Bioactive Saponins and Glycosides. V.[1] Acylated Polyhydroxyolean-12-ene Triterpene Oligoglycosides, Camelliasaponins A_1, A_2, B_1, B_2, C_1, and C_2, from the Seeds of *Camellia japonica* L.: Structures and Inhibitory Activity on Alcohol Absorption

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Acylated polyhydroxyolean-12-ene triterpene oligoglycosides, camelliasaponins A_1, A_2, B_1, B_2, C_1, and C_2, 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 B_1, B_2, C_1, and C_2 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 A_1; camelliasaponin B_1; camelliasaponin C_1; *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] *Aesculus 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 A_1 (**1**), A_2 (**2**), B_1 (**4**),[11] B_2 (**5**),[11] C_1 (**7**),[11] and C_2 (**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 B_1 (**4**), B_2 (**5**), C_1 (**7**), and C_2 (**8**) and desacyl-camelliasaponins B (**6**) and C (**9**) on ethanol ab-

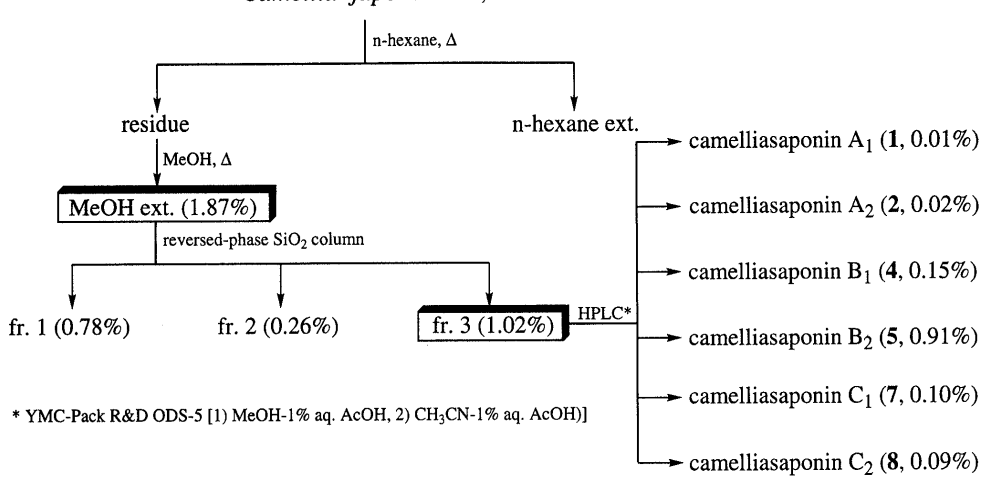

Chart 1

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

	Dose (mg/kg, *p.o.*)	*n*	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 A_1 (**1**, 0.01% from the seeds), A_2 (**2**, 0.02%), B_1 (**4**, 0.15%), B_2 (**5**, 0.91%), C_1 (**7**, 0.10%), and C_2 (**8**, 0.09%).

Camelliasaponins A_1 (1) and A_2 (2) Camelliasaponin A_1 (**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 $C_{58}H_{92}O_{25}$. 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 $C_{53}H_{86}O_{24}$ 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 ratio[14] on methanolysis with 9% hydrogen chloride. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-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.

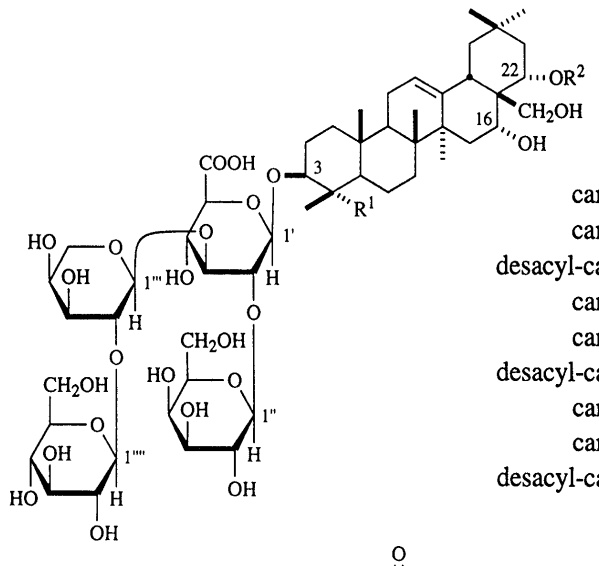
The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[15] of **1** showed signals assignable to an angeloyl group at δ 1.97 (s, 5-H_3), 2.09 (d, J=7.1 Hz, 4-H_3), 5.92 (dq-like, 3-H), together with the camelliagenin A part [δ 6.18 (dd, J=5.6, 12.2 Hz, 22-H)] and the tetrasaccharide moiety. The 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 glycyrrhizinic acid hydrolase[16] 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 ^{13}C-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 A_1 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 A_2 (**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 $C_{58}H_{92}O_{25}$, which was the same as that of camelliasaponin A_1 (**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 ^{13}C-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 ^{1}H-NMR (pyridine-d_5) spectrum of **2** showed signals due to the tigloyl group [δ 1.56 (d, J=6.9 Hz, 4-H_3), 1.88 (s, 5-H_3), 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-

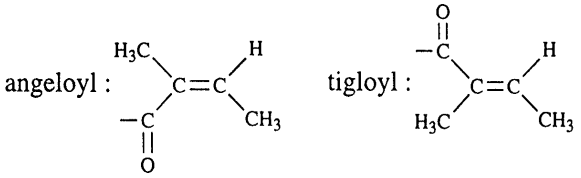
bon of the tigloyl group. Furthermore, a genuine aglycone 22-*O*-tigloylcamelliagenin A (**11**) was obtained on the enzymatic hydrolysis of **2** with glycyrrhizinic acid hydrolase. Finally, comparison of the ^{13}C-NMR data of **2** with those of **1** and **3** led us to confirm the structure of camelliasaponin A_2 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**).

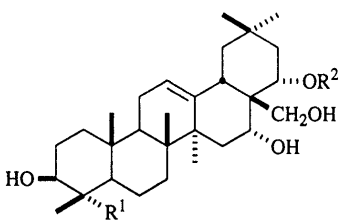
Camelliasaponins B_1 (4) and B_2 (5) Camelliasaponin B_1 (**4**) was isolated as colorless fine crystals with a mp of 209.6—211.1 °C, and it showed absorption bands due to hydroxyl, carboxyl, aldehyde, and *α*,*β*-unsaturated ester functions at 3430, 1735, 1719, 1660, 1650, and 1078 cm^{-1} in the IR spectrum. The positive-mode FAB-MS of **4** showed a quasimolecular ion peak at *m*/*z* 1247 $(M+2Na-H)^+$, and the molecular formula $C_{58}H_{90}O_{26}$ 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 ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[15] 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 ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Tables 2, 3) spectra[15] 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 ^{1}H-NMR and ^{13}C-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



	R^1	R^2
camelliasaponin A_1 (**1**)	CH_3	angeloyl
camelliasaponin A_2 (**2**)	CH_3	tigloyl
desacyl-camelliasaponin A (**3**)	CH_3	H
camelliasaponin B_1 (**4**)	CHO	angeloyl
camelliasaponin B_2 (**5**)	CHO	tigloyl
desacyl-camelliasaponin B (**6**)	CHO	H
camelliasaponin C_1 (**7**)	CH_2OH	angeloyl
camelliasaponin C_2 (**8**)	CH_2OH	tigloyl
desacyl-camelliasaponin C (**9**)	CH_2OH	H





	R^1	R^2
22-*O*-angeloylcamelliagenin A (**10**)	CH_3	angeloyl
22-*O*-tigloylcamelliagenin A (**11**)	CH_3	tigloyl
camelliagenin A (**12**)	CH_3	H
22-*O*-angeloylcamelliagenin B (**13**)	CHO	angeloyl
22-*O*-tigloylcamelliagenin B (**14**)	CHO	tigloyl
camelliagenin B (**15**)	CHO	H
22-*O*-angeloylcamelliagenin C (**16**)	CH_2OH	angeloyl
22-*O*-tigloylcamelliagenin C (**17**)	CH_2OH	tigloyl
camelliagenin C (**18**)	CH_2OH	H

Chart 2

Table 2. ^{13}C-NMR Data for Camelliasaponins A_1 (**1**), A_2 (**2**), B_1 (**4**), B_2 (**5**), C_1 (**7**), and C_2 (**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 B_1 was confirmed to be 22-*O*-angeloylcamelliagenin B 3-*O*-[*β*-D-galactopyranosyl (1→2)][*β*-D-glucopyranosyl (1→2)-α-L-arabinopyranosyl (1→3)]-*β*-D-glucopyranosiduronic acid (**4**).

Camelliasaponin B_2 (**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 ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 2) of **5** were superimposable on those of camelliasaponin B_1 (**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 ^{1}H-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 B_2 was determined to be 22-*O*-tigloylcamelliagenin B 3-*O*-[*β*-D-galactopyranosyl (1→2)][*β*-D-glucopyranosyl (1→2)-α-L-arabinopyranosyl (1→3)]-*β*-D-glucopyranosiduronic acid (**5**).

Camelliasaponins C_1 (7) and C_2 (8) Camelliasaponins C_1 (**7**) and C_2 (**8**) were also isolated as colorless fine crystals with a mp of 165.8—167.2 and 177.6—178.9 °C, respectively. Camelliasaponins C_1 (**7**) and C_2 (**8**) were found to have the same molecular formula, $C_{58}H_{92}O_{26}$, 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 ^{1}H-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 glycyrrhizinic acid hydrolase provided 22-*O*-angeloylcamelliagenin C (**16**) and 22-*O*-tigloylcamelliagenin C (**17**), respectively. Comparison of the ^{1}H-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 ($NaBH_4$) yielded **7** and **8**, quantitatively. Consequently, the structures of camelliasaponins C_1 (**5**) and C_2 (**6**) were clarified to be 22-*O*-angeloylcamelliagenin C 3-*O*-[*β*-D-galactopyranosyl (1→2)][*β*-D-glucopyranosyl (1→2)-α-L-arabinopyranoyl (1→3)]-*β*-D-glucopyranosiduronic acid (**5**) and 22-*O*-tigloylcamelliagenin C 3-*O*-[*β*-D-galactopyranosyl (1→2)][*β*-D-glucopyranosyl (1→2)-α-L-arabinopyranoyl (1→3)]-*β*-D-glucopyranosiduronic acid (**6**).

Inhibitory Activity of Camelliasaponins B_1 (4), B_2 (5), C_1 (7), and C_2 (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 B_1 (**4**), B_2 (**5**), C_1 (**7**), and C_2 (**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 3 h

Table 4. Inhibitory Activity of Camelliasaponins B_1 (**4**), B_2 (**5**), C_1 (**7**), and C_2 (**8**) and Desacyl-camelliasaponins B (**6**) and C(**9**)

	Dose (mg/kg, *p.o.*)	*n*	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 A_1 (1), A_2 (2), B_1 (4), B_2 (5), C_1 (7), and C_2 (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), $H_2O \rightarrow$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) CH_3CN–1% aqueous AcOH (2:3)] separation of fraction 3 (2.0 g) afforded camelliasaponins A_1 (**1**, 20 mg, 0.01%), A_2 (**2**, 40 mg, 0.02%), B_1 (**4**, 290 mg, 0.15%), B_2 (**5**, 1780 mg, 0.91%), C_1 (**7**, 200 mg, 0.10%), and C_2 (**8**, 190 mg, 0.09%).

Camelliasaponin A_1 (**1**): Colorless fine crystals from aqueous MeOH, mp 185.8—187.0 °C, $[\alpha]_D^{25}$ +22.9° (c=0.2, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{58}H_{92}NaO_{25}$ $(M+Na)^+$: 1211.5826. Found: 1211.5778. IR (KBr) cm^{-1}: 3432, 1730, 1662, 1647, 1078. ^{1}H-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-H_3×7), 1.97 (3H, s, Ang.-5-H_3), 2.09 (3H, d, J=7.1 Hz, Ang.-4-H_3), 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). ^{13}C-NMR: given in Table 2. Positive-mode FAB-MS m/z: 1211 $(M+Na)^+$.

Camelliasaponin A_2 (**2**): Colorless fine crystals from aqueous MeOH, mp 215.3—217.2 °C, $[\alpha]_D^{25}$ +8.0° (c=0.1, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{58}H_{92}NaO_{25}$ $(M+Na)^+$: 1211.5825. Found: 1211.5763. IR (KBr) cm^{-1}: 3453, 1736, 1686, 1647, 1076. ^{1}H-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-H_3×7), 1.56 (3H, d, J=6.9 Hz, Tig.-4-H_3), 1.88 (3H, s, Tig.-5-H_3), 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). ^{13}C-NMR: given in Table 2. Positive-mode FAB-MS m/z: 1211 $(M+Na)^+$.

Camelliasaponin B_1 (**4**): Colorless fine crystals from aqueous MeOH, mp 209.6—211.1 °C, $[\alpha]_D^{25}$ +23.7° (c=0.5, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{58}H_{89}Na_2O_{26}$ $(M+2Na-H)^+$: 1247.5438. Found: 1247.5353. IR (KBr) cm^{-1}: 3430, 1735, 1719, 1660, 1650, 1078. ^{1}H-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-H_3×7), 1.96 (3H, s, Ang.-5-H_3), 2.08 (3H, d, J=7.3 Hz, Ang.-4-H_3), 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). ^{13}C-NMR: given in Table 2. Positive-mode FAB-MS m/z: 1247 $(M+2Na-H)^+$.

Camelliasaponin B_2 (**5**): Colorless fine crystals from aqueous MeOH, mp 233.5—235.6 °C, $[\alpha]_D^{25}$ +20.7° (c=0.5, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{58}H_{90}NaO_{26}$ $(M+Na)^+$: 1225.5618. Found : 1225.5599. IR (KBr) cm^{-1}: 3432, 1740, 1721, 1686, 1647, 1078. ^{1}H-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-H_3×7), 1.59 (3H, d, J=7.0 Hz, Tig.-4-H_3), 1.88 (3H, s, Tig.-5-H_3), 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). ^{13}C-NMR: given in Table 2. Positive-mode FAB-MS m/z: 1225 $(M+Na)^+$.

Camelliasaponin C_1 (**7**): Colorless fine crystals from aqueous MeOH, mp 165.8—167.2 °C, $[\alpha]_D^{25}$ +4.3° (c=0.5, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{58}H_{92}NaO_{26}$ $(M+Na)^+$: 1227.5774. Found: 1227.5831. IR (KBr) cm^{-1}: 3416, 1730, 1686, 1648, 1078. ^{1}H-NMR (pyridine-d_5) δ: 0.92, 1.03 (6H each, both s, 25, 26, 24, 29-H_3×4), 1.27, 1.81 (3H each, both s, 30, 27-H_3×2), 1.96 (3H, s, Ang.-5-H_3), 2.07 (3H, d, J=6.9 Hz, Ang.-4-H_3), 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). ^{13}C-NMR: given in Table 2. Positive-mode FAB-MS m/z: 1227 $(M+Na)^+$.

Camelliasaponin C_2 (**8**): Colorless fine crystals from aqueous MeOH, mp 177.6—178.9 °C, $[\alpha]_D^{25}$ +8.8° (c=0.5, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{58}H_{92}NaO_{26}$ $(M+Na)^+$: 1227.5774. Found: 1227.5815. IR (KBr) cm^{-1}: 3432, 1736, 1686, 1647, 1078. ^{1}H-NMR (pyridine-d_5) δ: 0.92, 0.93 (3H each, both s, 25, 26-H_3×2), 1.03 (6H, s, 24, 29-H_3×2), 1.26, 1.81 (3H each, both s, 30, 27-H_3×2), 1.56 (3H, d, J=6.9 Hz, Tig.-4-H_3), 1.86 (3H, s, Tig.-5-H_3), 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). ^{13}C-NMR: given in Table 2. Positive-mode FAB-MS m/z: 1227 $(M+Na)^+$.

Alkaline Hydrolysis of Camelliasaponins A_1 (1) and A_2 (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, $CHCl_3$–MeOH–H_2O (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 $(CH_2)_2Cl_2$ (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–H_2O (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° (c=0.5, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{53}H_{86}NaO_{24}$ $(M+Na)^+$: 1129.5406. Found: 1129.5378. IR (KBr) cm^{-1}: 3424, 1736, 1655, 1640, 1078. ^{1}H-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-H_3 × 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). ^{13}C-NMR: given in Table 2. Positive-mode FAB-MS m/z: 1129 $(M+Na)^+$.

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 ^{1}H-NMR data with reported values.[3,17]

Enzymatic Hydrolysis of Camelliasaponin A_1 (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 ° (c=0.2, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{35}H_{57}O_5$ $(M+H)^+$: 557.4206. Found: 557.4216. IR (KBr) cm^{-1}: 3453, 1679, 1655. ^{1}H-NMR (pyridine-d_5) δ: 0.97, 1.08 (6H each, both s, 25, 26, 24, 29-H_3 × 4), 1.26, 1.32, 1.89 (3H each, all s, 30, 23, 27-H_3 × 3), 1.97 (3H, s, Ang.-5-H_3), 2.10 (3H, d, J=6.9 Hz, Ang.-4-H_3), 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). ^{13}C-NMR: given in Table 2. Positive-mode FAB-MS m/z: 557 $(M+Na)^+$.

Enzymatic Hydrolysis of Camelliasaponin A_2 (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° (c=0.1, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{35}H_{57}O_5$ $(M+H)^+$: 557.4216. Found : 557.4233. IR (KBr) cm^{-1}: 3460, 1679, 1643. ^{1}H-NMR (pyridine-d_5) δ: 0.97, 1.08 (6H each, both s, 25, 26, 24, 29-H_3 × 4), 1.27, 1.32, 1.89 (3H each, all s, 30, 23, 27-H_3 × 3), 1.56 (3H, d, J=6.9 Hz, Tig.-4-H_3), 1.89 (3H, s, Tig.-5-H_3), 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). ^{13}C-NMR: given in Table 2. Positive-mode FAB-MS m/z: 557 $(M+Na)^+$.

Alkaline Hydrolysis of Camelliasaponins B_1 (4) and B_2 (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–H_2O (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 $(CH_2)_2Cl_2$ (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° (c=0.5, MeOH). High-resolution negative-mode FAB-MS: Calcd for $C_{53}H_{83}O_{25}$ $(M-H)^-$: 1119.5224. Found : 1119.5226. IR (KBr) cm^{-1}: 3425, 1735, 1719, 1638, 1078. ^{1}H-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-H_3 × 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). ^{13}C-NMR: given in Table 2. Negative-mode FAB-MS m/z: 1119 $(M-H)^-$.

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 ^{1}H-NMR data with reported values.[3]

Enzymatic Hydrolysis of Camelliasaponins B_1 (4) and B_2 (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° (c=0.4, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{35}H_{55}O_5$ $(M+H)^+$: 571.3999. Found : 571.3976. IR (KBr) cm^{-1}: 3477, 1725, 1686, 1647. ^{1}H-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-H_3 × 6), 1.96 (3H, s, Ang.-5-H_3), 2.09 (3H, d, J=6.9 Hz, Ang.-4-H_3), 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). ^{13}C-NMR: given in Table 3. Positive-mode FAB-MS m/z: 571 $(M+H)^+$.

22-*O*-Tigloylcamelliagenin B (**14**): Colorless fine crystals from $CHCl_3$–MeOH, mp 170.3—172.9 °C, $[\alpha]_D^{25}$ +68.5° (c=0.5, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{35}H_{55}O_5$ $(M+H)^+$: 571.3999. Found: 571.4037. IR (KBr) cm^{-1}: 3425, 1725, 1674, 1649. ^{1}H-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-H_3 × 6), 1.56 (3H, d, J=7.3 Hz, Tig.-4-H_3), 1.87 (3H, s, Tig.-5-H_3), 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). ^{13}C-NMR: given in Table 3. Positive-mode FAB-MS m/z: 571 $(M+H)^+$.

Alkaline Hydrolysis of Camelliasaponins C_1 (7) and C_2 (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–H_2O (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)–$(CH_2)_2Cl_2$ (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° (c=0.5, MeOH). High-resolution negative-mode FAB-MS: Calcd for $C_{53}H_{85}O_{25}$ $(M-H)^-$: 1227.5380. Found : 1227.5409. IR (KBr) cm^{-1}: 3411, 1719, 1638, 1078. ^{1}H-NMR (pyridine-d_5) δ: 0.83, 0.86, 1.12, 1.79 (3H each, all s, 25, 26, 30, 27-H_3 × 4), 1.02 (6H, s, 24, 29-H_3 × 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}C-NMR: given in Table 2. Negative-mode FAB-MS m/z: 1121 $(M-H)^-$.

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}H-NMR data with reported values.[3)]

Enzymatic Hydrolysis of Camelliasaponins C_1 (7) and C_2 (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° (c=0.2, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{35}H_{55}O_6$ $(M+H)^+$: 573.4155. Found : 573.4113. IR (KBr) cm^{-1}: 3444, 1687, 1649. ^{1}H-NMR (pyridine-d_5) δ: 0.99, 1.10, 1.28, 1.82 (3H each, all s, 25, 29, 30, 27-H_3 × 7), 1.04 (6H, s, 24, 26-H_3 × 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}C-NMR: given in Table 3. Positive-mode FAB-MS m/z: 573 $(M+H)^+$.

22-*O*-Tigloylcamelliagenin C (**17**): Colorless fine crystals from $CHCl_3$–MeOH, mp 142.7—144.8 °C, $[\alpha]_D^{25}$ +44.0° (c=0.8, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{35}H_{55}O_6$ $(M+H)^+$: 573.4155. Found: 573.4146. IR (KBr) cm^{-1}: 3436, 1674, 1649. ^{1}H-NMR (pyridine-d_5) δ: 0.99, 1.10, 1.28, 1.82 (3H each, all s, 25, 29, 30, 27-H_3 × 7), 1.04 (6H, s, 24, 26-H_3 × 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}C-NMR: given in Table 3. Positive-mode FAB-MS m/z: 573 $(M+H)^+$.

$NaBH_4$ Reduction of Camelliasaponins B_1 (4) and B_2 (5) Giving Camelliasaponins C_1 (7) and C_2 (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 × 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 C_1 by TLC, IR, ^{1}H-NMR and ^{13}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 C_2 by TLC, IR, ^{1}H-NMR, and ^{13}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 administrated. Blood (*ca.* 0.4ml) 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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References and Notes

1) Part IV : Yoshikawa M., Murakami T., Ueda T., Matsuda H., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **44**, 1736—1743 (1996).
2) The flower: *a*) Itokawa H., Nakajima H., Ikuta A., Iitaka Y., *Phytochemistry*, **20**, 2539—2542 (1981); *b*) Nakajima H., Itokawa H., Ikuta A., *Yakugaku Zasshi*, **104**, 157—161 (1984); The leaves: *c*) Nagata T., Tsushida T., Hamaya E., Enoki N., Manabe S., Nishino C., *Agric. Biol. Chem.*, **49**, 1181—1186 (1985); *d*) Nishino C., Manabe S., Enoki N., Nagata T., Tsushida T., Hamaya E., *J. Chem. Soc., Chem. Commun.*, **1986**, 720—723; *e*) Numata A., Kitajima A., Katsuno T., Yamamoto K., Nagahama N., Takahashi C., Fujiki R., Nabae M., *Chem. Pharm. Bull.*, **35**, 3948—3951 (1987).
3) *a*) Ito S., Kodama M., Konoike M., *Tetrahedron Lett.*, **1967**, 591—596; *b*) Itokawa H., Sawada N., Murakami T., *ibid.*, **1967**, 597—601; *c*) *Idem*, *Yakugaku Zasshi*, **88**, 1463—1466 (1968); *d*) *Idem*, *Chem. Pharm. Bull.*, **17**, 474—480 (1969).
4) *a*) Yoshikawa M., Murakami T., Ueno T., Kadoya M., Matsuda H., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **43**, 350—352 (1995); *b*) Yamahara J., Matsuda H., Yamaguchi S., Shimoda H., Murakami N., Yoshikawa M., *Nat. Med.*, **49**, 76—83 (1995); *c*) Yamahara J., Miki A., Tsukamoto K., Murakami N., Yoshikawa M., *ibid.*, **49**, 84—87 (1995); *d*) Yoshikawa M., Ueda T., Muraoka O., Aoyama H., Matsuda H., Shimoda H., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **43**, 532—534 (1995); *e*) Yoshikawa M., Shimada H., Shimoda H., Matsuda H., Yamahara J., Murakami N., *ibid.*, **43**, 1245—1247 (1995); *f*) Yoshikawa M., Yamaguchi S., Nishisaka H., Yamahara J., Murakami N., *ibid.*, **43**, 1462—1465 (1995); *g*) Yamahara J., Matsuda H., Shimoda H., Wariishi N., Yagi N., Murakami N., Yoshikawa M., *Folia Pharmacol. Jpn.*, **105**, 365—379 (1995); *h*) Ikeda N., Fukuda T., Jyo H., Shimada Y., Murakami N., Saka M., Yoshikawa M., *Yakugaku Zasshi*, **114**, 138—147 (1996); *i*) Yoshikawa M., Yoshizumi S., Murakami T., Matsuda H., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **44**, 492—499 (1996).
5) *a*) Yoshikawa M., Matsuda H., Harada E., Murakami T., Wariishi N., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **42**, 1354—1356 (1994); *b*) Yoshikawa M., Harada E., Matsuda H., Murakami T., Yamahara J., Murakami N., *ibid.*, **41**, 2069—6071 (1993); *c*) Yoshikawa M., Yoshizumi S., Ueno T., Matsuda H., Murakami T., Yamahara J., Murakami N., *ibid.*, **43**, 1878—1882 (1995).
6) Yoshikawa M., Books of Abstracts, 210th American Chemical Society National Meeting, Chicago, August 1995, Part 1, p. 22.
7) *a*) Yoshikawa M., Harada E., Murakami T., Matsuda H., Wariishi N., Yamahara J., Murakami N., Kitagawa I., *Chem. Pharm. Bull.*, **42**, 1357—1359 (1994); *b*) Yoshikawa M., Murakami T., Matsuda H., Yamahara J., Murakami N., Kitagawa I., *ibid.*, **44**, 1454—1464 (1996).
8) *a*) Yoshikawa M., Murakami T., Ueno T., Kadoya M., Matsuda H., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **43**, 350—352 (1995); *b*) *Idem*, *ibid.*, **43**, 2115—2122 (1995); *c*) Yoshikawa M., Murakami T., Matsuda H., Ueno T., Kadoya M., Yamahara J., Murakami N., *ibid.*, **44**, 1305—1313 (1996).
9) *a*) Yashikawa M., Murakami T., Kadoya M., Matsuda H., Yamahara J., Muraoka O., Murakami N., *Heterocycles*, **41**, 1621—1626 (1995); *b*) Yoshikawa M., Murakami T., Kadoya M., Matsuda H., Muraoka O., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **44**, 1212—1217 (1996).
10) Murakami N., Murakami T., Kadoya M., Matsuda H., Yamahara J., Yoshikawa M., *Chem. Pharm. Bull.*, **44**, 469—471 (1996).
11) Yoshikawa M., Harada E., Murakami T., Matsuda H., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **42**, 742—744 (1994).
12) This work was partly reported in our preliminary communica-

tion.[11])
13) Yoshikawa K., Nakagawa M., Yamamoto R., Arihara S., Matsuura K., *Chem. Pharm. Bull.*, **40**, 1779—1782 (1992).
14) The proportions of carbohydrates were determined from the peak areas in GLC analysis.
15) The ^{1}H-NMR and ^{13}C-NMR spectra were assigned with the aid of homo and hetero correlation spectroscopy (^{1}H–^{1}H, ^{1}H–^{13}C COSY), homo and heteronuclear Hartmann-Hahn spectroscopy (^{1}H–^{1}H, ^{1}H-^{13}C HOHAHA), and distortionless enhancement by polarization transfer (DEPT) and HMBC.
16) *a*) Muro T., Kuramoto T., Imoto K., Okada S., *Agric. Biol. Chem.*, **50**, 687—692 (1986); *b*) Sakaki Y., Morita T., Kuramoto T., Mizutani K., Ikeda R., Tanaka O., *ibid.*, **52**, 207—210 (1988).
17) *a*) Khong P. W., Lewis K. G., *Aust. J. Chem.*, **28**, 165—172 (1975); *b*) Chakravarty A. K., Das B., Parkrashi S. C., *Phytochemistry*, **26**, 2348—2349 (1987).