Medicinal Flowers. XIV.[1] New Acylated Oleanane-Type Triterpene Oligoglycosides with Antiallergic Activity from Flower Buds of Chinese Tea Plant (*Camellia sinensis*)

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The methanolic extract from the flower buds of Chinese tea plant (*Camellia sinensis* (L.) O. KUNTZE) was found to inhibit release of β-hexosaminidase from RBL-2H3 cells. From the methanolic extract, six new acylated oleanane-type triterpene oligoglycosides, floratheasaponins D—I, were isolated together with 21 known compounds including floratheasaponins A—C. The chemical structures of floratheasaponins D—I were elucidated on the basis of chemical and physicochemical evidence. The principal constituents, floratheasaponins A—F, were found to show the inhibitory activity on the release of β-hexosaminidase from RBL-2H3 cells.

Key words floratheasaponin; *Camellia sinensis*; oleanane-type triterpene oligoglycoside; tea plant flower; degranulation inhibitor; medicinal flower

In the course of our characterization studies on the bioactive saponin constituents from *Camellia* (*C.*) *sinensis* (Theaceae), we have reported the isolation and structure elucidation of theasaponins A_1—A_5, C_1, E_1—E_{13}, F_1—F_3, G_1, G_2, and H_1,[2—6] assamsaponins A—I,[7,8] and camelliasaponins B_1 and C_1[9] from the seeds of Japanese *C. sinensis* (L.) O. KUNTZE and Sri Lankan *C. sinensis* L. var. *assamica* PERRE, and foliatheasaponins I—V[10] from the leaves of Japanese *C. sinensis*. Among the saponin constituents, theasaponin E_1 showed antisweet activity,[2] while theasaponins A_2, E_1, E_2, and E_5 and assamsaponins A, C, and D were found to exhibit potent inhibitory effects on gastric lesions in rats.[4,5] In addition, floratheasaponins A—C (**1**—**3**) were isolated from the flower buds of Japanese *C. sinensis* and these acylated polyhydroxyoleanane-type triterpene oligoglycosides showed inhibitory effects on serum triglyceride elevation in olive oil-treated mice.[11] As a continuation of our studies on bioactive constituents of medicinal flowers,[1,11—13] the methanolic extract of the flower buds of Chinese *C. sinensis* was found to inhibit an immediate allergic reaction by monitoring the release of β-hexosaminidase from rat basophilic leukemia (RBL-2H3) cells. From the methanolic extract, we have isolated six new acylated oleanane-type triterpene oligoglycosides, floratheasaponins D (**4**), E (**5**), F (**6**), G (**7**), H (**8**), and I (**9**), together with 21 known compounds including floratheasaponins A—C (**1**—**3**). Furthermore, we examined the inhibitory effects of principal floratheasaponins (**1**—**6**) on the release of β-hexosaminidase from RBL-2H3 cells. In this paper, we describe the isolation and structure elucidation of the new constituents (**4**—**9**) and the inhibitory effect of major floratheasaponins (**1**—**6**) from the flower buds of Chinese *C. sinensis* on the release of β-hexosaminidase from RBL-2H3 cells.[14]

The methanolic extract (38.5% from the dried flower buds of *C. sinensis* cultivated in Anhui province of China) with the inhibitory effect on the release of β-hexosaminidase from

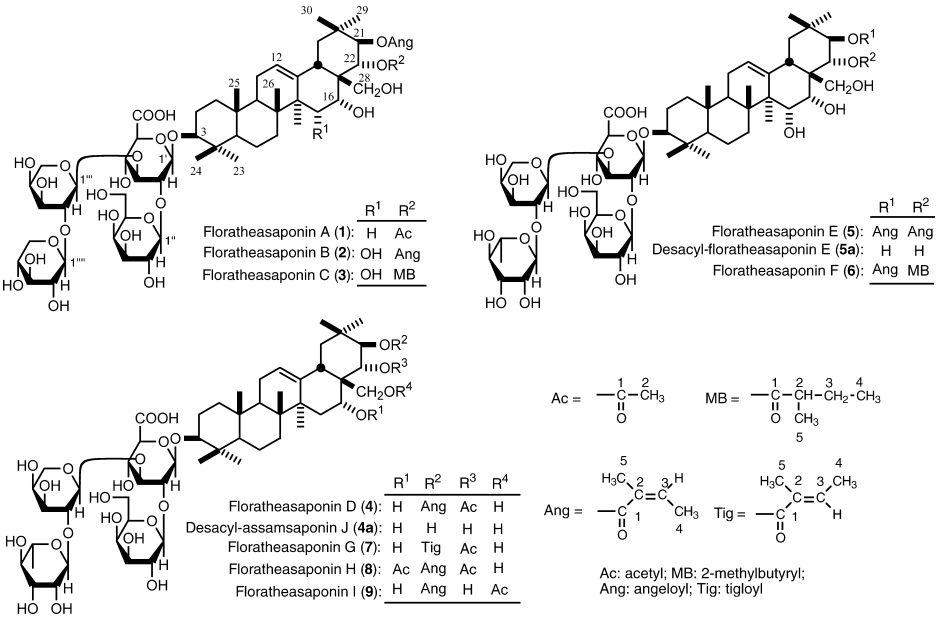

Chart 1

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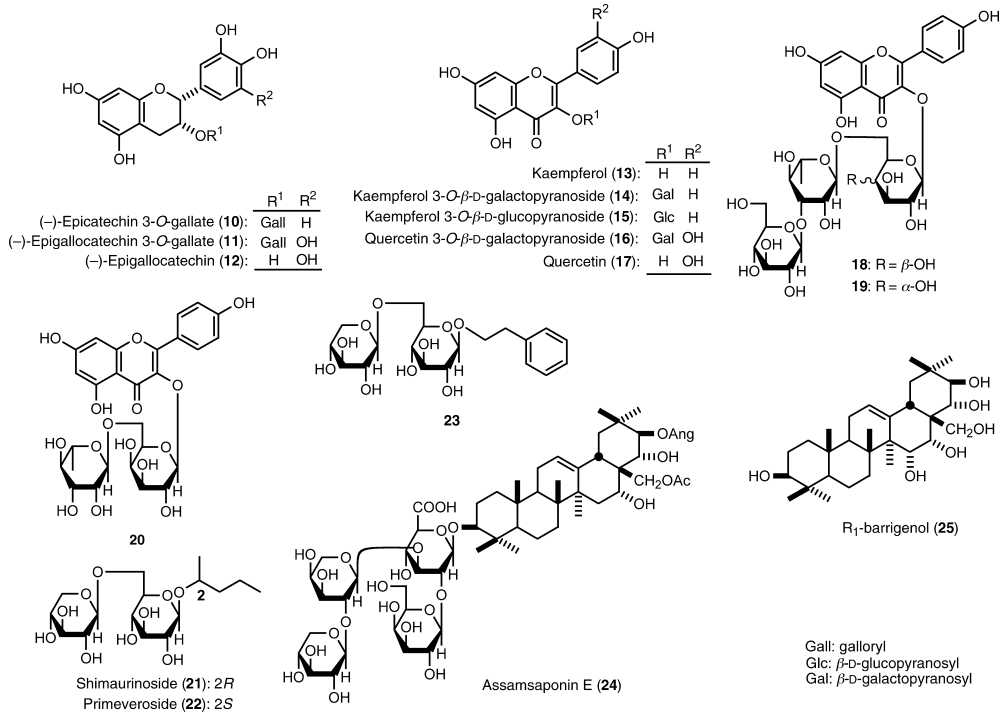

Chart 2

RBL-2H3 cells [inhibition (%): 43.5±2.4 (p<0.01) at 100 μg/ml] was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction (7.7%) and aqueous layer. The aqueous layer was further extracted with *n*-butanol (*n*-BuOH) to give *n*-BuOH- (12.8%) and H_2O- (18.1%) soluble fractions. The EtOAc- and *n*-BuOH-soluble fractions inhibited antigen-induced degranulation in RBL-2H3 cells [inhibition (%): 18.1±4.0 (p<0.05) at 100 μg/ml and 60.1±2.9 (p<0.01) at 30 μg/ml, respectively], but the H_2O-soluble fractions showed no activity [inhibition (%): −2.9±3.6]. The EtOAc-soluble fraction was subjected to normal-phase and reversed-phase column chromatographies, and finally HPLC to give 11 known compounds, (−)-epicatechin 3-*O*-gallate (**10**, 0.35%),[11,15,16] (−)-epigallocatechin 3-*O*-gallate (**11**, 0.16%),[16] (−)-epigallocatechin (**12**, 0.026%),[11,15,16] kaempferol (**13**, 0.17%),[17] kaempferol 3-*O*-β-D-galactopyranoside (**14**, 0.091%),[18,19] kaempferol 3-*O*-β-D-glucopyranoside (**15**, 0.011%),[17] quercetin 3-*O*-β-D-galactopyranoside (**16**, 0.023%),[19,20] quercetin (**17**, 0.0020%),[21] caffeine (0.76%),[15] gallic acid (0.33%),[15] and *trans*-cinnamic acid (0.0029%).[15] The *n*-BuOH-soluble fraction was also subjected to normal-phase and reversed-phase silica gel column chromatographies and repeated HPLC to give floratheasaponins D (**4**, 0.12%), E (**5**, 0.25%), F (**6**, 0.13%), G (**7**, 0.053%), H (**8**, 0.065%), and I (**9**, 0.0018%) together with 14 known compounds, floratheasaponins A (**1**, 0.013%),[11] B (**2**, 0.11%),[11] and C (**3**, 0.054%),[11] kaempferol 3-*O*-β-D-galactopyranoside (**14**, 0.074%),[18,19] kaempferol 3-*O*-β-D-glucopyranoside (**15**, 0.0064%),[17] quercetin 3-*O*-β-D-galactopyranoside (**16**, 0.024%),[19,20] kaempferol 3-*O*-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)-β-D-galactopyranoside (**18**, 0.38%),[22] kaempferol 3-*O*-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside (**19**, 0.36%),[22] kaempferol 3-*O*-α-L-rhamnopyranosyl(1→6)-β-D-galactopyranoside (**20**, 0.36%),[17] shimaurinoside (**21**, 0.058%),[23] primeveroside (**22**, 0.011%),[24] phenetyl alcohol β-D-xylopyranosyl(1→6)-glucopyranoside (**23**, 0.032%),[25] assamsaponin E (**24**, 0.015%),[7,8] and caffeine (0.36%).[15]

Structures of Floratheasaponins Floratheasaponin D (**4**) was isolated as colorless fine crystals of mp 203.0—206.0 °C (from $CHCl_3$–MeOH) with negative optical rotation ($[\alpha]_D^{21}$ −12.8° in MeOH). The IR spectrum of **4** showed absorption bands at 3475, 1735, 1719, 1654, and 1048 cm^{-1} due to hydroxyl, α,β-unsaturated ester, carboxyl, olefin, and ether functions. In the negative-ion FAB-MS of **4**, a quasi-molecular ion peak was observed at *m*/*z* 1229 $(M-H)^-$ in addition to a fragment ion peak at *m*/*z* 1067 $(M-H-C_6H_{10}O_5)^-$, whereas the positive ion FAB-MS showed quasi-molecular ion peaks at *m*/*z* 1253 $(M+Na)^+$ and *m*/*z* 1275 $(M+2Na-H)^+$. The molecular formula $C_{60}H_{94}O_{26}$ of **4** was determined by high-resolution MS measurement of the quasi-molecular ion peak $(M+Na)^+$. Treatment of **4** with 10% aqueous KOH–50% aqueous 1,4-dioxane (1 : 1) liberated a known compound, desacyl-assamsaponin J (**4a**),[8] and two organic acids, acetic acid and angelic acid, which were identified by HPLC analysis of their *p*-nitrobenzyl derivatives.[7,8] Acid hydrolysis of **4a** with 5% aqueous sulfuric acid (H_2SO_4)–1,4-dioxane (1 : 1, v/v) furnished L-arabinose, D-galactose, D-glucuronic acid, and L-rhamnose, whose absolute configurations (D- or L-) were identified by GLC analysis of their thiazolidine derivatives.[26—28] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **4**, which were assigned by various NMR experiments,[29] showed signals assignable to a theasapogenol B part: seven methyls [δ 0.83, 0.87, 1.09, 1.14, 1.29, 1.31, 1.82 (3H each, all s, H_3-25, 26, 29, 24, 23, 30, 27)], a methylene and four methines bearing an oxygen function [δ 3.32 (1H, dd-like, H-3), 3.37, 3.60 (1H each, both d, J=10.7 Hz, H_2-28), 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 glycopyra-

Table 1. ^{13}C-NMR (125 MHz) Data for **4**, **5**, **6**, **7**, **8**, and **9** (Measured in Pyridine-d_5)

Ccarbon	4	5	6	7	8	9	5a	Carbon	4	5	6	7	8	9	5a
1	38.9	39.1	39.1	38.9	38.8	38.9	39.1	22-*O*-Ac-1	171.0			171.1	170.5		
2	26.5	26.5	26.6	26.5	26.5	26.5	26.6	2	20.9			20.9	20.8		
3	89.7	89.8	89.8	89.9	89.8	89.9	89.8	22-*O*-Ang-1		168.2					
4	39.7	39.6	39.6	39.7	39.6	39.6	39.6	2		129.1					
5	55.9	55.6	55.7	55.9	55.8	55.9	55.7	3		136.6					
6	18.5	18.9	18.9	18.5	18.3	18.5	18.9	4		15.7					
7	33.2	36.8	36.7	33.2	33.1	33.2	36.7	5		20.6					
8	40.2	41.5	41.5	40.2	40.1	40.1	41.5	22-*O*-MB-1			176.7				
9	47.0	47.2	47.2	46.7	46.9	47.0	47.3	2			41.5				
10	37.0	37.0	37.0	36.9	36.8	36.8	37.1	3			26.9				
11	23.9	24.0	24.0	23.9	23.8	23.9	24.0	4			11.8				
12	123.9	125.5	125.5	124.0	125.2	124.2	124.5	5			16.6				
13	142.9	143.7	143.7	142.9	141.0	142.7	144.7	28-*O*-Ac-1						170.6	
14	41.8	48.4	48.5	41.8	41.2	41.8	47.4	2						20.7	
15	34.7	73.8	73.1	34.7	31.0	34.6	67.4	GlcA							
16	68.1	67.5	67.5	68.2	71.2	67.7	72.5	1′	105.6	105.4	105.4	105.4	105.5	105.4	105.4
17	48.1	47.8	47.8	48.0	46.9	47.1	48.1	2′	79.4	79.4	79.4	79.4	79.3	79.3	79.4
18	40.1	41.0	40.9	40.1	39.7	40.6	42.0	3′	83.0	83.0	83.0	83.0	82.8	82.8	83.0
19	47.2	46.9	46.9	47.3	47.3	47.4	47.9	4′	71.3	71.3	71.3	71.3	71.4	71.2	71.3
20	36.3	36.4	36.4	36.5	36.0	36.0	36.4	5′	77.4	77.3	77.6	77.6	77.6	78.1	77.6
21	79.0	78.6	78.7	79.4	78.4	81.3	78.5	6′	172.2	172.2	172.2	172.2	172.1	172.1	172.2
22	74.5	73.5	73.3	74.5	73.5	71.4	77.4	Gal							
23	28.1	28.1	28.1	28.1	28.1	28.1	28.1	1″	103.8	103.8	103.8	103.8	103.8	103.7	103.8
24	16.8	16.9	16.9	16.8	16.8	16.8	16.9	2″	73.5	73.5	73.5	73.5	73.5	73.5	73.4
25	15.7	15.8	15.8	15.7	15.6	15.8	15.8	3″	75.2	75.2	75.2	75.2	75.3	75.1	75.2
26	16.9	17.6	17.6	17.0	16.8	17.1	17.6	4″	70.0	70.0	70.0	70.0	70.0	70.0	70.0
27	27.5	21.2	21.1	27.5	27.0	27.4	21.1	5″	76.8	76.8	76.8	76.8	76.9	76.7	76.8
28	64.0	63.2	63.1	63.9	63.8	66.5	67.9	6″	62.5	62.5	62.5	62.5	62.5	62.4	62.5
29	29.5	29.5	29.5	29.6	29.4	29.7	30.6	Ara							
30	20.3	20.2	20.2	20.1	19.7	20.1	19.3	1‴	101.2	101.2	101.1	101.2	101.2	101.1	101.2
16-*O*-Ac-1					169.8			2‴	77.0	77.0	77.0	77.0	77.0	76.9	77.0
2					22.0			3‴	73.4	73.3	73.3	73.5	73.5	73.7	73.6
21-*O*-Ang-1	167.9	167.7	167.7		167.8	168.6		4‴	68.9	68.8	68.8	68.8	68.9	68.8	68.8
2	129.1	129.0	128.7		128.5	129.5		5‴	65.4	65.3	65.3	65.4	65.4	65.3	65.7
3	137.0	137.2	138.4		138.0	136.1		Rha							
4	15.9	15.9	16.0		15.9	15.7		1⁗	102.3	102.2	102.2	102.3	102.3	102.2	102.3
5	21.0	21.0	21.0		20.9	21.0		2⁗	72.5	72.5	72.4	72.5	72.5	72.5	72.5
21-*O*-Tig-1				168.0				3⁗	72.7	72.7	72.7	72.7	72.7	72.7	72.7
2				129.6				4⁗	74.0	74.0	74.0	74.0	74.0	73.9	74.0
3				136.8				5⁗	70.1	70.1	70.1	70.1	70.2	69.9	70.2
4				14.2				6⁗	18.3	18.3	18.3	18.3	18.4	18.3	18.3
5				12.4											

nosyl moieties {a β-D-glucuronopyranosyl [δ 4.97 (1H, d, J=7.4 Hz, H-1′)], a β-D-galactopyranosyl [δ 5.62 (1H, d, J=7.6 Hz, H-1″)], a α-L-arabinopyranosyl [δ 6.03 (1H, d, J=5.8 Hz, H-1‴)], a α-L-rhamnopyranosyl [δ 5.99 (1H, br s, H-1⁗)]} together with one acetyl and one angeloyl moieties [δ 1.92 (3H, s, H_3-Ac), 2.02 (3H, s-like, H_3-Ang-5), 2.10 (3H, d, J=7.0 Hz, H_3-Ang-4), 5.98 (1H, qd-like, H-Ang-3)]. The positions of the acetyl and angeloyl groups in **4** were clarified on the basis of a HMBC experiment. Thus, long-range correlations were observed between the following proton and carbon pairs: the 21-proton and the angeloyl carbonyl carbon (δ_C 167.9); the 22-proton and the acetyl carbonyl carbon (δ_C 171.0); the acetyl methyl and acetyl carbonyl carbon. Furthermore, comparison of the ^{13}C-NMR data for **4** with those for **4a** revealed acylation shifts around the 21- and 22-positions of the aglycon moiety. On the basis of the above-mentioned evidence, the structure of floratheasaponin D was determined to be 21-*O*-angeloyl-22-*O*-acetyltheasapogenol B 3-*O*-β-D-galactopyranosyl(1→2)[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**4**).

Floratheasaponins E (**5**) and F (**6**), which were obtained as colorless fine crystals [**5**: mp 204.0—207.0 °C; **6**: mp 210.0—213.0 °C (from $CHCl_3$–MeOH)] with negative optical rotation (**5**: $[\alpha]_D^{21}$ −9.3°; **6**: $[\alpha]_D^{21}$ −9.4° in MeOH), showed absorption bands due to hydroxyl, α,β-unsaturated ester, carboxyl, olefin, and ether functions in the IR spectrum [**5**: 3475, 1735, 1719, 1654, and 1046 cm^{-1}; **6**: 3453, 1735, 1722, 1654, and 1046 cm^{-1}]. The molecular formulas (**5**: $C_{63}H_{98}O_{27}$; **6**: $C_{63}H_{100}O_{27}$) were determined individually from the quasimolecular ion peaks [**5**: *m/z* 1285 $(M-H)^-$, *m/z* 1309 $(M+Na)^+$, and *m/z* 1331 $(M+2Na-H)^+$; **6**: *m/z* 1287 $(M-H)^-$, *m/z* 1311 $(M+Na)^+$, and *m/z* 1333 $(M+2Na-H)^+$] in the negative-ion and positive-ion FAB-MS and by high-resolution MS measurement. Treatment of **5** and **6** with 10% aqueous KOH–50% aqueous 1,4-dioxane (1 : 1) liberated desacyl-floratheasaponin E (**5a**) and organic acids, angelic acid (from **5** and **6**) and 2-methylbutylic acid (from **6**), which were identified by HPLC analysis of their *p*-nitrobenzyl derivatives.[7,8] Acid hydrolysis of **5a** with 5% aqueous H_2SO_4–1,4-dioxane (1 : 1, v/v) yielded a known triterpene, R_1-barrigenol (**25**),[11,30] as an aglycon, together with L-arabi-

nose, D-galactose, D-glucuronic acid, and L-rhamnose, which were identified by GLC analysis of their trimethylsilyl thiazolidine derivatives.[26—28]

The IR spectrum of **5a** showed absorption bands due to hydroxyl, carboxyl, olefin, and ether functions at 3453, 1718, 1647, and 1085 cm^{-1}. The molecular formula $C_{52}H_{84}O_{25}$ of **5a** was determined from the negative-ion and positive-ion FAB-MS [*m/z* 1121 $(M-H)^-$, *m/z* 1145 $(M+Na)^+$]. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[29] showed signals assignable to a R_1-barringenol part [δ: 0.67, 0.73, 1.07, 1.15, 1.26, 1.25, 1.48 (3H each, all s, H_3-25, 26, 29, 24, 23, 30, 27), 3.28 (1H, dd-like, H-3), 5.46 (1H, br s, H-12)], a β-D-glucopyranosiduronic acid moiety [δ 4.97 (1H, d, J=7.2 Hz, H-1′)], a β-D-galactopyranosyl moiety [δ 5.62 (1H, d, J=7.0 Hz, H-1″)], a α-L-arabinopyranosyl moiety [δ 6.04 (1H, d, J=6.8 Hz, H-1‴)], and a α-L-rhamnopyranosyl moiety [δ 5.47 (1H, br s, H-1⁗)]. The carbon and proton signals in the ^{1}H- and ^{13}C-NMR spectra of **5a** were superimposable on those of desacyl-floratheasaponin A, except for the signals due to the terminal α-L-rhamnopyranosyl moiety. The oligoglycoside structure at the 3-position in **5a** was elucidated by a HMBC experiment, which showed long-range correlations between the following proton and carbons: H-1′ and 3-C; H-1″ and C-2′; H-1‴ and C-3′; H-1⁗ and C-2‴. Consequently, the structure of desacyl-floratheasaponin E was characterized as R_1-barrigenol 3-*O*-β-D-galactopyranosyl(1→2)[α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**5a**).

The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[29] of **5** indicated the presence of a desacyl-floratheasaponin E part {seven methyls [δ 0.85, 1.02, 1.11, 1.15, 1.26, 1.33, 1.50 (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-like, H-3), 3.49, 3.74 (1H, each both d, J=10.7 Hz, H_2-28), 4.20 (1H, m, H-16), 4.40 (1H, m, H-15), 6.28 (1H, d, J=10.4 Hz, H-22), 6.67 (1H, d, J=10.4 Hz, H-21)], an olefin [δ 5.51 (1H, br s, H-12)], four glycopyranosyl moieties {a β-D-glucuronopyranosyl [δ 4.95 (1H, d, J=7.1 Hz, H-1′)], a β-D-galactopyranosyl [δ 5.61 (1H, d, J=7.1 Hz, H-1″)], a α-L-arabinopyranosyl [δ 6.02 (1H, d, J=5.8 Hz, H-1‴)], a α-L-rhamnopyranosyl [δ 5.99 (1H, br s, H-1⁗)]}} and two angeloyl moieties [δ 1.76, 2.01 (3H, each, both s-like, H_3-22-*O*-Ang-5, H_3-21-*O*-Ang-5), 1.96 (3H, d, J=7.0 Hz, H_3-22-*O*-Ang-4), 2.09 (3H, d, J=7.0 Hz, H_3-21-*O*-Ang-4), 5.81, 5.96 (1H each, both dq-like, H-22-*O*-Ang-3, H-21-*O*-Ang-3)]. The positions of the two angeloyl groups in **5** were clarified on the basis of a HMBC experiment, which showed long-range correlations between the 21-proton and angeloyl carbonyl carbon (δ_C 167.7) and between the 22-proton and another angeloyl carbonyl carbon (δ_C 168.2). Furthermore, comparison of the ^{13}C-NMR data for **5** with those for **5a** revealed acylation shifts around the 21- and 22-positions of the aglycon moiety. Consequently, the structure of floratheasaponin E was elucidated as 21,22-di-*O*-angeloyl-R_1-barrigenol 3-*O*-β-D-galactopyranosyl(1→2)[α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**5**).

On the other hand, the proton and carbon signals in the ^{1}H- and ^{13}C-NMR (Table 1) spectra[29] of **6** were superimposable on those of **5**, except for the signals due to the 22-acyl moiety. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR spectra of **6** showed signals assignable to an angeloyl moiety [δ 2.03 (3H, s-like, H_3-21-*O*-Ang-5), 2.09 (3H, dd, J=7.4, 1.3 Hz, H_3-22-*O*-Ang-4), 5.96 (1H, qd-like, H-21-*O*-Ang-3)] and a 2-methylbutyryl moiety [δ 0.70 (3H, t, J=7.3 Hz, H_3-4⁗), 1.02 (3H, d-like, H_3-5⁗), 1.25, 1.60 (1H each, both m, H_2-3⁗), 2.03 (1H, m, H-2⁗)] together with a desacyl-floratheasaponin E part. The positions of the angeloyl and 2-methylbutyryl groups in **6** were also clarified on the basis of a HMBC experiment, in which long-range correlations were observed between the 21-proton [δ 6.62 (d, J=10.4 Hz)] and angeloyl carbonyl carbon (δ_C 167.7) and between the 22-proton [δ 6.28 (d, J=10.4 Hz)] and 2-methylbutyryl carbonyl carbon (δ_C 176.6). On the basis of this evidence and observation of acylation shifts at the 21- and 22-positions, the structure of floratheasaponin F was elucidated as 21-*O*-angeloyl-22-*O*-2-methylbutyryl-R_1-barrigenol 3-*O*-β-D-galactopyranosyl(1→2)[α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**6**).

Floratheasaponin G (**7**), obtained as colorless fine crystals of mp 207.0—210.0 °C (from $CHCl_3$–MeOH) with negative optical rotation ($[\alpha]_D^{21}$ −16.5° in MeOH), showed absorption bands at 3424, 1735, 1719, 1658, and 1050 cm^{-1} in the IR spectrum. The negative- and positive-ion FAB-MS of **7** showed quasimolecular ion peaks at *m/z* 1229 $(M-H)^-$ and *m/z* 1253 $(M+Na)^+$ and the molecular formula $C_{60}H_{94}O_{26}$ was determined by high-resolution MS measurement. Treatment of **7** with 10% aqueous KOH–50% aqueous 1,4-dioxane (1 : 1) liberated desacyl-assamsaponin J (**4a**)[8] together with acetic acid and tiglic acid, which were identified by HPLC analysis of their *p*-nitrobenzyl derivatives.[8,9] The proton and carbon signals in the ^{1}H- and ^{13}C-NMR (Table 1) spectra[29] of **7** were superimposable on those of **4**, except for the signals due to the 21-acyl moiety. That is, the ^{1}H- (pyridine-d_5) and ^{13}C-NMR spectra of **7** showed signals assignable to one acetyl and one tigloyl moieties: [δ 2.10 (3H, s-like, H_3-Ac), 1.96 (3H, s, H_3-Tig-5), 1.66 (3H, dq-like, H_3-Tig-4), 7.09 (1H, qd-like, H-Tig-3)] together with a desacyl-assamsaponin J part. The positions of the acetyl and tigloyl groups in **7** were clarified on the basis of a HMBC experiment [long-range correlations: the 21-proton and the tigloyl carbonyl carbon (δ_C 168.0); the 22-proton and acetyl carbonyl carbon; the acetyl methyl and acetyl carbonyl carbon (δ_C 171.1)]. Furthermore, comparison of the ^{13}C-NMR data for **7** with those for **4a** revealed acylation shifts around the 21- and 22-positions. Consequently, the structure of floratheasaponin G was elucidated as 21-*O*-tigloyl-22-*O*-acetyltheasapogenol B 3-*O*-β-D-galactopyranosyl(1→2)[α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**7**).

Floratheasaponins H (**8**) and I (**9**), obtained as colorless fine crystals [**8**: mp 210.0—213.0 °C, **9**: mp 200.0—203.0 °C (from $CHCl_3$–MeOH)] with negative or positive optical rotation (**8**: $[\alpha]_D^{21}$ −21.0°, **9**: $[\alpha]_D^{21}$ +26.2° in MeOH), showed absorption bands due to hydroxyl, α,β-unsaturated ester, carboxyl, olefin, and ether functions [**8**: 3475, 1735, 1717, 1654, and 1055 cm^{-1}, **9**: 3475, 1735, 1719, 1654, and 1051 cm^{-1}] in the IR spectrum. The negative- and positive-ion FAB-MS of **8** and **9** showed quasimolecular ion peaks [**8**: *m/z* 1272 $(M-H)^-$, *m/z* 1295 $(M+Na)^+$, *m/z* 1317 $(M+2Na-H)^+$, **9**: *m/z* 1229 $(M-H)^-$, *m/z* 1253 $(M+Na)^+$] and the molecular formula $C_{62}H_{96}O_{27}$ of **8** and $C_{60}H_{94}O_{26}$ of **9** were determined

by high-resolution MS measurement. Treatment of **8** and **9** with 10% aqueous KOH–50% aqueous 1,4-dioxane (1 : 1) liberated desacyl-assamsaponin J (**4a**)[9] together with acetic acid and angelic acid, which were identified by HPLC analysis of their *p*-nitrobenzyl derivatives.[8,9] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[29] of **8** showed signals assignable a desacyl-assamsaponin J part together with two acetyl and one angeloyl groups [δ 2.15, 2.50 (3H each, both s, H_3-Ac-22, 16), 1.96 (3H, s-like, H_3-Ang-5), 2.06 (3H, dd, J=7.3, 1.5 Hz, H_3-Ang-4), 5.99 (1H, qd-like, H-Ang-3)]. The positions of the acetyl and angeloyl groups in **8** were clarified on the basis of a HMBC experiment, which showed long-range correlations between the following proton and carbon pairs: the 16-proton, the acetyl methyl and acetyl carbonyl carbon (δ_C 169.8): the 21-proton and the angeloyl carbonyl carbon (δ_C 167.8); the 22-proton, the acetyl methyl and another acetyl carbonyl carbon (δ_C 170.5). Furthermore, comparison of the ^{13}C-NMR data for **8** with those for **4** revealed an additional acylation shift around the 16-position of the aglycon moiety. Consequently, the structure of floratheasaponin H was elucidated as 21-*O*-angeloyl-16,22-di-*O*-acetyltheasapogenol B 3-*O*-β-D-galactopyranosyl(1→2)[α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**8**).

The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[29] of **9** showed signals due to an acetyl and angeloyl groups [δ 1.97 (3H, br s, H_3-Ang-5), 2.04 (3H, dq, J=7.3, 1.5 Hz, H_3-Ang-4), 2.11 (3H, br s, H_3-Ac), 5.91 (1H, qd-like, H-Ang-3)] together with a desacyl-assamsaponin J part. The positions of the acetyl and angeloyl groups in **9** were clarified on the basis of a HMBC experiment. Thus, long-range correlations were observed between the following proton and carbon pairs: the 21-proton and the angeloyl carbonyl carbon (δ_C 168.6); the 28-proton, the acetyl methyl and acetyl carbonyl carbon (δ_C 170.6). Furthermore, comparison of the ^{13}C-NMR data for **9** with those for **4a** revealed acylation shifts around the 21 and 28-positions of the aglycon moiety. Consequently, the structure of floratheasaponin I was elucidated as 21-*O*-angeloyl-28-*O*-acetyltheasapogenol B 3-*O*-β-D-galactopyranosyl(1→2)[α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**9**).

Inhibitory Effect of Floratheasaponins (1—6) on the Release of β-Hexosaminidase from RBL-2H3 Cells Histamine, which is released from mast cells stimulated by an antigen or a degranulation inducer, is usually determined as a degranulation marker in *in vitro* experiments on immediate allergic reactions. β-Hexosaminidase is also stored in secretory granules of mast cells, and is also released concomitantly with histamine when mast cells are immunologically activated.[31,32] Therefore, it is generally accepted that β-hexosaminidase is a degranulation marker of mast cells.

As a part of our characterization studies on the bioactive components of natural medicines, we previously reported several inhibitors of the release of β-hexosaminidase such as flavonoids,[33] diarylheptanoids,[34—36] sesquiterpenes,[37] diterpenes,[38] anthraquinones,[39] stilbenes,[40] phenanthrenes,[40] phenylpropanoids,[41] and alkaloids.[42,43] On the other hand, dammarane-type triterpene glycoside, ginsenoside Rh2, was reported to exhibit inhibitory activity on β-hexosaminidase release from RBL-2H3 cells and this activity was suggested to originate from cell membrane stabilizing action.[44] Acylated oleanane-type triterpene oligoglycosides, floratheasaponins A—F (**1**—**6**), isolated from the flower buds of *C. sinensis* were also expected to exhibit cell membrane stabilizing action. Therefore, we examined the effects of these triterpene oligoglycosides (**1**—**6**) on the release of β-hexosaminidase induced by dinitrophenylated bovine serum albumine (DNP-BSA) from RBL-2H3 cells sensitized with anti-DNP IgE. As shown in Table 2, all floratheasaponins (**1**—**6**) were found to show the inhibitory effects. Particularly, floratheasaponins B (**2**) and E (**5**) [inhibition (%): 59.8±3.6 (p<0.01) and 52.3±3.2 (p<0.01) at 3 μM, respectively] displayed strong activity, and their activities were stronger than those of two antiallergic compounds, tranilast and ketotifen fumarate [inhibition (%): 8.2 and 7.7 at 30 μM, respectively].[35] In addition, the effects of test compounds on β-hexosaminidase activity were examined to clarify whether their effects were due to the inhibition of enzyme activity or of degranulation. As a result, floratheasaponins A—F (**1**—**6**), did not affect the enzyme activity of β-hexosaminidase.

Table 2. Inhibitory Effects of Constituents from *C. sinensis* on the Release of β-Hexosaminidase from RBL-2H3 Cells

	Conc. (μM)	Inhibition (%)
Floratheasaponin A (**1**)	1	−1.9±4.8
	3	48.8±2.4**
	6	61.6±2.1**
Floratheasaponin B (**2**)	1	−0.5±4.1
	3	59.8±3.6**
	6	57.3±2.7**
Floratheasaponin C (**3**)	1	17.9±6.5
	3	15.2±3.8
	6	60.8±4.7**
Floratheasaponin D (**4**)	0.3	2.6±8.8
	1	1.3±9.1
	3	30.6±5.8*
Floratheasaponin E (**5**)	0.3	4.7±3.8
	1	11.1±2.4
	3	52.3±3.2**
Floratheasaponin F (**6**)	3	18.0±3.7**
	6	58.6±4.5**
Tranilast[36]	30	8.2±1.8
	100	22.4±2.5*
Ketorifen fumarate[36]	30	7.4±1.5
	100	27.6±2.2*

Values represent the means±S.E.M. Significantly different from the control group, * p<0.05, ** p<0.01.

In conclusion, six new acylated oleanane-type triterpene oligoglycosides, floratheasaponins D (**4**), E (**5**), F (**6**), G (**7**), H (**8**), and I (**9**), were isolated from the flower buds of Chinese *Camellia sinensis* and their structures were determined on the basis of chemical and physicochemical evidence. In addition, this study demonstrated that acylated polyhydroxyoleanane-type triterpene oligoglycosides such as floratheasaponins A—F (**1**—**6**) showed an inhibitory effect on β-hexosaminidase release from RBL-2H3 cells.

Experimental

The following instruments were used to obtain physical data: melting points, Yanaco micromelting points apparatus (MP-500D); specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); IR spectra, Shimadzu FTIR-8100 spectrometer; FAB-MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JNM-LA500 (500 MHz) spectrometer; ^{13}C-NMR spectra, JNM-LA500 (125 MHz) spectrometer with tetramethylsilane as an internal standard; HPLC detector, Shimadzu RID-6A

refractive index detector and Shimadzu SPD-10A UV-VIS detector.

The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150—350 mesh); reverse-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) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reverse phase); reverse-phase HPTLC, precoated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm); and detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 followed by heating.

Plant Material The flower buds of *Camellia sinensis* were cultivated in Anhui province of China and collected at November, 2005. The botanical identification was undertaken by one of authors (M. Y.). A voucher of the plant is on file in our laboratory.

Isolation of Floratheasaponins D—I (4—9) and Known Compounds from the Flowers of *C. sinensis* The Flower buds of *C. sinensis* (1.8 kg, Anhui province, China) were cut and extracted three times with MeOH under reflux. Evaporation of the solvent under reduced pressure provided the MeOH extract (693 g, 38.5%), which was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction (135 g, 7.7%) and aqueous layer. The aqueous layer was extracted with *n*-butanol (*n*-BuOH) to give *n*-BuOH- (226 g, 12.8%) and H_2O- (319 g, 18.1%) soluble fractions. The EtOAc-soluble fraction (100 g) was subjected to normal-phase silica gel column chromatography [2 kg, *n*-hexane–EtOAc (10 : 1→5 : 1→1 : 1, v/v)→EtOAc→$CHCl_3$–MeOH (10 : 1→5 : 1→1 : 1, v/v)→MeOH] to give seven fractions [Fr. 1 (12.8 g), Fr. 2 (6.9 g,), Fr. 3 (7.1 g), Fr. 4 (41.9 g), Fr. 5 (25.1 g), Fr. 6 (14.5 g), Fr. 7 (1.9 g)]. Fraction 3 (7.1 g) was separated by reversed-phase silica gel column chromatography [300 g, MeOH–H_2O (30 : 70→50 : 50→70 : 30, v/v)→MeOH] to give seven fractions [Fr. 3-1 (1.6 g), Fr. 3-2 (110 mg), Fr. 3-3 (182.1 g), Fr. 3-4 [=kaempferol (**13**, 223 mg, 0.017%)], Fr. 3-5 (258 mg), Fr. 3-6 (3.4 g), Fr. 3-7 (1.9 g)]. Fraction 3-2 (110 mg) was purified by HPLC [MeOH–H_2O (55 : 45, v/v)] to give cinnamoic acid (38 mg, 0.0029%). Fraction 3-3 (182 mg) was purified by HPLC [MeOH–H_2O (55 : 45, v/v)] to give quercetin (**17**, 26 mg, 0.0020%), kaempferol (**13**, 30 mg, 0.0023%). Fraction 4 (16.7 g) was separated by reversed-phase silica gel column chromatography [480 g, MeOH–H_2O (10 : 90, 30 : 70→50 : 50→70 : 30, v/v)→MeOH] to give six fractions [Fr. 4-1 (675 mg), Fr. 4-2 (1.9 g), Fr. 4-3 (9.0 g), Fr. 4-4 (2.4 g), Fr. 4-5 (429 mg), Fr. 4-6 (1.9 g)]. Fraction 4-1 (500 mg) was purified by HPLC [MeOH–H_2O (20 : 80, v/v)] to give gallic acid (125 mg, 0.33%). Fraction 4-2 (300 mg) was purified by HPLC [MeOH–H_2O (30 : 70, v/v)] to give (−)-epigallocatechin (**12**, 22 mg, 0.026%) and (−)-epigallocatechin 3-*O*-gallate (**11**, 57 mg, 0.19%). Fraction 4-3 (340 mg) was purified by HPLC [MeOH–H_2O (30 : 70, v/v)] to give (−)-epigallocatechin 3-*O*-gallate (**11**, 57 mg, 0.29%) and (−)-epicatechin 3-*O*-gallate (**10**, 39 mg, 0.19%). Fraction 5 (25.1 g) was separated by reversed-phase silica gel column chromatography [750 g, MeOH–H_2O (10 : 90→30 : 70→50 : 50→70 : 30, v/v)→MeOH] to give seven fractions [Fr. 5-1 (730 mg), Fr. 5-2 (358 mg), Fr. 5-3 [=caffeine (10 g, 0.76%)], Fr. 5-4 (1.2 g), Fr. 5-5 (3.4 g), Fr. 5-6 (2.1 g) , Fr. 5-7 (5.7 g)]. Fraction 5-1 (300 mg) was purified by HPLC [MeOH–H_2O (10 : 90, v/v)] to give gallic acid (13 mg, 0.0025%). Fraction 5-4 (500 mg) was purified by HPLC [MeOH–H_2O (60 : 40, v/v)] to give (−)-epigallocatechin 3-*O*-gallate (**11**, 64 mg, 0.012%). Fraction 5-5 (1.0 g) was purified by HPLC [1. MeOH–H_2O (50 : 50, v/v); 2. MeOH–H_2O (45 : 55, v/v)] to give kaempferol 3-*O*-*β*-D-galactopyranoside (**14**, 35 mg, 0.0091%) and kaempferol 3-*O*-*β*-D-glucopyranoside (**15**, 42 mg, 0.011%). The BuOH-soluble fraction (220 g) was subjected to normal-phase silica gel column chromatography [3 kg, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer→7 : 3 : 1, lower layer→6 : 4 : 1, v/v)→MeOH] to give six fractions [Fr. 1 (229 mg), Fr. 2 (10.3 g), Fr. 3 (8.2 g), Fr. 4 (15.8 g), Fr. 5 (124.8 g), Fr. 6 (60.0 g)]. Fraction 2 (5.0 g) was purified by reversed-phase silica gel column chromatography [150 g, MeOH–H_2O (30 : 70→50 : 50→70 : 30, v/v)→MeOH] to give Fr. 2-1 [=caffeine (3.0 g, 0.36%)], Fr. 2-2 (363 mg), Fr. 2-3 (100 mg), Fr. 2-4 (724 mg), Fr. 2-5 (603 mg). Fraction 3 (8.2 g) was separated by reversed-phase silica gel column chromatography [240 g, MeOH–H_2O (10 : 90→20 : 80→30 : 70→50 : 50→70 : 30, v/v)→MeOH] to give seven fractions [Fr. 3-1 (3.1 g), Fr. 3-2 (955 mg), Fr. 3-3 (692 mg), Fr. 3-4 (1.0 g), Fr. 3-5 (195 mg), Fr. 3-6 (2.0 g), Fr. 3-7 (555 mg)]. Fraction 3-3 (192 mg) was purified by HPLC [MeOH–H_2O (30 : 70, v/v)] to give caffeine (42 mg, 0.0024%). Fraction 3-4 (500 mg) was purified by HPLC [MeOH–H_2O (45 : 55, v/v)] to give kaempferol 3-*O*-*β*-D-galactopyranoside (**14**, 56 mg, 0.0065%) and kaempferol 3-*O*-*β*-D-glucopyranoside (**15**, 55 mg, 0.0064%). Fraction 3-5 (195 mg) was purified by HPLC [MeOH–H_2O (50 : 50, v/v)] to give kaempferol 3-*O*-*β*-D-galactopyranoside (**14**, 31 mg, 0.0018%). Fraction 4 (15.8 g) was separated by reversed-phase silica gel column chromatography [530 g, MeOH–H_2O (20 : 80→30 : 70→50 : 50→70 : 30, v/v)→MeOH] to give eight fractions [Fr. 4-1 (3.7 g), Fr. 4-2 (1.4 g), Fr. 4-3 (2.1 g), Fr. 4-4 (1.3 g), Fr. 4-5 (892 mg), Fr. 4-6 (1.1 g), Fr. 4-7 (3.4 g), Fr. 4-8 (783 mg)]. Fraction 4-3 (500 mg) was purified by HPLC [MeOH–H_2O (30 : 70, v/v)] to give primeveroside (**22**, 45 mg, 0.011%), phenetyl alcohol *β*-D-xylopyranosyl(1→6)-*β*-D-glucopyranoside (**23**, 13 mg, 0.003%), and shimaurinoside (**21**, 24 mg, 0.006%). Fraction 4-4 (825 mg) was purified by HPLC [MeOH–H_2O (45 : 55, v/v)] to give quercetin 3-*O*-*β*-D-galactopyranoside (**16**, 77.0 mg, 0.024%), kaempferol 3-*O*-*β*-D-galactopyranoside (**14**, 22 mg, 0.066%), and kaempferol 3-*O*-*α*-L-rhamnopyranosyl(1→6)-*β*-D-galactopyranoside (**20**, 37 mg, 0.011%). Fraction 5 (124.8 g) was separated by reversed-phase silica gel column chromatography [240 g, MeOH–H_2O (20 : 80→50 : 50→70 : 30, v/v)→MeOH] to give ten fractions [Fr. 5-1 (27.9 g), Fr. 5-2 (1.5 g), Fr. 5-3 (2.6 g), Fr. 5-4 (6.6 g), Fr. 5-5 (9.4 g), Fr. 5-6 (10.1 g), Fr. 5-7 (13.7 g), Fr. 5-8 (18.0 g), Fr. 5-9 (30.3 g), Fr. 5-10 (9.1 g)]. Fraction 5-4 (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 (**18**, 126 mg, 0.25%), kaempferol 3-*O*-*β*-D-glucopyranosyl(1→3)-*α*-L-rhamnopyranosyl(1→6)-*β*-D-glucopyranoside (**19**, 230.8 mg, 0.18%). Fraction 5-5 (500 mg) was purified by HPLC [MeOH–H_2O (45 : 55, v/v)] to give kaempferol 3-*O*-*β*-D-glucopyranosyl(1→3)-*α*-L-rhamnopyranosyl(1→6)-*β*-D-galactopyranoside (**18**, 118 mg, 0.13%) and kaempferol 3-*O*-*β*-D-glucopyranosyl(1→3)-*α*-L-rhamnopyranosyl(1→6)-*β*-D-glucopyranoside (**19**, 164 mg, 0.18%). Fraction 5-7 (500 mg) was purified by HPLC {1. MeOH–[H_2O : AcOH (99 : 1, v/v)] (65 : 35, v/v); 2. MeCN–[H_2O : AcOH (99 : 1, v/v)] (40 : 60, v/v)} to give floratheasaponin D (**4**, 72 mg, 0.12%) and floratheasaponin G (**7**, 67 mg, 0.053%). Fraction 5-8 (1 g) was purified by HPLC {1. MeOH–[H_2O : AcOH (99 : 1, v/v)] (70 : 30, v/v); 2. MeCN–[H_2O : AcOH (99 : 1, v/v)] (40 : 60, v/v)} to give floratheasaponine A (**1**, 35 mg, 0.013%), floratheasaponin H (**8**, 28 mg, 0.065%), floratheasaponin I (**9**, 117 mg, 0.018%), and assamsaponin E (**24**, 33 mg, 0.015%). Fraction 5-9 (1 g) was purified by HPLC {MeOH–[H_2O : AcOH (99 : 1, v/v)] (75 : 25, v/v)} to give floratheasaponine B (**2**, 61 mg, 0.11%), floratheasaponine E (**5**, 144 mg, 0.25%), floratheasaponine C (30 mg, 0.054%), and floratheasaponine F (**6**, 70 mg, 0.13%). The known compounds were identified by comparison of their physical data (MS, ^{1}H-NMR, ^{13}C-NMR) with reported values.

Floratheasaponins D (**4**): Colorless fine crystals from $CHCl_3$–MeOH, mp 203—206 °C, ($[\alpha]_D^{21}$ −12.8° (*c*=0.88, MeOH). IR (KBr): 3475, 1735, 1719, 1654, 1048 cm^{-1}. High-resolution positive-ion FAB-MS: Calcd for $C_{60}H_{94}O_{26}Na$ $(M+Na)^+$: 1253.5931. Found: 1253.5923. ^{1}H-NMR (500 MHz, pyridine-d_5) *δ*: 0.83, 0.87, 1.09, 1.14, 1.29, 1.31, 1.82 (3H each, all s, H_3-25, 26, 29, 24, 23, 30, 27), 1.92 (3H, s, Ac-H_3), 2.02 (3H, s, H_3-Ang-5), 2.10 (3H, d, *J*=7.0 Hz, H_3-Ang-4), 3.32 (1H, dd-like, H-3), 3.37, 3.60 (1H each, both d, *J*=10.7 Hz, H_2-28), 4.44 (1H, m, H-16), 4.97 (1H, d, *J*=7.4 Hz, H-1′), 5.40 (1H, br s, H-12), 5.62 (1H, d, *J*=7.6 Hz, H-1″), 5.98 (1H, dq-like, H-Ang-3), 5.99 (1H, br s, H-1‴), 6.03 (1H, d, *J*=5.8 Hz, H-1⁗), 6.15 (1H, d, *J*=10.4 Hz, H-22), 6.54 (1H, d, *J*=10.4 Hz, H-21), ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: *m/z* 1229 $(M-H)^-$, *m/z* 1067 $(M-H-C_6H_{10}O_5)^-$. Positive-ion FAB-MS: *m/z* 1253 $(M+Na)^+$, *m/z* 1275 $(M+2Na-H)^+$.

Floratheasaponins E (**5**): Colorless fine crystals from $CHCl_3$–MeOH, mp 204—207 °C, ($[\alpha]_D^{21}$ −9.3° (*c*=0.85, MeOH). IR (KBr): 3475, 1735, 1719, 1654, 1046 cm^{-1}. High-resolution positive-ion FAB-MS: Calcd for $C_{63}H_{98}O_{27}Na$ $(M+Na)^+$: 1309.6193. Found: 1309.6201. ^{1}H-NMR (500 MHz, pyridine-d_5) *δ*: 0.85, 1.02, 1.11, 1.15, 1.26, 1.33, 1.50 (3H each, all s, H_3-25, 26, 29, 24, 23, 30, 27), 1.76, 2.01 (3H, each, both s, H_3-22-*O*-Ang-5, H_3-21-*O*-Ang-5), 1.96 (3H, d, *J*=7.0 Hz, H_3-22-*O*-Ang-4), 2.09 (3H, d, *J*=7.0 Hz, H_3-21-*O*-Ang-4), 3.28 (1H, dd-like, H-3), 3.49, 3.74 (1H, each both d, *J*=10.7 Hz, H_2-28), 4.20 (1H, m, H-16), 4.40 (1H, m, H-15), 4.95 (1H, d, *J*=7.1 Hz, H-1′), 5.51 (1H, br s, H-12), 5.61 (1H, d, *J*=7.1 Hz, H-1″), 5.81, 5.96 (1H each, both dq-like, H-22-*O*-Ang-3, H-21-*O*-Ang-3), 5.99 (1H, br s, H-1‴), 6.02 (1H, d, *J*=5.8 Hz, H-1⁗), 6.28 (1H, d, *J*=10.4 Hz, H-22), 6.67 (1H, d, *J*=10.4 Hz, H-21), ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: 1285 $(M-H)^-$, *m/z* 1123 $(M-H-C_6H_{10}O_5)^-$. Positive-ion FAB-MS:*m/z* 1309 $(M+Na)^+$, *m/z* 1331 $(M+2Na-H)^+$.

Floratheasaponins F (**6**): Colorless fine crystals from $CHCl_3$–MeOH, mp 210—213 °C, ($[\alpha]_D^{21}$ −9.4° (*c*=0.78, MeOH). IR (KBr): 3453, 1735, 1722, 1654, 1046 cm^{-1}. High-resolution positive-ion FAB-MS: Calcd for $C_{63}H_{100}O_{27}Na$ $(M+Na)^+$: 1311.6350. Found: 1311.6365. ^{1}H-NMR (500 MHz, pyridine-d_5) *δ*: 0.70 (3H, td, *J*=7.3, 0.16 Hz, H_3-MB-4), 0.86, 1.03,

1.10, 1.13, 1.28, 1.30, 1.82 (3H each, all s, H_3-25, 26, 29, 24, 23, 30, 27), 1.02 (3H, d-like, H_3-MB-5), 1.25, 1.60 (1H each, both m, H_2-MB-3), 2.03 (1H, br s, H-MB-2), 2.03 (3H, br s, H_3-Ang-5), 2.09 (3H, dd, J=7.4, 1.3 Hz, H_3-Ang-4), 3.28 (1H, dd-like, H-3), 3.47, 3.73 (1H each, both d, J=10.7 Hz, H_2-28), 4.19 (1H, m, H-16), 4.40 (1H, m, H-15), 4.93 (1H, d, J=7.6 Hz, H-1′), 5.50 (1H, br s, H-12), 5.61 (1H, d, J=7.1 Hz, H-1″), 5.96 (1H, qd-like, H-Ang-3), 5.99 (1H, br s, H-1‴), 6.02 (1H, d, J=5.8 Hz, H-1⁗), 6.28 (1H, d, J=10.4 Hz, H-22), 6.62 (1H, d, J=10.4 Hz, H-21), ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: 1287 $(M-H)^-$. Positive-ion FAB-MS: *m/z* 1311 $(M+Na)^+$, *m/z* 1333 $(M+2Na-H)^+$.

Floratheasaponins G (**7**): Colorless fine crystals from $CHCl_3$–MeOH, mp 207—210 °C, ($[\alpha]_D^{21}$ −16.5° (*c*=0.22, MeOH). IR (KBr): 3424, 1735, 1719, 1658, 1050 cm^{-1}. High-resolution positive-ion FAB-MS: Calcd for $C_{60}H_{94}O_{26}Na$ $(M+Na)^+$: 1253.5931. Found: 1253.5940. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.83, 0.87, 1.10, 1.16, 1.25, 1.32, 1.82 (3H each, all s, H_3-25, 26, 29, 24, 23, 30, 27), 1.66 (3H, dq-like, H_3-MB-4), 1.96 (3H, s, H_3-MB-5), 2.10 (3H, s, H_3-Ac-2), 3.28 (1H, dd-like, H-3), 3.38, 3.62 (1H each, both d, J=10.7 Hz, H_2-28), 4.45 (1H, m, H-16), 4.93 (1H, d, J=7.4 Hz, H-1′), 5.40 (1H, br s, H-12), 5.61 (1H, d, J=7.6 Hz, H-1″), 5.97 (1H, br s, H-1⁗), 6.02 (1H, d, J=5.8 Hz, H-1‴), 6.21 (1H, d, J=10.1 Hz, H-22), 6.56 (1H, d, J=10.1 Hz, H-21), 7.09 (1H, qd-like, H-MB-3). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: *m/z* 1229 $(M-H)^-$. Positive-ion FAB-MS: *m/z* 1253 $(M+Na)^+$.

Floratheasaponins H (**8**): Colorless fine crystals from $CHCl_3$–MeOH, mp 210—213 °C, ($[\alpha]_D^{21}$ −21.0° (*c*=0.13, MeOH). IR (KBr): 3475, 1735, 1717, 1654, 1055 cm^{-1}. High-resolution positive-ion FAB-MS: Calcd for $C_{62}H_{96}O_{27}Na$ $(M+Na)^+$: 1295.6046. Found: 1295.6037. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.77, 0.78, 1.07, 1.15, 1.47, 1.47, 2.03 (3H each, all s, H_3-25, 26, 29, 24, 23, 30, 27), 1.96 (3H, br s, H_3-Ang-5), 2.06 (3H, dq, J=7.3, 1.5 Hz, H_3-Ang-4), 2.15, 2.50 (3H each, both s, H_3-Ac), 5.99 (1H, qd-like, H-Ang-3), 3.32 (1H, dd-like, H-3), 3.46, 3.59 (1H each, both d, J=10.4 Hz, H_2-28), 4.94 (1H, d, J=7.1 Hz, H-1′), 5.39 (1H, br s, H-12), 5.39 (1H, m, H-16), 5.63 (1H, d, J=7.3 Hz, H-1″), 5.98 (1H, br s, H-1⁗, 6.05 (1H, d, J=5.8 Hz, H-1‴), 6.11 (1H, d, J=10.1 Hz, H-22), 5.86 (1H, d, J=10.1 Hz, H-21) ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: *m/z* 1272 $(M-H)^-$. Positive-ion FAB-MS: *m/z* 1295 $(M+Na)^+$, *m/z* 1317 $(M+2Na-H)^+$.

Floratheasaponins I (**9**): Colorless fine crystals from $CHCl_3$–MeOH, mp 200—203 °C, ($[\alpha]_D^{21}$ +26.2° (*c*=0.26, MeOH). IR (KBr): 3475, 1735, 1719, 1654, 1051 cm^{-1}. High-resolution positive-ion FAB-MS: Calcd for $C_{60}H_{94}O_{26}Na$ $(M+Na)^+$: 1253.5931. Found: 1253.5935. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.83, 0.98, 1.08, 1.12, 1.26, 1.28, 1.99 (3H each, all s, H_3-25, 26, 29, 24, 23, 30, 27), 1.97 (3H, br s, H_3-Ang-5), 2.04 (3H, dq, J=7.3, 1.5 Hz, H_3-Ang-4), 2.11 (3H, s, H_3-Ac), 3.27 (1H, dd-like, H-3), 4.21 (2H, m, H_2-28), 4.43 (1H, m, H-16), 4.94 (1H, d, J=6.7 Hz, H-1′), 5.40 (1H, br s, H-12), 5.44 (1H, m, H-22), 5.57 (1H, d, J=7.3 Hz, H-1″), 5.91 (1H, qd-like, H-Ang-3), 5.93 (1H, br s, H-1⁗), 5.98 (1H, d, J=5.2 Hz, H-1‴), 6.39 (1H, d, J=9.5 Hz, H-21), ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: *m/z* 1229 $(M-H)^-$, *m/z* 1067 $(M-H-C_6H_{10}O_5)^-$. Positive-ion FAB-MS: *m/z* 1253 $(M+Na)^+$.

Alkaline Hydrolysis of Floratheasaponins D—I (4—9) A solution of Floratheasaponins D—I (**4**—**9**, 5—30 mg) was treated with 10% aqueous KOH–1,4-dioxane (1 : 1, v/v, 4—10 ml) and the whole was stirred at 37 °C for 1 h. Removal of the solvent under reduced pressure gave a reaction product. A part of the reaction product was dissolved in $(CH_2)_2Cl_2$ (2.0 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: MeCN–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 (t_R 8.5 min) from floratheasaponins D, G—I (**4**, **7**—**9**), tiglic acid (t_R 40.2 min) from floratheasaponin G (**7**), angelic acid (t_R 25.8 min) from floratheasaponins D—F, H, and I (**4**—**6**, **8**, **9**), and 2-methylbutylic acid (t_R 27.3 min) from floratheasaponins F (**6**). The rest of 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 [$CHCl_3$–MeOH–H_2O (10 : 3 : 1→6 : 4 : 1, v/v/v)] to give desacyl-assamsaponin J (**4a**, 4.5—14 mg) from floratheasaponins D, G—I (**4**, **7**—**9**) or desacyl-floratheasaponin E (**5a**, 17—20 mg) from floratheasaponins E and F (**5**, **6**).

Desacyl-floratheasaponin E (**5a**): Colorless fine crystals from $CHCl_3$–MeOH, mp 206—209 °C, ($[\alpha]_D^{26}$ +2.7° (*c*=0.67, MeOH). IR (KBr): 3453, 1718, 1647, 1085 cm^{-1}. High-resolution positive-ion FAB-MS: Calcd for $C_{52}H_{84}O_{25}Na$ $(M+Na)^+$: 1145.5356. Found: 1145.5348. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.67, 0.73, 1.07, 1.15, 1.25, 1.26, 1.48 (3H each, all s, H_3-25, 26, 29, 24, 30, 23, 27), 3.28 (1H, dd-like, H-3), 4.97 (1H, d, J=7.2 Hz, H-1′), 5.46 (1H, br s, H-12), 5.47 (1H, br s, H-1⁗), 5.62 (1H, d, J=7.0 Hz, H-1″), 6.04 (1H, d, J=6.8 Hz, H-1‴), ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: 1121 $(M-H)^-$. Positive-ion FAB-MS: *m/z* 1145 $(M+Na)^+$.

Acid Hydrolysis of Desacyl-floratheasaponin E (5a) A solution of **5a** (10 mg) in 5% aqueous H_2SO_4–1,4-dioxane was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and residue was removed by filtration. After removal of the solvent from the filtrate *in vacuo*, the residue was transferred to a Sep-Pak C18 cartridge with H_2O and MeOH. The H_2O eluate was concentrated and the residue was treated with L-cysteine methyl ester hydrochloride (1 mg) in pyridine (0.5 ml) at 60 °C for 2 h. After reaction, the solution was treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (0.1 ml) at 60 °C for 2 h. The supernatant was then subjected to GLC analysis to identify the derivatives of D-glucuronic acid (i), D-galactose (ii), L-arabinose (iii), L-rhamnose (iv); GLC conditions: column, Supeluco STB™-1, 30 m×0.25 mm (i.d.) capillary column; column temperature, 230 °C; carrier gas, N_2; t_R, (i) 22.9 min, (ii) 19.2 min, (iii) 10.4 min, (iv) 12.6 min, (v) 21.1 min (L-galactose), (vi) 11.6 min (D-arabinose). 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 (25, 3 mg).

Bioassay. Effects on the Release of β-Hexosaminidase from RBL-2H3 Cells Inhibitory effects on the release of β-hexosaminidase in RBL-2H3 (cell no. JCRB0023, obtained from Health Science Research Resources Bank, Osaka, Japan) were evaluated by a method reported previously.[33—43] Briefly, RBL-2H3 cells in 24-well plates (2×10^5 cells/well in MEM containing 10% FCS, penicillin 100 units/ml, streptomycin 100 μg/ml) were sensitized with anti-DNP IgE (0.45 μg/ml). The cells were washed with Siraganian buffer [119 mM NaCl, 5 mM KCl, 0.4 mM $MgCl_2$, 25 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), and 40 mM NaOH, pH 7.2] supplemented with 5.6 mM glucose, 1 mM $CaCl_2$, and 0.1% bovine serum albumin (BSA) (incubation buffer) and then incubated in 160 μl of the incubation buffer for 10 min at 37 °C. After that, 20 μl of test sample solution was added to each well and incubated for 10 min, followed by the addition of 20 μl of antigen (DNP-BSA, final concentration, 10 μg/ml) at 37 °C for 10 min to stimulate the cells to degranulate. The reaction was stopped by cooling in an ice bath for 10 min. The supernatant (50 μl) was transferred into 96-well plates and incubated with 50 μl of substrate (*p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide, 1 mM) in citrate buffer 0.1 M (pH 4.5) at 37 °C for 1 h. The reaction was stopped by adding 200 μl of stop solution (Na_2CO_3/$NaHCO_3$ 0.1 M, pH 10.0). The absorbance was measured with a microplate reader at 405 nm. The test sample was dissolved in dimethylsulfoxide (DMSO), and the solution was added to incubation buffer (final DMSO concentration. was 0.1%). The inhibition (%) of the release of β-hexosaminidase by the test samples was calculated using the following equation;

$$\text{inhibition (\%)}=[1-(T-B-N)/(C-N)]\times100$$

where the control (C) was DNP-BSA (+), test sample (−); test (T), DNP-BSA (+), test sample (+); blank (B), DNP-BSA (−), test sample (+); and normal (N), DNP-BSA (−), test sample (−).

To clarify that the anti allergic effects of samples we are due to the inhibition of β-hexosaminidase release, and not a false positive from the inhibition of β-hexosaminidase activity, the cell suspension in PBS was sonicated. The solution was then centrifuged, and the supernatant was diluted with the incubation buffer and adjusted to equal the enzyme activity of the degranulation tested above. The enzyme solution (45 μl) and test sample solution (5 μl) were transferred into a 96-well microplate and enzyme activity was examined as described above.

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

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