Medicinal Flowers. XXXI.[1] Acylated Oleanane-Type Triterpene Saponins, Sasanquasaponins I—V, with Antiallergic Activity from the Flower Buds of *Camellia sasanqua*

Hisashi Matsuda,[a] Seikou Nakamura,[a] Katsuyoshi Fujimoto,[a] Ryo Moriuchi,[a] Yuta Kimura,[a] Noriko Ikoma,[a] Yuki Hata,[a] Osamu Muraoka,[b] and Masayuki Yoshikawa*,[a]

[a] *Kyoto Pharmaceutical University; Misasagi, Yamashina-ku, Kyoto 607–8412, Japan: and* [b] *Pharmaceutical Research and Technology Institute, Kinki University; 3–4–1 Kowakae, Higashi-Osaka, Osaka 577–8502, Japan.*
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The methanolic extract and its 1-butanol-soluble fraction from the flower buds of *Camellia sasanqua* Thunb. were found to show inhibitory activities on the release of β-hexosaminidase from rat basophile leukemia (RBL-2H3) cells. From the 1-butanol-soluble fraction, five new acylated oleanane-type triterpene saponins, sasanquasaponins I—V, were isolated together with a known saponin and their chemical structures were elucidated on the basis of chemical and physicochemical evidence. The principal saponin constituents, sasanquasaponins I—III, with an acyl group at the 22-position of the aglycon part showed the inhibitory effects on the release of β-hexosaminidase and some structure–activity relationships were reported.

Key words *Camellia sasanqua*; sasanquasaponin; medicinal flower; antiallergic activity; degranulation inhibitor; rat basophile leukemia 2H3 cell

A Theaceae plant, *Camellia* (*C.*) *sasanqua* Thunb. (Japanese name "sazanka"), which is native to Japan, has been widely cultivated as an ornamental plant in Japanese garden. The flower buds of this plant are used for the similar purpose to those of *C. japonica* (Japanese name "tsubaki"), which has been used for the treatment of blood vomiting and bleeding due to internal and external injury, and also as antiinflamatory, tonic, and stomachic in Japanese folk medicine. As chemical constituents of this medicinal flower, several hydrolyzable tannins,[2] acylated anthocyanins,[3] and purin alkaloids[4] were reported. However, the pharmacological activities of the flower buds of this plant have not yet characterized. Recently, we have reported the isolation and structure elucidation of triterpene saponins from the flower buds of *C. japonica* L. (camelliosides A—D),[5,6] *C. sinensis* L. (chakasaponins I—VI, floratheasaponins A—J),[7—12] and *C. oleifera* Abel (yuchasaponins A—D).[13] Furthermore, those saponins were found to exhibit antiallergic, antidiabetic, antiobestic, gastroprotective, and platelet aggregation activities, *etc.*[5—13] As a continuation of our studies on bioactive constituents of the flower buds of *Camellia* species, we found that the methanolic extract and its 1-butanol-soluble fraction from the flower buds of *C. sasanqua* inhibited an immediate allergic reaction by monitoring the release of β-hexosaminidase from rat basophile leukemia (RBL-2H3) cells.[14] From the 1-butanol-soluble fraction, we have isolated new acylated oleanane-type triterpene saponins termed sasanquasaponins I (**1**), II (**2**), III (**3**), IV (**4**), and V (**5**), together with a known saponin, primulagenin A 3-*O*-[β-D-glucopyranosyl(1→2)][α-L-rhammopyranosyl(1→2)-β-D-galactopyranosyl(1→3)]-β-D-glucuronopyranoside (**6**) (Chart 1).[15] In addition, we examined the inhibitory effects of sasanquasaponins (**1**—**5**) and **6** on the release of β-hexosaminidase from RBL-2H3 cells. In this paper, we describe the isolation and structure elucida-

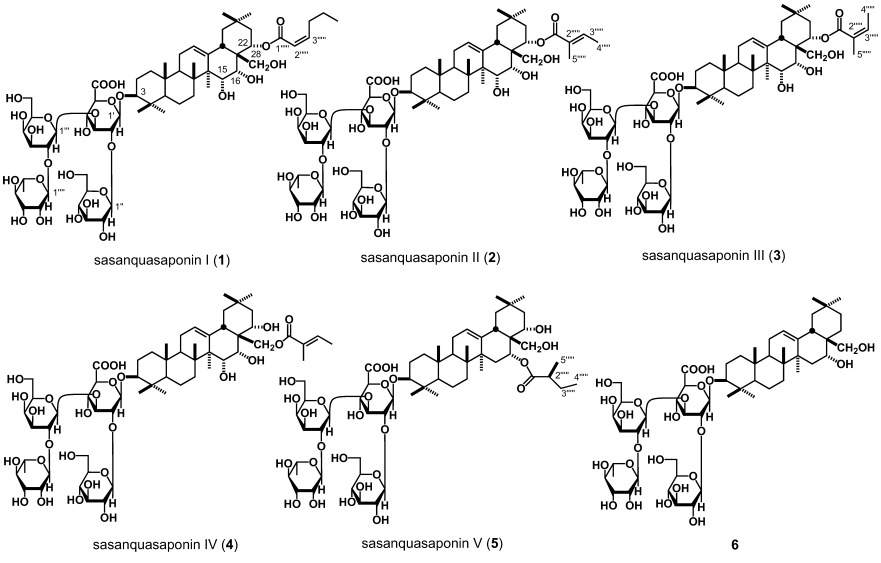

Chart 1. Saponin Constituents from the Flower Buds of *C. sasanqua*

* To whom correspondence should be addressed. e-mail: myoshika@mb.kyoto-phu.ac.jp

Table 1. ^{13}C-NMR (125 MHz) Data for **1**—**5**

Carbon	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]	**5**[b]
1	38.9	38.8	38.8	38.9	38.7	39.9
2	26.5	26.4	26.3	26.4	26.4	27.0
3	89.3	89.6	89.6	89.6	89.8	92.2
4	39.6	39.5	39.5	39.5	39.6	40.6
5	55.5	55.4	55.4	55.4	55.7	56.9
6	18.8	18.7	18.7	18.6	18.3	19.2
7	36.6	36.6	36.6	36.5	32.9	33.9
8	41.6	41.6	41.6	41.3	40.0	41.1
9	47.1	47.0	47.0	47.1	46.8	48.0
10	36.9	36.8	36.8	36.8	36.7	37.8
11	23.9	23.9	23.8	23.9	23.7	24.6
12	124.8	124.7	124.7	124.8	123.2	125.1
13	144.4	144.4	144.3	144.3	142.3	142.7
14	47.8	47.8	47.6	47.9	41.6	42.5
15	67.5	67.5	67.4	67.4	31.6	32.0
16	74.5	74.6	74.9	73.4	71.9	72.3
17	45.1	45.3	45.1	44.8	44.1	44.6
18	41.4	41.4	41.6	42.0	41.6	42.7
19	47.0	46.9	46.9	47.0	47.5	48.2
20	32.0	31.9	31.9	31.7	32.9	32.2
21	41.6	41.4	41.4	46.0	45.1	45.0
22	72.1	72.4	72.6	74.1	72.6	73.8
23	27.8	27.8	27.8	27.8	27.9	28.3
24	16.7	16.7	16.6	16.6	16.6	16.9
25	15.8	15.7	15.6	15.7	15.6	16.2
26	17.5	17.5	17.4	17.6	16.8	17.2
27	21.3	21.2	21.2	21.2	27.4	27.8
28	62.7	62.8	62.8	66.4	69.2	69.9
29	33.5	33.4	33.4	33.6	33.8	33.9
30	25.1	25.1	25.1	25.1	25.3	25.4
22-*O*-(*cis*-2-Hexenoyl)						
1‴″	166.5					
2‴″	121.0					
3‴″	149.3					
4‴″	31.1					
5‴″	22.3					
6‴″	13.9					
22- or 28-*O*-Tigloyl[c]						
1‴″		167.7		167.7		
2‴″		129.7		129.1		
3‴″		135.7		137.1		
4‴″		13.9		14.1		
5‴″		12.2		12.2		
22-*O*-Angeloyl						
1‴″			167.7			
2‴″			129.7			
3‴″			136.3			
4‴″			15.7			
5‴″			20.8			
16-*O*-(2-Methylbutyloyl)						
1‴″					175.8	177.6
2‴″					42.2	43.2
3‴″					27.1	27.8
4‴″					12.0	12.2
5‴″					16.5	16.5
3-*O*-*β*-D-Glucuronopyranosyl						
1′	105.4	105.3	105.3	105.4	105.9	105.9
2′	79.4	79.3	79.3	79.4	79.4	79.0
3′	82.4	82.5	82.5	82.4	82.5	81.1
4′	71.2	71.2	71.1	71.2	71.2	71.7
5′	77.1	76.9	76.9	77.1	77.0	77.0
6′	172.3	172.3	172.3	172.3	172.2	172.1
2′-*O*-*β*-D-Glucopyranosyl						
1″	102.6	102.6	102.5	102.6	102.7	102.6
2″	76.4	76.3	76.3	76.4	76.2	76.1
3″	78.4	78.3	78.3	78.4	78.4	78.3
4″	72.7	72.7	72.4	72.7	72.7	72.6
5″	78.3	78.1	78.1	78.3	78.2	78.2
6″	63.6	63.5	63.5	63.6	63.6	63.6
3′-*O*-*β*-D-Galactopyranosyl						
1‴	101.1	101.1	101.2	101.1	101.3	100.9
2‴	76.1	76.2	76.1	76.1	76.2	75.9
3‴	76.0	76.0	75.9	76.0	76.0	75.9
4‴	71.2	71.1	71.2	71.2	71.2	71.7
5‴	77.4	77.3	77.3	77.4	77.4	77.9
6‴	62.1	61.9	61.8	62.1	61.9	62.8
2‴′-*O*-*α*-L-Rhamnopyranosyl						
1‴′	102.3	102.3	102.2	102.3	102.4	102.1
2‴′	72.4	72.5	72.5	72.4	72.5	72.6
3‴′	72.5	72.5	72.5	72.5	72.5	72.3
4‴′	73.9	73.9	73.7	73.9	73.9	73.9
5‴′	69.8	69.7	69.7	69.8	69.8	70.3
6‴′	18.2	18.2	18.1	18.2	18.2	17.9

a) Measured in pyridine-d_5. *b*) Measured in CD_3OD. *c*) **2**: 22-*O*-Tig; **4**: 28-*O*-Tig.

tion of the new saponins (**1**—**5**) and the inhibitory effects of the saponin constituents on the release of *β*-hexosaminidase from RBL-2H3 cells.

The methanolic extract (10.41% from the fresh flower buds of *C. sasanqua* cultivated in Kyoto province in Japan) with the inhibitory effect on the release of *β*-hexosaminidase from RBL-2H3 cells [inhibition (%): 36.7±1.5 ($p<0.01$) at 100 *μ*g/ml] was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction (1.51%) and aqueous layer. The aqueous layer was further extracted with 1-butanol to give a 1-butanol- (3.90%) and an H_2O- (5.00%) soluble fractions. The 1-butanol-soluble fraction inhibited the degranulation in RBL-2H3 cells [inhibition (%) 53.3±2.9 ($p<0.01$) at 30 *μ*g/ml], but the EtOAc- and the H_2O-soluble fractions showed weak [inhibition (%): 15.2±2.2 ($p<0.01$) at 100 *μ*g/ml] or no activity, respectively. The 1-butanol-soluble fraction was subjected to HP-20 and reversed-phase ODS column chromatographies and repeated HPLC to give sasanquasaponins I (**1**, 0.0032%), II (**2**, 0.015%), III (**3**, 0.0057%), IV (**4**, 0.0010%), and V (**5**, 0.0012%) together with **6** (0.0037%).

Structures of Sasanquasaponins I—V (1—5) Sasanquasaponin I (**1**) was isolated as colorless fine crystals of mp 213.0—215.0 °C (from aqueous MeOH) with negative optical rotation ($[\alpha]_D^{20}$ −24.6° in MeOH). The IR spectrum of **1** showed absorption bands at 3370, 1720, 1698, 1075, and 1047 cm^{-1} due to hydroxy, *α*,*β*-unsaturated ester, carboxy and ether functions. In the positive-ion FAB-MS of **1**, a quasimolecular ion peak $(M+Na)^+$ was observed at *m*/*z* 1255 and the molecular formula $C_{60}H_{96}O_{26}$ was determined by high-resolution MS measurement of the quasimolecular ion peak. Treatment of **1** with 10% aqueous KOH–50% aqueous 1,4-dioxane (1 : 1) mixture liberated a known compound, desacyl-boninsaponin A (**1a**).[16] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **1**, which were assigned by various NMR experiments,[17] showed signals assignable to a A_1-

barrigenol part: seven methyls [δ 0.79, 1.01, 1.05, 1.07, 1.12, 1.27, 1.87 (all s, H_3-25, 26, 29, 24, 23, 30, 27)], a methylene [δ 3.59, 3.76 (both d, J=10.3 Hz, H_2-28)] and three methines bearing an oxygen function [δ 3.21 (dd, J=5.0, 12.0 Hz, H-3), 4.24 (d-like, H-15), 6.18 (dd, J=5.5, 11.7 Hz, H-22)], an olefin [δ 5.49 (br s, H-12)], and four glycopyranosyl moieties {β-D-glucuronopyranosyl [δ 4.84 (d, J=7.0 Hz, H-1′)], β-D-glucopyranosyl [δ 5.94 (d, J=7.6 Hz, H-1″)], β-D-galactopyranosyl [δ 6.26 (d, J=7.0 Hz, H-1‴)], and an α-L-rhamnopyranosyl [δ 6.24 (br s, H-1⁗)] together with a *cis*-2-hexenoyl group [δ 0.84 (t, J=7.6 Hz, H_3-6⁗)], 1.35 (m, H_2-5⁗), 2.71, 2.78 (both m, H_2-4⁗), 5.79 (d, J=11.0 Hz, H-2⁗), 6.09 (td, J=7.6, 11.0 Hz, H-3⁗)}.[18] The position of the acyl group and the structure of the oligoglycoside moiety were confirmed on the basis of a heteronuclear multiple bond connectivity spectroscopy (HMBC) experiment. Thus, the long-range correlations were observed between the following proton and carbon pairs: 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 **1** with those for **1a** revealed an acylation shift around the 22-position of the A_1-barrigenol part. On the basis of the above mentioned evidence, the chemical structure of sasanquasaponin I was determined to be 22-*O*-*cis*-2-hexenoyl-A_1-barrigenol 3-*O*-[β-D-glucopyranosyl(1→2)][α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→3)]-β-D-glucuronopyranoside (**1**).

Sasanquasaponins II (**2**), III (**3**), and IV (**4**), which were obtained as colorless fine crystals [**2**: mp 219.5—221.0 °C, **3**: mp 227.0—229.0 °C, **4**: mp 215.0—217.0 °C (from aqueous MeOH)] with negative optical rotation (**2**: $[\alpha]_D^{20}$ −18.2°, **3**: $[\alpha]_D^{21}$ −18.5°, **4**: $[\alpha]_D^{20}$ −31.9° in MeOH), showed absorption bands due to hydroxy, α,β-unsaturated ester, carboxy, and ether functions in their IR spectra. The common molecular formula $C_{59}H_{94}O_{26}$ of **2**, **3**, and **4** was determined individually from the quasimolecular ion peak [*m*/*z* 1241 $(M+Na)^+$] in the positive-ion FAB-MS and by high-resolution MS measurement. Alkaline treatment of **2**, **3**, and **4** yielded desacyl-boninsaponin A (**1a**) and organic acids (tiglic acid from **2** and **4**; angelic acid from **3**), which were identical by HPLC analysis of their *p*-nitrobenzyl derivatives.[19] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[17] of **2** indicated the presence of desacyl-boninsaponin A part and a tigloyl group [δ 1.43 (d, J=6.9 Hz, H_3-4⁗), 1.78 (s, H_3-5⁗), 6.84 (q, J=6.9 Hz, H-3⁗)]. On the other hand, the proton and carbon signals in the ^{1}H- and ^{13}C-NMR (Table 1) spectra[17] of **3** were superimposable on those of **2**, except for the signals due to the 22-acyl group of **3**. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR spectra of **3** showed signals assignable to an angeloyl group [δ 1.82 (s, H_3-5⁗), 2.02 (d, J=6.9 Hz, H_3-4⁗), 5.83 (q, J=6.9 Hz, H-3⁗)] together with a desacyl-boninsaponin A part. The positions of the both acyl groups in **2** and **3** were clarified by HMBC experiments, which showed long-range correlations between the 22-protons and the carbonyl carbons of the tigloyl and angeloyl groups. Furthermore, comparison of the ^{1}H- and ^{13}C-NMR data for **2** and **3** with those for **1a** revealed an acylation shift around the 22-postion of the A_1-barrigenol part. Consequently, the chemical structures of sasanquasaponins II (**2**) and III (**3**) were characterized to be as shown. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra of **4** was also similar to those of **2**, except for the signals due to the 22- and 28-positions. The position of tigloyl group in **4** was confirmed by a HMBC experiment, which showed a long-range correlation between the 28-methylene protons and the carbonyl carbon of tigloyl group. Comparison of the NMR data for **4** with those for **2** and **1a** led us to confirm the structure of **4** to be the 28-tigloyl isomer of **2**.

Sasanquasaponin V (**5**), obtained as colorless fine crystals (mp 224.0—226.0 °C from aqueous MeOH) with negative optical rotation ($[\alpha]_D^{21}$ −32.4° in MeOH), showed absorption bands due to hydroxy, ester, carboxy and ether functions in the IR spectrum (3370, 1730, 1717, 1078, 1047 cm^{-1}). The positive FAB-MS of **5** showed a quasimolecular ion peak at *m*/*z* 1227 $(M+Na)^+$ and the molecular formula $C_{59}H_{96}O_{25}$ was determined by high-resolution MS measurement. Alkaline hydrolysis of **5** provided ternstroemiaside A (**5a**)[20] and (*S*)-(+)-2-methylbutyric acid, which was identified by HPLC analysis using an optical rotation detector. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **5** showed signals due to a ternstroemiaside A part and (*S*)-(+)-2-methylbutyroyl group [δ 1.00 (t, J=7.6 Hz, H_3-4⁗), 1.30 (d, J=6.9 Hz, H_3-5⁗), 1.63, 1.94 (both m, H_2-3⁗), 2.59 (m, H-2⁗)]. The position of the acyl group in **5** was elucidated by a HMBC experiment and comparison the ^{13}C-NMR data for **5** with those for **5a**,[20] so that the structure of sasanquasaponin V (**5**) was characterized to be as shown.

Inhibitory Effects of Saponin Constituents (1—6) on the Release of β-Hexosaminidase from RBL-2H3 Cells In the course of our studies on antiallergic constituents from natural medicines,[21—32] we previously reported that some triterpenes and oleanane-type triterpene oligoglycosides showed inhibitory effects on histamine release from rat exudate cells induced by an antigen–antibody reaction[33,34] and on β-hexosaminidase release induced by dinitrophenylated bovine serum albumin (DNP-BSA) from RBL-2H3 cells sensitized with anti-DNP immunoglobulin E (IgE).[8,14] As a continuation of this study, we examined the effects of acylated oleanane-type triterpene saponins, sasanquasaponins, on the release of β-hexosaminidase from RBL-2H3 cells. As shown in Table 2, sasanquasaponins I—III (**1**—**3**) with the 22-acyl group were found to exhibit strong activities regardless of the structures of their acyl groups. Whereas sasanquasaponin V (**5**) with the 16-acyl group showed weak activity, and also sasanquasaponin IV (**4**) with the 28-acyl group and **6** showed no activity. As a result, the 22-acyl group in the saponin structures seems to be essential for the potent inhibitory effect on β-hexosaminidase release from RBL-2H3 cells.

In conclusion, five new acylated oleanane-type triterpene saponins, sasanquasaponins I—V (**1**—**5**), were isolated from the flower buds of *C. sasanqua* and their chemical structures were elucidated on the basis of chemical and physicochemical evidence. In addition, sasanquasaponins I—III (**1**—**3**) having the 22-acyl group were found to inhibit the β-hexosaminidase release from RBL-2H3.

Experimental

General Experimental Procedures The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); IR spectra, Shimadzu Fourier transform-infrared (FT-IR)-8100 spectrometer; electron ionization-mass spectra (EI-MS) and high resolution (HR)-EI-MS, JEOL JMS-GCMATE mass spectrometer; FAB-MS and HR-FAB-MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL JNM-EX 270 (270 MHz), JEOL JNM-LA 500 (500 MHz), and JEOL JNM-ECA 600 (600 MHz) spectrometers; ^{13}C-NMR spectra, JEOL

Table 2. Inhibitory Effects of Sasanquasaponins (**1**—**5**) and **6** from *C. sasanqua* on the Release of β-Hexosaminidase from RBL-2H3 Cells

	Conc.(μM)	Inhibition (%)
Sasanquasaponin I (**1**)	0.3	11.6±5.2
	1	41.3±2.9**
	3	36.5±1.4**
Sasanquasaponin II (**2**)	0.3	10.7±4.6
	1	17.9±5.6**
	3	40.2±1.7**
Sasanquasaponin III (**3**)	0.3	14.9±6.0
	1	24.4±3.0*
	3	51.6±3.0**
Sasanquasaponin IV (**4**)	1	9.4±4.5
	3	2.4±4.7
	6	−13.1±5.7
Sasanquasaponin V (**5**)	3	0.7±3.2
	6	27.0±7.2**
	10	39.4±4.4**
6	3	−5.6±4.1
	6	−8.3±6.3
	10	−4.6±2.1
Tranilast[32]	30	25.4±4.3**
	100	44.5±4.4**
Ketorifen fumarate[32]	30	10.8±5.4
	100	30.6±4.8**

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

JNM-EX 270 (68 MHz) JEOL JNM-LA (125 MHz), and JEOL JNM-ECA 600 (150 MHz) spectrometers with tetramethylsilane as an internal standard; HPLC detector, Shimadzu RID-10A refractive index detector; and HPLC column, COSMOSIL 5C18-MS-II (250×4.6 mm i.d.) and (250×20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

The following experimental materials 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) (ordinary 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); and detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 followed by heating.

Plant Material *C. sasanqua* was cultivated in the medicinal plant garden of Kyoto Pharmaceutical University and the flower buds were collected in February, 2008. A voucher of the plant is on file in our laboratory (2008. KPU-CS).

Extraction and Isolation The fresh flower buds (4.0 kg) were extracted three times with methanol under reflux for 3 h. Evaporation of the solvent under reduced pressure provided a methanolic extract (417.7 g, 10.41%). The MeOH extract (400 g) was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction (57.9 g, 1.51%) and an aqueous phase. The aqueous phase was further extracted with 1-butanol to give a 1-butanol-soluble fraction (149.5 g, 3.90%) and an H_2O-solbule fraction (191.7 g, 5.00%). A part of the 1-butanol-soluble fraction (143.5 g) was subjected to HP-20 column chromatography (H_2O–MeOH–acetone) to furnish an H_2O-eluted fraction (88.2 g, 2.40%), a MeOH-eluted fraction (54.1 g, 1.47%), and an acetone-eluted fraction (0.9 g, 0.024%). The MeOH-elutaed fraction (54.1 g) was subjected to reversed phase silica gel column chromatography [H_2O→MeOH–H_2O (10 : 90→20 : 80→30 : 40→40 : 60→50 : 50→60 : 40→70 : 30→80 : 20→90 : 10, v/v)→MeOH] to give seven fractions [Fr. 1 (0.83 g), Fr. 2 (3.28 g), Fr. 3 (27.96 g), Fr. 4 (6.14 g), Fr. 5 (8.89 g), Fr. 6 (0.88 g), Fr. 7 (0.64 g)]. Fraction 5 (8.89 g) was further separated by reversed phase silica gel column chromatography [MeOH : H_2O (50 : 50→60 : 40→70 : 30→80 : 20→90 : 10)→MeOH] to give eight fractions [Fr. 5-1, Fr. 5-2, Fr. 5-3, Fr. 5-4, Fr. 5-5 (3.69 g), Fr. 5-6 (3.05 g), Fr. 5-7, Fr. 5-8]. A part of fraction 5-5 (750 mg) was purified by HPLC [MeOH in 1% AcOH : H_2O (68 : 32)] to furnish sasanquasaponin II (**2**, 109 mg, 0.015%), III (**3**, 42 mg, 0.0057%), and **6** (28 mg, 0.0037 %). A part of fraction 5-6 (1.50 g) was purified by HPLC [MeOH in 1% AcOH : H_2O (73 : 27)] to give sasanquasaponin I (**1**, 57 mg, 0.0032%), IV (**4**, 18 mg, 0.0010%), and V (**5**, 23 mg, 0.0012%).

Sasanquasaponin I {=22-*O*-*cis*-2-Hexenoyl-A_1-barrigenol 3-*O*-[β-D-Glucopyranosyl(1→2)][α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→3)]-β-D-glucuronopyranoside, **1**}: Colorless fine crystals; mp 213.0—215.0 °C; $[\alpha]_D^{20}$ −24.6° (c=0.85, MeOH); IR (KBr) ν_{max}: 3370, 1720, 1698, 1075, 1047 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 600 MHz) δ: 0.79, 1.01, 1.05, 1.07, 1.12, 1.27, 1.87 (all s, H_3-25, 26, 29, 24, 23, 30, 27), 0.84 (t, J=7.6 Hz, H_3-6‴‴), 1.35 (m, H_2-5‴‴), 2.71, 2.78 (both m, H_2-4‴‴), 3.21 (1H, dd, J=5.0, 12.0 Hz, H-3), 3.59, 3.76 (both d, J=10.3 Hz, H_2-28), 4.24 (1H, d-like, H-15), 4.84 (d, J=7.0 Hz, H-1′), 5.49 (1H, br s, H-12), 5.79 (d, J=11.0 Hz, H-2‴‴), 5.94 (1H, d, J=7.6 Hz, H-1″), 6.09 (td, J=7.6, 11.0 Hz, H-3‴‴), 6.18 (1H, dd, J=5.5, 11.7 Hz, H-22), 6.24 (1H, br s, H-1‴‴), 6.26 (1H, d, J=7.0 Hz, H-1‴); ^{13}C-NMR: given in Table 1; positive-ion FAB-MS m/z: 1255 $[M+Na]^+$; high-resolution positive-ion FAB-MS m/z: 1255.6080 (Calcd for $C_{60}H_{96}O_{26}Na$ $[M+Na]^+$: m/z 1255.6088).

Sasanquasaponin II {=22-*O*-Tigloyl-A_1-barrigenol 3-*O*-[β-D-Glucopyranosyl(1→2)][α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→3)]-β-D-glucuronopyranoside, **2**}: Colorless fine crystals; mp 219.5—221.0 °C; $[\alpha]_D^{29}$ −18.2° (c=0.43, MeOH); IR (KBr) ν_{max}: 3370, 1720, 1684, 1075, 1045 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 600 MHz) δ: 0.80, 1.02, 1.06, 1.06, 1.14, 1.28, 1.87 (all s, H_3-25, 26, 29, 24, 23, 30, 27), 1.43 (3H, d, J=6.9 Hz, H_3-4‴‴), 1.78 (3H, s, H_3-5‴‴), 3.19 (1H, dd, J=5.0, 11.0 Hz, H-3), 3.61, 3.77 (both d, J=10.3 Hz, H_2-28), 4.24 (1H, d-like, H-15), 4.84 (1H, d, J=7.0 Hz, H-1′), 5.47 (1H, br s, H-12), 5.92 (1H, d, J=7.4 Hz, H-1″), 6.17 (1H, dd, J=5.5, 11.7 Hz, H-22), 6.21 (1H, d, J=7.6 Hz, H-1‴), 6.25 (1H, br s, H-1‴‴), 6.84 (1H, q, J=6.9 Hz, H-3‴‴); ^{13}C-NMR: given in Table 1; positive-ion FAB-MS m/z: 1241 $[M+Na]^+$; high-resolution positive-ion FAB-MS m/z: 1241.5923 (Calcd for $C_{59}H_{94}O_{26}Na$ $[M+Na]^+$: m/z 1241.5931).

Sasanquasaponin III {=22-*O*-Angeloyl-A_1-barrigenol 3-*O*-[β-D-Glucopyranosyl(1→2)][α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→3)]-β-D-glucuronopyranoside, **3**}: Colorless fine crystals; mp 227.0—229.0 °C; $[\alpha]_D^{21}$ −18.5° (c=0.72, MeOH); IR (KBr) ν_{max}: 3370, 1720, 1699, 1076, 1043 cm^{-1}: ^{1}H-NMR (pyridine-d_5, 600 MHz) δ: 0.79, 1.00, 1.05, 1.06, 1.12, 1.28, 1.86 (all s, H_3-25, 26, 29, 24, 23, 30, 27), 1.82 (3H, s, H_3- 5‴‴), 2.02 (3H, d, J=6.9 Hz, H_3-4‴‴), 3.18 (1H, dd, J=5.0, 11.0 Hz, H-3), 3.63, 3.79 (1H each, both d, J=10.3 Hz, H_2-28), 4.25 (1H, d-like, H-15), 4.89 (d, J=7.0 Hz, H-1′), 5.46 (1H, br s, H-12), 5.83 (1H, q, J=6.9 Hz, H-3‴‴), 5.93 (d, J=7.0 Hz, H-1″), 6.19 (1H, dd, J=5.5, 11.7 Hz, H-22), 6.22 (d, J=7.6 Hz, H-1‴), 6.25 (br s, H-1‴‴); ^{13}C-NMR: given in Table 1; positive-ion FAB-MS m/z: 1241 $[M+Na]^+$; high-resolution positive-ion FAB-MS m/z: 1241.5923 (Calcd for $C_{59}H_{94}O_{26}Na$ $[M+Na]^+$: m/z 1241.5931).

Sasanquasaponin IV {=28-*O*-Tigloyl-A_1-barrigenol 3-*O*-[β-D-Glucopyranosyl(1→2)][α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→3)]-β-D-glucuronopyranoside, **4**}: Colorless fine crystals; mp 215.0—217.0 °C; $[\alpha]_D^{21}$ −31.9° (c=0.17, MeOH); IR (KBr) ν_{max}: 3370, 1716, 1697, 1078, 1045 cm^{-1}: ^{1}H-NMR (pyridine-d_5, 600 MHz) δ: 0.79, 1.05, 1.09, 1.12, 1.15, 1.21, 1.87 (all s, H_3-25, 24, 29, 23, 26, 30, 27), 1.60 (3H, d, J=6.8 Hz, H_3-4‴‴), 1.84 (3H, s, H_3-5‴‴), 3.19 (1H, dd, J=5.0, 11.7 Hz, H-3), 4.38, 4.61 (1H each, both d, J=11.0 Hz, H_2-28), 4.35 (1H, d-like, H-15), 4.59 (1H, dd, J=5.5, 11.7 Hz, H-22), 4.91 (d, J=7.0 Hz, H-1′), 5.52 (1H, br s, H-12), 5.94 (d, J=7.4 Hz, H-1″), 6.25 (d, J=7.6 Hz, H-1‴), 6.26 (br s, H-1‴‴), 7.00 (1H, q, J=6.9 Hz, H-3‴‴); ^{13}C-NMR: given in Table 2; positive-ion FAB-MS m/z: 1241 $[M+Na]^+$; high-resolution positive-ion FAB-MS m/z: 1241.5933 (Calcd for $C_{59}H_{94}O_{26}Na$ $[M+Na]^+$: m/z 1241.5931).

Sasanquasaponin V {=16-*O*-(*S*)-(+)-2-Methylbutyroyl-camelliagenin A 3-*O*-[β-D-Glucopyranosyl(1→2)][α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→3)]-β-D-glucuronopyranoside, **5**}: Colorless fine crystals; mp 224.0—226.0 °C; $[\alpha]_D^{21}$ −32.4° (c=0.31, MeOH); IR (KBr) ν_{max}: 3370, 1730, 1717, 1078, 1047 cm^{-1}: ^{1}H-NMR (pyridine-d_5, 600 MHz) δ: 0.75, 0.78, 1.05, 1.10, 1.12, 1.16, 1.54 (all s, H_3-25, 26, 24, 29, 30, 23, 27), 1.00 (3H, t, J=7.6 Hz, H_3-4‴‴), 1.30 (3H, d, J=6.9 Hz, H_3-5‴‴), 1.63, 1.94 (1H each, both m, H_2-3‴‴), 2.59 (1H, m, H-2‴‴), 3.17 (1H, dd, J=4.9, 11.1 Hz, H-3), 3.66, 4.04 (1H each, both d, J=10.3 Hz, H_2-28), 4.09 (1H, dd like, H-22), 4.90 (d, J=7.0 Hz, H-1′), 5.32 (1H, br s, H-12), 5.94 (d, J=7.7 Hz, H-1″), 6.22 (m, H-16), 6.22 (d, J=7.6 Hz, H-1‴), 6.25 (br s, H-1‴‴); ^{13}C-NMR: given in Table 2; positive-ion FAB-MS m/z: 1227 $[M+Na]^+$; high-resolution positive-ion FAB-MS m/z: 1227.6143 (Calcd for $C_{59}H_{96}O_{25}Na$ $[M+Na]^+$: m/z 1227.6138).

Alkaline Hydrolysis of Sasanquasaponins I—V (1—5) A solution of sasanquasaponins I—V (**1**: 10 mg; **2**–**5**: each 6.0 mg) was treated with 10% aqueous KOH–1,4-dioxane (1 : 1, v/v, 1.0 ml) and the whole was stirred at 37 °C for 2 h, respectively. The reaction mixture was neutralized with Dowex HCR W2 (H^+ form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure yielded a crude product, whose most part was subjected to ordinary-phase silica gel column chro-

matography [$CHCl_3$–MeOH–H_2O (10 : 3 : 1→6 : 4 : 1, v/v/v)] to give **1a** (5.1 mg from **1**, 2.5 mg, 2.3 mg, 2.3 mg from **2**—**4**, respectively) or **5a** (2.4 mg from **5**). The desacyl-derivatives (**1a**, **5a**) were identified by comparison of their physical data with those of the reported values. A part (each 1.0 mg) of crude product obtained from **2**—**4** was dissolved in $(CH_2)_2Cl_2$ (0.5 ml), respectively. The solution was treated with *p*-nitrobenzyl-*N*-*N*′-diisopyopylisourea (4 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; column temperature: room temperature] to identify the *p*-nitrobenzyl esters of tiglic acid (t_R 30.0 min, from **2**, **4**) and angelic acid (t_R 33.9 min, from **3**). On the other hand, a part (1.0 mg) of crude product obtained from **5** was subjected to HPLC analysis [column: COSMOSIL 5C18-MS-II, 250×4.6 mm i.d.; mobile phase: MeOH in 1% AcOH–H_2O (50 : 50, v/v); detection: optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan)]; flow rate: 0.8 ml/min; column temperature: room temperature] to identify the (*S*)-(+)-2-methylbutyric acid. Identification of (*S*)-(+)-2-methylbutyric acid in the crude product obtained from **5** was carried out by comparison of its retention time and optical rotation with that of an authentic sample [t_R: 9.2 min (positive optical rotation), commercial sample (Sigma-Aldrich, Japan)].

Bioassay. Effects on the Release of β-Hexosaminidase from RBL-2H3 Cells The inhibitory effects of the test samples on the release of β-hexosaminidase from RBL-2H3 cells [Cell No. JCRB0023, obtained from Health Science Research Resources Bank (Osaka, Japan)] were evaluated by a method reported previously[21] with some modifications. Briefly, RBL-2H3 cells were dispensed into 48-well plates at a concentration of 4×104 cells/well using Eagle's minimum essential medium (MEM, Sigma) containing fetal calf serum (10%), penicillin (100 units/ml), streptomycin (100 μg/ml), and 0.45 μg/ml of anti-DNP IgE, and these were incubated overnight at 37 °C in 5% CO_2 for sensitization of the cells. Then the cells were washed twice with 200 μl of Siraganian buffer [119 mM NaCl, 5 mM KCl, 0.4 mM $MgCl_2$, 25 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES), and 40 mM NaOH, pH 7.2], and incubated in 80 μl of Siraganian buffer [5.6 mM glucose, 1 mM $CaCl_2$, and 0.1% bovine serum albmin (BSA) were added] for an additional 10 min at 37 °C. Aliquots (10 μl) of test sample solution were added to each well and incubated for 10 min, followed by the addition of 10 μl of antigen (DNP-BSA, final concentration 10 μg/ml) at 37 °C for 10 min to stimulate the cells to evoke allergic reactions (degranulation). The reaction was stopped by cooling in an ice bath for 10 min. The supernatant (40 μl) was transferred into a 96-well microplate and incubated with 40 μl of substrate (1 mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide) in 0.1 M citrate buffer (pH 4.5) at 37 °C for 2 h. The reaction was stopped by adding 200 μl of stop solution (0.1 M Na_2CO_3/$NaHCO_3$, pH 10.0). The absorbance was measured using a microplate reader at 405 nm. The test sample was dissolved in dimethylsulfoxide (DMSO), and the solution was added to Siraganian buffer (final DMSO concentration 0.1%).

The percent inhibition of the release of β-hexosaminidase by the test material was calculated using the following equation:

$$\text{inhibition (\%)} = [1-(T-B-N)/(C-N)]\times 100$$

Control (*C*): DNP-BSA (+), test sample (−); test (*T*): DNP-BSA (+), test sample (+); blank (*B*): DNP-BSA (−), test sample (+); normal (*N*): DNP-BSA (−), test sample (−).

Under these conditions, it was calculated that 10—15% of β-hexosaminidase was released from the cells in the control groups by determination of the total β-hexosaminidase activity after treatment with 0.05% Triton X-100.

In order to clarify that the antiallergic effects of samples were due to the inhibition on hexosaminidase release, but not the false positive by the inhibition of β-hexosaminidase activity, the following assay was carried out. The supernatant (36 μl) of the control group as an enzyme solution, the substrate solution (40 μl), and test sample solution (4 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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References and Notes

1) Part XXX: Morikawa T., Wang L. B., Ninomiya K., Nakamura S., Matsuda H., Muraoka O., Wu L. J., Yoshikawa M., *Chem. Pharm. Bull.*, **57**, 853—859 (2009).
2) Yoshida T., Chou T., Maruyama Y., Okuda T., *Chem. Pharm. Bull.*, **38**, 2681—2686 (1990).
3) Saito N., Yokoi M., Yamaji M., Honda T., *Phytochemistry*, **26**, 2761—2762 (1987).
4) Fujimori N., Ashihara H., *Phytochemistry*, **29**, 3513—3516 (1990).
5) Yoshikawa M., Morikawa T., Fujiwara E., Ohgushi T., Asao Y., Matsuda H., *Heterocycles*, **55**, 1653—1657 (2001).
6) Yoshikawa M., Morikawa T., Asao Y., Fujiwara E., Nakamura S., Matsuda H., *Chem. Pharm. Bull.*, **55**, 606—612 (2007).
7) Yoshikawa M., Morikawa T., Yamamoto K., Kato Y., Nagatomo A., Matsuda H., *J. Nat. Prod.*, **68**, 1360—1365 (2005).
8) Yoshikawa M., Nakamura S., Kato Y., Matsuhira K., Matsuda H., *Chem. Pharm. Bull.*, **55**, 598—605 (2007).
9) Yoshikawa M., Wang T., Sugimoto S., Nakamura S., Nagatomo A., Matsuda H., Harima S., *Yakugaku Zasshi*, **128**, 141—151 (2008).
10) Yoshikawa M., Sugimoto S., Nakamura S., Matsuda H., *Chem. Pharm. Bull.*, **56**, 1297—1303 (2008).
11) Sugimoto S., Yoshikawa M., Nakamura S., Matsuda H., *Heterocycles*, **78**, 1023—1029 (2009).
12) Yoshikawa M., Sugimoto S., Kato Y., Nakamura S., wang T., Yamashita C., Matsuda H., *Chem. Biodiv.*, **6**, 903—915 (2009).
13) Sugimoto S., Chi G., Kato Y., Nakamura S., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **57**, 269—275 (2009).
14) Morikawa T., Nakamura S., Kato Y., Muraoka O., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **55**, 293—298 (2007).
15) Ohtani K., Mavi S., Hostettmann K., *Phytochemistry*, **33**, 83—86 (1993).
16) Kitagawa I., Yoshikawa M., Kobayashi K., Imakura Y., Im K. S., Ikenishi Y., *Chem. Pharm. Bull.*, **28**, 296—300 (1980).
17) The ^{1}H- and ^{13}C-NMR spectra of **1**—**5** were assigned with the aid of distortionless enhancement by polarization transfer (DEPT), double quantum filter correlation spectroscopy (DQF COSY), heteronuclear multiple quantum coherence spectroscopy (HMQC), and heteronuclear multiple bond connectivity spectroscopy (HMBC) experiments.
18) Jiang Z., Gallard J.-F., Adeline M.-T., Dumontet V., Tri M. V., Sévenet T., Païs M., *J. Nat. Prod.*, **62**, 873—876 (1999).
19) Morikawa T., Li X., Nisida E., Nakamura S., Ninomiya K., Matsuda H., Oda Y., Muraoka O., Yoshikawa M., *Helv. Chim. Acta*, **93**, 573—587 (2010).
20) Shin M., Wang W., Nam K. I., Jo Y., Jung H. J., Im S. K., *J. Nat. Prod.*, **66**, 1351—1355 (2003).
21) Matsuda H., Morikawa T., Ueda K., Managi H., Yoshikawa M., *Bioorg. Med. Chem.*, **10**, 3123—3128 (2002).
22) Matsuda H., Morikawa T., Tao J., Ueda K., Yoshikawa M., *Chem. Pharm. Bull.*, **50**, 208—215 (2002).
23) Morikawa T., Matsuda H., Toguchida I., Veda K., Yoshikawa M., *J. Nat. Prod.*, **65**, 1468—1474 (2002).
24) Morikawa T., Matsuda H., Sakamoto Y., Ueda K., Yoshikawa M., *Chem. Pharm. Bull.*, **50**, 1045—1049 (2002).
25) Morikawa T., Tao J., Ueda K., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **51**, 62—67 (2003).
26) Tao J., Morikawa T., Ando S., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **51**, 654—662 (2003).
27) Matsuda H., Morikawa T., Xie H., Yoshikawa M., *Planta Med.*, **70**, 847—855 (2003).
28) Matsuda H., Morikawa T., Mangi H., Yoshikawa M., *Bioorg. Med. Chem. Lett.*, **13**, 3197—3202 (2003).
29) Matsuda H., Tewtrakul S., Morikawa T., Nakamura S., Yoshikawa M., *Bioorg. Med. Chem.*, **12**, 5891—5989 (2004).
30) Sun B., Morikawa T., Matsuda H., Tewtrakal S., Wu L. J., Harima S., Yoshikawa M., *J. Nat. Prod.*, **67**, 1464—1469 (2004).
31) Morikawa T., Sun B., Matasuda H., Wu L. J., Harima S., Yoshikawa M., *Chem. Pharm. Bull.*, **52**, 1194—1199 (2004).
32) Xu F., Matsuda H., Hata H., Sugawara K., Nakamura S., Yoshikawa M., *Chem. Pharm. Bull.*, **57**, 1089—1095 (2009).
33) Yoshikawa M., Shimada H., Komatsu H., Sakurama T., Nishida N., Shimoda H., Matsuda H., Tani T., *Chem. Pharm. Bull.*, **45**, 877—882 (1997).
34) Yoshizumi S., Murakami T., Kadoya M., Matsuda H., Yoshikawa M., *Yakugaku Zasshi*, **118**, 188—192 (1998).