C COSY). The fast atom bombardment (FAB) mass spectra were obtained in a glycerol matrix in positive-ion mode on a JEOL MStation.

Extraction and Isolation of Saponins from *A. sessiliflorus* Leaves. Air-dried *A. sessiliflorus* leaves (10 kg) were ground and extracted with 80 L of hot water for 1 h under reflux. The hot water extract was filtered and then concentrated under reduced pressure, and a powder was obtained by lyophilization. The powder extract (1.63 kg) was dissolved in water and extracted with ethyl acetate (AcOEt). The AcOEt layer was evaporated under reduced pressure, yielding an AcOEt-soluble portion weighing 21.3 g. Then, the aqueous layer was extracted with *n*-butanol (BuOH), and the removal of the solvent yielded an BuOH-soluble portion weighing 229.8 g and a water (H_2O)-soluble portion weighing 1369 g.

The AcOEt-soluble portion was chromatographed on a Sephadex LH-20 column with methanol (MeOH) to afford four fractions: A-1 (12.1 g), A-2 (3.3 g), A-3 (3.9 g), and A-4 (0.006 g). Fraction A-1 was subjected to silica gel column chromatography with chloroform ($CHCl_3$)/MeOH/H_2O (75:25:3, v/v). The eluate was separated into nine fractions. The eighth fraction (2.22 g) was subjected to silica gel column chromatography with $CHCl_3$/MeOH/AcOEt/H_2O (2:2:4:1, v/v) and subsequently to ODS column chromatography with 70% (v/v) MeOH, yielding saponin **1** (0.25 g).

A part of the BuOH-soluble portion (28.3 g) was chromatographed on a Sephadex LH-20 column with MeOH to afford four fractions: B-1 (24.1 g), B-2 (2.1 g), B-3 (0.8 g), and B-4 (0.2 g). Fraction B-1 was then subjected to silica gel column chromatography and eluted with $CHCl_3$/MeOH/H_2O (75:25:3, v/v), and the eluate was separated into three fractions. The second fraction (9.57 g) yielded saponin **2** (3.17 g). The third fraction (6.64 g) was subjected to ODS column chromatography with 65% (v/v) MeOH. The eluate was separated into five fractions. The fourth fraction yielded saponin **3** (0.58 g). The second fraction (0.59 g) was subjected to ODS column chromatography and eluted with 37.5% (v/v) acetonitrile (CH_3CN) and subsequently with 60% (v/v) MeOH, yielding saponin **4** (0.27 g).

Assay of Lipase Activity. The soybean oil emulsion was prepared by mechanical stirring of 1 g of soybean oil and 10 mL of 5% (w/v) gum arabic, which was dialyzed overnight against distilled water. Lipase activity was assayed by measuring the amount of free fatty acid liberated in the reaction mixture according to the method of Duncombe (*18*). As a standard assay to measure inhibitory activity, the saponins and substrate emulsion were mixed, and lipase solution was added. The tube was shaken at 37 °C at 125 strokes/min for 20 min. The free fatty acid released was extracted with $CHCl_3$ and mixed with a copper reagent containing 1 M triethanolamine, 1 M acetic acid, and 6.45% (w/v) copper(II) nitrate trihydrate [$Cu(NO_3)_2 \cdot 3H_2O$] (9:1:10, v/v). After excess copper reagent had been removed by filtration, 0.1% (w/v) sodium diethyldithiocarbamate in BuOH was added to chelate the copper in $CHCl_3$, and the color that subsequently developed was measured at 440 nm. The remaining lipase activity was expressed as a percentage of the lipase activity of the sample relative to the control lipase activity (*5*).

Assay of Amylase Activity. Amylase activity was assayed by measuring a reducing sugar formed in the reaction mixture using 3,5-dinitrosalicylic acid reagent. Ten micrograms of pancreatic juice was used as the enzyme, and 2 mg of starch was used as the substrate. Incubation was performed at 37 °C at 125 strokes/min for 20 min. The color that developed during the reaction was measured at 535 nm (*19*). The remaining amylase activity was expressed as a percentage of the amylase activity with the sample relative to the control amylase activity.

Table 1. ^{13}C Signals of Saponins **1**–**4**[a] (in Pyridine-d_5)

C	1	2	3	4
1	29.5	70.4	85.4	87.5
2	35.2	38.7	38.3	38.9
3	174.9	173	174.5	174.9
4	148.1	147.7	81.0	79.1
5	50.2	49.6	56.2	56.1
6	24.9	25.1	18.8	18.7
7	32.8	32.2	34.4	35.4
8	40.7	41.6	41.6	42.7
9	40.9	44.0	42.6	48.8
10	39.4	44.1	47.7	47.2
11	21.6	75.2[b]	23.7	67.6
12	25.8	33.4	25.5	36.6
13	38.3	35.2	38.5	37.5
14	43.1	42.1	43.2	42.7
15	30.1	29.5	30.4	30.3
16	32.1	32.1	32.3	32.2
17	56.9	56.7	56.9	56.9
18	49.7	49.5	49.7	49.4
19	47.3	47.6	47.4	46.8
20	150.8	150.1	150.8	150.4
21	30.8	30.7	30.8	30.8
22	36.7	36.7	36.9	36.9
23	113.6	113.9	24.7	24.9
24	23.7	23.5	32.7	32.8
25	20.5	19.1	19.2	19.2
26	16.2	17.9	17.0	17.8
27	14.7	13.7	14.8	15.1
28	176.9	175	174.9	175.5
29	110.1	110.7	110.1	110.2
30	19.4	18.8	19.4	19.4
28-*O*-Glc (inner)				
1	95.2	95.3	95.2	95.2
2	73.9	73.9	73.9	73.9
3	78.1	78.2	78.1	78.1
4	70.7	70.7	70.8	70.8
5	77.1	77.1	77.1	77.1
6	69.3	69.3	69.4	69.3
6-*O*-Glc (outer)				
1	105.0	105.1	105.1	105.0
2	75.2	75.2[b]	75.3	75.2
3	76.4	76.4	76.4	76.4
4	78.6	78.7	78.6	78.6
5	77.9	78.0	78.0	78.0
6	61.2	61.2	61.2	61.2
4-*O*-Rha				
1	102.6	102.7	102.7	102.6
2	72.5	72.5	72.5	72.5
3	72.7	72.7	72.7	72.7
4	74.0	74.0	74.0	74.0
5	70.2	70.3	70.3	70.2
6	18.5	18.5	18.5	18.5

[a] The assignment is based upon ^{1}H–^{13}C COSY, ^{1}H–^{1}H COSY, DEPT, HMQC, and HMBC experiments. [b] Overlapped signals.

Measurement of Hemolytic Activity. Rat peripheral blood was obtained from the tail vein, and erythrocytes were prepared by washing the rat blood with isotonic saline. Erythrocyte suspensions were then incubated with various amounts of saponins at 37 °C for 20 min and then were centrifuged at 500*g* for 5 min for precipitation. Aliquots of the supernatants were obtained, and the optical densities at 540 nm were measured to determine the percentage of hemoglobin released. The absorbance obtained by an addition of 1% (w/v) Triton X-100 was defined as 100%.

Preparation of Saponin-Rich Fraction. The hot water extract from *A. sessiliflorus* leaves was applied to a column of the highly porous

Table 2. ^{1}H NMR Signals of Saponins **1**–**4**[a] (in Pyridine-d_5)

position	1	2	3	4
aglycon				
1′	2.66[b]	3.70 (1H, d, J = 5.5)	4.45 (1H, dd, J = 3.7, 10.7)	5.07 (1H, dd, J = 2.0, 11.7)
1″	2.46 (1H, m)			
2′	1.87[b]	3.07 (1H, d, J = 14.4)	2.79 (1H, dd, J = 10.7, 13.9)	3.86 (1H, dd, J = 2.0, 13.6)
2″	1.87[b]	2.84[b]	2.71 (1H, dd, J = 3.7, 13.9)	2.83 (1H, dd, J = 11.7, 13.6)
5	2.09 (1H, br d, J = 13.4)	2.90 (1H, dd, J = 2.8, 13.4)	1.69[b]	1.80[b]
6′	1.71[b]	1.83 (1H, m)	1.71[b]	1.69[b]
6″	1.30 (1H, m)	1.40[b]	1.35[b]	1.32[b]
7′	1.38[b]	1.33 (1H, m)	1.36[b]	1.43[b]
7″	1.20[b]	1.12[b]	1.27[b]	1.32[b]
9	1.67[b]	2.70 (1H, d, J = 9.8)	1.82[b]	2.03[b]
11′	1.43[b]	4.49 (1H, ddd, J = 8.3, 9.0, 9.0)	1.47[b]	4.20[b]
11″	1.21[b]		1.13[b]	
12′	1.85[b]	2.46 (1H ddd, J = 4.0, 8.3, 12.2)	1.81[b]	2.22 (1H, dd, J = 8.4, 11.9)
12″	1.20[b]	1.71[b]	1.24[b]	1.47[b]
13	2.67[b]	2.84[b]	2.67[b]	2.82 (1H, dd, J = 8.4, 12.7)
15′	1.99 (1H, br dd, J = 12.2, 12.2)	1.91[b]	2.07 (1H, m)	2.01[b]
15″	1.19[b]	1.08[b]	1.20[b]	1.25[b]
16′	2.64[b]	2.61 (1H, ddd, J = 3.1, 3.1, 12.5)	2.65[b]	2.68 (1H, br d, J = 12.9)
16″	1.51[b]	1.47[b]	1.53 (1H, ddd, J = 3.3, 13.3, 13.3)	1.54[b]
18	1.75[b]	1.67[b]	1.70[b]	1.78[b]
19	3.38 (1H, ddd, J = 4.5, 10.7, 10.7)	3.37 (1H, ddd, J = 4.6, 10.7, 10.7)	3.37 (1H, ddd, J = 4.7, 10.6, 10.6)	3.36 (1H, ddd, J = 4.3, 10.6, 10.6)
21′	2.14[b]	2.17[b]	2.15[b]	2.14 (1H, m)
21″	1.41[b]	1.41[b]	1.40[b]	1.41[b]
22′	2.20 (1H, br dd, J = 11.2, 11.2)	2.19[b]	2.21[b]	2.40 (1H, m)
22″	1.49[b]	1.48[b]	1.49[b]	1.56[b]
23′	4.93 (1H, br s)	5.15 (1H, br s)	1.14 (3H, s)	1.20 (3H, s)
23″	4.86 (1H, br s)	5.02 (1H, br s)		
24	1.77 (3H, s)	1.89 (3H, s)	1.40 (3H, s)	1.49 (3H, s)
25	0.82 (3H, s)	1.01 (3H, s)	1.07 (3H, s)	1.37 (3H, s)
26	1.16 (3H, s)	1.11 (3H, s)	1.14 (3H, s)	1.20 (3H, s)
27	1.05 (3H, s)	1.01 (3H, s)	1.08 (3H, s)	1.14 (3H, s)
29′	4.88 (1H, br s)	4.86 (1H, br d, J = 1.8)	4.88 (1H, br s)	4.81 (1H, br s)
29″	4.74 (1H, br s)	4.60 (1H, br s)	4.74 (1H, br s)	4.64 (1H, br s)
30	1.72 (3H, s)	1.64 (3H, s)	1.76 (3H, s)	1.71 (3H, s)
Glc (inner)				
1	6.34 (1H, d, J = 8.3)	6.35 (1H, d, J = 8.3)	6.38 (1H, d, J = 8.3)	6.34 (1H, d, J = 8.2)
2	4.10[b]	4.13[b]	4.11[b]	4.11[b]
3	4.40 (1H, dd, J = 9.2, 9.3)	4.42 (1H, dd, J = 9.2, 9.5)	4.43 (1H, dd, J = 8.9, 9.5)	4.42 (1H, dd, J = 9.3, 9.3)
4	4.33 (1H, dd, J = 9.3, 9.9)	4.33 (1H, dd, J = 9.2, 9.5)	4.35 (1H, dd, J = 8.5, 8.9)	4.34 (1H, dd, J = 9.2, 9.3)
5	3.63 (1H, br d, J = 9.9)	3.65 (1H, ddd, J = 2.6, 4.9, 9.5)	3.65 (1H, br d, J = 8.5)	3.65 (1H, m)
6	4.68[b]	4.71 (1H, br dd, J = 2.6, 11.3)	4.70[b]	4.68 (1H, br d, J = 11.3)
	4.29 (1H, dd, J = 4.6, 11.3)	4.30 (1H, dd, J = 4.9, 11.3)	4.30 (1H, dd, J = 5.6, 11.0)	4.29 (1H, dd, J = 4.5, 11.3)
Glc (outer)				
1	4.94 (1H, d, J = 8.4)	4.94 (1H, d, J = 7.7)	4.96 (1H, d, J = 7.9)	4.96 (1H, d, J = 7.9)
2	3.94 (1H, dd, J = 8.4, 8.7)	3.95 (1H, dd, J = 7.7, 9.2)	3.95 (1H, dd, J = 7.9, 9.0)	3.94 (1H, dd, J = 7.9, 9.3)
3	4.14 (1H, dd, J = 8.7, 9.0)	4.15 (1H, dd, J = 9.0, 9.2)	4.15 (1H, dd, J = 9.0, 9.0)	4.14 (1H, dd, J = 9.2, 9.3)
4	4.23 (1H, dd, J = 9.0, 9.0)	4.24 (1H, dd, J = 9.0, 9.0)	4.25 (1H, dd, J = 8.9, 9.0)	4.23 (1H, dd, J = 9.2, 9.2)
5	4.10[b]	4.12[b]	4.11[b]	4.11[b]
6	4.19 (1H, br d, J = 11.3)	4.20 (1H, br d, J = 11.4)	4.20 (1H, br d, J = 11.0)	4.20 (1H, br d, J = 11.5)
	4.07 (1H, dd, J = 4.0, 11.3)	4.09 (1H, dd, J = 4.2, 11.4)	4.09 (1H, dd, J = 3.4, 12.2)	4.08 (1H, dd, J = 3.4, 11.5)
Rha				
1	5.85 (1H, br s)	5.86 (1H, br s)	5.87 (1H, br s)	5.86 (1H, br s)
2	4.68[b]	4.68 (1H, br dd, J = 1.5, 3.4)	4.69[b]	4.68 (1H, m)
3	4.56 (1H, dd, J = 3.1, 9.2)	4.56 (1H, dd, J = 3.4, 9.2)	4.57 (1H, dd, J = 3.4, 9.1)	4.55 (1H, dd, J = 6.5, 9.2)
4	4.34 (1H, dd, J = 9.2, 9.5)	4.34 (1H, dd, J = 9.2, 9.5)	4.35 (1H, dd, J = 8.5, 9.1)	4.32 (1H, dd, J = 8.6, 9.2)
5	4.96 (1H, m)	4.97 (1H, m)	4.99 (1H, m)	4.98 (1H, m)
6	1.70 (3H, d, J = 6.4)	1.72 (3H, d, J = 5.5)	1.71 (3H, d, J = 6.4)	1.69 (3H, d, J = 5.5)

[a] Multiplicity and J values (hertz) are in parentheses. [b] Overlapped signals. d value is determined from ^{1}H–^{13}C COSY and ^{1}H–^{1}H COSY.

polymer resin Dianon HP 20. After washing with excesses of H_2O and 30% (v/v) ethanol (EtOH), the column was eluted with 50% (v/v) EtOH. The 50% (v/v) EtOH eluate was further applied to an ion-exchange resin IEC SP-825 column. After washing with excesses of H_2O and 30% (v/v) EtOH, the saponin-rich fraction was obtained by 60% (v/v) EtOH elution.

Determination of Saponin Concentrations by HPLC. The saponin concentrations in the hot water extract and saponin-rich fraction from *A. sessiliflorus* leaves were determined using an HPLC system that consisted of an SCI-10A system controller, a PU-980 intelligent pump, and a UV-970 UV−vis intelligent detector (JASCO Corp., Tokyo, Japan). The column was a TSK-GEL ODS-80 Ts (75 × 4.6 mm i.d., TOSOH Corp., Tokyo, Japan). The mobile phase consisted of A, 0.1% (v/v) trifluoroacetic acid (TFA) in H_2O, and B, 0.1% (v/v) TFA in CH_3CN solutions. Elution was performed by A:B = 75:25, v/v, and for the initial 3 min and a gradient of A:B = 75:25, v/v, and A:B = 40:60, v/v, from 3 to 20 min, and A:B = 40:60, v/v, from 20 min to 30 min with a flow rate of 0.5 mL/min at 40 °C. UV absorption at 203 nm was used for detection. The weight percentage of each saponin (w/w) was determined by an absolute quantification. Saponins isolated were weighed and dissolved in a mixture of 0.1% (v/v) TFA in H_2O and 0.1% (v/v) TFA in CH_3CN (1:1, v/v). They were serially diluted with an equal volume of the dissolving mixture. Among saponins, the slopes for the calibration curves were different, but good linear relationships between the weights of the saponins and their responses at 203 nm were obtained with correlation coefficients of 0.99.

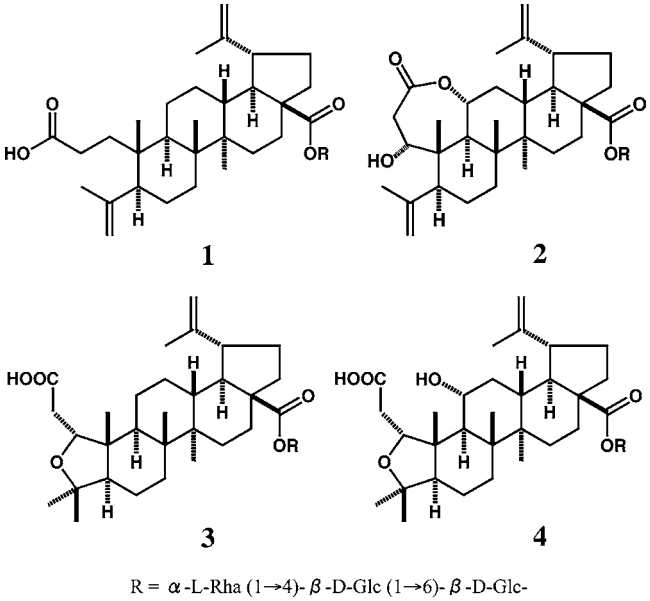

Figure 1. Chemical structures of saponins in the hot water extract from *A. sessiliflorus* leaves: **1**, sessiloside; **2**, chiisanoside; **3**, 11-deoxyisochiisanoside; **4**, isochiisanoside.

Diet Compositions. The control diet, AIN-93G base, was purchased from Oriental Yeast Co. Ltd. (Tokyo, Japan) and composed of 20% (w/w) casein, 53% (w/w) cornstarch, 10% (w/w) sucrose, 7% (w/w) soybean oil, 5% (w/w) cellulose powder, 3% (w/w) mineral mixture, 1% (w/w) vitamin mixture, 0.3% (w/w) cysteine, and 0.25% (w/w) choline. The high-fat diet contained 60% (w/w) lard instead of 7% (w/w) soybean oil by omitting the 10% (w/w) sucrose and 43% (w/w) cornstarch from the AIN-93G base diet. To avoid autoxidation of the fat components, 0.0014% (w/w) of *tert*-butylhydroquinone was included. The saponin-rich fraction was added to the high-fat diet at final concentrations of 1 and 0.5% (w/w). The calories per 100 g of diets were 394 kcal for the control diet, 676 kcal for the high-fat diet, and 672 and 677 kcal for the 1 and 0.5% saponin-supplemented diets, respectively.

Estimation of Body Weight in Mice Fed a High-Fat Diet. Four-week-old female ICR mice were fed for 1 week on a diet of commercial chow ad libitum and divided into four groups matched by body weight. Each group was fed the diet for 4 weeks. The body weight and the amount of food consumed by each mouse were measured twice a week. This study was performed according to the guidelines of accordance with *Standards Relating to the Care and Management of Experimental Animals* (notification 6, March 27, 1980, of the Prime Minister's Office, Japan).

RESULTS

Structural Determination of Saponins from *A. sessiliflorus* Leaves. On the basis of the evidence obtained from IR spectra, NMR spectra (**Tables 1** and **2**), and FAB mass spectra, as well as a comparison of the optical rotations with the reported values, saponins **2**, **3**, and **4** from the BuOH-soluble fraction were identified as chiisanoside, 11-deoxyisochiisanoside, and isochiisanoside (*12*−*14*), respectively (**Figure 1**).

Saponin **1** was obtained from the AcOEt-soluble portion as a white powder. The IR spectra of **1** showed the absorption bands due to hydroxyl (3236 cm^{-1}) and carboxyl and ester (1750 and 1708 cm^{-1}, respectively) groups. Its molecular formula was determined to be $C_{48}H_{76}O_{18}$ by an HR-positive FAB mass spectrum at *m*/*z* 941.5165 $[M + H]^+$ (calcd for $C_{48}H_{77}O_{18}$, 941.5110). The ^1H and ^{13}C NMR signals of **1** were superimposable onto those of 11-deoxyisochiisanoside, **3**, except for the signals due to the A ring moiety (**Tables 1** and **2**). The C_1−C_5, C_{23}−C_{24}, C_{10}, and C_{25} of **1** showed ^{13}C NMR signals

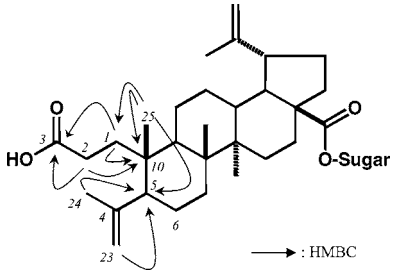

Figure 2. HMBC correlations of sessiloside (**1**) in pyridine-d_5.

due to an isopropenyl group at δ 148.1 (C_4), δ 113.6 (C_{23}), and δ 23.7 (C_{24}), an ethylene group at δ 29.5 (C_1) and δ 35.2 (C_2) placed between a tetrasubstituted sp^3 carbon atom at δ 39.4 (C_{10}) and a carboxyl group at δ 174.9 (C_3), a tertiary methyl group at δ 20.5 (C_{25}), and a methine group at δ 50.2 (C_5) adjacent to a methylene group at δ 24.9 (C_6) and ^1H NMR signals due to an isopropenyl group at δ 4.93 (1H, br s, $H_{23'}$), δ 4.86 (1H, br s, $H_{23''}$), and δ 1.77 (3H, s, H_{24}), an ethylene group at δ 2.66 ($H_{1'}$), δ 2.46 (1H, m, $H_{1''}$), δ 1.87 ($H_{2'}$), and δ 1.87 ($H_{2''}$), a tertiary methyl group at δ 0.82 (3H, s, H_{25}), and a methine group at δ 2.09 (1H, br d, J = 13.4, H_5) adjacent to a methylene group at δ 171 ($H_{6'}$) and δ 1.30 (1 H, m, $H_{6''}$). The HMBC spectrum of **1** showed correlations between 23-H_2/C_5, 24-H_3/C_5, 25-H_3/C_5, 1-H_2/C_{10}, 2-H_2/C_{10}, 25-H_3/C_{10}, 25-H_3/C_1, 1-H_2/C_3, and 2-H_2/C_3, respectively (**Figure 2**). Thus, the structure of **1** was 3,4-*seco*-4(23),20,(29)-lupadiene-3,28-dioic acid 28-*O*-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside. This is a novel saponin and was designated sessiloside, a saponin from *A. sessiliflorus* (**Figure 1**).

The weight percentages of chiisanoside, 11-deoxyisochiisanoside, isochiisanoside, and sessiloside in the hot water extract of leaves from *A. sessiliflorus* were determined to be 4.1, 1.0, 0.5, and 0.4% (w/w), respectively.

Inhibitory Activity of Hot Water Extract and Saponins from *A. sessiliflorus* Leaves on Lipase. A hot water extract of *A. sessiliflorus* leaves did not inhibit porcine pancreatic lipase at the concentration of 1 mg/mL but was gradually inhibitory at higher concentrations. A reduction of ∼30% of the lipase activity on the soybean oil emulsion was observed using 4 mg/mL (**Figure 3**, left). The effect of saponins from *A. sessiliflorus* on lipase was also examined using hyperin, the major flavonoid in *A. sessiliflorus*, as a control (**Figure 3**, right) (*20*). Hyperin did not inhibit the activity of lipase, but saponins were inhibitory. Sessiloside and chiisanoside inhibited the lipase activity in a dose-dependent manner, and their IC_{50} values were 0.36 and 0.75 mg/mL, respectively; these values were 10 and 5 times lower than that of the hot water extract. However, 11-deoxyisochiisanoside and isochiisanoside, both of which have a 1,4-ether structure instead of the alkene structure of C-4 (**Figure 1**), exhibited less inhibitory activity, and in the case of 11-deoxyisochiisanoside, no dose dependency was observed.

Inhibitory Activity of Saponins from *A. sessiliflorus* Leaves on Amylase. It is well-known that many of the secondary products from plant materials including tannins have an action to precipitate proteins. Effects of sessiloside and chiisanoside on amylase activity in pancreatic juice were examined by comparison with that of *m*-galloylgallic acid, a representative polyphenol compound. When *m*-galloylgallic acid, which is 10 times more inhibitory of pancreatic lipase activity than sessiloside, was added to pancreatic juice, amylase activity was also inhibited with a similar dose dependency (**Figure 4**, left). In contrast, neither sessiloside nor chiisanoside inhibited the activity of amylase, but instead slightly enhanced it (**Figure 4**, right).

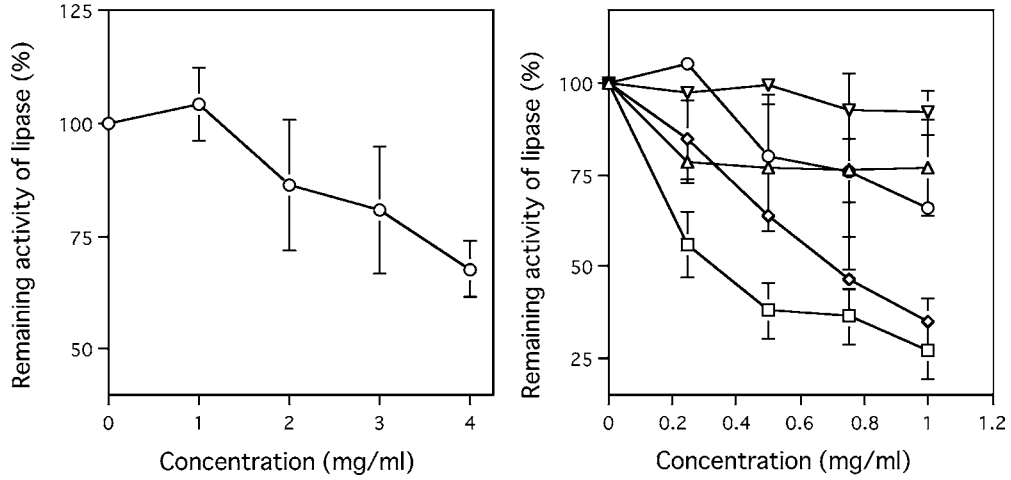

Figure 3. Inhibitory activity of hot water extract (left) and saponins (right) from *A. sessiliflorus* leaves on pancreatic lipase: sessiloside (□); chiisanoside (◇); 11-deoxyisochiisanoside (△); hyperine (▽); isochiisanoside (○). Values are means ± SEM of three experiments.

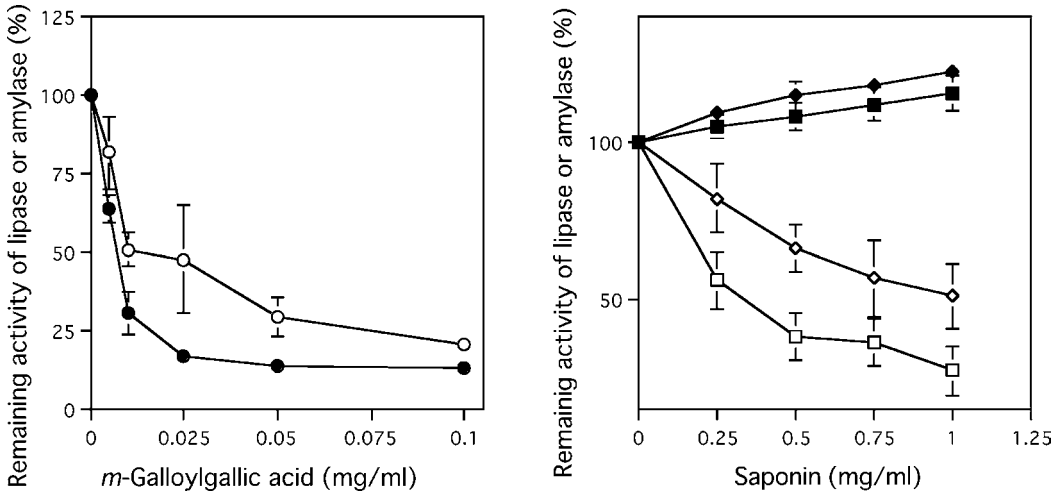

Figure 4. Inhibitory activity of saponins from *A. sessiliflorus* on pancreatic amylase. Effects of *m*-galloylgallic acid (left) and chiisanoside (◇) and sessiloside (□) (right) on the respective activities of pancreatic amylase and lipase were determined. Open symbols indicate lipase activity, and solid symbols indicate amylase activity. Values are means ± SEM of three experiments.

Effect of Saponin-Rich Fraction on Body Weight Gain in Mice Fed a High-Fat Diet. Saponin-rich fraction, in which the concentrations of chiisanoside, 11-deoxyisochiisanoside, isochiisanoside, and sessiloside were 42.6, 3.9, 1.8, and 2.4% (w/w), respectively, was prepared from the hot water extract from *A. sessiliflorus* leaves (**Figure 5**). Consuming a high-fat diet for 4 weeks caused significant increases in body weight gain compared to that of the control group. Supplementations of the saponin-rich fraction to high-fat diet, especially 0.5% supplementation, significantly suppressed the increase in body weight (**Figure 6**). There was no difference in feed comsumption between high-fat groups with or without saponin supplementations (data not shown).

Hemolytic Activity. The hemolytic activities of sessiloside and chiisanoside were evaluated using rat erythrocytes. Soya saponin, which belongs to the oleanene-type of triterpene oligoglycosides, was used as a control. As shown in **Figure 7**, only sessiloside showed hemolytic activity at a dose of 0.2 mg/mL, that is, 2×10^{-4} M, which is 10 times higher than the amount of soya saponin used here.

DISCUSSION

When the body has sufficient energy stores, the circulating triacylglycerols are deposited in adipose tissue for the storage of energy. To reduce the deposit of triacylglycerols, we searched for lipase inhibitors from among more than 400 kinds of foodstuffs and natural products such as Chinese medicines and herbs. Plant materials were infused with hot water, and the freeze-dried infusions were screened for their ability to inhibit the lipase activity of porcine pancreatic juice. About one-fourth of the infiltrates were found to inhibit pancreatic lipase activity (data not shown). It is well-known that polyphenols from plant materials have an affinity for association with proteins, primarily through hydrophobic bonding as well as hydrogen bonding. Thus, the hot water extracts from various plant materials exhibit inhibitory activity for enzymes due to the aggregation of enzyme protein (*21*). In fact, when *m*-galloylgallic acid, which was used here as a representative polyphenol compound, was added to pancreatic juice, both lipase and amylase activity were inhibited with a similar dose dependency (**Figure 4**, left). Thus, many inhibitions by plant material extracts would be ascribed to this mechanism. Saponins are a series of compounds that are composed of sugars attached to a steroid or triterpene, and they act as natural surfactants and/or detergents. These glycoconjugates are characteristic of the genus *Acanthopanax* (*11*−*16*). The isolated saponins, sessiloside and chiisanoside, that inhibited pancreatic lipase activity did not inhibit pancreatic amylase (**Figure 4**, right), suggesting that these saponins do not inhibit

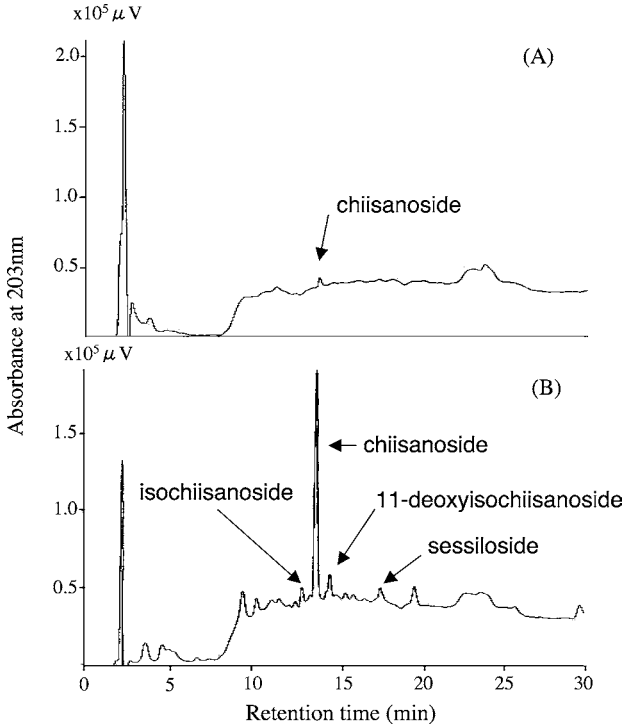

Figure 5. HPLC profiles of hot water extract (**A**) and saponin-rich fraction (**B**) from *A. sessiliflorus* leaves. The same amounts (21.9 mg) of hot water extract and saponin-rich fraction were weighed and dissolved in the mixture of 0.1% (v/v) TFA in H_2O and 0.1% (v/v) TFA in CH_3CN (1:1, v/v). Aliquots were used for HPLC analyses. Retention times for isochiisanoside, chiisanoside, 11-deoxyisochiisanoside, and sessiloside were 11.3, 13.9, 14.7, and 17.8 min, respectively.

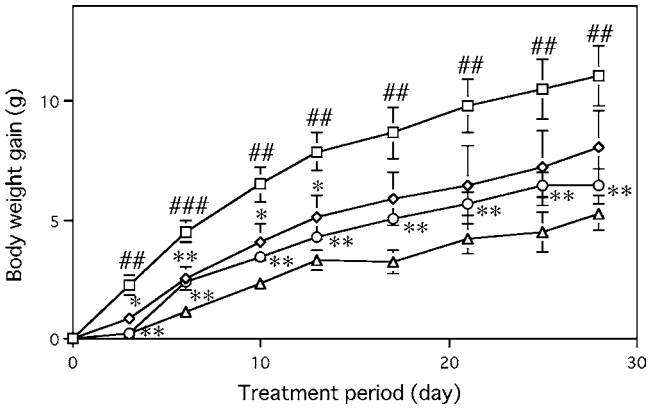

Figure 6. Effects of saponin-rich fraction on body weight gain in mice fed a high-fat diet: control diet groups (△) ($n = 8$); high-fat diet group (□) ($n = 11$); high-fat plus 1% (◇) ($n = 9$) and 0.5% saponin-rich fraction (○) ($n = 9$). Values are mean ± SEM. Data were analyzed by one-way ANOVA, and then differences among means were analyzed using Fisher's PLSD test. Differences were considered to be significant at $P < 0.05$: #, significantly different from the control diet group; ##, $P < 0.01$; ###, $P < 0.001$; ∗, significantly different from the high-fat diet group; ∗, $P < 0.05$; ∗∗, $P < 0.01$.

lipase activity simply by the aggregation of protein, that is, by what is referred to as tannin-like activity, but by affecting a surface property of the substrate emulsion due to their detergent properties. If it is a mechanism for lipase inhibition, saponin glycoconjugates are expected to act in the gastrointestinal tract, where lipase and dietary fats come across. In the preliminary experiment, we found that 5% (w/w) supplementation of the high-fat diet with a hot water extract from *A. sessiliflorus* leaves

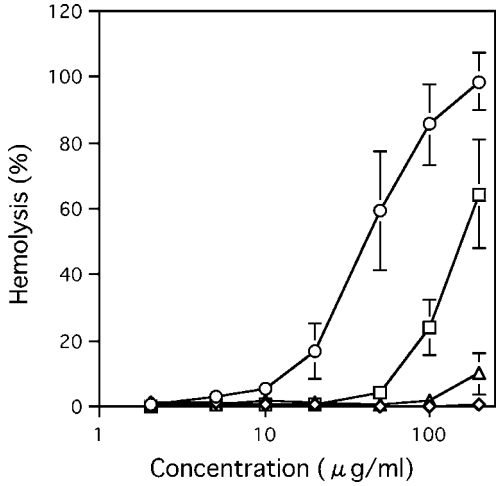

Figure 7. Hemolytic activity of saponins from *A. sessiliflorus* leaves: sessiloside (□); chiisanoside (◇); 11-deoxyisochiisanoside (△); soya saponin (○). Hemolytic activity was measured as lytic activity against red blood cells from rat blood samples. Hemolytic activity was expressed as a percentage of hemoglobin release at OD 540 nm of the sample relative to the control hemolytic activity. Absorbance obtained by 1% (w/v) Triton X-100 was 100%. Values are mean ± SEM of five experiments.

was the most effective to reduce body weight gain of mice due to high-fat intake (data not shown). In support, 0.5% (w/w) supplementation of the saponin-rich fraction, in which the saponin concentration was increased ∼10 times, resulted in significant decrease in body weight gain of mice (**Figures 5** and **6**). Liver weights were not different among four groups (data not shown); however, the reason 0.5% supplementation was more effective in reducing body weight than 1% supplementation is not clear. There might be an optimum concentration of saponin to reduce body weight.

Chiisanoside, which is a major saponin in *A. sessiliflorus*, has been isolated from *Acanthopanax chiisanensis* (*12*) and *Acanthopanax divaricatus* (*13*), and its metabolism by human intestinal bacteria and biological activities such as an antirotaviral activity were examined (*22*). 11-Deoxyisochiisanoside and isochiisanoside were also isolated from the leaves of *A. divaricatus* (*13*), *Acanthopanax senticosus* forma *inermis* (*14*), and *A. divaricatus* var. *sachunesis* (*15*), respectively. In this work, we found a novel saponin compound, referred to as sessiloside, from a hot water extract of *A. sessiliflorus*. To the best of our knowledge, the present study is the first to characterize the inhibitory activity on lipase of these lupane-type saponins. At present, however, the reason lupane-type saponins having a 1,4-ether structure are weak and chiisanoside and a novel compound, sessiloside, having an alkene structure of C-4 are strong in lipase inhibition is not known.

It has been reported that soya saponin (*17*), tea saponin (*23*), and the saponin of *Platycodi radix* (*24*, *25*), all belonging to the family of oleanene-type triterpenoid saponins, exert inhibitory effects upon pancreatic lipase and suppress the increase of body weight due to a high-fat diet. Recently, saponins from *Dioscorea nipponica* Makino root (*26*), belonging among the steroid-type saponins, have also been found to exert lipase-inhibiting effects, both in vitro and in vivo. In general, saponin is known to be toxic due to its hemolytic activity. Within the range examined, a novel saponin from *A. sessiliflorus*, sessiloside, caused only slight hemolysis, whereas chiisanoside did not lead to any hemolysis (**Figure 7**).

Lupane-type saponins including sessiloside from the leaves of *A. sessiliflorus* inhibited the activity of pancreatic lipase in

vitro and reduced body weight gain significantly in mice that ate a high-fat diet supplemented with the saponin-rich fraction without symptoms of diarrhea. Consequently, it is suggested that lupane-type saponins from the leaves of *A. sessiliflorus* would be a candidate for a mild and safe treatment to prevent and reduce obesity.

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LITERATURE CITED

(1) Gargouri, Y.; Ransac, S.; Verger, R. Covalent inhibition of digestive lipases: an in vitro study. *Biochim. Biophys. Acta* **1997**, *1344*, 6−37.

(2) Hadvary, P.; Sidler, W.; Meister, W.; Vetter, W.; Wolfer, H. The lipase inhibitor tetrahydrolipstatin binds covalently to the putative activity site serine of pancreatic lipase. *J. Biol. Chem.* **1991**, *266*, 2021−2025.

(3) Hadvary, P.; Lengsfeld, H.; Wolfer, H. Inhibition of pancreatic lipase *in vitro* by the covalent inhibitor tetrahydrolipstatin. *Biochem. J.* **1988**, *256*, 357−361.

(4) Drent, M. L.; van der Veen, E. A. Lipase inhibition: a novel concept in the treatment of obesity. *Int. J. Obes.* **1993**, *17*, 241−244.

(5) Satouchi, K.; Hirano, K.; Fujino, O.; Ikoma, M.; Tanaka, T.; Kitamura, K. Lipoxygenase-1 from soybean seed inhibiting the activity of pancreatic lipase. *Biosci., Biotechnol., Biochem.* **1998**, *62*, 1498−1503.

(6) Tsujita, T.; Matsuura, Y.; Okuda, H. Studies on the inhibition of pancreatic and carboxylester lipases by protamine. *J. Lipid Res.* **1996**, *37*, 1481−1487.

(7) Yamamoto, M.; Shimura, S.; Itoh, Y.; Ohsaka, T.; Egawa, M.; Inoue, S. Anti-obesity effects of lipase inhibitor CT-II, an extract from edible herbs, Nomame herba, on rats fed a high-fat diet. *Int. J. Obes.* **2000**, *24*, 758−764.

(8) Kawaguchi, K.; Mizuno, T.; Aida, K.; Uchino, K. Hesperidin as an inhibitor of lipases from porcine pancreas and *Pseudomonas*. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 102−104.

(9) Bitou, N.; Ninomiya, M.; Tsujita, T.; Okuda, H. Screening of lipase inhibitors from marine algae. *Lipids* **1999**, *34*, 441−445.

(10) Nakai, M.; Fukui, Y.; Asami, S.; Toyoda-Ono, Y.; Iwashita, T.; Shibata, H.; Mitsunaga, T.; Hashimoto, F.; Kiso, Y. Inhibitory effects of oolong tea polyphenols on pancreatic lipase in vitro. *J. Agric. Food Chem.* **2005**, *53*, 4593−4598.

(11) Sawada, H.; Miyakoshi, M.; Isoda, S.; Ida, Y.; Shoji, J. Saponins from leaves of *Acathopanax sieboldianus. Phytochemistry* **1993**, *34*, 1117−1121.

(12) Hahn, D.-R.; Kasai, R.; Kim, J.-H.; Taniyasu, S.; Tanaka, O. A new glycosyl ester of a 3,4-seco-triterpene from Korean medicinal plant, *Acanthopanax chiisanensis* (Araliaceae). *Chem. Pharm. Bull.* **1984**, *32*, 1244−1247.

(13) Shirasuna, K.; Miyakoshi, M.; Mimoto, S.; Isoda, S.; Satoh, Y.; Hirai, Y.; Ida, Y.; Shoji, J. Lupane triterpenoid glycosyl esters from leaves of *Acanthopanax divaricatus*. *Phytochemistry* **1997**, *45*, 579−584.

(14) Park, S.-Y.; Chang, S.-Y.; Yook, C.-S.; Nohara, T. New 3,4-seco-lupane-type triterpene glycosides from *Acanthopanax senticosus* forma *inermis*. *J. Nat. Prod.* **2000**, *63*, 1630−1633.

(15) Park, S.-Y.; Yook, C.-S.; Nohara, T. A novel 3,4-*seco*-migrated-lupane glycoside with a seven-membered B-ring from *Acanthopanax divaricatus* var. *sachunensis*. *Tetrahedron Lett.* **2001**, *42*, 2825−2828.

(16) Park, S.-Y.; Choi, H.-S.; Yook, C.-S. Nohara, T. A new lupane glycoside from the leaves of *Acanthopanax koreanum*. *Chem. Pharm. Bull.* **2005**, *53*, 97−99.

(17) Takahashi, Y.-K.; Ohminami, H.; Okuda, H.; Kitagawa, I.; Yoshikawa, M.; Arichi, S.; Hayashi, T. Effect of soya saponins on gold thioglucose (GTG)-induced obese mice. *Int. J. Obes.* **1986**, *10*, 293−302.

(18) Duncombe, W. G. The colorimetric micro-determination of long-chain fatty acids. *Biochem. J.* **1963**, *88*, 7−10.

(19) Bernfeld, P. Amylases α and β. *Methods Enzymol.* **1955**, *1*, 147−158.

(20) Lee, S.; Kim, B.-K.; Cho, S.-H.; Shin, K.-H. Phytochemical constituents from the fruits of *A. sessiliflorus*. *Arch. Pharm. Res.* **2002**, *25*, 280−284.

(21) Loomis, W. D. Overcoming problems of phenolics and quinines in the isolation of plant enzymes and organelles. *Methods Enzymol.* **1974**, *16*, 528−544.

(22) Bae, E.-A.; Yook, C.-S.; Oh, O.-J.; Chang, S.-Y.; Nohara, T.; Kim, D.-H. Metabolism of chiisanoside from *Acanthopanax divaricatus* var. *albeofructus* by human intestinal bacteria and its relation to some biological activity. *Biol. Pharm. Bull.* **2001**, *24*, 582−585.

(23) Han, L.-K.; Kimura, Y.; Kawashima, M.; Taniyama, T.; Hayashi, T.; Zheng, Y.-N.; Okuda, H. Anti-obesity effects in rodents of dietary tea saponin, a lipase inhibitor. *Int. J. Obes.* **2001**, *25*, 1459−1464.

(24) Han, L.-K.; Xu, B.-J.; Kimura, Y.; Zheng, Y.-N.; Okuda, H. *Platycodi radix* affects lipid metabolism in mice with high fat diet-induced obesity. *J. Nutr.* **2000**, *130*, 2760−2764.

(25) Han, L.-K.; Zheng, Y.-N.; Xu, B.-J.; Okuda, H.; Kimura, Y. Saponins from *Platycodi radix* ameliorate high fat diet-induced obesity in mice. *J. Nutr.* **2002**, *132*, 2241−2245.

(26) Kwon, C.-S.; Sohn, H. Y.; Kim, S. H.; Kim, J. H.; Son, K. H.; Lee, J. S.; Lim, J. K.; Kim, J.-S. Anti-obesity effect of *Dioscorea nipponica* Makino with lipase-inhibitory activity in rodents. *Biosci., Biotechnol., Biochem.* **2003**, *67*, 1451−1456.

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