Medicinal Flowers. XXII[1] Structures of Chakasaponins V and VI, Chakanoside I, and Chakaflavonoside A from Flower Buds of Chinese Tea Plant (*Camellia sinensis*)

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Received May 10, 2008; accepted July 2, 2008; published online July 3, 2008

Two acylated oleanane-type triterpene oligoglycosides, chakasaponins V and VI, an aromatic glycoside, chakanoside I, and an acylated flavonol oligoglycoside, chakaflavonoside A, were isolated from the flower buds of Chinese tea plant [*Camellia sinensis* (L.) O. Kuntze]. The chemical structures of those new glycosides were elucidated on the basis of chemical and physicochemical evidence.

Key words chakasaponin; chakanoside I; chakaflavonoside A; *Camellia sinensis*; medicinal flower; tea plant

Tea flowers, the flower buds of *Camellia* (*C.*) *sinensis* (L.) O. Kuntze, are used as a food garnish in Japanese-style dishes (*e.g.*, batabata-cha in Shimane prefecture) or drinks in Japan (*e.g.*, hanaban-cha in Shimane and Kouchi prefectures or botebote-cha in Niigata prefecture). However, the chemical constituents and biological activities of tea flowers have not yet characterized. Previously, we have reported the isolation and structure elucidation of floratheasaponins A (**5**), B (**6**), and C (**7**) from Japanese tea flowers, the flower buds of Japanese *C. sinensis*.[2] Floratheasaponins (**5**—**7**) were found to show inhibitory effects on serum triglyceride elevation in olive oil-treated mice,[2] on ethanol- and indomethacin-induced gastric mucosal lesions in rats,[3] and on serum glucose elevation in sucrose-loaded rats.[3] From the flower buds of Chinese tea plant cultivated in Anhui province, floratheasaponins D (**8**), E (**9**), F (**10**), G (**11**), H, and I (**12**), were isolated together with floratheasaponins A (**5**), B (**6**), and C (**7**).[4] The principal floratheasaponins (**5**—**10**) were found to exhibit inhibitory activities on the release of β-hexosaminidase from RBL-2H3 cells.[4] Furthermore, we have developed qualitative and quantitative methods using HPLC for the principal floratheasaponins in tea flowers cultivated in Japan, China, India, and Sri Lanka.[3] In the course of our studies on the saponin compositions of tea flowers cultivated in various provinces of China, we found that floratheasaponins were not contained in the tea flowers cultivated in Fujian province, from which chakasaponins I (**13**), II (**14**), and III (**15**) were isolated and their structures were determined.[5] Chakasaponins (**13**—**15**) significantly showed accelerating effects on gastrointestinal transit and inhibitory effects against pancreatic lipase.[5] As a continuation of our studies on bioactive constituents of medicinal flowers[6—11] and tea plants,[12—19] we examined the constituents of the tea flowers cultivated in Sichuan and Fujian provinces of China. In this paper, we describe the isolation and structure elucidation of two new acylated oleanane-type triterpene oligoglycosides termed chakasaponins V (**1**) and VI (**2**) and a new aromatic glycoside called chakanoside I (**3**) from the tea flowers cultivated in Sichuan province and a new flavonol oligoglycoside named chakaflavonoside A (**4**) from the tea flowers cultivated in Fujian province.

Isolation of Chakasaponins V and VI, and Chakanoside I The methanolic extract (33.6% from the dried flower buds of *C. sinensis* cultivated in Sichuan province of China) was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction (3.3%) and aqueous layer. The aqueous layer was further extracted with 1-butanol (1-BuOH) to give 1-BuOH- (12.7%) and H_2O- (17.6%) soluble fractions. The 1-BuOH-soluble fraction was subjected to normal-phase and reversed-phase silica gel column chromatographies and repeated HPLC to give chakasaponins V (**1**, 0.10%) and VI (**2**, 0.039%), and chakanoside I (**3**, 0.0010%) together with 12 known saponins, floratheasaponins A (**5**, 0.17%),[2] B (**6**, 0.29%),[2] C (**7**, 0.018%),[2] D (**8**, 0.13%),[4] E (**9**, 0.032%),[4] F (**10**, 0.031%),[4] G (**11**, 0.17%),[4] and I (**12**, 0.063%),[4] chakasaponins I (**13**, 0.14%),[5] II (**14**, 0.14%),[5] and III (**15**, 0.079%),[5] assamsaponin E (**16**, 0.070%),[12] nine known flavonoids, kaempferol (**17**, 0.0024%),[20] astragalin (**18**, 0.0030%),[20] trifolin (**19**, 0.0037%),[21] kaempferol 3-*O*-(2″-*O*-*p*-*trans*-coumaroyl)-β-D-glucopyranoside (**20**, 0.00073%),[22] kaempferol 3-*O*-[α-L-rhamnopyranosyl(1—6)-β-D-glucopyranoside (**21**, 0.0014%),[23] kaempferol 3-*O*-[α-L-rhamnopyranosyl(1—6)-β-D-galactopyranoside (**22**, 0.0017%),[23] kaempferol 3-*O*-β-D-glucopyranosyl(1—3)-α-L-rhamnopyranosyl(1—6)-β-D-glucopyranoside (**23**, 0.018%),[24] kaempferol 3-*O*-β-D-glcopyranosyl(1—3)-α-L-rhamnopyranosyl(1—6)-β-D-galactopyranoside (**24**, 0.051%),[24] and 2-methyl-5,7-dihydroxychromone 7-*O*-β-D-glucopyranoside (**25**, 0.0016%),[25] a catechin, (−)-epicatechin (**26**, 0.0014%),[26] six known aromatic glycosides, 1-(*R*)-phenylethyl β-D-glucopyranoside (**27**, 0.020%),[27] benzyl β-D-glucopyranoside (**28**, 0.0016%),[28] 2-*O*-β-D-glucopyranosyl-(1*R*)-phenylethylene glycol (**29**, 0.00016%),[29] *trans*-*p*-coumaroyl-β-D-glucopyranoside (**30**, 0.00061%),[30] *trans*-*p*-feruloyl-β-D-glucopyranoside (**31**, 0.00027%),[31] and sachaliside I (=triandirin, **32**, 0.00030%),[32] three other compounds, icariside B_5 (**33**, 0.00056%),[33] 1-(*S*)-methylbutyl-β-D-glucopyranoside (0.0019%),[34] and caffeine (0.0050%).

Isolation of Chakaflavonoside A The 1-BuOH-soluble fraction (16.4% from the dried flower buds)[5] from the flower buds of *C. sinensis* cultivated in Fujian province was separated by normal- and reversed-phase silica gel column chromatographies and repeated HPLC to give chakaflavonoside A (**4**, 0.053%).

Structures of Chakasaponins V and VI, Chakanoside I,

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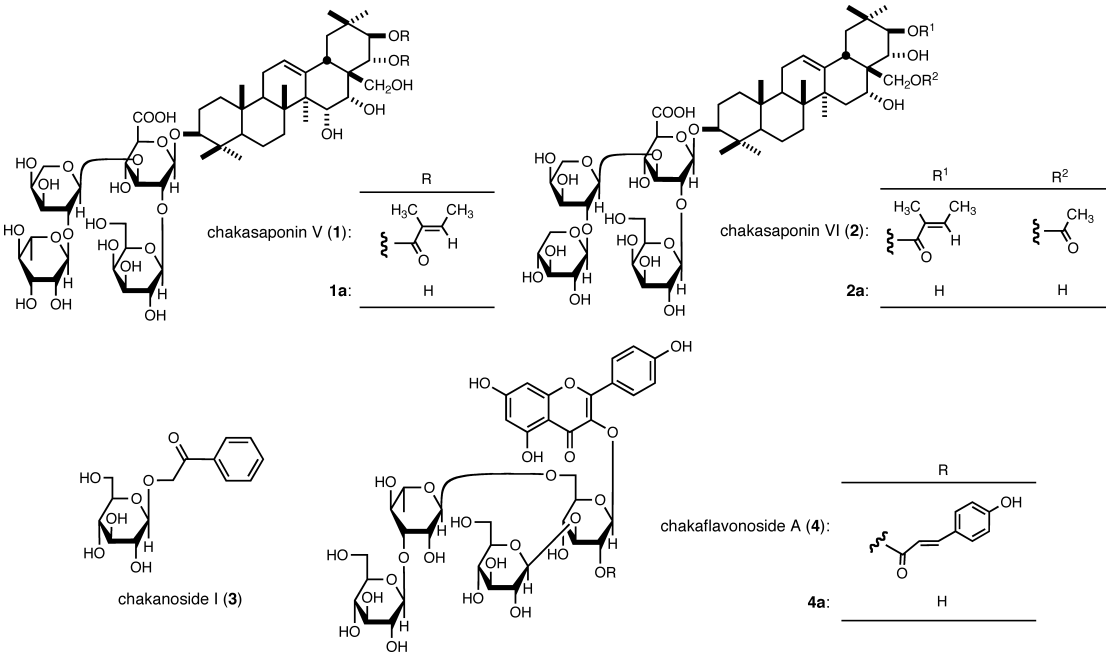

Chart 1

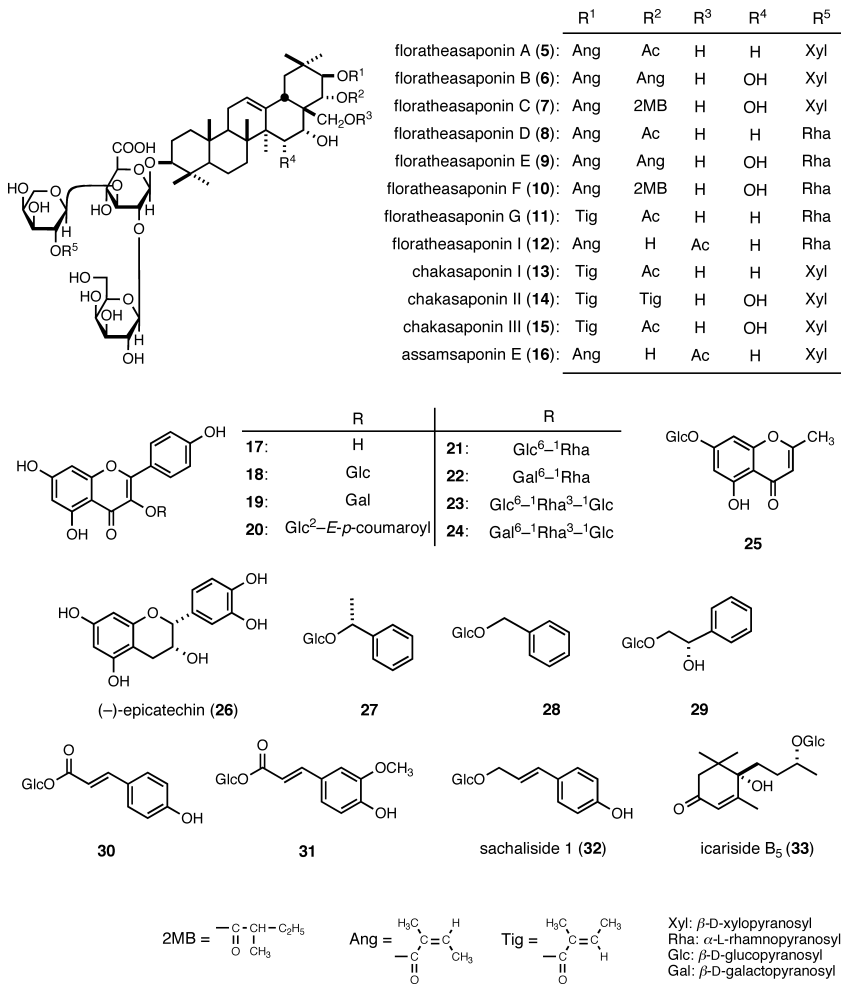

Chart 2. Isolated Known Constituents from the Flower Buds of *C. sinensis*

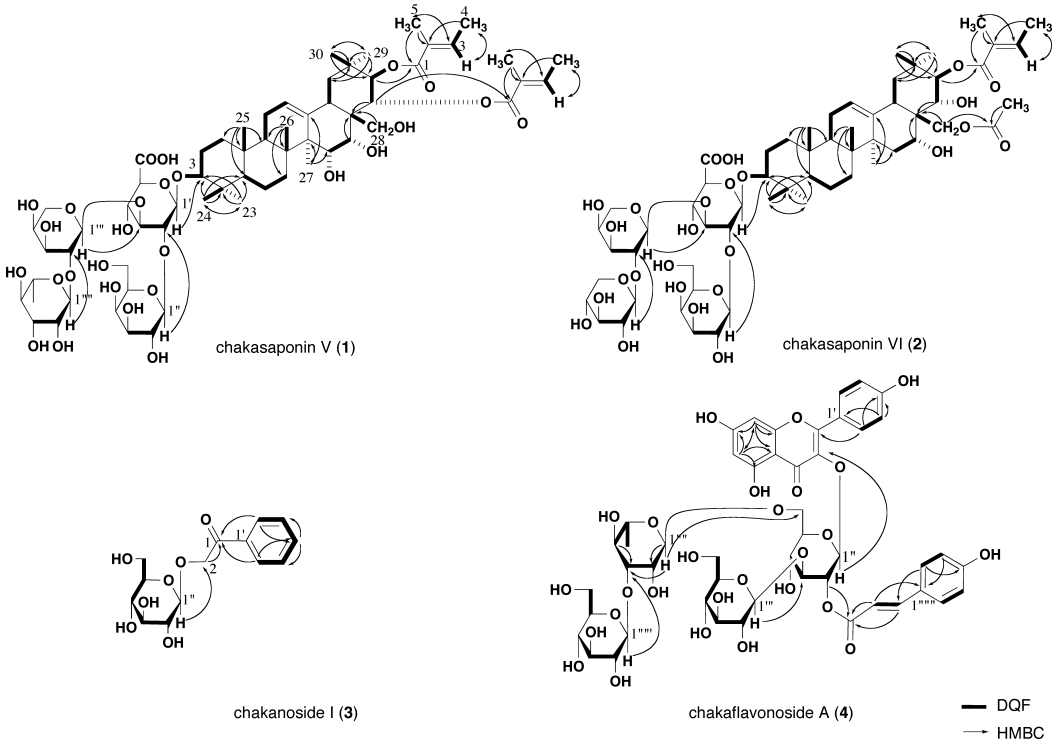

Fig. 1. Significant DQF COSY and HMBC Correlations for New Constituents (**1**—**4**)

and Chakaflavonoside A Chakasaponin V (**1**) was isolated as colorless fine crystals with mp 204.0—207.0 °C (from $CHCl_3$–MeOH) and exhibited a positive optical rotation ($[\alpha]_D^{21}$ +10.3° in MeOH). The IR spectrum of **1** showed absorption bands at 1716 and 1641 cm^{-1} ascribable to carboxyl and α,β-unsaturated ester functions and broad bands at 3541, 1080, and 1048 cm^{-1} suggestive of an oligoglycoside structure. In the positive-ion matrix-assisted laser desorption/ionization (MALDI)-MS of **1**, a quasimolecular ion peak was observed at *m/z* 1309 $(M+Na)^+$, and high-resolution (HR) MALDI-MS analysis of the quasimolecular ion peak revealed the molecular formula of **1** to be $C_{63}H_{98}O_{27}$. On alkaline hydrolysis of **1** with 10% aqueous KOH–50% aqueous 1,4-dioxane (1 : 1), desacyl-floratheasaponin E (**1a**)[4] was obtained together with tiglic acid, which was identified by HPLC analysis of the *p*-nitrobenzyl derivative.[13,35] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **1**, which were assigned by various NMR experiment,[36] showed signals assignable to a desacyl-floratheasaponin E part {seven methyls [δ 0.74, 0.92, 1.04, 1.08, 1.18, 1.25, 1.77 (3H each, all s, H_3-25, 26, 29, 24, 23, 30, 27)], four glycopyranosyl moieties [δ 4.84 (1H, d, J=7.6 Hz, H-1′), δ 5.58 (1H, d, J=6.8 Hz, H-1″), δ 6.05 (1H, d, J=7.2 Hz, H-1‴), δ 5.96 (1H, br s, H-1⁗)], and two tigloyl moieties [δ 1.23, 1.52 (3H each, both d, J=6.8 Hz, H_3-22-*O*-Tig-4, H_3-21-*O*-Tig-4), δ 1.64 (3H, s, H_3-22-*O*-Tig-5) δ 1.82 (3H, br s, H_3-21-*O*-Tig-5), δ 6.70, 6.98 (1H each, both dq-like, H-22-*O*-Tig-3, H-21-*O*-Tig-3)]}. The positions of the two tigloyl groups in **1** were characterized on the basis of an HMBC experiment, in which long-range correlations were observed between the 21,22-protons and tigloyl carbonyl carbons. In addition, comparison of the ^{13}C-NMR data for **1** with those of **1a** revealed acylation shifts around the 21- and 22-positions. On the basis of those findings, the structure of chakasaponin V was determined to be 21,22-di-*O*-tigloyl-R_1-barrigenol 3-*O*-[β-D-galactopyranosyl(1—2)][α-L-rhamnopyranosyl(1—2)-α-L-arabinopyranosyl(1—3)]-β-D-glucopyranosiduronic acid (**1**).

Chakasaponin VI (**2**) was also obtained as colorless fine crystals with mp 201.0—203.0 °C (from $CHCl_3$–MeOH) and a negative optical rotation ($[\alpha]_D^{20}$ −12.5° in MeOH). The IR spectrum of **2** showed absorption bands at 3452, 1714, 1647, 1078, and 1048 cm^{-1}, ascribable to hydroxyl, carbonyl, α,β-unsaturated ester, and ether functions. The molecular formula, $C_{59}H_{92}O_{26}$, of **2** was determined from the positive- and negative-ion fast atom bombardment (FAB)-MS [*m/z* 1239 $(M+Na)^+$, and *m/z* 1215 $(M-H)^-$] and by positive-ion HR-FAB-MS measurement. Alkaline hydrolysis of **2** liberated desacyl-assamsaponin E (**2a**)[12] together with tiglic acid and acetic acid, which were identified by HPLC analysis of their *p*-nitrobenzyl derivatives. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) indicated the presence of a desacyl-assamsaponin E part and a tigloyl [δ 1.50 (3H, d, J=6.9 Hz, H_3-4), 1.74 (3H, s, H_3-5), 6.92 (1H, qd-like, H-3)] and an acetyl [δ 1.92 (3H, s)] moieties. Finally, the positions of two acyl groups in **2** were determined by the HMBC experiment, which showed long-range correlations between the 21-proton and the tigloyl carbonyl carbon and between the 28-protons and the acetyl carbonyl carbon. Consequently, the structure of chakasaponin VI was elucidated as 21-*O*-tigloyl-28-*O*-acetyl-theasapogenol B 3-*O*-[β-D-galactopyranosyl(1—2)][β-D-xylopyranosyl(1—2)-α-L-arabinopyranosyl(1—3)]-β-D-glucopyranosiduronic acid (**2**).

Chakanoside I (**3**), obtained as a white powder with a negative optical rotation ($[\alpha]_D^{26}$ −31.9° in MeOH), showed absorption bands at 3414, 1694, and 1078 cm^{-1} due to hydroxyl, carbonyl, and ether functions in the IR spectrum. The

Table 1. ^{13}C-NMR (150 MHz) Data of Chakasaponins V (**1**) and VI (**2**)

C-	**1**	**2**	C-	**1**	**2**
1	38.8	38.6	GlcA		
2	26.4	26.3	1′	105.5	105.5
3	89.6	89.5	2′	78.8	78.7
4	39.4	39.4	3′	83.7	83.6
5	55.3	55.5	4′	72.3	70.9
6	18.6	18.2	5′	76.4	77.0
7	36.5	32.9	6′	171.9	172.0
8	41.2	39.8	Gal		
9	46.9	46.7	1″	103.3	103.2
10	36.8	36.1	2″	73.6	73.5
11	23.8	23.7	3″	74.9	74.9
12	125.2	123.3	4″	69.8	69.8
13	143.5	142.5	5″	76.2	76.3
14	47.6	41.6	6″	61.9	61.8
15	74.1	34.4	Ara		
16	67.4	67.5	1‴	101.0	101.5
17	48.4	47.0	2‴	77.0	82.0
18	40.7	40.7	3‴	73.5	73.2
19	46.7	46.9	4‴	68.9	68.3
20	36.3	36.5	5‴	65.7	65.8
21	78.8	81.4	Rha		
22	73.5	71.0	1⁗	102.2	
23	27.7	27.8	2⁗	72.3	
24	16.6	16.6	3⁗	72.5	
25	15.6	15.4	4⁗	74.1	
26	17.4	16.8	5⁗	69.9	
27	21.0	27.2	6⁗	18.1	
28	62.6	66.2	Xyl		
29	29.3	29.5	1⁗		106.6
30	19.9	19.9	2⁗		75.6
21-*O*-Tig			3⁗		77.9
1	168.0	168.4	4⁗		70.5
2	129.2	129.6	5⁗		67.2
3	136.8	136.0			
4	14.0	14.0			
5	12.2	12.2			
22-*O*-Tig					
1	168.1				
2	129.0				
3	137.9				
4	13.8				
5	12.1				
28-*O*-Ac					
1		170.0			
2		20.6			

Measured in pyridine-d_5.

Table 2. ^{13}C-NMR (125 MHz) Data of Chakanoside I (**3**)

C-	**3**	C-	**3**
1	199.6	Glc	
2	72.4	1″	103.2
1′	137.5	2″	74.0
2′	128.7	3″	76.9
3′	129.6	4″	70.6
4′	135.6	5″	76.7
5′	129.6	6″	62.7
6′	128.7		

Measured in D_2O.

molecular formula, $C_{14}H_{18}O_7$, of **3** was characterized by the positive-ion FAB-MS [*m/z* 321 $(M+Na)^+$] and by HR-FAB-MS measurement. Acid hydrolysis of **3** with 1.0 M HCl liberated D-glucose, which was identified by HPLC using an optical rotation detector.[37] The ^{1}H-NMR (D_2O) and ^{13}C-NMR (Table 2) spectra of **3** showed signals due to a methylene bearing an oxygen function [δ 5.01, 5.18 (1H each, both d, J=16.8 Hz, H_2-2)] and a benzoyl group together with a β-D-glucopyranosyl moiety [δ 4.45 (1H, d, J=6.9 Hz, H-1″)]. The HMBC experiment on **3** showed long-range correlations between the 1″-proton and 2-methylene carbon and between the 2-methylene protons and 1-carbonyl carbon, so that the structure of chakanoside I was elucidated as shown.

Chakaflavonoside A (**4**) was isolated as a yellow powder with a negative optical rotation ($[\alpha]_D^{24}$ −96.7° in MeOH). The IR spectrum of **4** showed absorption bands at 3430, 1712, 1655, 1606, and 1076 cm^{-1} ascribable to hydroxyl, ester, chelated carbonyl, aromatic ring, and ether functions. In the UV spectrum of **4**, absorption maxima were observed at 316 (log ε 4.52), 267 (4.36), and 207 (4.57) nm, suggestive of a flavonol structure. The molecular formula, $C_{48}H_{56}O_{27}$, of **4** was determined by the positive-ion FAB-MS [*m/z* 1087 $(M+Na)^+$] and by HR-FAB-MS measurement. The negative-ion FAB-MS of **4** showed a quasimolecular ion peak at *m/z* 1063 $(M-H)^-$ together with fragment ion peaks at *m/z* 1047 $(M-OH)^-$, *m/z* 917 $[M-C_9H_7O_2$ (*p*-coumaroyl)$]^-$, *m/z* 901 $[M-C_6H_{11}O_5$ (Glc)$]^-$, *m/z* 755 $[M-C_{12}H_{21}O_9$ (Glc-Rha)$]^-$, *m/z* 591 $[M-C_{12}H_{21}O_9$ (Glc-Rha)$-C_6H_{11}O_5$ (Glc)$]^-$, and *m/z* 285 (Kaempferol$-H)^-$, which were derived by cleavage of the glycosidic linkages. The alkaline hydrolysis of **4** provided desacyl-chakaflavonoside A (**4a**) and *trans-p*-coumaric acid. Acid hydrolysis of **4** with 1.0 M aqueous HCl furnished D-glucose and L-rhamnose, which were identified by HPLC analysis using an optical rotation detector.[37] The molecular formula, $C_{39}H_{50}O_{25}$, of **4a** was determined by the positive- and negative-ion FAB-MS [*m/z* 941 $(M+Na)^+$, *m/z* 917 $(M-H)^-$, *m/z* 755 $(M-C_6H_{11}O_5)^-$, *m/z* 285 (Kaempferol$-H)^-$] and by HR-FAB-MS measurement. The carbon and proton signals due to the aglycon moiety in the ^{1}H- and ^{13}C-NMR spectra of **4a** were superimposable to those of kaempferol 3-*O*-glycosides.[23] Namely, the ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 3) spectra of **4a** showed signals due to a kaempferol part [δ 6.19 (1H, d, J=2.0 Hz, H-6), 6.39 (1H, d, J=2.0 Hz, H-8), 8.03 (2H, d, J=8.2 Hz, H_2-2′, 6′), 6.78 (2H, d, J=8.2 Hz, H_2-3′, 6′)], three β-D-glucopyranosyl [δ 5.15 (1H, d, J=6.9 Hz, H-1″), 4.61 (1H, d, J=7.6 Hz, H-1‴) and 4.36 (1H, d, J=7.6 Hz, H-1⁗)] and an α-L-rhamnopyranosyl [δ 4.54 (1H, s, H-1⁗)] moieties. Enzymatic hydrolysis of **4a** with naringinase liberated kaempferol. The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 3) spectra of **4** showed signals due to a desacyl-chakaflavonoside part together with *trans-p*-coumaroyl group [δ 6.34, 7.65 (1H each, both d, J=15.9 Hz, *p*-coumaroyl-2, 3), 7.41, 6.76 (2H each, both d, J=8.7 Hz, *p*-coumaroyl-2′, 6′, 3′, 5′)]. The oligoglycoside structure bonding to the 3-position was characterized by HMBC experiment, which showed long-range correlations between the following protons and carbons: H-1″ and C-3, H-1‴ and C-3″, H-1⁗ and C-6″, H-1⁗′ and C-3⁗′. The position of the *E-p*-coumaroyl group was also determined by HMBC experiment, which showed a long-range correlation between the 2″-proton and the *p*-coumaroyl carbonyl carbon. Furthermore, comparison of the ^{13}C-NMR data for **4** with those of **4a** revealed an acylation shift around the 2″-position. On the basis of those findings, the structure of chakaflavonoside A was determined to be kaempferol 3-*O*-[β-D-glucopyranosyl(1—3)] [β-D-glucopy-

Table 3. ^{13}C-NMR (150 MHz) Data of Chakaflavonoside A (**4**) and Desacyl-Chakaflavonoside A (**4a**)

C-	4	4a	C-	4	4a
2	159.0	159.4	Glc-1″	100.8	104.2
3	134.8	135.5	2″	74.5	75.4
4	179.0	179.3	3″	84.7	87.4
5	163.0	163.0	4″	69.5	70.1
6	99.9	100.0	5″	76.8	76.8
7	165.6	166.0	6″	68.5	68.9
8	94.9	95.0	Glc-1‴	104.8	105.1
9	158.4	158.6	2‴	74.8	75.2
10	105.9	105.6	3‴	77.5	77.5
1′	122.8	122.7	4‴	70.8	70.8
2′	132.3	132.4	5‴	78.1	78.1
3′	116.5	116.2	6‴	62.1	62.0
4′	161.3	161.5	Rha-1⁗	102.3	102.4
5′	116.5	116.2	2⁗	71.3	71.3
6′	132.3	132.4	3⁗	83.1	83.1
acyl-1‴″	127.3		4⁗	72.5	72.5
2‴″	131.3		5⁗	70.2	69.4
3‴″	116.8		6⁗	18.0	18.0
4‴″	161.2		Glc-1‴″	105.9	105.6
5‴″	116.8		2‴″	75.4	75.6
6‴″	131.3		3‴″	77.6	77.8
7‴″	147.2		4‴″	71.4	71.6
8‴″	115.2		5‴″	77.5	77.5
9‴″	168.6		6‴″	62.5	62.7

Measured in CD_3OD

ranosyl(1—3)-α-L-rhamnopyranosyl(1—6)]-(2-*O*-*trans*-*p*-coumaroyl)-β-D-glucopyranoside (**4**).[38,39]

In conclusion, two acylated oleanane-type triterpene oligoglycosides, chakasaponins V (**1**) and VI (**2**), an aromatic glycoside, chakanoside I (**3**), and an acylated flavonol oligoglycoside, chakaflavonoside A (**4**), were isolated from the flower buds of *C. sinensis* grown in China (Sichuan province). Furthermore, both floratheasaponins A—I, which were only isolated from the flower buds of *C. sinensis* grown in Japan and China (Anhui province), and chakasaponins I—III, which were only isolated from the flower buds of *C. sinensis* grown in China (Fujian province), were obtained from the flower buds of *C. sinensis* grown in China (Sichuan province). This finding is interesting from the perspective of chemotaxonomy of *C. sinensis*.

Experimental

The following instruments were used to obtain physical and spectroscopic data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); IR spectra, Shimadzu FTIR-8100 spectrometer; MALDI-MS and HR-MS, Applied Biosystems Voyager-DE STR; FAB-MS and HR-MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL EX-270 (270 MHz), JNM-LA500 (500 MHz), and JEOL ECA-600K (600 MHz) spectrometers; ^{13}C-NMR spectra, JEOL EX-270 (68 MHz) JNM-LA500 (125 MHz), and JEOL ECA-600K (150 MHz) spectrometers with tetramethylsilane as an internal standard; and HPLC detector, Shimadzu RID-6A refractive index and SPD-10A*vp* UV–VIS detectors. HPLC column, COSMOSIL 5C_{18}-MS-II {[250×4.6 mm i.d. (5 μm) for analytical purpose] and [250×20 mm i.d. (5 μm) for preparative purpose], Nacalai tesque} and Develosil C30-UG-5 {[250×4.6 mm i.d. (5 μm) for analytical purpose] and [250×20 mm i.d. (5 μm) for preparative purpose], Nomura chemical} columns were used.

The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150—350 mesh); reverse-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100—200 mesh); TLC, precoated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reverse phase); reversed-phase HPTLC, precoated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm); and detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 followed by heating.

Plant Material The flower buds of *Camellia sinensis*, which were cultivated in Fujian and Sichuan province of China, were collected in 2006. The botanical identification was undertaken by one of authors (M. Y.).

Isolation of Chakasaponins V and VI and Chakanoside I The dried flower buds of *C. sinensis* (1.5 kg, Sichuan province, China) were finely cut and extracted three times with MeOH under reflux. Evaporation of the solvent under reduced pressure provided a MeOH extract (503.2 g, 33.6%). The MeOH extract (490 g) was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction (48.4 g, 3.3%) and aqueous phase, which was extracted with 1-BuOH to give 1-BuOH- (186.2 g, 12.7%) and H_2O- (255.4 g, 17.6%) soluble fractions.

The 1-BuOH-soluble fraction (180 g) was subjected to normal-phase silica gel column chromatography [3.5 kg, $CHCl_3$→$CHCl_3$: MeOH : H_2O (10 : 3 : 1→7 : 3 : 1→6 : 4 : 1)→MeOH] to give five fractions [Fr. 1, Fr. 2, Fr. 3 (15.3 g), Fr. 4 (100.1 g), Fr. 5]. Fraction 3 (15.3 g) was separated by reversed-phase silica gel column chromatography [450 g, MeOH : H_2O (5 : 95→10 : 90→20 : 80→30 : 70→40 : 60→50 : 50→60 : 40→70 : 30→MeOH→$CHCl_3$)] to yield 13 fractions [Fr. 3-1, Fr. 3-2, Fr. 3-3, Fr. 3-4, Fr. 3-5 (1.7 g), Fr. 3-6, Fr. 3-7, Fr. 3-8 (0.6 g), Fr. 3-9, Fr. 3-10 (0.5 g), Fr. 3-11 (0.3 g), Fr. 3-12, Fr. 3-13]. Fraction 3-5 (1.7 g) was subjected to normal-phase silica gel column chromatography [6.0 g, $CHCl_3$: MeOH : H_2O (20 : 3 : 1→10 : 3 : 1→6 : 4 : 1)] to give seven fractions [Fr. 3-5-1 (170 mg), Fr. 3-5-2 (484 mg), Fr. 3-5-3 (247 mg), Fr. 3-5-4 (273 mg), Fr. 3-5-5 (225 mg), Fr. 3-5-6, Fr. 3-5-7]. Fr. 3-5-1 (170 mg), Fr. 3-5-2 (484 mg), and Fr. 3-5-3 (247 mg) were purified by HPLC [MeOH–H_2O (30 : 70, v/v), COSMOSIL 5C_{18}-MS-II] to give chakanoside I (**3**, 15.1 mg), 2-methyl 5,7-dihydroxychromone 7-*O*-β-D-glucopyranoside (**25**, 19.9 mg), 1-(*R*)-phenylethyl β-D-glucopyranoside (**27**, 276 mg), benzyl β-D-glucopyranoside (**28**, 23.2 mg), caffeine (70.4 mg), and 1-(*S*)-methylbutyl-β-D-glucopyranoside (27.3 mg). Fr. 3-5-4 (273 mg) was separated by HPLC [MeOH–H_2O (25 : 75, v/v), COSMOSIL 5C_{18}-MS-II] to give six fractions {Fr. 3-5-4-1, Fr. 3-5-4-2 (29.1 mg), Fr. 3-5-4-3 (31.4 mg), Fr. 3-5-4-4 [=icariside B_5 (**33**, 8.1 mg)]}. Fr. 3-5-4-2 (29.1 mg) was further purified by HPLC [MeOH–H_2O (25 : 75, v/v), Develosil C30-UG-5] to give 2-*O*-β-D-glucosyl-(1*R*)-phenylethylene glycol (**29**, 2.3 mg), *trans*-*p*-coumaroyl-β-D-glucopyranoside (**30**, 5.5 mg), and *trans*-*p*-feruloyl-β-D-glucopyranoside (**31**, 3.9 mg). Fr. 3-5-4-3 (31.4 mg) was separated by HPLC [MeOH–H_2O (25 : 75, v/v), Develosil C30-UG-5] to give 2-methyl-5,7-dihydroxychromone 7-*O*-β-D-glucopyranoside (**25**, 2.8 mg) and sachaliside 1 (**32**, 2.4 mg). Fr. 3-5-5 (225 mg) was separated by HPLC {[1] [MeOH–H_2O (30 : 70, v/v), COSMOSIL 5C_{18}-MS-II] [2] [MeOH–H_2O (25 : 75, v/v), Develosil C30-UG-5]} to give (−)-epicatechin (**26**, 19.9 mg), *trans*-*p*-coumaroyl-β-D-glucopyranoside (**30**, 3.3 mg), and sachaliside 1 (**32**, 3.7 mg). Fr. 3-8 (0.6 g) was separated by HPLC [MeOH–H_2O (45 : 55, v/v), COSMOSIL 5C_{18}-MS-II] to give astragalin (**18**, 42.9 mg) and trifolin (**19**, 52.5 mg). Fr. 3-10 (0.5 g) was separated by HPLC [MeOH–H_2O (60 : 40, v/v), COSMOSIL 5C_{18}-MS-II] to give kaempferol (**17**, 34.2 mg). Fr. 3-11 (0.3 g) was purified by HPLC [MeOH–H_2O (65 : 35, v/v), COSMOSIL 5C_{18}-MS-II] to give kaempferol 3-*O*-β-D-(2″-*O*-*p*-*trans*-coumaroyl)glucopyranoside (**20**, 10.5 mg). Fr. 4 (100 g) was subjected to reversed-phase silica gel column chromatography [2.5 kg, MeOH : H_2O (50 : 50→70 : 30→MeOH)] to give ten fractions [Fr. 4-1, Fr. 4-2, Fr. 4-3, Fr. 4-4 (7.1 g), Fr. 4-5 (2.1 g), Fr. 4-6 (13.1 g), Fr. 4-7 (8.6 g), Fr. 4-8 (2.7 g), Fr. 4-9 (8.9 g), and Fr. 4-10]. Fr. 4-4 (7.1 g) was separated by reversed-phase silica gel column chromatography [240 g, MeOH : H_2O (45 : 55→50 : 50→55 : 45→60 : 40→70 : 30→80 : 20→MeOH) to give seven fractions [Fr. 4-4-1, Fr. 4-4-2 (1.4 g), Fr. 4-4-3 (1.0 g), Fr. 4-4-4, Fr. 4-4-5, Fr. 4-4-6, Fr. 4-4-7]. Fr. 4-4-2 (0.5 g) and Fr. 4-4-3 (0.5 g) was separated by HPLC [MeOH–H_2O (50 : 50, v/v), COSMOSIL 5C_{18}-MS-II] to give kaempferol 3-*O*-α-L-rhamnopyranosyl(1—6)-β-D-glucopyranoside (**21**, 12.3 mg), kaempferol 3-*O*-α-L-rhamnopyranosyl(1—6)-β-D-galactopyranoside (**22**, 9.5 mg), kaempferol 3-*O*-β-D-glucopyranosyl(1—3)-α-L-rhamnopyranosyl-(1—6)-β-D-glucopyranoside (**23**, 71.3 mg), and kaempferol 3-*O*-β-D-glucopyranosyl(1—3)-α-L-rhamnopyranosyl(1—6)-β-D-galactopyranoside (**24**, 262 mg). Fr. 4-5 (0.5 g) was separated by HPLC {{MeOH–[H_2O : AcOH (99 : 1, v/v)] (70 : 30, v/v), [1] COSMOSIL 5C_{18}-MS-II [2] Develosil C30-UG-5} to give floratheasaponin G (**11**, 11.9 mg), chakasaponin I (**13**, 30.2 mg), and chakasaponin III (**15**, 16.2 mg). Fr. 4-6 (0.7 g) was purified by HPLC {MeOH–[H_2O : AcOH (99 : 1, v/v)] (70 : 30, v/v), Develosil C30-UG-5} to give floratheasaponin A (**5**, 19.2 mg), floratheasaponin C (**7**, 8.0 mg), floratheasaponin G (**11**, 11.5 mg), chakasaponin I (**13**, 46.1 mg), and chakasaponin III (**15**, 30.2 mg). Fr. 4-7 (0.5 g) was purified by HPLC {MeOH–[H_2O : AcOH (99 : 1, v/v)] ([1] 70 : 30 [2] 75 : 25, v/v), Develosil

C30-UG-5} to give floratheasaponin B (**6**, 9.6 mg), floratheasaponin D (**8**, 6.8 mg), floratheasaponin G (**11**, 63.1 mg), floratheasaponin I (**13**, 15.6 mg), and chakasaponin II (**14**, 58.8 mg). Fr. 4-8 (1.0 g) was separated by HPLC {MeOH–[H_2O : AcOH (99 : 1, v/v)] (75 : 25, v/v), [1] COSMOSIL 5C_{18}-MS-II [2] Develosil C30-UG-5} to give chakasaponin V (**1**, 70.7 mg), floratheasaponin A (**5**, 60.0 mg), floratheasaponin E (**9**, 94.6 mg), floratheasaponin I (**12**, 81.6 mg), chakasaponin I (**13**, 11.7 mg), and chakasaponin II (**14**, 54.2 mg). Fr. 4-9 (1.0 g) was separated by HPLC {[1] MeOH–[H_2O : AcOH (99 : 1, v/v)] (75 : 25, v/v) [2] MeOH–MeCN–[H_2O : AcOH (99 : 1, v/v)] (42 : 18 : 40, v/v/v), Develosil C30-UG-5} to give chakasaponin V (**1**, 70.2 mg), chakasaponin VI (**2**, 34.6 mg), floratheasaponin B (**6**, 247 mg), floratheasaponin C (**7**, 7.0 mg), floratheasaponin F (**10**, 12.0 mg), and assamsaponin E (**16**, 62.6 mg).

The known compounds except for caffeine were identified by comparison of their physical data ($[\alpha]_D$, IR, MS, ^{1}H-, and ^{13}C-NMR) with reported values. The obtained caffeine was identified by comparison of the MS, ^{1}H-, and ^{13}C-NMR data with those of commercial product.

Isolation of Chakaflavonoside A The 1-BuOH-soluble fraction (142 g, from the flower buds of *C. sinensis* cultivated in Fujian province) was subjected to normal-phase silica gel column chromatography [3 kg, $CHCl_3$→$CHCl_3$: MeOH : H_2O (30 : 10 : 1→10 : 3 : 1→7 : 3 : 1→6 : 4 : 1)→MeOH] to give five fractions [Fr. 1, Fr. 2, Fr. 3, Fr. 4 (115.0 g), Fr. 5]. Fr.4 (115 g) was subjected to reversed-phase silica gel column chromatography [530 g, MeOH : H_2O (10 : 90→30 : 70→50 : 50→70 : 30→MeOH)] to give nine fractions [Fr. 4-1, Fr. 4-2, Fr. 4-3, Fr. 4-4, Fr. 4-5, Fr. 4-6 (12.5 g), Fr. 4-7, Fr. 4-8, and Fr. 4-9]. Fraction 4-6 (12.5 g) was subjected by reversed-phase silica gel column chromatography [370 g, MeOH : H_2O (50 : 50→60 : 40→MeOH) to give five fractions [Fr. 4-6-1, Fr. 4-6-2 (3.8 g), Fr. 4-6-3, Fr. 4-6-4, Fr. 4-6-5]. Fr. 4-6-2 (1.0 g) was further separated by HPLC [MeOH–H_2O (55 : 45, v/v), COSMOSIL 5C_{18}-MS-II] to give chakaflavonoside A (**4**, 101 mg).

Chakasaponin V (**1**): Colorless fine crystals from $CHCl_3$–MeOH; mp 204.0—207.0 °C; $[\alpha]_D^{20}$ +10.3° (c=1.3, MeOH); IR (KBr) ν_{max} 3541, 2980, 1716, 1641, 1080, 1048 cm^{-1}; ^{1}H-NMR (600 MHz, pyridine-d_5) δ: 0.74, 0.92, 1.04, 1.08, 1.18, 1.25, 1.77 (3H each, all s, H_3-25, 26, 29, 24, 23, 30, 27), 1.23, 1.52 (3H each, both d, J=6.8Hz, H_3-22-*O*-Tig-4, H_3-21-*O*-Tig-4), 1.64 (3H, s, H_3-22-*O*-Tig-5) 1.82 (3H, br s, H_3-21-*O*-Tig-5), 3.18 (1H, br d, J=*ca.* 11 Hz, H-3), 3.37, 3.68 (1H each, both br d, J=*ca.* 10 Hz, H_2-28), 4.10 (1H, m, H-16), 4.40 (1H, m, H-15), 4.84 (1H, d, J=7.6 Hz, H-1′), 5.43 (1H, br s, H-12), 5.58 (1H, d, J=6.8 Hz, H-1″), 5.96 (1H, br s, H-1‴), 6.05 (1H, d, J=7.2 Hz, H-1‴), 6.24 (1H, d, J=10.3 Hz, H-22), 6.64 (1H, d, J=10.3 Hz, H-21), 6.70, 6.98 (1H each, both dq-like, H-22-*O*-Tig-3, H-21-*O*-Tig-3); ^{13}C-NMR (150 MHz, pyridine-d_5) δ_C: given in Table 1; positive-ion MALDI-MS: m/z 1309 (M+Na)$^+$; HR-MALDI-MS: m/z 1309.6175 [Calcd for $C_{63}H_{98}O_{27}$Na (M+Na)$^+$: m/z 1309.6188].

Chakasaponin VI (**2**): Colorless fine crystals from $CHCl_3$–MeOH; mp 201.0—203.0 °C; $[\alpha]_D^{20}$ −12.5° (c=1.2, MeOH); IR (KBr) ν_{max} 3452, 2980, 1714, 1646, 1078 cm^{-1}; ^{1}H-NMR (600 MHz, pyridine-d_5) δ: 0.70, 0.87, 0.99, 1.02, 1.17, 1.22, 1.71 (3H each, all s, H_3-25, 26, 29, 24, 23, 30, 27), 1.50 (3H, d, J=6.9Hz, H_3-21-*O*-Tig-4), 1.74 (3H, br s, H_3-21-*O*-Tig-5), 1.56, 1.80 (1H each, both m, H_2-15), 1.92 (3H, s, H_3-28), 3.16 (1H, dd, J=4.1, 11.0 Hz, H-3), 4.18, 4.20 (1H each, both m, H_2-28), 4.40 (1H, m, H-22), 4.66 (1H, m, H-16), 4.83 (1H, d, J=7.6 Hz, H-1′), 4.92 (1H, d, J=7.6 Hz, H-1‴), 5.36 (1H, br s, H-12), 5.60 (1H, d, J=7.2 Hz, H-1″), 5.67 (1H, d, J=7.6 Hz, H-1‴), 6.33 (1H, d, J=10.8 Hz, H-21), 6.92 (1H, dq-like, H-21-*O*-Tig-3); ^{13}C-NMR (150 MHz, pyridine-d_5) δ_C: given in Table 1; positive-ion FAB-MS: m/z 1239 (M+Na)$^+$; negative-ion FAB-MS: m/z 1215 (M−H)$^-$, m/z 1083 (M−H−$C_5H_8O_4$)$^-$, m/z 951 (M−H−$C_{10}H_{16}O_8$)$^-$, m/z 789 (M−H−$C_{16}H_{26}O_{13}$)$^-$; HR-FAB-MS: m/z 1239.5767 [Calcd for $C_{59}H_{96}O_{26}$Na (M+Na)$^+$: 1239.5775].

Chakanoside I (**3**): A white powder; $[\alpha]_D^{26}$ −31.9° (c=0.69, MeOH); IR (KBr) ν_{max} 3414, 1694, 1078, 1044 cm^{-1}; ^{1}H-NMR (500 MHz, D_2O) δ: 4.45 (1H, d, J=6.9 Hz, H-1′), 5.01, 5.18 (1H each, both d, J=16.8 Hz, H-2), 7.40 (2H, d, J=7.3 Hz, H-3, 5), 7.56 (1H, t-like, J=7.3, H-4), 7.78 (1H, d, J=7.3 Hz, H-2, 6); ^{13}C-NMR (125 MHz, D_2O) δ_C: given in Table 2; positive-ion FAB-MS: m/z 321 (M+Na)$^+$; HR-FAB-MS: m/z 321.0953 [Calcd for $C_{14}H_{18}O_7$Na (M+Na)$^+$: 321.0950].

Chakaflavonoside A (**4**): A yellow powder; $[\alpha]_D^{24}$ −96.7° (c=3.4, MeOH); IR (KBr) ν_{max} 3430, 2926, 1712, 1655, 1606, 1076 cm^{-1}; UV [MeOH, nm, (log ε)]: 316 (4.52), 267 (4.36), 207 (4.57)]; ^{1}H-NMR (methanol-d_4, 500 MHz) δ: 1.07 (3H, d, J=6.1 Hz, H-6‴), 4.36 (1H, d, J=7.6 Hz, H-1⁗), 4.38 (1H, d, J=7.7 Hz, H-1‴), 4.54 (1H, d, J=1.4 Hz, H-1‴), 5.52 (1H, d, J=8.0 Hz, H-1″), 6.11 (1H, d, J=2.0 Hz, H-6), 6.30 (1H, d, J=2.0 Hz, H-8), 6.34 (1H, d, J=15.9 Hz, H-8⁗), 6.76 (2H, d, J=8.7 Hz, H-3⁗, 5⁗), 6.85 (2H, d, J=8.9 Hz, H-3′, 5′), 7.41 (2H, d, J=8.7 Hz, H-2⁗, 6⁗), 7.65 (1H, d, J=15.9 Hz, H-7⁗), 7.93 (2H, d, J=8.9 Hz, H-2′, 6′); ^{13}C-NMR (methanol-d_4, 125 MHz) δ_C: give in Table 3. Positive-ion FAB-MS m/z 1087 (M+Na)$^+$; negative-ion FAB-MS m/z 1063 (M−H)$^-$, 1047 (M−OH)$^-$, 917 (M−*trans*-*p*-coumaroyl acid)$^-$, 901 (M−$C_6H_{11}O_5$)$^-$, 755 (M−$C_{12}H_{21}O_9$)$^-$, 591 (M−$C_{18}H_{32}O_{14}$)$^-$, 285 (M−$C_{24}H_{43}O_{19}$−*trans*-*p*-coumaroyl acid)$^-$; HR-FAB-MS: m/z 1087.2907 [Calcd for $C_{48}H_{56}O_{27}$Na (M+Na)$^+$, 1087.2900].

Alkaline Hydrolysis of Chakasaponins V (1) and VI (2) A solution of chakasaponin V (**1**) and VI (**2**) (2.5 mg each) in 50% aqueous 1,4-dioxane (0.5 ml) was treated with 10% aqueous KOH (0.5 ml), respectively, and the whole was stirred at 37 °C for 1 h. The reaction mixture was neutralized with Dowex HCR W2 (H^+ form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure yielded a reaction product. A part of the reaction product was dissolved in $(CH_2)_2Cl_2$ (2 ml) and the solution was treated with *p*-nitrobenzyl-*N*,*N*′-diisopropylisourea (10 mg), then the whole was stirred at 80 °C for 1 h. The reaction solution was subjected to HPLC analysis [column: YMC-Pack ODS-A, 250×4.6 mm i.d.; mobile phase: MeCN–H_2O (50 : 50, v/v); detection: UV (254 nm); flow rate: 1.0 ml/min] to identify the *p*-nitrobenzyl ester of acetic acid (t_R 8.9 min) from **2** and the *p*-nitrobenzyl ester of tiglic acid (t_R 24.4 min) from **1** and **2**. The rest of the reaction product was subjected to normal-phase silica gel column chromatography [50 mg, $CHCl_3$–MeOH–H_2O (10 : 3 : 1 lower layer→6 : 4 : 1)] to give desacyl-floratheasaponin E (**1a**, 1.4 mg) from **1** or desacyl-assamsaponin E (**2a**, 1.0 mg) from **2**. The desacyl-derivatives (**1a**, **2a**) were identified by comparison of their MS, ^{1}H-, and ^{13}C-NMR with reported values.

Alkaline Hydrolysis of Chakaflavonoside A (4) A solution of chakaflavonoside A (**4**) (15.7 mg) in 50% aqueous 1,4-dioxane (2.0 ml) was treated with 10% aqueous KOH (2.0 ml) and the whole was stirred at 60 °C for 4 h. The reaction mixture was neutralized with Dowex HCR W2 (H^+ form) and the resin was removed by filtration. A part of the reaction product was subjected to HPLC analysis [column: YMC-Pack ODS-A, 250×4.6 mm i.d.; mobile phase: MeCN–H_2O (45 : 55, v/v); detection: UV (254 nm); flow rate: 1.0 ml/min] to identify *trans*-*p*-coumaroyl acid (t_R 10.7 min). The rest of the reaction mixture was purified by normal-phase silica gel column chromatography [0.7 g, $CHCl_3$–MeOH–H_2O (7 : 3 : 1 lower-layer→6 : 4 : 1, v/v/v)] to give desacyl-chakaflavonoside A (**4a**, 10.2 mg).

Desacyl-chakaflavonoside A (**4a**): A yellow powder; $[\alpha]_D^{26}$ +4.0° (c=0.68, MeOH); IR (KBr) ν_{max} 3451, 2918, 1718, 1686, 1078 cm^{-1}; UV [MeOH, nm, (log ε)]: 267 (4.14), 204 (4.33)]; ^{1}H-NMR (methanol-d_4, 600 MHz) δ: 1.08 (3H, d, J=6.2 Hz, H-6⁗), 4.36 (1H, d, J=7.6 Hz, H-1⁗), 4.54 (1H, s, H-1‴), 4.61 (1H, d, J=7.6 Hz, H-1‴), 5.15 (1H, d, J=6.9 Hz, H-1″), 6.19 (1H, d, J=2.0 Hz, H-6), 6.39 (1H, d, J=2.0 Hz, H-8), 6.78 (2H, d, J=8.2 Hz, H-3′, 5′), 8.03 (2H, d, J=8.2 Hz, H-2′, 6′); ^{13}C-NMR (methanol-d_4, 150 MHz) δ_C: give in Table 3; positive-ion FAB-MS m/z 941 (M+Na)$^+$; negative-ion FAB-MS m/z 917 (M−H)$^-$, 755 (M−$C_6H_{11}O_5$)$^-$, 285 (M−$C_{24}H_{43}O_{19}$)$^-$; HR-FAB-MS: m/z 941.2532 [Calcd for $C_{39}H_{50}O_{25}$Na (M+Na)$^+$, 941.2539].

Acid Hydrolysis of Chakanoside I (3) and Chakaflavonoside A (4) A solution of **3** and **4** (1 mg each) in 1.0 M HCl (1.0 ml) was heated under reflux for 3 h, respectively. After cooling, the reaction mixture was poured into ice-water and neutralized with Amberlite IRA-400 (OH^- form), and the resin was removed by filtration. Then, the filtrate was extracted with EtOAc. The aqueous layer was subjected to HPLC analysis {column: Kaseisorb LC NH_2-60-5, 250×4.6 mm i.d. (Tokyo Kasei Co., Ltd., Tokyo, Japan); mobile phase: MeCN–H_2O (75 : 25, v/v); detection: optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan)]; flow rate: 0.8 ml/min} to identify D-glucose (from **3** and **4**) and D-xylose (from **4**), which were confirmed by comparison of their retention times with those of the authentic samples; t_R: 8.6 min (D-glucose, positive optical rotation); t_R: 6.2 min (L-rhamnose, negative optical rotation).

Enzymatic Hydrolyses of Desacyl-chakaflavonoside A (4a) A solution of **4a** (3.9 mg) in 0.2 M acetate buffer (1.0 ml, pH: 3.8) was treated with naringinase (13.0 mg, from *Aspergillus*, Wako Pure Chemical Ind., Osaka, Japan) and the solution was stirred at 37 °C for 15 h. The reaction mixture was added 1.0 ml of EtOH and then centrifuged at 4000 rpm for 10 min, and the supernatant solution was concentrated under reduced pressure to give a residue. The residue was purified by HPLC [MeOH–H_2O (60 : 40, v/v), COSMOSIL 5C_{18}-MS-II] to furnish kaempferol (1.0 mg). The obtained compound, kaempferol, was identified by comparison of their ^{1}H-, ^{13}C-NMR, and MS data with those of an authentic sample.

References and Notes

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