

Bioactive Saponins and Glycosides. V.¹⁾ Acylated Polyhydroxyolean-12-ene Triterpene Oligoglycosides, Camelliasaponins A₁, A₂, B₁, B₂, C₁, and C₂, from the Seeds of *Camellia japonica* L.: Structures and Inhibitory Activity on Alcohol Absorption

Masayuki YOSHIKAWA,* Toshiyuki MURAKAMI, Satoshi YOSHIKAWA, Nobutoshi MURAKAMI, Johji YAMAHARA, and Hisashi MATSUDA

Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607, Japan.

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Acylated polyhydroxyolean-12-ene triterpene oligoglycosides, camelliasaponins A₁, A₂, B₁, B₂, C₁, and C₂, were isolated from the seeds of *Camellia japonica* L. The structures of six camelliasaponins were elucidated on the basis of chemical and physicochemical evidence. Camelliasaponins B₁, B₂, C₁, and C₂ were found to exhibit inhibitory activity on ethanol absorption. By comparison of the inhibitory activities for camelliasaponins with those for desacyl-camelliasaponins, acyl groups such as the angeloyl or tigloyl group were found to be essential to exerting the activity.

Key words alcohol absorption inhibitor; camelliasaponin A₁; camelliasaponin B₁; camelliasaponin C₁; *Camellia japonica*; acylated polyhydroxyolean-12-ene oligoglycoside

The seeds of *Camellia japonica* L. (camellia, Theaceae, Japanese named "tsubaki") have been used as a stomachic and anti-inflammatory in Japanese folk medicine and also as an oil material, while the flower of this plant has been prescribed in Chinese traditional preparations for the treatment of hematemesis and "Oketsu" syndrome (blood stagnation). The chemical constituents of the flower and leaves of camellia have been studied extensively, and many compounds such as flavonols, sterols, 28-nor-oleanene triterpenes, and their oligoglycosides have been reported.²⁾ In regard to the seeds of camellia, three sapogenols, camelliagenins A (12), B (15), and C (18), which were obtained by alkaline hydrolysis followed by acid hydrolysis of the glycoside mixture from the fruit, have been characterized.³⁾

In the course of our studies on the bioactive constituents of natural medicines,⁴⁾ we have reported many saponins from *Aralia elata* SEEM. (Japanese angelica tree, Araliaceae, root cortex, bark, and young shoot),⁵⁾ *Sapindus mukurossi* (Japanese soapnut tree, Sapindaceae, pericarps),⁶⁾ *Aescu-*

lus hippocastanum L. (horse chestnut tree, Hippocastanaceae, seeds),⁷⁾ *Polygala senega* L. var. *latifolia* TORREY et GRAY (Japanese senega snakeroot, Polygalaceae, roots),⁸⁾ *Beta vulgaris* L. (sugar beet, Chenopodiaceae, root and leaves),⁹⁾ and *Gymnema sylvestre* R. B_R (gymnema, Asclepiadaceae, leaves),¹⁰⁾ which exhibited inhibitory activity on ethanol absorption and a hypoglycemic effect. As a continuing study to find saponin constituents with inhibitory activity on ethanol absorption, we have found that the methanolic extract obtained from the defatted seeds of camellia has an inhibitory activity on ethanol absorption in rats. Through bioassay-guided separation, we have isolated six acylated polyhydroxyolean-12-ene triterpene oligoglycosides named camelliasaponins A₁ (1), A₂ (2), B₁ (4),¹¹⁾ B₂ (5),¹¹⁾ C₁ (7),¹¹⁾ and C₂ (8)¹¹⁾ from the seeds of camellia. In this paper, we present a full account of the structural elucidation of camelliasaponins (1, 2, 4, 5, 7, 8), as well as the inhibitory activity of camelliasaponins B₁ (4), B₂ (5), C₁ (7), and C₂ (8) and desacyl-camelliasaponins B (6) and C (9) on ethanol ab-

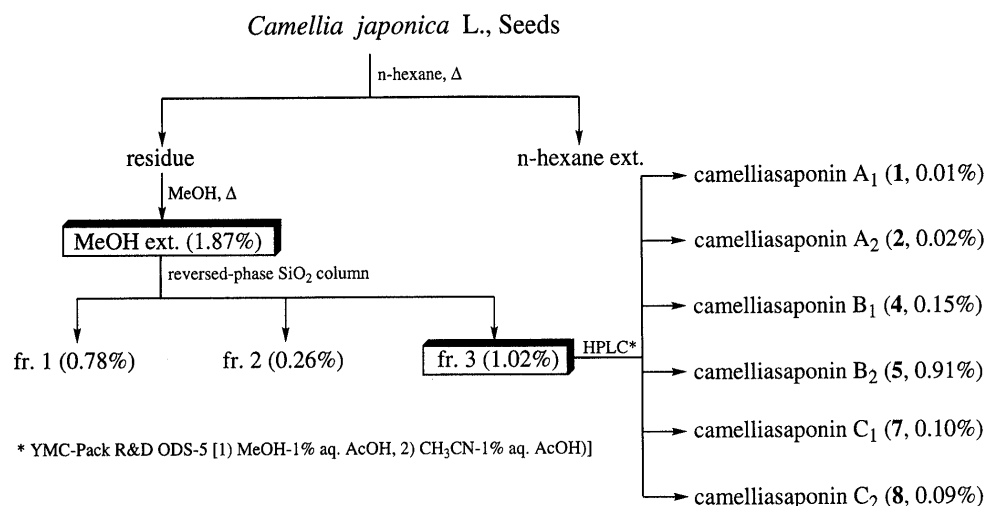


Chart 1

* To whom correspondence should be addressed.

Table 1. Inhibitory Activity of the Methanol Extract and the Fractions from Camellia Seeds

	Dose (mg/kg, <i>p.o.</i>)	<i>n</i>	Ethanol concentration in blood (mg/ml)		
			1 h	2 h	3 h
Control		5	0.711 ± 0.063	—	0.133 ± 0.014
The MeOH extract	500	5	0.125 ± 0.031 ^{a)}	—	0.257 ± 0.066
Control		4	0.580 ± 0.009	0.264 ± 0.014	0.013 ± 0.008
Fr. 1	200	4	0.576 ± 0.013	0.273 ± 0.011	0.016 ± 0.006
Fr. 2	200	4	0.560 ± 0.010	0.250 ± 0.009	0.013 ± 0.000
Fr. 3 (saponin fraction)	200	4	0.261 ± 0.039 ^{a)}	0.167 ± 0.054	0.008 ± 0.001

a) $p < 0.01$.

sorption.¹²⁾

The seeds of camellia, which were collected in Kyoto Prefecture, were defatted with *n*-hexane and then extracted with methanol under reflux. Since the methanolic extract was found to inhibit ethanol absorption in rats after a single oral administration of 500 mg/kg dose, it was subjected to a bioassay-guided separation and purification through the procedures shown in Chart 1 and Table 1. Thus, the methanolic extract was separated by reversed-phase silica-gel column chromatography to give the saponin fraction (fraction 3). The saponin fraction showed potent inhibitory activity at a lower dose (200 mg/kg), while other fractions (fractions 1 and 2) were found to lack this activity. The saponin fraction was subjected to repeated HPLC separation to furnish camelliasaponins A₁ (**1**, 0.01% from the seeds), A₂ (**2**, 0.02%), B₁ (**4**, 0.15%), B₂ (**5**, 0.91%), C₁ (**7**, 0.10%), and C₂ (**8**, 0.09%).

Camelliasaponins A₁ (1) and A₂ (2) Camelliasaponin A₁ (**1**) was isolated as colorless fine crystals of mp 185.8–187.0 °C. The IR spectrum of **1** showed absorption bands at 1730, 1662, and 1647 cm⁻¹ assignable to carboxyl and α,β -unsaturated esters, and broad bands at 3432 and 1078 cm⁻¹ suggestive of a glycosidic structure. In the positive-mode FAB-MS of **1**, a quasimolecular ion peak was observed at m/z 1211 (M+Na)⁺, and high-resolution MS analysis revealed the molecular formula of **1** to be C₅₈H₉₂O₂₅. Alkaline hydrolysis of **1** with 10% aqueous potassium hydroxide–50% aqueous dioxane (1 : 1) provided desacyl-camelliasaponin A (**3**) and angelic acid. The organic acid was derived to the *p*-nitrobenzyl ester,¹³⁾ which was identified by HPLC analysis.

Desacyl-camelliasaponin A (**3**), whose molecular formula C₅₃H₈₆O₂₄ was determined by positive-mode FAB-MS [m/z 1129 (M+Na)⁺] and high-resolution MS measurement, liberated camelliagenin A (**12**) together with the methyl glycosides of D-glucuronic acid, D-galactose, L-arabinose, and D-glucose in a 1:1:1:1 ratio¹⁴⁾ on methanolysis with 9% hydrogen chloride. The ¹H-NMR (pyridine-*d*₅) and ¹³C-NMR (Table 2) spectra of **3**, which were assigned by various NMR experiments,¹⁵⁾ showed signals due to the camelliagenin A moiety [δ 4.48 (dd-like, 22-H)], and the tetrasaccharide moiety consisted of β -D-glucuronic acid [δ 4.94 (d, $J=7.6$ Hz, 1'-H)], β -D-galactopyranosyl [δ 5.65 (d, $J=7.6$ Hz, 1''-H)], α -L-arabinopyranosyl [δ 5.74 (d, $J=5.6$ Hz, 1'''-H)], and β -D-glucopyranosyl [δ 5.15 (d, $J=6.9$ Hz, 1''''-H)] parts. The tetrasaccharide structure bonding to the 3-position of **12** was clarified by a heteronuclear multiple bond

connectivity (HMBC) experiment. Thus, long-range correlations were observed between the 1'-proton of the glucuronic acid part and the 3-carbon of the camelliagenin A moiety, between the 1''-proton of the galactopyranosyl part and the 2'-carbon of the glucuronic acid part, between the 1'''-proton of the arabinopyranosyl part and the 3'-carbon of the glucuronic acid part, and between the 1''''-proton of the glucopyranosyl part and the 2''''-carbon of the arabinopyranosyl part. On the basis of the above-mentioned evidence, the structure of desacyl-camelliasaponin A (**3**) was elucidated as shown.

The ¹H-NMR (pyridine-*d*₅) and ¹³C-NMR (Table 2) spectra¹⁵⁾ of **1** showed signals assignable to an angeloyl group at δ 1.97 (s, 5-H₃), 2.09 (d, $J=7.1$ Hz, 4-H₃), 5.92 (dq-like, 3-H), together with the camelliagenin A part [δ 6.18 (dd, $J=5.6, 12.2$ Hz, 22-H)] and the tetrasaccharide moiety. The position of the angeloyl group in **1** was clarified by HMBC, which showed a long-range correlation between the 22-proton of the camelliagenin A moiety and the 1-carbonyl carbon of the angeloyl group. Furthermore, enzymatic hydrolysis of **1** with glycyrrhizic acid hydrolase¹⁶⁾ furnished a genuine aglycone 22-*O*-angeloylcamelliagenin A (**10**), which showed signals due to the 22-proton [δ 6.25 (dd, $J=5.6, 12.2$ Hz)] bearing the angeloyl group. Finally, comparison of the ¹³C-NMR data for **1** with those for **3** revealed an acylation shift around the 22-position of the camelliagenin A moiety of **1**. Consequently, the structure of camelliasaponin A₁ was determined to be 22-*O*-angeloylcamelliagenin A 3-*O*-[β -D-galactopyranosyl (1→2)][β -D-glucopyranosyl (1→2)- α -L-arabinopyranosyl (1→3)]- β -D-glucopyranosiduronic acid (**1**).

Camelliasaponin A₂ (**2**), also obtained as colorless fine crystals of mp 215.3–217.2 °C, liberated desacyl-camelliasaponin A (**3**) and tiglic acid upon alkaline hydrolysis. The molecular formula C₅₈H₉₂O₂₅, which was the same as that of camelliasaponin A₁ (**1**), was determined from the positive-mode FAB-MS [m/z 1211 (M+Na)⁺] and by high-resolution MS measurement. The carbon signals in the ¹³C-NMR (Table 2) spectrum of **2** were shown to be superimposable on those of **1**, except for some signals assignable to an acyl group. The ¹H-NMR (pyridine-*d*₅) spectrum of **2** showed signals due to the tigloyl group [δ 1.56 (d, $J=6.9$ Hz, 4-H₃), 1.88 (s, 5-H₃), 7.01 (dq-like, 3-H)] bonding to the 22-hydroxyl group of the camelliagenin A moiety [δ 6.13 (dd, $J=4.7, 12.6$ Hz, 22-H)]. In the HMBC of **2**, a long-range correlation was observed between the 22-proton and the 1-carbonyl car-

bon of the tigloyl group. Furthermore, a genuine aglycone 22-*O*-tigloylcamelliagenin A (**11**) was obtained on the enzymatic hydrolysis of **2** with glycyrrhizinic acid hydrolase. Finally, comparison of the ¹³C-NMR data of **2** with those of **1** and **3** led us to confirm the structure of camelliasaponin A₂ as 22-*O*-tigloylcamelliagenin A 3-*O*-[β-D-galactopyranosyl (1→2)][β-D-glucopyranosyl (1→2)-α-L-arabinopyranosyl (1→3)]-β-D-glucopyranosiduronic acid (**2**).

Camelliasaponins B₁ (4) and B₂ (5) Camelliasaponin B₁ (**4**) was isolated as colorless fine crystals with a mp of 209.6–211.1 °C, and it showed absorption bands due to hydroxyl, carboxyl, aldehyde, and α,β-unsaturated ester functions at 3430, 1735, 1719, 1660, 1650, and 1078 cm⁻¹ in the IR spectrum. The positive-mode FAB-MS of **4** showed a quasimolecular ion peak at *m/z* 1247 (M + 2Na - H)⁺, and the molecular formula C₅₈H₉₀O₂₆ was determined by high-resolution MS measurement. On the alkaline hydrolysis of **4**, desacyl-camelliasaponin B (**6**) and angelic acid were obtained. Methanolysis of **6** liberated camelliagenin B (**15**) together with the methyl glycosides of D-glucuronic acid, D-galactose, L-arabinose, and D-glucose. The ¹H-NMR (pyridine-*d*₅) and ¹³C-NMR

(Table 2) spectra¹⁵ of **6** showed signals due to a camelliagenin B moiety [δ 4.59 (dd, *J*=6.0, 12.2 Hz, 22-H), 9.85 (s, 23-H)] and the same tetrasaccharide moiety [δ 4.84 (d, *J*=6.9 Hz, 1'-H), 5.07 (d-like, 1'''-H), 5.59 (d, *J*=7.6 Hz, 1''-H), and 5.84 (d, *J*=6.0 Hz, 1''''-H)] as desacyl-camelliasaponin A (**3**). Furthermore, the HMBC of **6** showed long-range correlations between the following protons and carbons: 1'-H and 3-C; 1''-H and 2'-C; 1'''-H and 3'-C; and 1''''-H and 2'''-C. Based on these findings, the structure of desacyl-camelliasaponin B (**6**) was determined as shown.

Enzymatic hydrolysis of **4** with glycyrrhizinic acid hydrolase yielded 22-*O*-angeloylcamelliagenin B (**13**). The ¹H-NMR (pyridine-*d*₅) and ¹³C-NMR (Tables 2, 3) spectra¹⁵ of **4** and **13** showed signals assignable to the 22-proton bearing an angeloyl group [**4**: δ 6.15 (dd, *J*=5.6, 12.5 Hz, 22-H); **13**: δ 6.18 (dd, *J*=5.6, 12.2 Hz, 22-H)]. In the HMBC of **4** and **13**, a long-range correlation was observed between the 22-proton and carbonyl carbon of the angeloyl group. Furthermore, comparison of the ¹H-NMR and ¹³C-NMR spectra of **4** and **13** with those of **6** and **15** disclosed an acylation shift around the 22-position of **4** and **13**. Consequently, the structure of

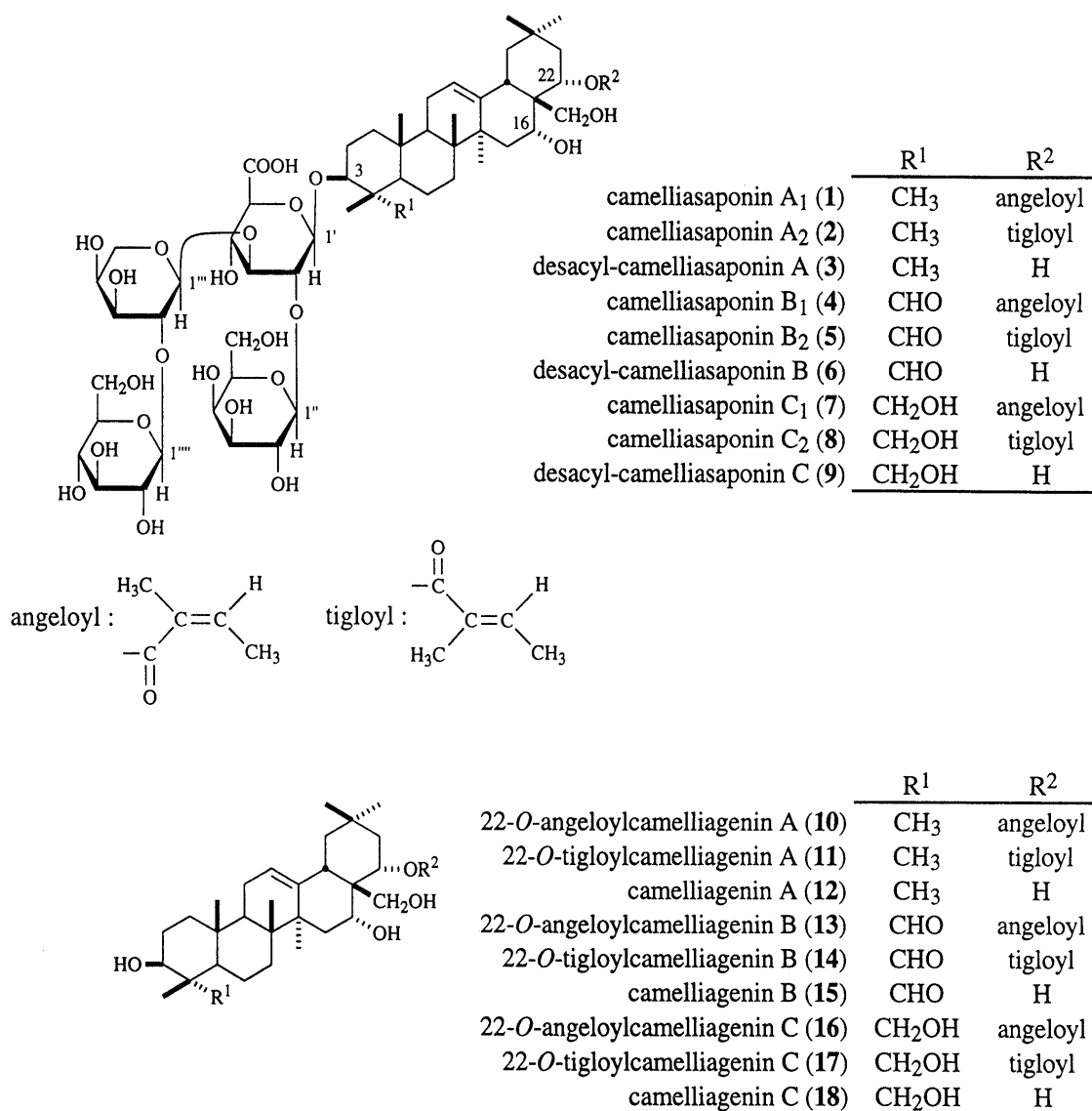


Chart 2

Table 2. ^{13}C -NMR Data for Camelliasaponins A₁ (1), A₂ (2), B₁ (4), B₂ (5), C₁ (7), and C₂ (8) and Desacylcamelliasaponins A (3), B (6), and C (9)

	1	2	3	4	5	6	7	8	9
C-1	39.6	39.6	39.6	38.3	38.3	38.3	38.8	38.8	38.8
C-2	26.6	26.6	26.5	25.2	25.2	25.2	25.5	25.6	25.6
C-3	84.3	84.3	84.3	84.2	84.2	84.2	83.1	83.1	83.1
C-4	40.2	40.2	40.0	55.1	55.1	55.1	43.6	43.5	43.5
C-5	55.9	55.8	55.8	48.5	48.5	48.4	48.3	48.3	48.3
C-6	18.5	18.5	18.5	20.4	20.4	20.4	18.2	18.2	18.2
C-7	33.3	33.3	33.2	32.5	32.5	32.4	32.9	32.9	32.9
C-8	40.2	40.2	40.1	40.4	40.4	40.3	40.2	40.2	40.2
C-9	47.0	47.0	47.0	46.9	46.9	46.9	47.1	47.1	47.1
C-10	36.9	36.9	36.8	36.1	36.1	36.1	36.8	36.8	36.8
C-11	23.9	23.9	23.8	23.8	23.8	23.8	23.9	23.9	23.9
C-12	122.7	122.7	122.7	122.4	122.3	122.3	122.7	122.7	122.7
C-13	143.8	143.8	144.3	143.8	143.9	144.3	143.8	143.8	144.3
C-14	41.8	41.9	42.2	41.7	41.7	42.2	41.7	41.9	42.2
C-15	35.2	35.2	34.7	35.1	35.1	34.6	35.2	35.2	34.7
C-16	70.3	70.1	68.5	70.2	70.0	68.4	69.9	70.0	68.6
C-17	44.9	45.2	44.8	44.9	45.1	44.8	44.9	45.1	44.8
C-18	41.1	41.1	42.6	41.1	41.0	42.5	41.0	41.0	42.6
C-19	47.5	47.5	47.9	47.5	47.5	47.8	47.5	47.5	47.9
C-20	32.1	32.0	31.7	32.1	32.5	31.8	32.0	32.0	31.8
C-21	41.8	41.8	46.0	41.8	41.9	45.9	41.7	41.8	46.0
C-22	73.2	73.2	74.4	73.2	73.2	74.3	73.2	73.2	74.4
C-23	28.1	28.1	28.1	209.7	209.7	209.7	64.9	64.9	64.9
C-24	16.8	16.9	16.1	11.0	11.1	11.0	13.5	13.6	13.5
C-25	15.7	15.7	15.7	15.8	15.8	15.8	16.2	16.2	16.2
C-26	16.9	17.0	16.9	16.9	16.9	16.8	17.0	17.0	17.0
C-27	27.6	27.6	27.5	27.6	27.5	27.4	27.6	27.6	27.5
C-28	63.9	63.9	70.4	63.9	63.8	70.1	63.9	63.9	70.3
C-29	33.5	33.5	33.7	33.5	33.5	33.7	33.4	33.5	33.7
C-30	25.2	25.2	25.4	25.2	25.7	25.4	25.2	25.2	25.5

	1	2	3	4	5	6	7	8	9
GlcA-1'	105.6	105.6	105.6	104.1	104.1	103.9	104.1	104.2	104.1
GlcA-2'	79.2	79.2	79.2	78.5	78.5	78.4	78.7	78.7	78.7
GlcA-3'	89.6	89.1	86.9	84.5	84.5	84.5	85.1	85.1	85.1
GlcA-4'	71.3	71.3	71.2	71.0	71.0	70.9	71.2	71.2	71.2
GlcA-5'	77.3	77.3	77.2	77.3	77.2	77.2	77.2	77.3	77.2
GlcA-6'	171.9	172.5	171.9	171.8	171.8	171.8	171.8	172.0	171.9
Gal-1''	103.7	103.7	103.7	103.6	103.6	103.5	103.5	103.5	103.5
Gal-2''	73.9	74.0	73.9	73.7	73.7	73.7	73.8	73.8	73.8
Gal-3''	75.0	75.0	75.0	75.2	75.2	75.1	75.1	75.1	75.1
Gal-4''	69.9	70.0	69.9	70.3	70.3	70.2	70.2	70.1	69.9
Gal-5''	76.5	76.5	76.5	76.6	76.6	76.5	76.5	76.6	76.5
Gal-6''	62.0	62.0	61.9	62.2	62.2	62.1	61.9	62.0	61.9
Ara-1'''	101.8	101.8	101.8	101.7	101.7	101.6	101.8	101.8	101.8
Ara-2'''	81.3	81.3	81.2	81.3	81.3	81.3	81.2	81.2	81.2
Ara-3'''	72.6	72.6	72.5	72.4	72.4	72.4	72.4	72.5	72.4
Ara-4'''	67.7	67.7	67.7	67.6	67.6	67.6	67.6	67.6	67.6
Ara-5'''	65.0	64.9	65.0	64.9	64.9	64.9	64.9	64.9	64.9
Glc-1''''	105.9	105.9	105.8	106.0	106.1	105.9	105.9	105.9	105.9
Glc-2''''	75.7	75.4	75.7	75.9	75.9	75.8	75.8	75.8	75.8
Glc-3''''	78.4	78.4	78.4	78.4	78.4	78.4	78.4	78.4	78.4
Glc-4''''	71.4	71.4	71.4	71.6	71.6	71.6	71.6	71.6	71.6
Glc-5''''	78.4	78.4	78.3	78.4	78.4	78.4	78.4	78.4	78.4
Glc-6''''	62.6	62.6	62.6	62.7	62.7	62.7	62.7	62.7	62.7
Ang or Tig-1	168.1	168.0		168.0	167.9		168.0	168.0	
Ang or Tig-2	129.6	130.1		129.6	130.1		129.6	130.1	
Ang or Tig-3	136.4	136.3		136.4	136.2		136.4	136.3	
Ang or Tig-4	15.8	14.1		15.8	14.0		15.8	14.1	
Ang or Tig-5	20.9	12.3		20.9	12.3		20.9	12.3	

68 MHz, pyridine-*d*₅, δ_{C} .

Table 3. ^{13}C -NMR Data for 22-*O*-Angeloyl and Tigloylcamelliagenins B (13, 14) and C (16, 17)

	13	14	16	17		13	14	16	17
C-1	38.8	38.8	39.0	39.1	C-21	42.0	41.7	41.8	41.8
C-2	27.2	27.2	27.8	27.8	C-22	73.1	73.2	73.2	73.2
C-3	71.8	71.7	73.8	73.8	C-23	207.2	207.2	68.5	68.5
C-4	56.3	56.3	42.9	42.9	C-24	9.7	9.7	13.1	13.0
C-5	47.9	47.8	49.0	49.0	C-25	16.0	16.0	16.3	16.3
C-6	21.1	21.0	18.7	18.7	C-26	17.0	17.0	17.1	17.1
C-7	32.6	32.6	33.0	33.0	C-27	27.6	27.6	27.5	27.6
C-8	40.6	40.5	40.2	40.3	C-28	63.9	63.9	64.0	63.9
C-9	47.1	47.1	47.2	47.2	C-29	33.5	33.5	33.4	33.5
C-10	36.1	36.1	37.2	37.2	C-30	25.2	25.2	25.2	25.2
C-11	23.9	23.9	24.2	24.0	Ang.-1	168.0		168.0	
C-12	122.4	122.3	122.7	122.7	Ang.-2	129.6		129.6	
C-13	143.9	143.9	143.8	143.8	Ang.-3	136.5		136.3	
C-14	42.0	42.0	41.9	41.9	Ang.-4	15.8		15.8	
C-15	35.1	35.1	35.2	35.2	Ang.-5	20.9		20.9	
C-16	70.2	70.0	70.3	70.1	Tig.-1		168.0		167.9
C-17	45.0	45.1	44.9	45.2	Tig.-2		130.1		130.1
C-18	41.1	41.1	41.1	41.1	Tig.-3		136.2		136.2
C-19	47.6	47.5	47.5	47.5	Tig.-4		14.1		14.1
C-20	32.1	32.0	32.1	32.0	Tig.-5		12.3		12.3

camelliasaponin B₁ was confirmed to be 22-*O*-angeloylcamelliagenin B 3-*O*-[β -D-galactopyranosyl (1 \rightarrow 2)][β -D-glucopyranosyl (1 \rightarrow 2)]- α -L-arabinopyranosyl (1 \rightarrow 3)]- β -D-glucopyranosiduronic acid (4).

Camelliasaponin B₂ (5), obtained as colorless fine crystals of mp 233.5–235.6 °C, liberated desacyl-camelliasaponin B (6) and tiglic acid upon the alkaline hydrolysis. The carbon and proton signals in the ^1H -NMR (pyridine-*d*₅) and ^{13}C -NMR (Table 2) of 5 were superimposable on those of camelliasaponin B₁ (4), except for the signals due to the acyl group. The enzymatic hydrolysis of 5 furnished 22-*O*-tigloylcamelliagenin B (14). Based on comparison of the ^1H -NMR and ^{13}C -NMR spectra of 5 and 14 with those of 6 and 15, and on examination of the HMBC of 5, which showed a correlation between the 22-proton and the carbonyl carbon of the tigloyl group, the structure of camelliasaponin B₂ was determined to be 22-*O*-tigloylcamelliagenin B 3-*O*-[β -D-galactopyranosyl (1 \rightarrow 2)][β -D-glucopyranosyl (1 \rightarrow 2)]- α -L-arabinopyranosyl (1 \rightarrow 3)]- β -D-glucopyranosiduronic acid (5).

Camelliasaponins C₁ (7) and C₂ (8) Camelliasaponins C₁ (7) and C₂ (8) were also isolated as colorless fine crystals with a mp of 165.8–167.2 and 177.6–178.9 °C, respectively. Camelliasaponins C₁ (7) and C₂ (8) were found to have the same molecular formula, C₅₈H₉₂O₂₆, which was determined from the quasimolecular ion peak in their positive-mode FAB-MS [*m/z* 1227 (M+Na)⁺] and by high-resolution MS measurement. Alkaline hydrolysis of 7 with 10% aqueous potassium hydroxide–50% aqueous dioxane furnished desacyl-camelliasaponin C (9) and angelic acid, while 9 and tiglic acid were obtained from 8 on the alkaline hydrolysis.

Desacyl-camelliasaponin C (9) liberated camelliagenin C (18), methyl glucuronide, methyl galactoside, methyl arabinoside, and methyl glucoside. The proton and carbon signals due to the tetrasaccharide moiety in the ^1H -NMR (pyridine-*d*₅) and ^{13}C -NMR (Table 2) spectra of 9 were similar to those of 3 and 6. The HMBC experiment of 9

showed long-range correlations between the following protons and carbons: 1'-H and 3-C; 1''-H and 2'-C; 1'''-H and 3'-C; 1''''-H and 2'''-C. Consequently, the structure of desacyl-camelliasaponin C (9) was characterized as shown.

Enzymatic hydrolysis of 7 and 8 with glycyrrhizic acid hydrolase provided 22-*O*-angeloylcamelliagenin C (16) and 22-*O*-tigloylcamelliagenin C (17), respectively. Comparison of the ^1H -NMR (pyridine-*d*₅) and ^{13}C -NMR (Tables 2, 3) spectra of 7, 8, 16, and 17 with those for 9 and 18 revealed an acylation shift around the 22-position of the camelliagenin C moiety, and their HMBC experiments showed a long-range correlation between the 22-proton and the carbonyl carbon of the tigloyl group. Finally, the structures of 7 and 8 were established by their chemical correlations with 4 and 5, respectively. Namely, reduction of the aldehyde group in 4 and 5 with sodium borohydride (NaBH₄) yielded 7 and 8, quantitatively. Consequently, the structures of camelliasaponins C₁ (5) and C₂ (6) were clarified to be 22-*O*-angeloylcamelliagenin C 3-*O*-[β -D-galactopyranosyl (1 \rightarrow 2)][β -D-glucopyranosyl (1 \rightarrow 2)]- α -L-arabinopyranosyl (1 \rightarrow 3)]- β -D-glucopyranosiduronic acid (5) and 22-*O*-tigloylcamelliagenin C 3-*O*-[β -D-galactopyranosyl (1 \rightarrow 2)][β -D-glucopyranosyl (1 \rightarrow 2)]- α -L-arabinopyranosyl (1 \rightarrow 3)]- β -D-glucopyranosiduronic acid (6).

Inhibitory Activity of Camelliasaponins B₁ (4), B₂ (5), C₁ (7), and C₂ (8) and Desacyl-camelliasaponins B (6) and C (9) on Ethanol Absorption As shown in Table 4, we have examined the inhibitory activity of camelliasaponins B₁ (4), B₂ (5), C₁ (7), and C₂ (8), which were obtained in a sufficient amount for the bioassay test from the seeds, on ethanol absorption in rats. Furthermore, in order to obtain evidence of the structure–activity relationships, the inhibitory activity of desacyl-camelliasaponins B (6) and C (9) were examined.

The tested saponins were dissolved in water and were orally administered to rats. At 1h thereafter, 20% aqueous ethanol was administered orally. The ethanol concentration in the blood, which was collected at 1, 2, and 3h

Table 4. Inhibitory Activity of Camelliasaponins B₁ (4), B₂ (5), C₁ (7), and C₂ (8) and Desacyl-camelliasaponins B (6) and C(9)

	Dose (mg/kg, <i>p.o.</i>)	<i>n</i>	Ethanol concentration in blood (mg/ml)		
			1 h	2 h	3 h
Control		10	0.57 ± 0.01	0.17 ± 0.02	0.03 ± 0.00
Camelliasaponin B ₁ (4)	100	6	0.10 ± 0.06**	0.09 ± 0.03**	0.03 ± 0.00
Camelliasaponin B ₂ (5)	100	5	0.43 ± 0.05*	0.22 ± 0.01	0.04 ± 0.01
Desacyl-camelliasaponin B (6)	100	5	0.58 ± 0.02	0.27 ± 0.02	0.03 ± 0.00
Camelliasaponin C ₁ (7)	100	7	0.35 ± 0.05**	0.19 ± 0.03	0.02 ± 0.00
Camelliasaponin C ₂ (8)	100	8	0.32 ± 0.08**	0.12 ± 0.03	0.04 ± 0.01
Desacyl-camelliasaponin C (9)	100	5	0.58 ± 0.01	0.24 ± 0.01	0.03 ± 0.01

* $p < 0.05$, ** $p < 0.01$.

after ethanol administration, was assayed by the enzyme method. All camelliasaponins (4, 5, 7, 8) showed inhibitory activity on ethanol absorption after a single oral administration at the dose of 100 mg/kg. Particularly, 4 exhibited the most potent inhibitory activity. On the other hand, desacyl-camelliasaponins B (6) and C (9) were found to lack the activity. This evidence revealed that the acyl group in camelliasaponins was essential for the inhibitory activity on ethanol absorption.

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper.¹⁾

Isolation of Camelliasaponins A₁ (1), A₂ (2), B₁ (4), B₂ (5), C₁ (7), and C₂ (8) The seeds of *Camellia japonica* L. (10 kg, collected in Kyoto Prefecture) were crushed and extracted with *n*-hexane under reflux. After removal of the solvent by filtration, the residue was further extracted three times with MeOH under reflux. Evaporation of the solvent under reduced pressure provided the MeOH extract (187 g), which was subjected to reversed-phase silica-gel column chromatography [Chromatorex DM1020T (Fuji Silysia Chemical Ltd., 2 kg), H₂O→MeOH] to give three fractions [fr. 1 (77.6 g), fr. 2 (26.2 g), and fr. 3 (101.9 g, saponin fraction)]. Repeated HPLC [column: YMC-Pack R&D ODS-5 (250 × 25 mm i.d.), solvent: 1) MeOH–1% aqueous AcOH (3:1); 2) CH₃CN–1% aqueous AcOH (2:3)] separation of fraction 3 (2.0 g) afforded camelliasaponins A₁ (1, 20 mg, 0.01%), A₂ (2, 40 mg, 0.02%), B₁ (4, 290 mg, 0.15%), B₂ (5, 1780 mg, 0.91%), C₁ (7, 200 mg, 0.10%), and C₂ (8, 190 mg, 0.09%).

Camelliasaponin A₁ (1): Colorless fine crystals from aqueous MeOH, mp 185.8–187.0°C, $[\alpha]_D^{25} + 22.9^\circ$ ($c=0.2$, MeOH). High-resolution positive-mode FAB-MS: Calcd for C₅₈H₉₂NaO₂₅ (M + Na)⁺: 1211.5826. Found: 1211.5778. IR (KBr) cm⁻¹: 3432, 1730, 1662, 1647, 1078. ¹H-NMR (pyridine-*d*₅) δ: 0.83, 0.90, 1.05, 1.10, 1.28, 1.29, 1.86 (3H each, all s, 25, 26, 29, 24, 30, 23, 27-H₃ × 7), 1.97 (3H, s, Ang.-5-H₃), 2.09 (3H, d, $J=7.1$ Hz, Ang.-4-H₃), 4.94 (1H, d, $J=6.8$ Hz, 1'-H), 5.12 (1H, d, $J=6.9$ Hz, 1''-H), 5.65 (1H, d, $J=7.1$ Hz, 1'''-H), 5.75 (1H, d, $J=6.0$ Hz, 1''''-H), 5.92 (1H, dq-like, Ang.-3-H), 6.18 (1H, dd, $J=5.6$, 12.2 Hz, 22-H). ¹³C-NMR: given in Table 2. Positive-mode FAB-MS *m/z*: 1211 (M + Na)⁺.

Camelliasaponin A₂ (2): Colorless fine crystals from aqueous MeOH, mp 215.3–217.2°C, $[\alpha]_D^{25} + 8.0^\circ$ ($c=0.1$, MeOH). High-resolution positive-mode FAB-MS: Calcd for C₅₈H₉₂NaO₂₅ (M + Na)⁺: 1211.5825. Found: 1211.5763. IR (KBr) cm⁻¹: 3453, 1736, 1686, 1647, 1076. ¹H-NMR (pyridine-*d*₅) δ: 0.84, 0.90, 1.05, 1.11, 1.27, 1.29, 1.86 (3H each, all s, 25, 26, 29, 24, 30, 23, 27-H₃ × 7), 1.56 (3H, d, $J=6.9$ Hz, Tig.-4-H₃), 1.88 (3H, s, Tig.-5-H₃), 4.95 (1H, d, $J=6.9$ Hz, 1'-H), 5.13 (1H, d, $J=6.6$ Hz, 1''-H), 5.65 (1H, d, $J=7.3$ Hz, 1'''-H), 5.77 (1H, d-like, 1''''-H), 6.13 (1H, dd, $J=4.7$, 12.6 Hz, 22-H), 7.01 (1H, dq-like, Tig.-3-H). ¹³C-NMR: given in Table 2. Positive-mode FAB-MS *m/z*: 1211 (M + Na)⁺.

Camelliasaponin B₁ (4): Colorless fine crystals from aqueous MeOH, mp 209.6–211.1°C, $[\alpha]_D^{25} + 23.7^\circ$ ($c=0.5$, MeOH). High-resolution positive-mode FAB-MS: Calcd for C₅₈H₈₉Na₂O₂₆ (M + 2Na – H)⁺: 1247.5438. Found: 1247.5353. IR (KBr) cm⁻¹: 3430, 1735, 1719, 1660,

1650, 1078. ¹H-NMR (pyridine-*d*₅) δ: 0.82, 0.85, 1.05, 1.28, 1.43, 1.81 (3H each, all s, 25, 26, 29, 30, 23, 27-H₃ × 7), 1.96 (3H, s, Ang.-5-H₃), 2.08 (3H, d, $J=7.3$ Hz, Ang.-4-H₃), 4.85 (1H, d, $J=7.2$ Hz, 1'-H), 5.19 (1H, d, $J=6.3$ Hz, 1''-H), 5.62 (1H, d, $J=7.6$ Hz, 1'''-H), 5.76 (1H, d, $J=5.9$ Hz, 1''''-H), 5.94 (1H, dq-like, Ang.-3-H), 6.15 (1H, dd, $J=5.6$, 12.2 Hz, 22-H), 9.87 (1H, s, 23-H). ¹³C-NMR: given in Table 2. Positive-mode FAB-MS *m/z*: 1247 (M + 2Na – H)⁺.

Camelliasaponin B₂ (5): Colorless fine crystals from aqueous MeOH, mp 233.5–235.6°C, $[\alpha]_D^{25} + 20.7^\circ$ ($c=0.5$, MeOH). High-resolution positive-mode FAB-MS: Calcd for C₅₈H₉₀NaO₂₆ (M + Na)⁺: 1225.5618. Found: 1225.5599. IR (KBr) cm⁻¹: 3432, 1740, 1721, 1686, 1647, 1078. ¹H-NMR (pyridine-*d*₅) δ: 0.82, 0.85, 1.05, 1.26, 1.43, 1.86 (3H each, all s, 25, 26, 29, 30, 23, 27-H₃ × 7), 1.59 (3H, d, $J=7.0$ Hz, Tig.-4-H₃), 1.88 (3H, s, Tig.-5-H₃), 4.85 (1H, d, $J=7.6$ Hz, 1'-H), 5.11 (1H, d-like, 1''-H), 5.61 (1H, d, $J=7.6$ Hz, 1'''-H), 5.76 (1H, d, $J=5.0$ Hz, 1''''-H), 6.13 (1H, dd, $J=5.6$, 11.6 Hz, 22-H), 7.00 (1H, dq-like, Tig.-3-H), 9.87 (1H, s, 23-H). ¹³C-NMR: given in Table 2. Positive-mode FAB-MS *m/z*: 1225 (M + Na)⁺.

Camelliasaponin C₁ (7): Colorless fine crystals from aqueous MeOH, mp 165.8–167.2°C, $[\alpha]_D^{25} + 4.3^\circ$ ($c=0.5$, MeOH). High-resolution positive-mode FAB-MS: Calcd for C₅₈H₉₂NaO₂₆ (M + Na)⁺: 1227.5774. Found: 1227.5831. IR (KBr) cm⁻¹: 3416, 1730, 1686, 1648, 1078. ¹H-NMR (pyridine-*d*₅) δ: 0.92, 1.03 (6H each, both s, 25, 26, 24, 29-H₃ × 4), 1.27, 1.81 (3H each, both s, 30, 27-H₃ × 2), 1.96 (3H, s, Ang.-5-H₃), 2.07 (3H, d, $J=6.9$ Hz, Ang.-4-H₃), 5.05 (1H, d, $J=7.6$ Hz, 1'-H), 5.10 (1H, d, $J=6.3$ Hz, 1''-H), 5.74 (1H, d, $J=7.9$ Hz, 1'''-H), 5.79 (1H, d-like, 1''''-H), 5.95 (1H, dq-like, Ang.-3-H), 6.16 (1H, dd, $J=5.6$, 11.5 Hz, 22-H). ¹³C-NMR: given in Table 2. Positive-mode FAB-MS *m/z*: 1227 (M + Na)⁺.

Camelliasaponin C₂ (8): Colorless fine crystals from aqueous MeOH, mp 177.6–178.9°C, $[\alpha]_D^{25} + 8.8^\circ$ ($c=0.5$, MeOH). High-resolution positive-mode FAB-MS: Calcd for C₅₈H₉₂NaO₂₆ (M + Na)⁺: 1227.5774. Found: 1227.5815. IR (KBr) cm⁻¹: 3432, 1736, 1686, 1647, 1078. ¹H-NMR (pyridine-*d*₅) δ: 0.92, 0.93 (3H each, both s, 25, 26-H₃ × 2), 1.03 (6H, s, 24, 29-H₃ × 2), 1.26, 1.81 (3H each, both s, 30, 27-H₃ × 2), 1.56 (3H, d, $J=6.9$ Hz, Tig.-4-H₃), 1.86 (3H, s, Tig.-5-H₃), 5.06 (1H, d, $J=7.6$ Hz, 1'-H), 5.12 (1H, d, $J=6.9$ Hz, 1''-H), 5.75 (1H, d, $J=7.9$ Hz, 1'''-H), 5.76 (1H, d, $J=5.0$ Hz, 1''''-H), 6.14 (1H, dd, $J=5.9$, 11.9 Hz, 22-H), 7.01 (1H, dq-like, Tig.-3-H). ¹³C-NMR: given in Table 2. Positive-mode FAB-MS *m/z*: 1227 (M + Na)⁺.

Alkaline Hydrolysis of Camelliasaponins A₁ (1) and A₂ (2) Giving Desacyl-camelliasaponin A (3) A solution of 1 and 2 (20 mg each) in 50% aqueous dioxane (0.5 ml) was treated with 10% aqueous KOH (0.5 ml), respectively, and the whole mixture was stirred at 37°C for 1 h. The reaction mixture was neutralized with Dowex HCR W × 2 (H⁺ form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure yielded a residue, which was separated by silica-gel column chromatography [1 g, CHCl₃–MeOH–H₂O (6:4:1)] to give desacyl-camelliasaponin A (3, 17 mg from 1, 18 mg from 2) and an organic acid fraction (2 mg from 1, 2 mg from 2).

A solution of the organic acid fraction (1 mg each) in (CH₂)₂Cl₂ (2 ml) was treated with *p*-nitrobenzyl-*N,N'*-diisopropylisourea (10 mg), and the whole mixture was stirred at 80°C for 1 h. The reaction mixture was subjected to HPLC analysis to identify the *p*-nitrobenzyl esters of angelic acid (a) and tiglic acid (b). HPLC conditions: TSK gel ODS-Prep (250 × 4.6 mm i.d.); solvent, MeOH–H₂O (7:3); flow rate, 1.0 ml/min; a (from 1), 17.0 min; b (from 2), 18.5 min.

Desacyl-camelliasaponin A (**3**): Colorless fine crystals from CHCl_3 -aqueous MeOH, mp 228.5–230.0 °C, $[\alpha]_D^{25} -2.1^\circ$ ($c=0.5$, MeOH). High-resolution positive-mode FAB-MS: Calcd for $\text{C}_{53}\text{H}_{86}\text{NaO}_{24}$ ($\text{M} + \text{Na}$)⁺: 1129.5406. Found: 1129.5378. IR (KBr) cm^{-1} : 3424, 1736, 1655, 1640, 1078. ¹H-NMR (pyridine-*d*₅) δ : 0.83, 0.90, 1.01, 1.10, 1.11, 1.27, 1.86 (3H each, all s, 25, 26, 24, 29, 30, 23, 27-H₃ × 7), 4.48 (1H, dd-like, 22-H), 4.94 (1H, d, $J=7.6$ Hz, 1'-H), 5.15 (1H, d, $J=6.9$ Hz, 1''-H), 5.65 (1H, d, $J=7.6$ Hz, 1''-H), 5.74 (1H, d, $J=5.6$ Hz, 1'''-H). ¹³C-NMR: given in Table 2. Positive-mode FAB-MS m/z : 1129 ($\text{M} + \text{Na}$)⁺.

Methanolysis of Desacyl-camelliasaponin A (3) a) Methanolysis of **3** (5 mg) in 9% HCl-dry MeOH (0.5 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 powder and the insoluble part was removed by filtration. After removal of the solvent from the filtrate under reduced pressure, the residue was dissolved in pyridine (0.01 ml) and *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 0.02 ml), and the whole mixture was left standing for 1 h. The reaction mixture was subjected to GLC analysis to identify the trimethylsilyl (TMS) derivatives of methyl glucuronide (c), methyl galactoside (d), methyl arabinoside (e), and methyl glucoside (f). GLC conditions: column, CBR1-M25-025 [0.25 mm (i.d.) × 25 m]; injector temp., 140 °C; detector temp., 280 °C, column temp., 140–240 °C; 5 °C/min; initial time, 5 min; He flow rate, 15 ml/min; t_R : c, 18.0, 21.0, 21.3 min; d, 19.0, 19.7 min; e, 12.0, 12.7 min; f, 20.6 min.

b) A solution of **3** (10 mg) in 9% HCl-dry MeOH (1 ml) was heated under reflux for 1 h. The reaction mixture was neutralized with Amberlite IRA-400 (OH⁻ form) and the resin was removed by filtration. After evaporation of the solvent from the filtrate under reduced pressure, the residue was purified by silica-gel column chromatography [2 g, CHCl_3 -MeOH (40:1)] to give camelliagenin A (**12**, 3 mg), which was identified by comparison of the melting point, $[\alpha]_D$, IR, and ¹H-NMR data with reported values.^{3,17)}

Enzymatic Hydrolysis of Camelliasaponin A₁ (1) Giving 22-O-Angeloylcamelliagenin A (10) A solution of **1** (5 mg) in 0.1 M acetate buffer (pH 4.4, 0.5 ml) was treated with glycyrrhizinic acid hydrolase (0.5 ml) and the whole mixture was stirred at 44 °C for 3 h. After treatment of the reaction mixture with EtOH, the whole mixture was evaporated to dryness under reduced pressure and the residue was purified by silica-gel column chromatography [1 g, CHCl_3 -MeOH (10:1)] to yield **10** (2 mg).

22-O-Angeloylcamelliagenin A (**10**): Colorless fine crystals from CHCl_3 -MeOH, mp 151.2–153.0 °C, $[\alpha]_D^{25} +14.0^\circ$ ($c=0.2$, MeOH). High-resolution positive-mode FAB-MS: Calcd for $\text{C}_{35}\text{H}_{55}\text{O}_5$ ($\text{M} + \text{H}$)⁺: 557.4206. Found: 557.4216. IR (KBr) cm^{-1} : 3453, 1679, 1655. ¹H-NMR (pyridine-*d*₅) δ : 0.97, 1.08 (6H each, both s, 25, 26, 24, 29-H₃ × 4), 1.26, 1.32, 1.89 (3H each, all s, 30, 23, 27-H₃ × 3), 1.97 (3H, s, Ang.-5-H₃), 2.10 (3H, d, $J=6.9$ Hz, Ang.-4-H₃), 4.67 (1H, br s, 16-H), 5.92 (1H, dq-like, Ang.-3-H), 6.25 (1H, dd, $J=5.6$, 12.2 Hz, 22-H). ¹³C-NMR: given in Table 2. Positive-mode FAB-MS m/z : 557 ($\text{M} + \text{Na}$)⁺.

Enzymatic Hydrolysis of Camelliasaponin A₂ (2) Giving 22-O-Tigloylcamelliagenin A (11) A solution of **2** (5 mg) in 0.1 M acetate buffer (pH 4.4, 0.5 ml) was treated with glycyrrhizinic acid hydrolase (0.5 ml), and the whole mixture was stirred at 44 °C for 3 h. After treatment of the reaction mixture with EtOH, the whole mixture was evaporated to dryness under reduced pressure and the residue was purified by silica-gel column chromatography [1 g, CHCl_3 -MeOH (10:1)] to give **11** (3 mg).

22-O-Tigloylcamelliagenin A (**11**): Colorless fine crystals from CHCl_3 -MeOH, mp 148.0–149.8 °C, $[\alpha]_D^{25} +20.0^\circ$ ($c=0.1$, MeOH). High-resolution positive-mode FAB-MS: Calcd for $\text{C}_{35}\text{H}_{55}\text{O}_5$ ($\text{M} + \text{H}$)⁺: 557.4216. Found: 557.4233. IR (KBr) cm^{-1} : 3460, 1679, 1643. ¹H-NMR (pyridine-*d*₅) δ : 0.97, 1.08 (6H each, both s, 25, 26, 24, 29-H₃ × 4), 1.27, 1.32, 1.89 (3H each, all s, 30, 23, 27-H₃ × 3), 1.56 (3H, d, $J=6.9$ Hz, Tig.-4-H₃), 1.89 (3H, s, Tig.-5-H₃), 4.67 (1H, br s, 16-H), 6.24 (1H, dd, $J=6.6$, 12.2 Hz, 22-H), 7.00 (1H, dq-like, Tig.-3-H). ¹³C-NMR: given in Table 2. Positive-mode FAB-MS m/z : 557 ($\text{M} + \text{Na}$)⁺.

Alkaline Hydrolysis of Camelliasaponins B₁ (4) and B₂ (5) Giving Desacyl-camelliasaponin B (6) A solution of **4** and **5** (100 mg each) in 50% aqueous dioxane (5 ml) was treated with 10% aqueous KOH (5 ml), respectively, and the whole mixture was stirred at 37 °C for 1 h. After neutralization of the reaction mixture, the filtrate was worked-up as described above to give a residue, which was purified by silica-gel column chromatography [3 g, CHCl_3 -MeOH-H₂O (6:4:1)] to furnish **6** (87 mg from **4**, 88 mg from **5**) and the organic acid fraction (11 mg from **4**, 10 mg from **5**).

The organic acid fraction (1 mg) was dissolved in $(\text{CH}_2)_2\text{Cl}_2$ (2 ml) containing *p*-nitrobenzyl-*N,N'*-diisopropylisourea (10 mg), and the solution was stirred at 80 °C for 1 h. The reaction mixture was subjected to HPLC analysis (the same condition as described above) to identify the *p*-nitrobenzyl esters of the organic acid (angelic acid from **4**, tiglic acid from **5**), as described above.

Desacyl-camelliasaponin B (**6**): Colorless fine crystals from CHCl_3 -aqueous MeOH, mp 235.3–237.1 °C, $[\alpha]_D^{25} +18.4^\circ$ ($c=0.5$, MeOH). High-resolution negative-mode FAB-MS: Calcd for $\text{C}_{53}\text{H}_{83}\text{O}_{25}$ ($\text{M} - \text{H}$)⁻: 1119.5224. Found: 1119.5226. IR (KBr) cm^{-1} : 3425, 1735, 1719, 1638, 1078. ¹H-NMR (pyridine-*d*₅) δ : 0.83, 0.85, 1.04, 1.13, 1.41, 1.77 (3H each, all s, 25, 26, 29, 24, 30, 27-H₃ × 6), 4.59 (1H, dd, $J=6.0$, 12.2 Hz, 22-H), 4.84 (1H, d, $J=6.9$ Hz, 1'-H), 5.07 (1H, d-like, 1''-H), 5.59 (1H, d, $J=7.6$ Hz, 1''-H), 5.84 (1H, d, $J=6.0$ Hz, 1'''-H), 9.85 (1H, s, 23-H). ¹³C-NMR: given in Table 2. Negative-mode FAB-MS m/z : 1119 ($\text{M} - \text{H}$)⁻.

Methanolysis of Desacyl-camelliasaponin B (6) 1) A solution of **6** (5 mg) in 9% HCl-dry MeOH (0.5 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 powder and filtered. Removal of the solvent from the filtrate under reduced pressure gave a residue, which was dissolved in pyridine (0.01 ml) and BSTFA (0.02 ml), and the whole mixture was left standing for 1 h. The reaction solution was subjected to gas-liquid chromatography (GLC) analysis (the same condition as described above) to identify the TMS derivatives of methyl glucuronide, methyl galactoside, methyl arabinoside, and methyl glucoside.

2) A solution of **6** (50 mg) in 9% HCl-dry MeOH (1 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with IRA-400 (OH⁻ form) and filtered. After removal of the solvent from the filtrate under reduced pressure, the residue was purified by silica-gel column chromatography [2 g, CHCl_3 -MeOH (40:1)] to give camelliagenin B (19 mg), which was identified by comparison of the melting point, $[\alpha]_D$, IR, and ¹H-NMR data with reported values.³⁾

Enzymatic Hydrolysis of Camelliasaponins B₁ (4) and B₂ (5) Giving 22-O-Angeloyl and Tigloylcamelliagenin B (13, 14) A solution of **4** or **5** (30 mg each) in 0.1 M acetate buffer (pH 4.4, 0.5 ml) was treated with glycyrrhizinic acid hydrolase (0.5 ml), respectively, and the whole mixture was stirred at 44 °C for 3 h. After treatment of the reaction mixture with EtOH, the whole mixture was evaporated to dryness under reduced pressure and the residue was purified by silica-gel column chromatography [6 g, CHCl_3 -MeOH (10:1)] to give **13** (13 mg) from **4** and **14** (12 mg) from **5**.

22-O-Angeloylcamelliagenin B (**13**): Colorless fine crystals from CHCl_3 -MeOH, mp 172.1–174.0 °C, $[\alpha]_D^{25} +45.0^\circ$ ($c=0.4$, MeOH). High-resolution positive-mode FAB-MS: Calcd for $\text{C}_{35}\text{H}_{55}\text{O}_5$ ($\text{M} + \text{H}$)⁺: 571.3999. Found: 571.3976. IR (KBr) cm^{-1} : 3477, 1725, 1686, 1647. ¹H-NMR (pyridine-*d*₅) δ : 0.93, 0.97, 1.06, 1.29, 1.38, 1.85 (3H each, all s, 25, 26, 29, 24, 30, 27-H₃ × 6), 1.96 (3H, s, Ang.-5-H₃), 2.09 (3H, d, $J=6.9$ Hz, Ang.-4-H₃), 5.93 (1H, dq-like, Ang.-3-H), 6.18 (1H, dd, $J=5.6$, 12.2 Hz, 22-H), 9.61 (1H, s, 23-H). ¹³C-NMR: given in Table 3. Positive-mode FAB-MS m/z : 571 ($\text{M} + \text{H}$)⁺.

22-O-Tigloylcamelliagenin B (**14**): Colorless fine crystals from CHCl_3 -MeOH, mp 170.3–172.9 °C, $[\alpha]_D^{25} +68.5^\circ$ ($c=0.5$, MeOH). High-resolution positive-mode FAB-MS: Calcd for $\text{C}_{35}\text{H}_{55}\text{O}_5$ ($\text{M} + \text{H}$)⁺: 571.3999. Found: 571.4037. IR (KBr) cm^{-1} : 3425, 1725, 1674, 1649. ¹H-NMR (pyridine-*d*₅) δ : 0.93, 0.97, 1.06, 1.29, 1.37, 1.85 (3H each, all s, 25, 26, 29, 24, 30, 27-H₃ × 6), 1.56 (3H, d, $J=7.3$ Hz, Tig.-4-H₃), 1.87 (3H, s, Tig.-5-H₃), 6.14 (1H, dd, $J=5.6$, 12.2 Hz, 22-H), 6.96 (1H, dq-like, Tig.-3-H), 9.61 (1H, s, 23-H). ¹³C-NMR: given in Table 3. Positive-mode FAB-MS m/z : 571 ($\text{M} + \text{H}$)⁺.

Alkaline Hydrolysis of Camelliasaponins C₁ (7) and C₂ (8) Giving Desacyl-camelliasaponin C (9) A solution of **7** and **8** (100 mg each) in 50% aqueous dioxane (5 ml) was treated with 10% aqueous KOH (5 ml), respectively, and the whole mixture was stirred at 37 °C for 1 h. The reaction mixture was neutralized with Dowex HCR W × 2 (H⁺ form) and then filtered. The filtrate was worked-up as described above to give a product, which was purified by silica-gel column chromatography [3 g, CHCl_3 -MeOH-H₂O (6:4:1)] to give **9** (88 mg from **7**, 90 mg from **8**) and the organic acid fraction (11 mg from **7**, 10 mg from **8**).

The organic acid fraction (1 mg each) was treated with *p*-nitrobenzyl-*N,N'*-diisopropylisourea (10 mg)- $(\text{CH}_2)_2\text{Cl}_2$ (2 ml), and the whole mixture was stirred at 80 °C for 1 h. The reaction product was subjected to HPLC analysis (the same condition as described above) to identify the *p*-nitrobenzyl ester of the organic acid (angelic acid from **7**, tiglic

acid from **8**) as described above.

Desacyl-camelliasaponin C (9): Colorless fine crystals from CHCl_3 -aqueous MeOH, mp 214.5–216.2 °C, $[\alpha]_D^{25} + 1.9^\circ$ ($c=0.5$, MeOH). High-resolution negative-mode FAB-MS: Calcd for $\text{C}_{53}\text{H}_{85}\text{O}_{25}$ ($\text{M}-\text{H}^-$): 1227.5380. Found: 1227.5409. IR (KBr) cm^{-1} : 3411, 1719, 1638, 1078. $^1\text{H-NMR}$ (pyridine- d_5) δ : 0.83, 0.86, 1.12, 1.79 (3H each, all s, 25, 26, 30, 27- $\text{H}_3 \times 4$), 1.02 (6H, s, 24, 29- $\text{H}_3 \times 2$), 4.60 (1H, dd-like, 22-H), 5.06 (1H, d, $J=7.6$ Hz, 1'-H), 5.12 (1H, d, $J=7.2$ Hz, 1''-H), 5.74 (1H, d, $J=7.9$ Hz, 1'''-H), 5.76 (1H, d, $J=5.6$ Hz, 1''''-H). $^{13}\text{C-NMR}$: given in Table 2. Negative-mode FAB-MS m/z : 1121 ($\text{M}-\text{H}^-$).

Methanolysis of Desacyl-camelliasaponin C (9) a) A solution of **9** (5 mg) in 9% HCl-dry MeOH (0.5 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and filtered. After work-up of the filtrate as described above, the product was treated with BSTFA and pyridine, and the reaction was left standing for 1 h. The reaction product was subjected to GLC analysis to identify the TMS derivatives of methyl glucuronide, methyl galactoside, methyl arabinoside, and methyl glucoside as described above.

b) A solution of **9** (50 mg) in 9% HCl-dry MeOH (1 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with IRA-400 (OH^- form) and filtered. After work-up of the filtrate in the usual manner, the product was purified by silica-gel column chromatography [2 g, CHCl_3 -MeOH (40:1)] to provide camelliagenin **C (18)**, 18 mg, which was identified by comparison of the melting point, $[\alpha]_D$, IR, and $^1\text{H-NMR}$ data with reported values.³⁾

Enzymatic Hydrolysis of Camelliasaponins C₁ (7) and C₂ (8) Giving 22-O-Angeloylcamelliagenin C (16) and 22-O-Tigloylcamelliagenin C (17) A solution of **7** (20 mg) and **8** (30 mg) in 0.1 M acetate buffer (pH 4.4, 0.5 ml) was treated with glycyrrhizinic acid hydrolase (0.5 ml), respectively, and the whole mixture was stirred at 44 °C for 3 h. Work-up of the reaction mixture as described above furnished a product, which was purified by silica-gel column chromatography [6 g, CHCl_3 -MeOH (10:1)] to give **16** (8 mg) from **7** and **17** (13 mg) from **8**.

22-O-Angeloylcamelliagenin C (16): Colorless fine crystals from CHCl_3 -MeOH, mp 129.6–130.2 °C, $[\alpha]_D^{25} + 39.0^\circ$ ($c=0.2$, MeOH). High-resolution positive-mode FAB-MS: Calcd for $\text{C}_{35}\text{H}_{55}\text{O}_6$ ($\text{M}+\text{H}^+$): 573.4155. Found: 573.4113. IR (KBr) cm^{-1} : 3444, 1687, 1649. $^1\text{H-NMR}$ (pyridine- d_5) δ : 0.99, 1.10, 1.28, 1.82 (3H each, all s, 25, 29, 30, 27- $\text{H}_3 \times 7$), 1.04 (6H, s, 24, 26- $\text{H}_3 \times 2$), 1.96 (3H, s, Ang.-5- H_3), 2.09 (3H, d, $J=6.9$ Hz, Ang.-4- H_3), 3.72, 4.16 (2H, ABq, $J=10.2$ Hz, 23-H), 5.93 (1H, dq-like, Ang.-3-H), 6.16 (1H, dd, $J=5.6, 12.2$ Hz, 22-H). $^{13}\text{C-NMR}$: given in Table 3. Positive-mode FAB-MS m/z : 573 ($\text{M}+\text{H}^+$).

22-O-Tigloylcamelliagenin C (17): Colorless fine crystals from CHCl_3 -MeOH, mp 142.7–144.8 °C, $[\alpha]_D^{25} + 44.0^\circ$ ($c=0.8$, MeOH). High-resolution positive-mode FAB-MS: Calcd for $\text{C}_{35}\text{H}_{55}\text{O}_6$ ($\text{M}+\text{H}^+$): 573.4155. Found: 573.4146. IR (KBr) cm^{-1} : 3436, 1674, 1649. $^1\text{H-NMR}$ (pyridine- d_5) δ : 0.99, 1.10, 1.28, 1.82 (3H each, all s, 25, 29, 30, 27- $\text{H}_3 \times 7$), 1.04 (6H, s, 24, 26- $\text{H}_3 \times 2$), 1.56 (3H, d, $J=6.9$ Hz, Tig.-4- H_3), 1.87 (3H, s, Tig.-5- H_3), 3.71, 4.16 (2H, ABq, $J=10.5$ Hz, 23-H), 6.15 (1H, dd, $J=5.6, 11.9$ Hz, 22-H), 6.99 (1H, dq-like, Tig.-3-H). $^{13}\text{C-NMR}$: given in Table 3. Positive-mode FAB-MS m/z : 573 ($\text{M}+\text{H}^+$).

NaBH_4 Reduction of Camelliasaponins B₁ (4) and B₂ (5) Giving Camelliasaponins C₁ (7) and C₂ (8) A solution of **4** (75 mg) in EtOH (18.0 ml) was treated with NaBH_4 (75 mg), and the whole mixture was stirred at 0 °C for 10 min. The reaction mixture was neutralized with Dowex HCR W $\times 2$ (H^+ form) and the resin was removed by filtration. Removal of the solvent from the filtrate under reduced pressure gave **7** (75 mg), which was identified with an authentic camelliasaponin C₁ by TLC, IR, $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectral comparisons.

A solution of **5** (10 mg) in EtOH (2.5 ml) was treated with NaBH_4 (10 mg), and the whole mixture was stirred at 0 °C for 10 min. Work-up of the reaction mixture as described above furnished **8** (10 mg), which was also identified with an authentic camelliasaponin C₂ by TLC, IR, $^1\text{H-NMR}$, and $^{13}\text{C-NMR}$ spectral comparisons.

Bioassay for Inhibitory Effect on Ethanol Absorption The methods of bioassay testing are the same as those used for several saponins described in our previous papers.^{1,7,8)} Male Wistar rats (Kiwa Laboratory Animals, Ltd., Wakayama) weighing 170–180 g were used. The testing saponins were dissolved in water (5 ml/kg), and then orally administered to the rats. One hour thereafter, 20% aqueous ethanol (5 ml/kg) was orally administered. Blood (ca. 0.4 ml) was collected from the carotid at 1, 2 and 3 h after ethanol administration. The ethanol concentration in each blood sample was assayed by the enzyme method using a blood alcohol test "BMY" (Boehringer-Mannheim Yamanouchi). Statistical

significance was estimated by analysis of variance (ANOVA) followed by Dunnett's test.

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