Floratheasaponins A−C, Acylated Oleanane-Type Triterpene Oligoglycosides with Anti-hyperlipidemic Activities from Flowers of the Tea Plant (*Camellia sinensis*)[1]

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The methanolic extract and its *n*-butanol-soluble fraction from the flowers of the tea plant (*Camellia sinensis*) were found to suppress serum triglyceride elevation in olive oil-treated mice. From the *n*-butanol-soluble fraction, three new acylated oleanane-type triterpene oligoglycosides, floratheasaponins A−C (**1**−**3**), were isolated together with several flavonol glycosides and catechins. The structures of **1**−**3** were elucidated on the basis of chemical and physicochemical evidence as 21-*O*-angeloyl-22-*O*-acetylTheasapogenol B 3-*O*-[*β*-D-galactopyranosyl(1→2)][*β*-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-*β*-D-glucopyranosiduronic acid, 21,22-di-*O*-angeloyl-R$_1$-barrigenol 3-*O*-[*β*-D-galactopyranosyl(1→2)][*β*-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl(1→3)]-*β*-D-glucopyranosiduronic acid, and 21-*O*-angeloyl-22-*O*-2-methylbutyryl-R$_1$-barrigenol 3-*O*-[*β*-D-galactopyranosyl(1→2)][*β*-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-*β*-D-glucopyranosiduronic acid, respectively. Floratheasaponins (**1**−**3**) showed inhibitory effects on serum triglyceride elevation, with their activities being more potent than those of theasaponins E$_1$ (**4**) and E$_2$ (**5**) obtained previously from the seeds of *C. sinensis*.

During the course of characterization studies on the bioactive saponin constituents from *Camellia* species,[2,3] we have reported the isolation and structure elucidation of the following saponins with antisweet, gastroprotective, gastric-emptying inhibitory, and gastrointestinal transit accelerating activities from the tea plant: theasaponins E$_1$ (**4**) and E$_2$ (**5**) from the seeds of *Camellia sinensis* (L.) O. Kuntze[4] and assamsaponins A−J from the seeds and leaves of *C. sinensis* var. *assamica* Pierre.[5,6] In addition, the following compounds were isolated as methyl esters from tea plants: 3-*O*-{*β*-D-galactopyranosyl(1→2)-[*β*-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-*β*-D-glucuronopyranosyl}-21-*O*-cinnamoyl-16,22-di-*O*-acetylbarringtogenol C (from the leaves of *C. sinensis*)[7] and TR-saponins A−C (from the roots of *C. sinensis* var. *assamica*).[8] However, the chemical constituents as well as the pharmacological properties of the flowers of *C. sinensis* have yet to be characterized. In a continuing study on bioactive constituents of medicinal flowers,[1,9,10] we found that the methanolic extract from the flowers of *C. sinensis* and its *n*-butanol (BuOH)-soluble fraction showed an inhibitory effect on serum triglyceride (TG) elevation in olive oil-treated mice. From the BuOH-soluble fraction, we isolated three new acylated oleanane-type triterpene oligoglycosides, floratheasaponins A−C (**1**−**3**), together with six known flavonol glycosides and three epicatechins. This paper deals with structure elucidation of floratheasaponins (**1**−**3**) as well as the anti-hyperlipidemic activities of **1**−**5**.

Results and Discussion

As shown in Table 1, the methanolic extract from the flowers of *C. sinensis* (34.1% from the flowers) significantly suppressed serum TG elevation 2 h after administration of olive oil at doses of 500−1000 mg/kg, p.o. The methanolic extract was partitioned into an EtOAc and water mixture to give an EtOAc-soluble fraction (5.0%) and an aqueous phase. The aqueous phase was further extracted with BuOH to give a BuOH-soluble fraction (15.8%) and a H$_2$O-soluble fraction (13.3%). The EtOAc- and BuOH-soluble fractions (500 mg/kg, p.o.) significantly suppressed the increase of serum TG level 2 h after administration of olive oil, and their activities were more potent than that of a hypolipidemic drug, clofibrate.

The most active fraction (the BuOH-soluble fraction) was subjected to normal- and reversed-phase column chromatography and finally HPLC to give floratheasaponins A (**1**, 0.34% from the dried flowers), B (**2**, 0.42%), and C (**3**, 0.24%) together with kaempferol 3-*O*-*β*-D-galactopyranoside (0.018%),[1] kaempferol 3-*O*-*β*-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)-*β*-D-galactopyranoside (0.12%),[12] kaempferol 3-*O*-*β*-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)-*β*-D-glucopyranoside (0.13%),[12] quercetin 3-*O*-*β*-D-galactopyranoside (0.018%),[11,13] rutin (0.011%),[14,15] myricetin 3-*O*-*β*-D-galactopyranoside (0.0030%),[16] (−)-epicatechin (0.0062%),[15,17] (−)-epicatechin 3-*O*-gallate (0.045%),[17] and (−)-epigallocatechin 3-*O*-gallate (0.026%).[17]

Floratheasaponin A (**1**) was obtained as colorless fine crystals from CHCl$_3$−MeOH with mp 201−203 °C and exhibited a negative optical rotation ([α]$^{26}_D$ −3.3°). The IR spectrum of **1** showed absorption bands at 1719 and 1655 cm^{-1} ascribable to carbonyl and α,*β*-unsaturated ester functions, and broad bands at 3505, 1078, and 1048 cm^{-1}, suggestive of an oligoglycoside structure. In the positive- and negative-ion FABMS of **1**, quasimolecular ion peaks were observed at *m/z* 1239 [M + Na]$^+$ and 1215 [M − H]$^-$, and HRFABMS analysis revealed the molecular formula of **1** to be C$_{59}$H$_{92}$O$_{26}$. The fragmentation patterns in the negative-ion FABMS of **1** indicated the loss of monopentose (*m/z* 1083 [M − C$_5$H$_9$O$_4$]$^-$), dipentoses (*m/z* 951 [M − C$_{10}$H$_{17}$O$_8$]$^-$), and monopentose and monohexose (*m/z* 921 [M − C$_{11}$H$_{19}$O$_9$]$^-$) units. On alkaline hydrolysis of **1** with 10% aqueous KOH−50% aqueous 1,4-dioxane (1:1, v/v), desacyl-assamsaponin E (**1a**)[5] was obtained together with two organic acids, acetic acid and angelic acid, which were identified by HPLC analysis of their *p*-nitrobenzyl deriva-

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Chart 1

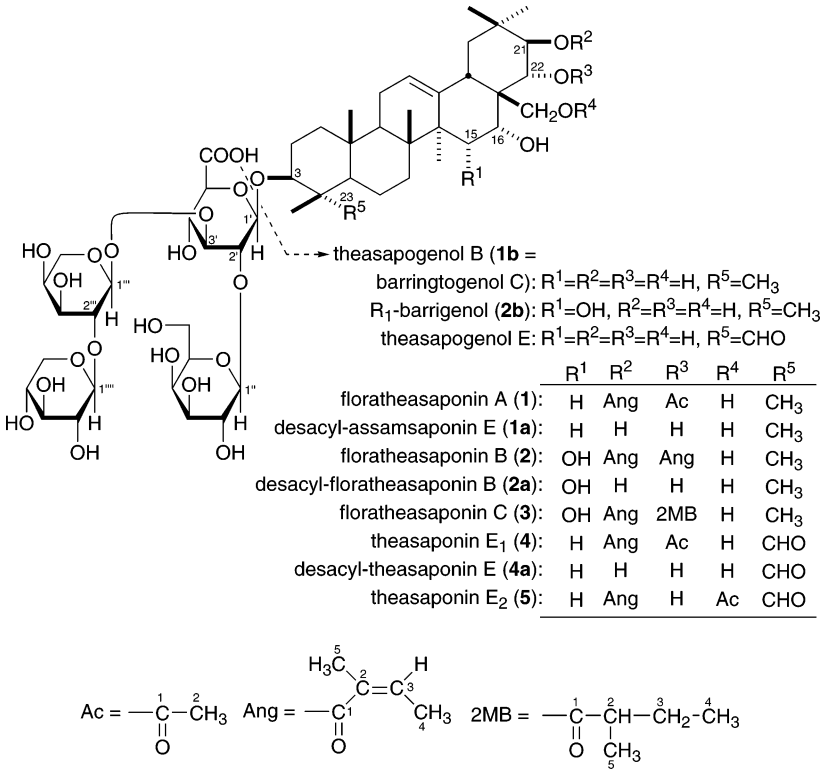

Table 1. Inhibitory Effects of the Methanolic Extract and EtOAc-, BuOH-, and H_2O-Soluble Fractions from the Flowers of *Camellia sinensis* on Serum Triglyceride Elevation in Olive Oil-Treated Mice

			serum triglyceride (mg/dL)[a,b]		
treatment	dose (mg/kg, p.o.)	*N*	2.0 h	4.0 h	6.0 h
normal		9	129.6 ± 12.3**	132.3 ± 14.4**	119.3 ± 12.5*
control		12	470.8 ± 36.7	327.1 ± 30.8	224.9 ± 17.1
MeOH ext.	250	12	486.5 ± 34.8	273.9 ± 25.4	202.8 ± 21.2
	500	12	255.6 ± 36.2**	281.1 ± 27.5	185.7 ± 13.6
	1000	11	134.4 ± 12.5**	293.2 ± 46.9	338.5 ± 40.2*
normal		8	144.0 ± 11.2**	118.6 ± 5.6**	114.1 ± 6.6**
control		12	476.4 ± 28.3	337.3 ± 32.1	213.8 ± 16.6
EtOAc-soluble fraction	250	8	418.8 ± 37.8	353.4 ± 58.8	214.4 ± 23.0
	500	8	246.8 ± 41.0**	280.4 ± 34.5	170.2 ± 18.3
BuOH-soluble fraction	250	8	360.4 ± 42.9	304.9 ± 23.9	202.6 ± 13.0
	500	8	159.3 ± 31.0**	308.5 ± 54.3	309.2 ± 21.6**
H_2O-soluble fraction	250	8	475.3 ± 60.2	263.3 ± 20.3	214.1 ± 12.5
	500	8	541.1 ± 31.4	358.9 ± 49.0	267.3 ± 37.8
normal		10	140.0 ± 7.4**	124.6 ± 9.3*	147.6 ± 7.5
control		10	439.4 ± 34.5	267.5 ± 22.4	244.8 ± 22.0
clofibrate	125	10	377.5 ± 22.8	295.0 ± 40.7	333.4 ± 29.4
	250	10	441.2 ± 25.4	340.5 ± 58.6	443.3 ± 59.2**
	500	10	318.2 ± 18.2**	239.7 ± 28.7	373.5 ± 28.5*

[a] Values represent the means ± SEM. [b] Significantly different from the control group, *$p < 0.05$, **$p < 0.01$.

tives.[5,6] The ^{1}H (pyridine-d_5) and ^{13}C NMR (Table 2) spectra of **1**, which were assigned by various NMR experiments,[18] showed signals assignable to seven methyls [δ 0.84, 0.87, 1.09, 1.13, 1.82 (3H each, all s, H_3-25, 26, 29, 24, 27), 1.30 (6H, s, H_3-23, 30)], a methylene and four methines bearing an oxygen function [δ 3.31 (1H, dd, J = 4.3, 11.6 Hz, H-3), 3.37, 3.60 (1H each, both d, J = 10.7 Hz, H2−28), 4.44 (1H, m, H-16), 6.15 (1H, d, J = 10.4 Hz, H-22), 6.54 (1H, d, J = 10.4 Hz, H-21)], an olefin [δ 5.40 (1H, br s, H-12)], and four glycopyranosyl moieties [δ 4.94 (1H, d, J = 7.4 Hz, H-1′), 5.01 (1H, d, J = 7.3 Hz, H-1‴), 5.72 (1H, d, J = 7.0 Hz, H-1″), 5.72 (1H, d, J = 7.0 Hz, H-1⁗)] together with two acyl moieties {an acetyl group [δ 1.92 (3H, s, H_3-2⁗′)] and an angeloyl group [δ 2.02 (3H, s, H_3-5⁗′), 2.10 (3H, d, J = 7.0 Hz, H_3-4⁗′), 5.98 (1H, dq-like, H-3⁗′)]}. The positions of the two acyl groups in **1** were clarified on the basis of a HMBC experiment. Thus, long-range correlations were observed between the 21-proton and the carbonyl carbon of the angeloyl moiety (δ_C 167.8, C-1⁗′) and between the 22-proton and the carbonyl carbon of the acetyl unit (δ_C 171.0, C-1⁗″). On the basis of the above-mentioned evidence, the structure of floratheasaponin A was determined to be 21-*O*-angeloyl-22-*O*-acetyltheasapogenol B 3-*O*-[β-D-galactopyranosyl(1→2)][β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**1**).

Floratheasaponin B (**2**) was also obtained as colorless fine crystals from $CHCl_3$−MeOH with mp 214−216 °C and a negative optical rotation ($[\alpha]^{26}_D$ −1.4°). The IR spectrum of **2** showed absorption bands at 3431, 1716, 1651, 1084, and 1043 cm^{-1}, ascribable to hydroxyl, carbonyl, α,β-unsaturated ester, and ether functions. The molecular formula, $C_{62}H_{96}O_{27}$, of **2** was determined from the positive-

Table 2. ^{13}C NMR (125 MHz, pyridine-d_5) Data of Floratheasaponins A (**1**), B (**2**), and C (**3**)

carbon	**1**	**2**	**3**	carbon	**1**	**2**	**3**
1	38.9	39.1	39.1	GlcA			
2	26.5	26.6	26.6	1′	105.6	105.6	105.6
3	89.7	89.6	89.6	2′	79.1	79.1	79.1
4	39.6	39.6	39.6	3′	84.1	84.1	84.0
5	55.9	55.6	55.7	4′	71.0	71.1	71.1
6	18.5	18.9	18.9	5′	77.2	77.2	77.2
7	33.2	36.7	36.7	6′	172.0	172.2	172.0
8	40.2	41.5	41.5	Gal			
9	47.0	47.2	47.2	1″	103.5	103.5	103.5
10	36.8	37.0	37.0	2″	73.7	73.7	73.8
11	23.9	24.0	24.0	3″	75.1	75.1	75.1
12	123.9	125.5	125.5	4″	70.1	70.1	70.1
13	142.9	143.7	143.7	5″	76.4	76.4	76.4
14	41.7	48.4	48.5	6″	62.0	62.0	62.0
15	34.7	73.8	73.1	Ara			
16	68.1	67.5	67.5	1‴	101.7	101.7	101.7
17	48.1	47.8	47.8	2‴	81.9	81.9	81.9
18	40.1	41.0	40.9	3‴	73.3	73.3	73.3
19	47.2	46.9	46.9	4‴	68.2	68.3	68.3
20	36.3	36.4	36.4	5‴	65.9	65.9	65.9
21	78.9	78.6	78.7	Xyl			
22	74.5	73.5	73.3	1⁗	106.8	106.8	106.8
23	28.1	28.1	28.1	2⁗	75.6	75.7	75.7
24	16.8	16.8	16.9	3⁗	78.2	78.2	78.2
25	15.7	15.8	15.8	4⁗	70.7	70.8	70.8
26	16.9	17.6	17.6	5⁗	67.4	67.4	67.4
27	27.5	21.2	21.2				
28	64.0	63.2	63.1				
29	29.5	29.5	29.5				
30	20.3	20.2	20.2				
21-*O*-Ang							
1⁗′	167.8	167.7	167.6				
2⁗′	129.1	129.0	128.7				
3⁗′	137.0	137.2	138.4				
4⁗′	15.9	15.9	16.0				
5⁗′	21.0	21.0	21.0				
22-*O*-Ac							
1⁗″	171.0						
2⁗″	20.9						
22-*O*-Ang							
1⁗″		168.2					
2⁗″		129.1					
3⁗″		136.6					
4⁗″		15.7					
5⁗″		20.6					
22-*O*-2MB							
1⁗″			176.7				
2⁗″			41.5				
3⁗″			26.9				
4⁗″			11.8				
5⁗″			16.6				

and negative-ion FABMS (*m/z* 1295 $[M + Na]^+$ and 1271 $[M - H]^-$) and by HRFABMS. Treatment of **2** with 10% aqueous KOH−50% aqueous 1,4-dioxane (1:1, v/v) liberated desacyl-floratheasaponin B (**2a**) and angelic acid, which were identified by HPLC analysis of its *p*-nitrobenzyl derivative.[5,6] Acid hydrolysis of **2a** with 5% aqueous H_2SO_4−1,4-dioxane (1:1, v/v) yielded R_1-barrigenol (**2b**),[19,20] as an aglycon, together with D-glucuronic acid, D-galactose, L-arabinose, and D-xylose, which were identified by GLC analysis of their trimethylsilyl thiazolidine derivatives.[5,6] The ^1H (pyridine-d_5) and ^{13}C NMR (Table 2) spectra[18] of **2** indicated the presence of the following functions: aglycon part {seven methyls [δ 0.86, 1.03, 1.11, 1.13, 1.27, 1.33, 1.83 (3H each, all s, H_3-25, 26, 29, 24, 23, 30, 27)], a methylene, and five methines bearing an oxygen function [δ 3.28 (1H, dd, J = 4.9, 11.5 Hz, H-3), 3.48, 3.74 (1H each, both d, J = 11.3 Hz, H_2-28), 4.20 (1H, m, H-16), 4.40 (1H, m, H-15), 6.27 (1H, d, J = 10.4 Hz, H-22), 6.67 (1H, d, J = 10.4 Hz, H-21)], an olefin [δ 5.52 (1H, br s, H-12)]}, four glycopyranosyl moieties [δ 4.92 (1H, d, J = 7.3 Hz, H-1′), 5.00 (1H, d, J = 7.6 Hz, H-1⁗), 5.70 (1H, d, J = 7.2 Hz, H-1″), 5.72 (1H, d, J = 7.2 Hz, H-1‴)]}, and two angeloyl moieties [δ 1.76, 2.01 (3H each, both s, H_3-5⁗′, 5⁗′), 1.97 (3H, d, J = 7.4 Hz, H_3-4⁗′), 2.09 (3H, d, J = 7.1 Hz, H_3-4⁗″), 5.81, 5.98 (1H each, both dq-like, H-3⁗′, 3⁗″)]. The oligoglycoside structure and positions of acyl groups in **2** were characterized by HMBC experiments. Accordingly, long-range correlations were observed between the following proton and carbon pairs (H-21 and C-1⁗′; H-22 and C-1⁗″; H-1′ and C-3; H-1″ and C-2′; H-1‴ and C-3′; H-1⁗ and C-2‴). Furthermore, comparison of the ^{13}C NMR data for **2** with those for **2a** revealed two acylation shifts around the 21- and 22-positions of the R_1-barrigenol moiety. Consequently, the structure of floratheasaponin B was determined to be 21,22-di-*O*-angeloyl-R_1-barrigenol 3-*O*-[β-D-galactopyranosyl(1→2)][β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**2**).

Floratheasaponin C (**3**), $[\alpha]^{25}_D$ +4.6° (MeOH), was also obtained as colorless fine crystals from $CHCl_3$−MeOH with mp 220−222 °C. The positive- and negative-ion FABMS of **3** showed quasimolecular and fragment ion peaks at *m/z* 1297 $[M + Na]^+$, 1273 $[M - H]^-$, 1141 $[M - C_5H_9O_4]^-$, 1111 $[M - C_6H_{11}O_5]^-$, and 1009 $[M - C_{10}H_{17}O_8]^-$. The HR-FABMS revealed the molecular formula of **3** to be $C_{62}H_{98}O_{27}$, and the IR spectrum showed absorption bands at 3453, 1717, 1653, 1078, and 1048 cm^{-1}, ascribable to hydroxyl, carbonyl, α,β-unsaturated ester, and ether functions. Alkaline hydrolysis of **3** with 10% aqueous KOH−50% aqueous 1,4-dioxane (1:1, v/v) liberated **2a** and two organic acids, angelic acid and 2-methylbutylic acid, which were identified by HPLC analysis of its *p*-nitrobenzyl derivative.[5,6] The proton and carbon signals in the ^1H (pyridine-d_5) and ^{13}C NMR (Table 2) spectra[18] of **3** were superimposable on those of **2**, except for the signals due to the 22-acyl moiety {seven methyls [δ 0.86, 1.03, 1.10, 1.13, 1.28, 1.31, 1.83 (3H each, all s, H_3-25, 26, 29, 24, 23, 30, 27)], a methylene and five methines bearing an oxygen function [δ 3.30 (1H, dd, J = 4.3, 11.0 Hz, H-3), 3.47, 3.75 (1H each, both d, J = 10.7 Hz, H_2-28), 4.19 (1H, m, H-16), 4.38 (1H, m, H-15), 6.20 (1H, d, J = 10.0 Hz, H-22), 6.62 (1H, d, J = 10.0 Hz, H-21)], an olefin [δ 5.51 (1H, br s, H-12)]}, and four glycopyranosyl moieties [δ 4.93 (1H, d, J = 7.3 Hz, H-1′), 5.01 (1H, d, J = 7.3 Hz, H-1⁗′), 5.70 (1H, d, J = 7.1 Hz, H-1″), 5.71 (1H, d, J = 7.1 Hz, H-1‴)] together with an angeloyl unit [δ 2.04 (3H, s, H_3-5⁗″), 2.16 (3H, d, J = 6.7 Hz, H_3-4⁗″), 6.07 (1H, dq-like, H-3⁗″)] and 2-methylbutyryl moieties [δ 0.70 (3H, t, J = 7.4 Hz, H_3-4⁗‴), 1.02 (3H, d, J = 6.7 Hz, H_3-5⁗‴), 1.25, 1.60 (1H each, both m, H_2-3⁗‴), 2.10 (1H, m, H-2⁗‴)]. Finally, the positions of two acyl groups in **3** were characterized by the HMBC experiments, in which long-range correlations were observed between H-21 and C-1⁗″ and H-22 and C-1⁗‴. Consequently, the structure of floratheasaponin C was elucidated as 21-*O*-angeloyl-22-*O*-2-methylbutyryl-R_1-barrigenol 3-*O*-[β-D-galactopyranosyl(1→2)][β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**3**).

The effects of the floratheasaponins A−C (**1**−**3**) on serum TG elevation in olive oil-treated mice were examined. As shown in Tables 1 and 3, a reference compound, clofibrate, which reduces TG through peroxisome proliferator-activated receptor-α (PPARα)-mediated stimulation of fatty acid oxidation, increased lipoprotein lipase synthesis and reduced expression of apolipoprotein C-III as a mechanism of action[21] and showed a weak effect, and a potent lipase inhibitor, orlistat,[22] showed a potent effect in this assay model. Floratheasaponins A−C (**1**−**3**) significantly suppressed the increase in serum TG levels 2 h after admin-

Table 3. Inhibitory Effects of Floratheasaponins A (**1**), B (**2**), and C (**3**), Theasaponins E_1 (**4**) and E_2 (**5**), and Desacyl-theasaponin E (**4a**) on Serum Triglyceride Elevation in Olive Oil-Treated Mice

			serum triglyceride (mg/dL)[a,b]		
treatment	dose (mg/kg, p.o.)	*N*	2.0 h	4.0 h	6.0 h
normal		9	140.8 ± 5.9**	92.1 ± 3.9**	83.6 ± 4.2
control		9	566.6 ± 22.2	325.9 ± 31.4	180.8 ± 9.8
floratheasaponin A (**1**)	25	6	411.0 ± 34.3	251.8 ± 65.8	135.7 ± 32.0
	50	6	387.4 ± 74.7*	392.9 ± 27.3	256.8 ± 38.5
	100	6	158.3 ± 31.9**	242.0 ± 58.4	322.3 ± 54.1**
floratheasaponin B (**2**)	25	6	411.7 ± 50.6	256.5 ± 47.1	164.7 ± 26.9
	50	6	316.9 ± 63.0**	415.6 ± 36.9	254.3 ± 20.3
	100	6	161.9 ± 11.9**	195.0 ± 23.2	267.2 ± 30.1
floratheasaponin C (**3**)	25	6	348.5 ± 83.7**	260.4 ± 57.8	188.4 ± 28.3
	50	6	204.1 ± 40.3**	238.6 ± 24.2	204.6 ± 23.4
	100	6	143.1 ± 11.3**	144.8 ± 25.3**	244.0 ± 51.7
normal		5	178.8 ± 16.9**	132.9 ± 9.8*	103.1 ± 5.6*
control		6	575.5 ± 26.4	368.6 ± 22.6	243.9 ± 25.8
theasaponin E_1 (**4**)	25	7	578.5 ± 78.4	430.7 ± 78.1	233.9 ± 23.9
	50	7	373.9 ± 72.9	312.2 ± 46.4	267.2 ± 43.6
	100	7	342.3 ± 56.5*	389.5 ± 56.9	294.9 ± 39.4
normal		6	146.6 ± 15.3**	143.2 ± 9.6*	145.8 ± 12.0
control		5	506.7 ± 39.5	422.8 ± 35.5	331.6 ± 43.8
theasaponin E_2 (**5**)	50	7	552.9 ± 44.8	507.5 ± 82.0	361.3 ± 58.2
	100	7	509.4 ± 82.9	507.0 ± 59.1	396.4 ± 55.1
normal		6	122.6 ± 17.4**	127.1 ± 21.3**	141.8 ± 22.4
control		6	414.4 ± 34.2	311.8 ± 42.1	247.0 ± 30.9
desacyl-theasaponin E (**4a**)	50	7	468.7 ± 53.4	410.5 ± 44.6	332.3 ± 40.5
	100	7	440.8 ± 60.6	363.2 ± 40.1	314.3 ± 35.8
normal		10	154.3 ± 9.3**	138.0 ± 9.8**	138.1 ± 12.3**
control		10	387.1 ± 39.2	320.4 ± 61.3	276.5 ± 35.1
orlistat	6.25	10	266.4 ± 31.1*	179.3 ± 17.2*	155.6 ± 13.2**
	12.5	10	187.9 ± 25.5**	176.0 ± 29.5**	189.7 ± 28.8*
	25	10	158.9 ± 28.7**	132.2 ± 10.5**	140.1 ± 13.7**

[a] Values represent the means ± SEM. [b] Significantly different from the control group, $*p < 0.05$, $**p < 0.01$.

istration of olive oil at doses of 25−100 mg/kg, p.o. (Table 3). Compound **3** was the most potent, and the activities of **1**−**3** were greater than those of theasaponins E_1 (**4**) and E_2 (**5**) and their desacyl derivative, desacyl-theasaponin E (**4a**), which were isolated from the seed of this plant material.[4,23] The difference between **1** and **4** is a methyl group instead of an aldehyde group at the 23-position. These findings suggest that the 21,22-acyl groups are essential and the 23-aldehyde group is not preferable for activity.

The methanolic extract, BuOH-soluble fraction, and floratheasaponins A−C (**1**−**3**) inhibited the increase in serum TG levels at 2 h, but the levels increase later. In our previous study, gastric-emptying inhibitory activity of a saponin mixture and theasaponin E_1 (**4**) from *C. sinensis* var. *assamica* was reported.[6] The present results suggested that **1**−**3** might have the same effect as **4** as their mechanism of action. Recently, Han et al. reported that the theasaponin mixture including **4** and **5** showed an anti-obese effect in high-fat diet-treated mice (in vivo) and pancreatic lipase inhibitory activity (in vitro).[24,25] Therefore, **1**−**3** may inhibit pancreatic lipase activity. Although other mechanisms of action including anti-obese effect of **1**−**3** in high-fat diet-treated mice should be studied further, all of the evidence in the present study suggests that **1**−**3** may be more useful for the prevention of obesity than **4** and **5**.

Experimental Section

General Experimental Procedures. The following instruments were used to obtain 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 NMR spectra, JEOL JNM-LA500 (500 MHz) spectrometer; ^{13}C NMR spectra, JEOL JNM-LA500 (125 MHz) spectrometer with tetramethylsilane as an internal standard; FABMS and HRFABMS, JEOL JMS-SX 102A mass spectrometer; HPLC detector, Shimadzu RID-6A refractive index and SPD-10A UV−vis detectors; HPLC column, YMC-Pack ODS-A (250 × 4.6 mm i.d.) and (250 × 20 mm i.d.) columns for analytical and preparative purposes, respectively.

The following experimental conditions were used for chromatography: normal-phase silica gel column chromatography, silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150−350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100−200 mesh); TLC, precoated TLC plates with silica gel 60F_{254} (Merck, 0.25 mm) (normal-phase) and silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed-phase); reversed-phase HPTLC, precoated 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.

Plant Material. The flowers of *Camellia sinensis* were collected at a tea plantation in Shiga Prefecture, Japan, in January 2003, and identified by one of the authors (M.Y.). A voucher of the plant is on file in our laboratory (2003.01. Japan-01).

Extraction and Isolation. The dried flowers of *C. sinensis* (1.1 kg) were cut finely and extracted three times with methanol (10 L) under reflux for 3 h. Evaporation of the solvent under reduced pressure provided a methanolic extract (375 g, 34.1% from the flowers), and an aliquot (300 g) was partitioned into an EtOAc−H_2O (1:1, v/v) mixture to furnish an EtOAc-soluble fraction (43.7 g, 5.0%) and an aqueous phase. The aqueous phase was further extracted with BuOH to give a BuOH-soluble fraction (139.2 g, 15.8%) and an H_2O-soluble fraction (117.1 g, 13.3%). The BuOH-soluble fraction (95.2 g) was subjected to normal-phase silica gel column chromatography [3.0 kg, $CHCl_3$−MeOH−H_2O (10:3:1, lower layer → 7:3:1, lower layer → 6:4:1, v/v/v) → MeOH] to give 10 fractions [fractions 1 (0.45 g), 2 (6.38 g), 3 (0.48 g), 4 (3.85 g), 5 (9.82 g), 6 (35.20 g), 7 (5.40 g), 8 (6.25 g), 9 (9.24 g), and 10 (14.80 g)]. Fraction 5 (9.82 g) was subjected to reversed-phase silica gel column chromatography [240 g, MeOH−H_2O (20:80 → 30:70 → 50:50 → 70:30, v/v) → MeOH] to afford eight fractions

[fractions 5-1 (1.44 g), 5-2 (1.29 g), 5-3 (1.05 g), 5-4 (0.83 g), 5-5 (0.40 g), 5-6 (0.33 g), 5-7 (0.31 g), and 5-8 (1.40 g)]. Fraction 5-2 (500 mg) was purified by HPLC [MeOH−H_2O (25:75, v/v)] to furnish (−)-epicatechin (15 mg, 0.0062%). Fraction 5-4 (500 mg) was further separated by HPLC [MeOH−H_2O (40:60, v/v)] to afford kaempferol 3-*O*-*β*-D-galactopyranoside (63 mg, 0.018%) and quercetin 3-*O*-*β*-D-galactopyranoside (66 mg, 0.018%). Fraction 6 (35.2 g) was subjected to reversed-phase silica gel column chromatography [700 g, MeOH−H_2O (50:50 → 70:30, v/v) → MeOH] to afford six fractions [fractions 6-1 (2.56 g), 6-2 (0.23 g), 6-3 (2.16 g), 6-4 (0.40 g), 6-5 (12.00 g), and 6-6 (1.23 g)]. Fraction 6-1 (500 mg) was purified by HPLC [MeOH−H_2O (35:65, v/v)] to furnish (−)-epicatechin 3-*O*-gallate (54 mg, 0.045%). Fraction 6-2 (500 mg) was further separated by HPLC [MeOH−H_2O (40:60, v/v)] to furnish myricetin 3-*O*-*β*-D-galactopyranoside (17 mg, 0.0030%). Fraction 6-3 (500 mg) was purified by HPLC [MeOH−H_2O (40:60, v/v)] to give kaempferol 3-*O*-*β*-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)-*β*-D-galactopyranoside (160 mg, 0.12%), kaempferol 3-*O*-*β*-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)-*β*-D-glucopyranoside (173 mg, 0.13%), and rutin (15 mg, 0.011%). Fraction 6-5 (1.0 g) was further separated by HPLC [MeOH−1% aqueous acetic acid (25:75, v/v)] to furnish floratheasaponins A (**1**, 171 mg, 0.34%), B (**2**, 211 mg, 0.42%), and C (**3**, 121 mg, 0.24%). Fraction 8 (6.25 g) was further separated by reversed-phase silica gel column chromatography [200 g, MeOH−H_2O (30:70 → 40:60 → 60:40, v/v) → MeOH] to afford 13 fractions [fractions 8-1 (1.18 g), 8-2 (0.46 g), 8-3 (0.61 g), 8-4 (0.15 g), 8-5 (0.98 g), 8-6 (0.57 g), 8-7 (0.18 g), 8-8 (0.26 g), 8-9 (0.42 g), 8-10 (1.18 g), 8-11 (0.15 g), 8-12 (1.48 g), and 8-13 (0.08 g)]. Fraction 8-3 (500 mg) was purified by HPLC [MeOH−H_2O (40:60, v/v)] to give (−)-epigallocatechin 3-*O*-gallate (113 mg, 0.026%). The known compounds were identified by comparison of their physical data ($[\alpha]_D$, IR, ^1H NMR, ^{13}C NMR, MS] with reported values,[1,12−14,16,17] and rutin and (−)-epicatechin were identified by comparison of their physical data with those of commercially obtained samples.[15]

Floratheasaponin A (1): colorless fine crystals from $CHCl_3$−MeOH, mp 201−203 °C; $[\alpha]^{26}_D$ −3.3° (*c* 0.50, MeOH); IR (KBr) ν_{max} 3505, 1719, 1655, 1078, 1048 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) δ 0.84, 0.87, 1.09, 1.13, 1.82 (3H each, all s, H_3-25, 26, 29, 24, 27), 1.30 (6H, s, H_3-23, 30), 1.92 (3H, s, H_3-2‴″), 2.02 (3H, s, H_3-5‴″), 2.10 (3H, d, J = 7.0 Hz, H_3-4‴″), 3.31 (1H, dd, J = 4.3, 11.6 Hz, H-3), 3.37, 3.60 (1H each, both d, J = 10.7 Hz, H_2-28), 4.44 (1H, m, H-16), 4.94 (1H, d, J = 7.4 Hz, H-1′), 5.01 (1H, d, J = 7.3 Hz, H-1‴), 5.40 (1H, br s, H-12), 5.72 (1H, d, J = 7.0 Hz, H-1″), 5.72 (1H, d, J = 7.0 Hz, H-1‴), 5.98 (1H, dq-like, H-3‴″), 6.15 (1H, d, J = 10.4 Hz, H-22), 6.54 (1H, d, J = 10.4 Hz, H-21); ^{13}C NMR data, see Table 2; positive-ion FABMS *m/z* 1239 $[M + Na]^+$; negative-ion FABMS *m/z* 1215 $[M - H]^-$, 1083 $[M - C_5H_9O_4]^-$, 951 $[M - C_{10}H_{17}O_8]^-$, 921 $[M - C_{11}H_{19}O_9]^-$; HRFABMS *m/z* 1239.5764 (calcd for $C_{59}H_{92}O_{26}Na$ $[M + Na]^+$, 1239.5774).

Floratheasaponin B (2): colorless fine crystals from $CHCl_3$−MeOH, mp 214−216 °C; $[\alpha]^{26}_D$ −1.4° (*c* 0.50, MeOH); IR (KBr) ν_{max} 3431, 1716, 1651, 1084, 1043 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) δ 0.86, 1.03, 1.11, 1.13, 1.27, 1.33, 1.83 (3H each, all s, H_3-25, 26, 29, 24, 23, 30, 27), 1.76, 2.01 (3H each, both s, H_3-5‴″, 5‴″), 1.97 (3H, d, J = 7.4 Hz, H_3-4‴″), 2.09 (3H, d, J = 7.1 Hz, H_3-4‴″), 3.28 (1H, dd, J = 4.9, 11.5 Hz, H-3), 3.48, 3.74 (1H each, both d, J = 11.3 Hz, H_2-28), 4.20 (1H, m, H-16), 4.40 (1H, m, H-15), 4.92 (1H, d, J = 7.3 Hz, H-1′), 5.00 (1H, d, J = 7.6 Hz, H-1‴), 5.52 (1H, br s, H-12), 5.70 (1H, d, J = 7.2 Hz, H-1″), 5.72 (1H, d, J = 7.2 Hz, H-1‴), 5.81, 5.98 (1H each, both dq-like, H-3‴″, 3‴″), 6.27 (1H, d, J = 10.4 Hz, H-22), 6.67 (1H, d, J = 10.4 Hz, H-21); ^{13}C NMR data, see Table 2; positive-ion FABMS *m/z* 1295 $[M + Na]^+$; negative-ion FABMS *m/z* 1271 $[M - H]^-$, 1109 $[M - C_6H_{11}O_5]^-$; HRFABMS *m/z* 1295.6029 (calcd for $C_{62}H_{96}O_{27}Na$ $[M + Na]^+$, 1295.6037).

Floratheasaponin C (3): colorless fine crystals from $CHCl_3$−MeOH, mp 220−222 °C; $[\alpha]^{25}_D$ +4.6° (*c* 0.50, MeOH); IR (KBr) ν_{max} 3453, 1717, 1653, 1078, 1048 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) δ 0.70 (3H, t, J = 7.4 Hz, H_3-4‴″), 0.86, 1.03, 1.10, 1.13, 1.28, 1.31, 1.83 (3H each, all s, H_3-25, 26, 29, 24, 23, 30, 27), 1.25, 1.60 (1H each, both m, H_2-3‴″), 2.04 (3H, s, H_3-5‴″), 2.10 (1H, m, H-2‴″), 2.16 (3H, d, J = 6.7 Hz, H_3-4‴″), 3.30 (1H, dd, J = 4.3, 11.0 Hz, H-3), 3.47, 3.75 (1H each, both d, J = 10.7 Hz, H_2-28), 4.19 (1H, m, H-16), 4.38 (1H, m, H-15), 4.93 (1H, d, J = 7.3 Hz, H-1′), 5.01 (1H, d, J = 7.3 Hz, H-1‴), 5.51 (1H, br s, H-12), 5.70 (1H, d, J = 7.1 Hz, H-1″), 5.71 (1H, d, J = 7.1 Hz, H-1‴), 6.07 (1H, dq-like, H-3‴″), 6.20 (1H, d, J = 10.0 Hz, H-22), 6.62 (1H, d, J = 10.0 Hz, H-21); ^{13}C NMR data, see Table 2; positive-ion FABMS *m/z* 1297 $[M + Na]^+$; negative-ion FABMS *m/z* 1273 $[M - H]^-$, 1141 $[M - C_5H_9O_4]^-$, 1111 $[M - C_6H_{11}O_5]^-$, 1009 $[M - C_{10}H_{17}O_8]^-$; HRFABMS *m/z* 1297.6187 (calcd for $C_{62}H_{98}O_{27}Na$ $[M + Na]^+$, 1297.6194).

Alkaline Hydrolysis of Floratheaponins A (1), B (2), and C (3). A solution of each floratheaponin (**1**, 10 mg; **2**, 20 mg; **3**, 10 mg) in 50% aqueous 1,4-dioxane (2.0 mL) was treated with 10% aqueous KOH (2.0 mL), and the whole was stirred at 37 °C for 1 h. Removal of the solvent under reduced pressure gave a reaction mixture. A part of the reaction mixture was dissolved in $(CH_2)_2Cl_2$ (2 mL), and the solution was treated with *p*-nitrobenzyl-*N*-*N*′-diisopyopylisourea (10 mg). Then the whole was stirred at 80 °C for 1 h. The reaction solution was subjected to HPLC analysis [column: YMC-Pack ODS-A, 250 × 4.6 mm i.d.; mobile phase: CH_3CN-H_2O (50:50, v/v); detection: UV (254 nm); flow rate: 1.0 mL/min] to identify the *p*-nitrobenzyl esters of acetic acid (**a**, t_R 9.6 min) from **1**, angelic acid (**b**, t_R 30.6 min) from **1**−**3**, and 2-methylbutyric acid (**c**, t_R 32.1 min) from **3**. The rest of each 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 yielded a product, which was subjected to ordinary-phase silica gel column chromatography [2.0 g, $CHCl_3$−MeOH−H_2O (7:3:1, v/v/v, lower layer)] to give desacyl-assamsaponin E[5] (**1a**, 6 mg, from **1**) and **2a** (16 mg, from **2**; 6 mg from **3**).

Compound 2a: colorless fine crystals from $CHCl_3$−MeOH, mp 218−220 °C; $[\alpha]^{25}_D$ −19.4° (*c* 0.12, MeOH); IR (KBr) ν_{max} 3432, 1725, 1607, 1078, 1046 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) δ 0.87, 1.04, 1.14, 1.26, 1.32, 1.34, 1.83 (3H each, all s, $-CH_3$), 3.29 (1H, dd-like, H-3), 4.93 (1H, d, J = 7.2 Hz, H-1′), 5.01 (1H, d, J = 7.2 Hz, H-1‴), 5.46 (1H, br s, H-12), 5.71 (2H, br d, J = ca. 7 Hz, H-1″ and 1‴); ^{13}C NMR (pyridine-d_5, 125 MHz) δ_C 39.1 (C-1), 26.6 (C-2), 89.6 (C-3), 39.6 (C-4), 55.7 (C-5), 18.9 (C-6), 36.7 (C-7), 41.5 (C-8), 47.3 (C-9), 37.1 (C-10), 24.0 (C-11), 124.5 (C-12), 144.7 (C-13), 47.4 (C-14), 72.6 (C-15), 67.4 (C-16), 48.1 (C-17), 42.0 (C-18), 47.9 (C-19), 36.4 (C-20), 78.4 (C-21), 77.4 (C-22), 28.1 (C-23), 16.9 (C-24), 15.9 (C-25), 17.6 (C-26), 21.7 (C-28), 30.5 (C-29), 19.3 (C-30), 105.5 (C-1′), 79.2 (C-2′), 83.8 (C-3′), 70.9 (C-4′), 76.8 (C-5′), 172.1 (C-6′), 103.5 (C-1″), 73.8 (C-2″), 75.2 (C-3″), 70.2 (C-4″), 76.4 (C-5″), 62.0 (C-6″), 101.6 (C-1‴), 81.8 (C-2‴), 73.3 (C-3‴), 68.3 (C-4‴), 65.2 (C-5‴), 106.7 (C-1‴″), 75.6 (C-2‴″), 78.2 (C-3‴″), 70.8 (C-4‴″), 67.4 (C-5‴″); positive-ion FABMS *m/z* 1131 $[M + Na]^+$; negative-ion FABMS *m/z* 1107 $[M - H]^-$, 975 $[M - C_5H_9O_4]^-$, 843 $[M - C_{10}H_{17}O_8]^-$; HRFABMS *m/z* 1131.5205 (calcd for $C_{52}H_{84}O_{25}Na$ $[M + Na]^+$, 1131.5199).

Acid Hydrolysis of 2a. A solution of **2a** (10 mg) in 5% aqueous H_2SO_4−1,4-dioxane (1:1, v/v, 2 mL) was heated under refux for 1 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and the resin was filtered. On removal of the solvent from the filtrate under reduced pressure, the residue was passed through a Sep-Pack C18 cartridge by elution with H_2O and then MeOH. The H_2O eluate was concentrated and the residue was treated with L-cysteine methyl ester hydrochloride (0.01 mL) in pyridine (0.02 mL) at 60 °C for 1 h. After this reaction, the solution was treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (0.01 mL) at 60 °C for 1 h. The supernatant was then subjected to GLC analysis [column: Supelco STB-1, 0.25 mm i.d. × 30 m; column temperature: 230 °C; detector temperature: 230 °C; injector temperature: 230 °C; He gas flow rate: 15 mL/min] to identify the derivatives of D-glucuronic acid (i, t_R 26.5 min), D-galactose (ii, t_R 25.6 min), L-arabinose (iii, t_R 15.1 min), and D-xylose (iv, t_R 19.3 min). The MeOH eluate was purified by normal-phase silica gel column chromatography [200 mg,

$CHCl_3$–MeOH–H_2O (10:3:1, lower layer, v/v/v)] to give R_1-barrigenol[19,20] (**2b**, 3 mg).

Bioassay Method. Male ddY mice weighing about 25–30 g were purchased from Kiwa Laboratory Animal Co., Ltd., Wakayama, Japan. The animals were housed at a constant temperature of 23 ± 2 °C and were fed a standard laboratory chow (MF, Oriental Yeast Co., Ltd., Tokyo, Japan). The animals were fasted for 24–26 h prior to the beginning of the experiment, but were allowed free access to tap water. All of the experiments were performed with conscious mice unless otherwise noted. The experimental protocol was approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

Inhibitory Effect on Serum TG Elevation in Olive Oil-Treated Mice. Each test sample was administered orally to fasted mice, and olive oil (5 mL/kg) was administered p.o. 30 min thereafter. Blood was collected from the infraorbital venous plexus 2, 4, and 6 h after olive oil treatment. Serum TG was determined by enzymatic method using a triglyceride E test (Wako Pure Chemical Industries Ltd., Osaka, Japan).[26]

Statistics. Values were expressed as means ± SEM. One-way analysis of variance followed by Dunnett's test was used for statistical analysis.

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Supporting Information Available: Structures of known constituents from the flowers of the tea plant (*C. sinensis*). This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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