Bioactive Saponins and Glycosides. XXV.[1] Acylated Oleanane-Type Triterpene Saponins from the Seeds of Tea Plant (*Camellia sinensis*)

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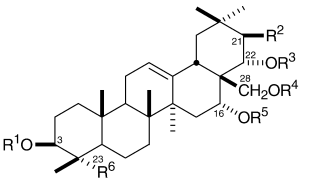
Seven new acylated oleanane-type triterpene oligoglycosides, theasaponins A_4 (1), A_5 (2), C_1 (3), E_8 (4), E_9 (5), G_1 (6), and H_1 (7), were isolated from the seeds of Japanese tea plant (*Camellia sinensis*). The structures of 1—7 were elucidated on the basis of chemical and physicochemical evidence.

Key words *Camellia sinensis*; Theaceae; triterpene oligoglycoside; theasaponin; tea seed

During the course of our characterization studies on the bioactive saponin constituents from tea plant (Theaceae), we have reported the isolation and structure elucidation of theasaponins A_1—A_3, E_1—E_7, and F_1—F_3 from the seeds of Japanese tea plant [*Camellia sinensis* (L.) O. KUNTZE (*C. sinensis* L. var. *sinensis*)][2—4] and assamsaponins A—I from the seeds and leaves of Sri Lankan tea plant (*C. sinensis* L. var. *assamica* PIERRE),[5,6] as well as their anti-sweet, gastric emptying, and gastroprotective activities and accelerating effect on gastrointestinal transit. Recently, floratheasaponins A—C with anti-hyperlipidemic activities were also isolated from the flower part of Japanese tea plant.[7] Our continuing search led to the additional isolation of seven new acylated oleanane-type triterpene oligoglycosides, theasaponins A_4 (**1**), A_5 (**2**), C_1 (**3**), E_8 (**4**), E_9 (**5**), G_1 (**6**), and H_1 (**7**), from the seeds of Japanese tea plant. This paper deals with the structure elucidation of seven new saponins (**1**—**7**).

The seeds of tea plant, which were cultivated in Shizuoka prefecture, Japan, were defatted with hexane and then the residues were extracted with methanol. The methanolic extract was deposited with dimethylether and the precipitation was subjected to Diaion HP-20 column chromatography (H_2O→MeOH→$CHCl_3$) to give the saponin fraction (=the methanol-eluted fraction, 6.3%), which was described previously.[3] The saponin fraction was further purified by HPLC to give **1** (0.005%), **2** (0.010%), **3** (0.031%), **4** (0.007%), **5** (0.013%), **6** (0.005%), and **7** (0.010%).

Structures of Theasaponins A_4 (1), A_5 (2), and C_1 (3) Theasaponin A_4 (**1**) was obtained as colorless fine crystals from $CHCl_3$–MeOH with mp 236.1—236.9 °C, and exhibited a positive optical rotation ($[\alpha]_D^{27}$ +25.8° in MeOH). The IR spectrum of **1** showed absorption bands at 1718 and 1646 cm^{-1} ascribable to carbonyl and α,β-unsaturated ester functions, and broad bands at 3432 and 1075 cm^{-1}, suggestive of an oligoglycoside structure. In the positive- and negative-ion FAB-MS of **1**, quasimolecular ion peaks were observed at *m/z* 1243 $(M+Na)^+$ and 1219 $(M-H)^-$, and high-resolution positive-ion FAB-MS analysis revealed the molecular formula of **1** to be $C_{58}H_{92}O_{27}$. On alkaline hydrolysis of **1** with 10% aqueous KOH–50% aqueous 1,4-dioxane (1 : 1, v/v), desacyl-theasaponin A (**1a**) was obtained together with angelic acid, which was identified by HPLC analysis of its *p*-



	R^1	R^2	R^3	R^4	R^5	R^6
Theasaponin A_4 (**1**):	S_1	OAng	H	H	H	CH_2OH
Theasaponin A_5 (**2**):	S_1	OAng	H	Ac	H	CH_2OH
Desacyl-theasaponin A (**1a**):	S_1	OH	H	H	H	CH_2OH
Theasapogenol A (**1b**):	H	OH	H	H	H	CH_2OH
Theasaponin C_1 (**3**):	S_2	H	Ang	H	H	CH_2OH
Desacyl-theasaponin C (**3a**):	S_2	H	H	H	H	CH_2OH
Theasapogenol C (**3b**):	H	H	H	H	H	CH_2OH
Theasaponin E_8 (**4**):	S_2	OTig	H	H	Ac	CHO
Theasaponin E_9 (**5**):	S_2	OTig	H	Ac	Ac	CHO
Desacyl-theasaponin E (**4a**):	S_2	OH	H	H	H	CHO
Theasaponin G_1 (**6**):	S_2	H	H	Ang	H	CHO
Desacyl-assamsaponin A (**6a**):	S_2	H	H	H	H	CHO
Camelliagenin B (**6b**):	H	H	H	H	H	CHO
Theasaponin H_1 (**7**):	S_2	H	Ang	H	H	$COOCH_3$
Desacyl-theasaponin H (**7a**):	S_2	H	H	H	H	$COOCH_3$

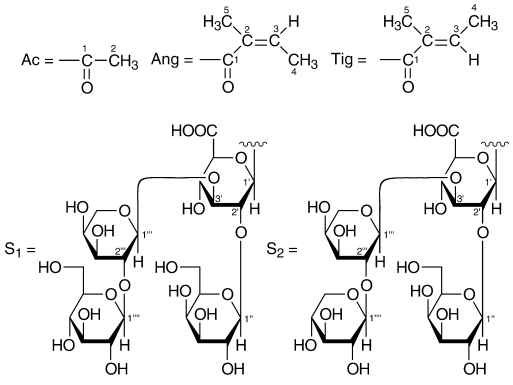


Chart 1

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nitrobenzyl derivative.[1,3—7] Acid hydrolysis of **1a** with 5% aqueous H_2SO_4–1,4-dioxane (1 : 1, v/v) yielded theasapogenol A (**1b**),[8] as an aglycon, together with D-glucuronic acid, D-galactose, L-arabinose, and D-glucose, which were identified by GLC analysis of their trimethylsilyl thiazolidine derivatives.[1,3—7] The ^{1}H- (pyridine-d_5) and ^{13}C-NMR (Tables 1, 2) spectra of **1**, which were assigned by various NMR experiments,[9] showed signals assignable to six methyls [δ 0.89, 0.90, 1.05, 1.10, 1.32, 1.80 (3H each, all s, 26, 25, 24, 29, 30, 27-H_3)], two methylenes and four methines bearing an oxygen function {δ 3.69, 3.96 (1H each, both d, J=10.0 Hz, 28-H_2), [3.77 (1H, d, J=10.1 Hz), 4.42 (1H, m), 23-H_2], 4.14 (1H, m, 3-H), 4.80 (1H, d, J=10.1 Hz, 22-H), 4.84 (1H, br s, 16-H), 6.47 (1H, d, J=10.1 Hz, 21-H)}, an olefin [δ 5.37 (1H, br s, 12-H)], and four glycopyranosyl moieties [δ 5.08 (1H, d, J=7.7 Hz, 1′-H), 5.14 (1H, d, J=7.1 Hz, 1⁗-H), 5.81 (1H, d, J=6.7 Hz, 1″-H), 5.84 (1H, d, J=5.8 Hz, 1‴-H)] together with an angeloyl group [δ 1.98 (3H, s, Ang-5-H_3), 2.05 (3H, d, J=7.0 Hz, Ang-4-H_3), 5.90 (1H, dq-like, Ang-3-H)]. The positions of the angeloyl and oligosugar moieties, and the oligoglycoside structure of **1** were clarified on the basis of a HMBC experiment, which showed long-range correlations between the following proton and carbon pairs [21-H and the angeloyl carbonyl carbon (δ_C 168.7); 1′-H and 3-C; 1″-H and 2′-C; 1‴-H and 3′-H; 1⁗-H and 2‴-C]. Furthermore, comparison of the ^{13}C-NMR data for **1** with those for **1a** revealed an acylation shift around the 21-position of the aglycon moiety [**1a**: δ_C 78.7 (21-C), 36.4 (20-C), 77.3 (22-C); **1**: δ_C 81.7 (21-C), 36.1 (20-C), 73.1 (22-C)]. On the basis of the above-mentioned evidence, the structure of theasaponin A_4 was determined to be 21-*O*-angeloyltheasapogenol A 3-*O*-β-D-galactopyranosyl(1→2)[β-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucuronopyranoside (**1**).

Theasaponin A_5 (**2**) was also obtained as colorless fine crystals from $CHCl_3$–MeOH with mp 233.0—234.2 °C and positive optical rotation ($[\alpha]_D^{27}$ +34.5° in MeOH). The IR spectrum of **2** showed absorption bands at 3432, 1719, 1647, 1078 cm^{-1}, ascribable to hydroxyl, carbonyl, α,β-unsaturated ester, and ether functions. The molecular formula, $C_{60}H_{94}O_{28}$, of **2** was determined from the positive- and negative-ion FAB-MS [m/z 1285 $(M+Na)^+$ and 1261 $(M-H)^-$] and by high-resolution positive-ion FAB-MS. The fragmentation patterns in the negative-ion FAB-MS of **2** indicated the loss of mono-hexose [m/z 1099 $(M-C_6H_{11}O_5)^-$] and mono-pentose and mono-hexose [m/z 967 $(M-C_{11}H_{19}O_9)^-$]. Treatment of **2** with 10% aqueous KOH–50% aqueous 1,4-dioxane (1 : 1, v/v) liberated **1a** and two organic acids, acetic acid and angelic acid, which were identified by HPLC analysis of those *p*-nitrobenzyl derivatives.[1,3—7] The ^{1}H- (pyridine-d_5) and ^{13}C-NMR[9] (Tables 1, 2) spectra of **2** indicated the presence of the following functions: a theasapogenol A part {{six methyls [δ 0.91, 1.01, 1.04, 1.10, 1.30, 1.76 (3H each, all s, 25, 26, 24, 29, 30, 27-H_3)], two methylenes and four methines bearing an oxygen function {δ [3.77 (1H, d, J=10.1 Hz), 4.40 (1H, m), 23-H_2], 4.14 (1H, m, 3-H), 4.25 (2H, m, 28-H_2), 4.47 (1H, d, J=10.4 Hz, 22-H), 4.72 (1H, br s, 16-H), 6.48 (1H, d, J=10.4 Hz, 21-H)}, and an olefin [δ 5.45 (1H, br s, 12-H)]}}, four glycopyranosyl moieties [δ 5.07 (1H, d, J=7.4 Hz, 1′-H), 5.13 (1H, d, J=6.8 Hz, 1⁗-H), 5.79 (1H, d, J=7.7 Hz, 1″-H), 5.83 (1H, d, J=6.1 Hz, 1‴-H)]}, and an acetyl and an angeloyl moieties [δ 1.97 (3H, s, Ang-5-H_3), 1.99 (3H, s, Ac-H_3), 2.05 (3H, d, J=7.1 Hz, Ang-4-H_3), 5.90 (1H, dq-like, Ang-3-H)]. The HMBC experiment on **2** showed long-range correlations between the 21-proton and the angeloyl carbonyl carbon (δ_C 168.6) and between the 28-protons and the acetyl carbonyl carbon (δ_C 170.1). Furthermore, comparison of the ^{13}C-NMR data for **2** with those for **1** revealed an acetylation shift around the 28-position of the aglycon moiety. Consequently, the structure of theasaponin A_5 was determined to be 21-*O*-angeloyl-28-*O*-acetyltheasapogenol A 3-*O*-β-D-galactopyranosyl(1→2)[β-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucuronopyranoside (**2**).

Theasaponin C_1 (**3**), $[\alpha]_D^{27}$ +7.9° (MeOH), was also obtained as colorless fine crystals from $CHCl_3$–MeOH with mp 223.1—224.3 °C. The positive- and negative-ion FAB-MS of **3** showed quasimolecular ion peaks at m/z 1197 $(M+Na)^+$ and m/z 1173 $(M-H)^-$, respectively. The high-resolution positive-ion FAB-MS of **3** revealed the molecular formula to be $C_{57}H_{90}O_{25}$. Alkaline hydrolysis of **3** with 10% aqueous KOH–50% aqueous 1,4-dioxane (1 : 1, v/v) liberated desacyltheasaponin C (**3a**) and an organic acid, angelic acid, which was identified by HPLC analysis of this *p*-nitrobenzyl derivative.[1,3—7] Acid hydrolysis of **3a** with 5% aqueous H_2SO_4–1,4-dioxane (1 : 1, v/v) yielded theasapogenol C (=camelliagenin C, **3b**)[10] together with D-glucuronic acid, D-galactose, L-arabinose, and D-xylose, which were identified by GLC analysis of their trimethylsilyl thiazolidine derivatives.[1,3—7] The proton and carbon signals in the ^{1}H- (pyridine-d_5) and ^{13}C-NMR[9] (Tables 1, 2) spectra of **3** indicated the presence of a theasapogenol C part {{six methyls [δ 0.90, 0.90, 1.03, 1.08, 1.29, 1.83 (3H each, all s, 25, 26, 29, 24, 30, 27-H_3)], two methylenes and three methines bearing an oxygen function {δ 3.55, 3.70 (1H each, both d, J=11.0 Hz, 28-H_2), [3.76 (1H, d, J=10.4 Hz), 4.39 (1H, m), 23-H_2], 4.14 (1H, dd-like, 3-H), 4.61 (1H, br s, 16-H), 6.21 (1H, dd, J=5.5, 12.6 Hz, 22-H)}, and an olefin [δ 5.38 (1H, br s, 12-H)]}}, four glycopyranosyl moieties [δ 5.02 (1H, d, J=7.7 Hz, 1⁗-H), 5.05 (1H, d, J=6.7 Hz, 1′-H), 5.77 (1H, d, J=6.5 Hz, 1‴-H), 5.88 (1H, d, J=7.4 Hz, 1″-H)], and an angeloyl moiety [δ 1.96 (3H, s, Ang-5-H_3), 2.09 (3H, d, J=7.0 Hz, Ang-4-H_3), 5.92 (1H, dq-like, Ang-3-H)]. In the HMBC experiment on **3**, long-range correlations were observed between the following proton and carbon pairs; 22-H and the angeloyl carbonyl carbon (δ_C 168.0); 1′-H and 3-C; 1″-H and 2′-C; 1‴-H and 3′-C; 1⁗-H and 2‴-C. On the basis of above-mentioned evidence, the structure of theasaponin C_1 was elucidated to be 22-*O*-angeloyltheasapogenol C 3-*O*-β-D-galactopyranosyl(1→2)[β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucuronopyranoside (**3**).

Structures of Theasaponins E_8 (4), E_9 (5), G_1 (6), and H_1 (7) Theasaponin E_8 (**4**) was isolated as colorless fine crystals of mp 216.4—217.1 °C (from $CHCl_3$–MeOH) with positive optical rotation ($[\alpha]_D^{27}$ +23.6° in MeOH). In the positive- and negative-ion FAB-MS of **4**, quasimolecular ion peaks were observed at m/z 1253 $(M+Na)^+$ and m/z 1229 $(M-H)^-$, respectively, and high-resolution positive-ion FAB-MS revealed the molecular formula to be $C_{59}H_{90}O_{27}$. Alkaline hydrolysis of **4** with 10% aqueous potassium hydroxide (KOH)–50% aqueous 1,4-dioxane (1 : 1, v/v) provided desacyl-theasaponin E (**4a**)[2] together with two organic acids,

Table 1. ^{13}C-NMR (125 MHz) Data for the Aglycon and Acyl Moieties in **1**—**7**, **1a**, **3a**, and **7a** (Pyridine-d_5)

C-	**1**	**1a**	**2**	**3**	**3a**	**4**	**5**	**6**	**7**	**7a**	C-	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	38.8	38.8	38.8	38.7	38.8	38.1	38.1	38.1	38.6	38.6	16-*O*-Ac							
2	25.1	25.5	25.6	25.5	25.5	25.3	25.3	25.2	26.1	26.1	1				170.1	169.8		
3	83.7	83.0	82.9	83.1	83.1	84.6	84.7	84.7	85.6	85.7	2				22.2	22.1		
4	43.5	43.5	43.5	43.5	43.5	55.1	55.0	55.0	53.8	53.8	21-*O*-Ang							
5	48.2	48.2	48.2	48.2	48.2	48.4	48.5	48.5	52.3	52.2	1	168.7	168.6					
6	18.2	18.2	18.2	18.2	18.2	20.3	20.3	20.3	21.0	21.0	2	129.6	129.6					
7	32.7	32.9	32.9	32.8	32.8	32.3	32.3	32.3	32.7	32.7	3	135.8	136.1					
8	40.1	40.1	40.1	40.1	40.1	40.2	40.3	40.2	40.3	40.3	4	15.9	15.9					
9	47.0	47.1	47.1	47.0	47.1	46.7	46.2	46.8	47.1	47.1	5	21.1	21.0					
10	36.8	36.8	36.8	36.8	36.8	36.0	36.0	36.0	36.5	36.5	21-*O*-Tig							
11	23.8	23.9	24.0	23.9	23.9	23.7	23.8	23.6	23.8	23.7	1				168.3	168.1		
12	123.1	123.9	123.9	123.1	123.1	124.1	124.8	123.1	123.1	122.4	2				129.7	129.6		
13	143.5	144.0	142.7	143.7	144.3	142.0	141.1	142.5	143.7	144.3	3				136.3	136.5		
14	41.9	42.0	41.8	41.6	42.2	41.4	41.4	41.6	41.7	42.1	4				14.1	14.1		
15	34.4	34.4	34.7	35.2	34.8	30.9	30.9	31.6	35.1	34.7	5				12.4	12.4		
16	67.9	67.8	67.7	70.1	68.4	71.5	70.8	71.0	70.1	68.2	22-*O*-Ang							
17	48.2	47.3	47.1	44.8	44.8	47.3	46.7	44.2	44.8	44.7	1			168.0				168.0
18	40.5	41.2	40.6	40.9	42.5	39.7	40.1	44.8	40.9	42.7	2			129.5				129.5
19	47.4	47.3	47.3	47.4	47.8	47.6	47.1	47.3	47.4	47.8	3			136.6				136.5
20	36.1	36.4	36.1	32.1	31.8	36.2	36.1	31.7	32.1	31.8	4			15.9				15.8
21	81.7	78.7	81.3	41.7	45.9	80.8	80.3	41.5	41.7	46.0	5			21.0				21.0
22	73.1	77.3	71.2	73.0	74.4	70.8	69.8	72.4	73.0	74.3	28-*O*-Ac							
23	64.7	64.7	64.7	64.8	64.8	210.1	210.3	210.3	178.1	178.1	1		170.1			170.5		
24	13.6	13.6	13.6	13.6	13.6	11.1	11.1	11.2	12.3	12.3	2		20.7			20.7		
25	16.2	16.2	16.2	16.2	16.3	15.7	15.8	15.8	16.1	16.1	28-*O*-Ang							
26	17.0	17.0	17.0	16.9	16.9	16.7	16.8	16.6	16.6	16.6	1						167.2	
27	27.4	27.4	27.4	27.6	27.5	27.0	27.0	27.0	27.5	27.4	2						128.7	
28	66.0	68.4	66.5	63.6	70.2	64.6	65.9	69.3	63.7	70.1	3						138.0	
29	29.9	30.6	29.7	33.5	33.7	30.0	29.8	33.4	33.5	33.7	4						15.9	
30	20.4	19.5	20.2	25.2	25.5	20.0	19.7	25.2	25.2	25.5	5						21.3	
$COO\underline{C}H_3$									52.2	52.2								

Table 2. ^{13}C-NMR (125 MHz) Data for the Tetraglycoside Moieties in **1**—**7**, **1a**, **3a**, and **7a** (Pyridine-d_5)

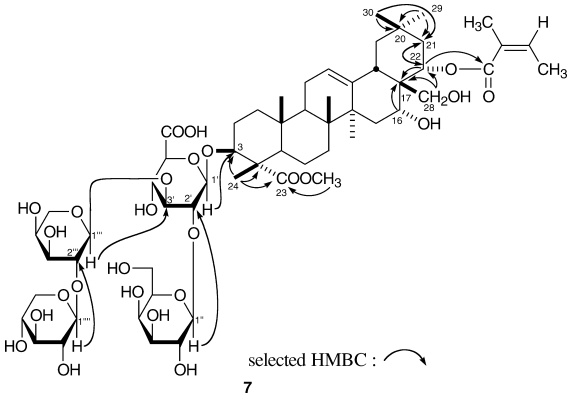
C-	**1**	**1a**	**2**	**3**	**3a**	**4**	**5**	**6**	**7**	**7a**
β-D-Glucopyranosiduronic acid										
1′	104.2	104.2	104.1	104.1	104.1	104.3	104.3	104.3	104.7	104.8
2′	78.5	78.4	78.7	78.5	78.5	78.3	78.3	78.3	78.5	78.5
3′	84.7	85.1	84.9	84.6	84.6	84.1	84.1	84.1	84.3	84.3
4′	70.8	71.3	71.2	71.0	71.0	70.8	70.8	70.8	71.0	70.9
5′	77.3	77.3	77.2	77.4	77.4	77.4	77.4	77.4	77.2	77.2
6′	171.9	172.0	172.0	172.0	172.0	172.0	172.0	171.9	172.0	172.0
β-D-Galactopyranosyl										
1″	103.6	103.5	103.5	103.2	103.2	103.3	103.3	103.3	103.2	103.4
2″	73.8	73.8	73.8	73.8	73.8	73.7	73.7	73.7	73.6	73.6
3″	75.1	75.1	75.1	75.3	75.3	75.4	75.4	75.4	75.7	75.7
4″	69.9	69.9	69.9	70.1	70.1	70.5	70.5	70.5	70.7	70.7
5″	76.5	76.5	76.5	76.5	76.5	76.5	76.5	76.6	76.4	76.4
6″	61.9	61.8	61.9	61.9	61.9	62.1	62.1	62.1	61.8	61.8
α-L-Arabinopyranosyl										
1‴	101.2	101.8	101.7	101.7	101.7	101.7	101.7	101.7	101.6	101.7
2‴	81.2	81.1	81.3	82.3	82.3	82.3	82.3	82.3	82.4	82.4
3‴	72.3	72.3	72.3	73.4	73.4	73.4	73.3	73.3	73.3	73.3
4‴	67.5	67.4	67.5	68.3	68.3	68.3	68.3	68.3	68.3	68.2
5‴	64.7	64.7	64.7	66.0	66.0	66.0	66.0	66.0	66.0	66.0
β-D-Glucopyranosyl										
1⁗	106.0	105.9	105.9							
2⁗	75.9	75.9	75.8							
3⁗	78.5	78.4	78.4							
4⁗	71.2	71.5	71.5							
5⁗	78.5	78.4	78.4							
6⁗	62.7	62.7	62.7							
β-D-Xylopyranosyl										
1⁗				107.1	107.1	107.1	107.1	107.1	107.1	107.2
2⁗				75.9	75.9	75.9	75.9	75.9	76.0	76.0
3⁗				78.3	78.3	78.3	78.3	78.3	78.2	78.2
4⁗				70.8	70.8	70.8	70.7	70.8	70.8	70.8
5⁗				67.5	67.5	67.5	67.5	67.5	67.5	67.5

acetic acid and tiglic acid, which were identified by HPLC analysis of those *p*-nitrobenzyl derivatives.[1,3—7] The ^{1}H-(pyridine-d_5) and ^{13}C-NMR[9] (Tables 1, 2) spectra of **4** showed signals assignable to six methyls [δ 0.71, 0.76, 1.13, 1.31, 1.46, 1.48 (3H each, all s, 26, 25, 29, 30, 27, 24-H$_3$)], a methylene and four methines bearing an oxygen function [δ 3.65, 3.94 (1H each, both d, J=10.4 Hz, 28-H$_2$), 3.97 (1H, dd-like, 3-H), 4.84 (1H, d, J=9.5 Hz, 22-H), 5.94 (1H, br s, 16-H), 5.97 (1H, d, J=9.5 Hz, 21-H)], an olefin [δ 5.35 (1H, br s, 12-H)], an aldehyde [δ 9.96 (1H, s, 23-H)], and four glycopyranosyl moieties {a β-D-glucuronopyranosyl [δ 4.82 (1H, d, J=7.4 Hz, 1′-H)], a β-D-xylopyranosyl [δ 5.02 (1H, d, J=7.2 Hz, 1‴-H)], a β-D-galactopyranosyl [δ 5.78 (1H, d, J=7.3 Hz, 1″-H)], and an α-L-arabinopyranosyl [δ 5.81 (1H, d, J=6.7 Hz,1⁗-H)]}, together with an acetyl and a tigloyl groups [δ 1.60 (3H, d, J=7.0 Hz, Tig-4-H$_3$), 1.84 (3H, s, Tig-5-H$_3$), 2.55 (3H, s, Ac-H$_3$), 6.97 (1H, dq-like, Tig-3-H)]. The positions of two acyl groups in **4** were clarified by the HMBC experiment. Thus, long-range correlations were observed between the 16-proton and the carbonyl carbon of the acetyl part (δ_C 170.1) and between the 21-proton and the carbonyl carbon of the tigloyl part (δ_C 168.3). On the basis of this evidence, the structure of theasaponin E$_8$ was determined to be 16-*O*-acetyl-21-*O*-tigloyltheasapogenol E 3-*O*-β-D-galactopyranosyl(1→2)[β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucuronopyranoside (**4**).

Theasaponin E$_9$ (**5**) was also obtained as colorless fine crystals from CHCl$_3$–MeOH with mp 192.0—193.4 °C and positive optical rotation ([α]$_D^{27}$ +26.5° in MeOH). The molecular formula, $C_{61}H_{92}O_{28}$, of **5** was determined from the positive- and negative-ion FAB-MS [m/z 1295 (M+Na)$^+$ and m/z 1271 (M−H)$^-$] and by high-resolution positive-ion FAB-MS. Treatment of **5** with 10% aqueous KOH–50% aqueous 1,4-dioxane (1 : 1, v/v) liberated **4a** and two organic acids, acetic acid and tiglic acid, which were identified by HPLC analysis of those *p*-nitrobenzyl derivatives.[1,3—7] The ^{1}H-(pyridine-d_5) and ^{13}C-NMR[9] (Tables 1, 2) spectra of **5** indicated the presence of the following functions: a theasapogenol E part [δ 0.78, 0.87, 1.12, 1.27, 1.45, 1.48 (3H each, all s, 25, 26, 29, 30, 27, 24-H$_3$), 3.94 (1H, dd-like, 3-H), 4.28 (2H, m, 28-H$_2$), 4.45 (1H, d, J=10.1 Hz, 22-H), 5.43 (1H, br s, 12-H), 5.86 (1H, br s, 16-H), 5.93 (1H, d, J=10.1 Hz, 21-H), 9.96 (1H, s, 23-H)], a tetrasaccharide moiety [δ 4.82 (1H, d, J=7.4 Hz, 1′-H), 5.01 (1H, d, J=7.3 Hz, 1⁗-H), 5.77 (1H, d, J=8.0 Hz, 1″-H), 5.78 (1H, d, J=6.1 Hz, 1‴-H)], and two acetyl and a tigloyl moieties [δ 1.58 (3H, d, J=7.0 Hz, Tig-4-H$_3$), 1.82 (3H, s, Tig-5-H$_3$), 2.02, 2.54 (3H each, both s, Ac-H$_3$), 6.96 (1H, dq-like, Tig-3-H)]. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra of **5** were shown to be superimposable on those of **4**, except for the signals due to an acetyl group. Comparison of the ^{13}C-NMR data for **5** with those for **4** revealed an acetylation shift around the 28-position of the theasapogenol E moiety. This evidence was supported by the HMBC experiment of **5**, in which long-range correlations were observed between the 16-proton and the acetyl carbonyl carbon (δ_C 169.8), between the 21-proton and the tigloyl carbonyl carbon (δ_C 168.1), and between the 28-protons and the acetyl carbonyl carbon (δ_C 170.5). Consequently, the structure of theasaponin E$_9$ was determined to be 16,28-di-*O*-acetyl-21-*O*-tigloyltheasapogenol E 3-*O*-β-D-galactopyranosyl(1→2)[β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucuronopyranoside (**5**).

Theasaponin G$_1$ (**6**), [α]$_D^{27}$ +36.3° (MeOH), was also obtained as colorless fine crystals from CHCl$_3$–MeOH with mp 227.1—228.2 °C. The positive- and negative-ion FAB-MS of **6** showed quasimolecular ion peaks at m/z 1195 (M+Na)$^+$ and m/z 1171 (M−H)$^-$, respectively. The high-resolution positive-ion FAB-MS of **6** revealed the molecular formula to be $C_{57}H_{88}O_{25}$. The IR spectrum of **6** showed absorption bands at 3453, 1743, 1645, and 1086 cm^{-1}, ascribable to hydroxyl, carbonyl, α,β-unsaturated ester, and ether functions. Alkaline hydrolysis of **6** with 10% aqueous KOH–50% aqueous 1,4-dioxane (1 : 1, v/v) liberated desacyl-assamsaponin A (**6a**)[6] and angelic acid, which was identified by HPLC analysis of its *p*-nitrobenzyl derivative.[1,3—7] The proton and carbon signals in the ^{1}H- (pyridine-d_5) and ^{13}C-NMR[8] (Tables 1, 2) spectra of **6** indicated the presence of the following functions: an aglycon part {six methyls [δ 0.74, 0.77, 1.06, 1.11, 1.43, 1.47 (3H each, all s, 26, 25, 29, 30, 27, 24-H$_3$)], a methylene and three methines bearing an oxygen function [δ 3.65, 4.03 (1H, d, J=10.4 Hz, 28-H$_2$), 3.96 (1H, dd, J=3.7, 10.7 Hz, 3-H), 4.58 (1H, m, 22-H), 4.81 (1H, br s, 16-H)], an olefin [δ 5.31 (1H, br s, 12-H)], and an aldehyde [δ 9.96 (1H, s, 23-H)]} together with four glycopyranosyl moieties [δ 4.83 (1H, d, J=7.3 Hz, 1′-H), 5.01 (1H, d, J=7.4 Hz, 1⁗-H), 5.77 (1H, d, J=7.7 Hz, 1″-H), 5.79 (1H, d, J=6.1 Hz, 1‴-H)], and an angeloyl moiety [δ 2.13 (3H, s, Ang-5-H$_3$), 2.14 (3H, d, J=6.5 Hz, Ang-4-H$_3$), 5.95 (1H, dq-like, Ang-3-H)]. The proton and carbon signals of the aglycon part in the ^{1}H- and ^{13}C-NMR data of **6** were similar to those of camelliagenin B (**6b**),[11,12] whereas the proton and carbon signals due to the tetrasaccharide moiety were superimposable on those of **3**, **4**, and **5**. The position of an angeloyl group in **6** was characterized by the HMBC experiment, in which a long-range correlation was observed between the 28-protons and the angeloyl carbonyl carbon (δ_C 167.2). On the basis of this evidence, the structure of theasaponin G$_1$ was elucidated to be 28-*O*-angeloylcamelliagenin B 3-*O*-β-D-galactopyranosyl(1→2)[β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucuronopyranoside (**6**).

Theasaponin H$_1$ (**7**) was obtained as colorless fine crystals from CHCl$_3$–MeOH with mp 218.0—219.4 °C, and exhibited a positive optical rotation ([α]$_D^{27}$ +10.0° in MeOH). The IR spectrum of **7** showed absorption bands at 1717 and 1638 cm^{-1} ascribable to carbonyl and α,β-unsaturated ester functions, and broad bands at 3453 and 1080 cm^{-1}, suggestive of an oligoglycoside structure. In the positive- and negative-ion FAB-MS of **7**, quasimolecular ion peaks were observed at m/z 1225 (M+Na)$^+$ and 1201 (M−H)$^-$, and high-resolution positive-ion FAB-MS analysis revealed the molecular formula of **7** to be $C_{58}H_{90}O_{26}$. The fragmentation patterns in the negative-ion FAB-MS of **7** indicated the loss of mono-pentose [m/z 1069 (M−$C_5H_9O_4$)$^-$], di-pentoses [m/z 937 (M−$C_{10}H_{17}O_8$)$^-$], and mono-pentose and mono-hexose [m/z 907 (M−$C_{11}H_{19}O_9$)$^-$]. On alkaline hydrolysis of **7** with 10% aqueous KOH–50% aqueous 1,4-dioxane (1 : 1, v/v), desacyl-theasaponin H (**7a**) was obtained together with angelic acid, which was identified by HPLC analysis of its *p*-nitrobenzyl derivative.[1,3—7] The ^{1}H- (pyridine-d_5) and ^{13}C-NMR[9] (Tables 1, 2) spectra of **7** showed signals assignable to six methyls [δ 0.80, 0.83, 1.04, 1.29, 1.52, 1.79 (3H each, all s, 25, 26, 29, 30, 24, 27-H$_3$)], a methylene and three me-



7

Fig. 1. Selected HMBC Correlations of **7**

thines bearing an oxygen function [δ 3.55, 3.72 (1H, d, J=10.4 Hz, 28-H_2), 4.36 (1H, m, 3-H), 4.61 (1H, br s, 16-H), 6.21 (1H, dd, J=5.5, 11.9 Hz, 22-H)], a methoxycarbonyl group [δ 3.68 (3H, s)], an olefin [δ 5.35 (1H, br s, 12-H)], and four glycopyranosyl moieties [δ 4.97 (1H, d, J=7.4 Hz, 1′-H), 4.99 (1H, d, J=7.6 Hz, 1‴-H), 5.76 (1H, d, J=7.6 Hz, 1″-H), 5.79 (1H, d, J=6.1 Hz, 1‴-H)] together with an angeloyl group [δ 1.95 (3H, s, Ang-5-H_3), 2.09 (3H, d, J=7.3 Hz, Ang-4-H_3), 5.92 (1H, dq-like, Ang-3-H)]. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR data of **7** and **7a** were similar to those of **3** and **3a**, respectively, except for the signals due to the 23-aldehyde group. The oligoglycoside structures and positions of an angeloyl moiety and functional groups in the aglycon moiety were characterized by the HMBC experiment on **7**, which showed long-range correlations between the following proton and carbon pairs [24-H_3, 1′-H and 3-C; 24-H_3, 23-methoxycarbonyl methyl proton and 4-C; 16-H, 22-H, 28-H_2 and 17-C; 29-H_3, 30-H_3 and 20-C, 21-C; 21-H_2, 28-H_2 and 22-C; 22-H and 21-C, angeloyl carbonyl carbon (δ_C 168.0); 24-H_3 and 23-C; 1″-H and 2′-C; 1‴-H and 3′-C; 1⁗-H and 2‴-C (Fig. 1)]. Finally, reduction of **7a** with sodium borohydride ($NaBH_4$) in EtOH liberated **3a**,[13] so that the structures of the new aglycon part and the tetraglycoside moiety were confirmed. Consequently, the structure of theasaponin H_1 (**7**) was determined to be as shown.

Experimental

The following instruments were used to obtain physical data: melting points, Yanagimoto micro hot-stage apparatus (uncorrected); specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); UV spectra, Shimadzu UV-1600 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; FAB-MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JNM-LA500 (500 MHz) spectrometer; ^{13}C-NMR spectra, JNM-LA500 (125 MHz) spectrometer with tetramethylsilane as an internal standard; and HPLC detector, Shimadzu RID-6A refractive index and SPD-10A*vp* UV–VIS detectors.

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, Diaion HP-20 (Nippon Rensui); TLC, precoated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reverse phase); reverse-phase HPTLC, precoated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm); and detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 followed by heating.

Plant Material The seeds of *C. sinensis* were cultivated in Shizuoka prefecture, Japan and identified by one of the authors (Masayuki Yoshikawa).[3] A voucher of this plant material is on file in our laboratory.

Extraction and Isolation Fractions 2 (0.43 g), 5 (2.20 g), 6 (0.96 g), and 8 (0.97 g) obtained from the saponin fraction (=methanol-eluted fraction) of the seeds of *C. sinensis* (1.0 kg, cultivated in Shizuoka prefecture, Japan) as reported previously.[3] Fraction 2 (0.43 g) was purified by HPLC [YMC-pack ODS-A, 250×20 mm i.d., CH_3CN–1% aqueous AcOH (40 : 60, v/v)] to give five fractions {Fr. 2-1 (=theasaponin E_6, 34 mg, 0.013%), Fr. 2-2 (20 mg), Fr. 2-3 [=theasaponin E_8 (**4**, 17 mg, 0.007%)], Fr. 2-4 (24 mg), and Fr. 2-5 (=theasaponin E_7, 95 mg, 0.037%)}. Fraction 5 (2.20 g) was separated by HPLC [YMC-Pack ODS-A, 250×20 mm i.d., CH_3CN–1% aqueous AcOH (40 : 60, v/v)] to give 12 fractions {Fr. 5-1 [=theasaponin A_4 (**1**, 13 mg, 0.005%)], Fr. 5-2 (=theasaponin A_1, 53 mg, 0.021%), Fr. 5-3 (=theasaponin F_1, 23 mg, 0.009%), Fr. 5-4 (14 mg), Fr. 5-5 (37 mg), Fr. 5-6 (164 mg), Fr. 5-7 (100 mg), Fr. 5-8 (328 mg), Fr. 5-9 (=theasaponin A_3, 148 mg, 0.059%), Fr. 5-10 (200 mg), Fr. 5-11 (645 mg), and Fr. 5-12 (85 mg)}. Fraction 5-8 (328 mg) was subjected to HPLC [Develosil C30-UG-5, 250×20 mm i.d., CH_3CN–MeOH–1% aqueous AcOH (35 : 16 : 49, v/v/v)] to give four fractions {Fr. 5-8-1 (=camelliasaponin C_1, 10 mg, 0.004%), Fr. 5-8-2 [=theasaponin C_1 (**3**, 77 mg, 0.031%)], Fr. 5-8-3 (=theasaponin F_2, 54 mg, 0.021%), Fr. 5-8-4 (26 mg)}. Fraction 5-12 (85 mg) was further purified by HPLC [YMC-Pack ODS-A, 250×20 mm i.d., CH_3CN–MeOH–1% aqueous AcOH (37 : 16 : 47, v/v/v)] to give two fractions {Fr. 5-12-1 (20 mg) and Fr. 5-12-2 [=theasaponin E_9 (**5**, 32 mg, 0.013%)]}. Fraction 6 (960 mg) was subjected to HPLC [YMC-pack ODS-A, 250×20 mm i.d., CH_3CN–1% aqueous AcOH (43 : 57, v/v)] to give nine fractions [Fr. 6-1 (16 mg), Fr. 6-2 (=assamsaponin I, 56 mg, 0.022%) and Fr. 6-3 (=assamsaponin C, 323 mg, 0.13%), Fr. 6-4 (65 mg), Fr. 6-5 (=floratheasaponin A, 39 mg, 0.016%), Fr. 6-6 (15 mg), Fr. 6-7 (75 mg), Fr. 6-8 (20 mg), and Fr. 6-9 (=theasaponin E_5, 126 mg, 0.050%)]. Fraction 6-7 (75 mg) was further purified by HPLC [YMC-Pack ODS-A, 250×20 mm i.d., CH_3CN–MeOH–1% aqueous AcOH (39 : 16 : 45, v/v/v)] to give two fractions {Fr. 6-7-1 [=theasaponin H_1 (**7**, 25 mg, 0.010%)] and Fr. 6-7-2 (19 mg)}. Fraction 8 (0.97 g) was subjected to HPLC [YMC-Pack ODS-A, 250×20 mm i.d., CH_3CN–1% aqueous AcOH (43 : 57, v/v)] to give five fractions [Fr. 8-1 (=theasaponin A_2, 323 mg, 0.13%), Fr. 8-2 (=theasaponin F_3, 136 mg, 0.054%), Fr. 8-3 (46 mg), Fr. 8-4 (84 mg), and Fr. 8-5 (82 mg)]. Fraction 8-4 (84 mg) was further purified by HPLC [YMC-Pack ODS-A, 250×20 mm i.d., CH_3CN–MeOH–1% aqueous AcOH (35 : 16 : 49, v/v/v)] to give two fractions {Fr. 8-4-1 [=theasaponin G_1 (**6**, 13 mg, 0.005%)] and Fr. 8-4-2 [=theasaponin A_5 (**2**, 19 mg, 0.010%)]}.

Theasaponin A_4 (**1**): Colorless fine crystals (from $CHCl_3$–MeOH), mp 236.1—236.9 °C, $[\alpha]_D^{27}$ +25.8° (c=0.50, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{58}H_{92}O_{27}Na$ $(M+Na)^+$: 1243.5724. Found: 1243.5714. IR (KBr): 3432, 1718, 1646, 1075 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5): δ 0.89, 0.90, 1.05, 1.10, 1.32, 1.80 (3H each, all s, 26, 25, 24, 29, 30, 27-H_3), 1.98 (3H, s, Ang-5-H_3), 2.05 (3H, d, J=7.0 Hz, Ang-4-H_3), 2.92 (1H, dd-like, 18-H), 3.69, 3.96 (1H each, both d, J=10.0 Hz, 28-H_2), [3.77 (1H, d, J=10.1 Hz), 4.42 (1H, m), 23-H_2], 4.14 (1H, m, 3-H), 4.80 (1H, d, J=10.1 Hz, 22-H), 4.84 (1H, br s, 16-H), 5.08 (1H, d, J=7.7 Hz, 1′-H), 5.14 (1H, d, J=7.1 Hz, 1⁗-H), 5.37 (1H, br s, 12-H), 5.81 (1H, d, J=6.7 Hz, 1″-H), 5.84 (1H, d, J=5.8 Hz 1‴-H) 5.90 (1H, dq-like, Ang-3-H), 6.47 (1H, d, J=10.1 Hz, 21-H). ^{13}C-NMR data (125 MHz, pyridine-d_5) δ_C: given in Tables 1, 2. Positive-ion FAB-MS m/z: 1243 $(M+Na)^+$. Negative-ion FAB-MS m/z: 1219 $(M-H)^-$, 1057 $(M-C_6H_{11}O_5)^-$, 925 $(M-C_{11}H_{19}O_9)^-$.

Theasaponin A_5 (**2**): Colorless fine crystals (from $CHCl_3$–MeOH), mp 233.0—234.2 °C, $[\alpha]_D^{27}$ +34.5° (c=0.30, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{60}H_{94}O_{28}Na$ $(M+Na)^+$: 1285.5829. Found: 1285.5837. IR (KBr): 3432, 1719, 1647, 1078 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5): δ 0.91, 1.01, 1.04, 1.10, 1.30, 1.76 (3H each, all s, 25, 26, 24, 29, 30, 27-H_3), 1.97 (3H, s, Ang-5-H_3), 1.99 (3H, s, Ac-H_3), 2.05 (3H, d, J=7.1 Hz, Ang-4-H_3), 2.83 (1H, dd-like, 18-H), [3.77 (1H, d, J=10.1 Hz), 4.40 (1H, m), 23-H_2], 4.14 (1H, m, 3-H), 4.25 (2H, m, 28-H_2), 4.47 (1H, d, J=10.4 Hz, 22-H), 4.72 (1H, br s, 16-H), 5.07 (1H, d, J=7.4 Hz, 1′-H), 5.13 (1H, d, J=6.8 Hz, 1⁗-H), 5.45 (1H, br s, 12-H), 5.79 (1H, d, J=7.7 Hz, 1″-H), 5.83 (1H, d, J=6.1 Hz, 1‴-H), 5.90 (1H, dq-like, Ang-3-H), 6.48 (1H, d, J=10.4 Hz, 21-H). ^{13}C-NMR data (125 MHz, pyridine-d_5) δ_C: given in Tables 1, 2. Positive-ion FAB-MS m/z: 1285 $(M+Na)^+$. Negative-ion FAB-MS m/z: 1261 $(M-H)^-$, 1099 $(M-C_6H_{11}O_5)^-$, 967 $(M-C_{11}H_{19}O_9)^-$.

Theasaponin C_1 (**3**): Colorless fine crystals (from $CHCl_3$–MeOH), mp 223.1—224.3 °C, $[\alpha]_D^{27}$ +7.9° (c=2.00, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{57}H_{90}O_{25}Na$ $(M+Na)^+$: 1197.5669. Found: 1197.5657. IR (KBr): 3453, 1742, 1085 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5): δ 0.90, 0.90, 1.03, 1.08, 1.29, 1.83 (3H each, all s, 25, 26, 29, 24, 30, 27-H_3), 1.96 (3H, s, Ang-5-H_3), 2.09 (3H, d, J=7.0 Hz, Ang-4-H_3), 2.80, 2.85 (1H each, both m, 21-H_2), 3.04 (1H, dd-like, 18-H), 3.55, 3.70 (1H

each, both d, J=11.0 Hz, 28-H_2), [3.76 (1H, d, J=10.4 Hz), 4.39 (1H, m), 23-H_2], 4.14 (1H, dd-like, 3-H), 4.61 (1H, br s, 16-H), 5.02 (1H, d, J=7.7 Hz, 1‴-H), 5.05 (1H, d, J=6.7 Hz, 1′-H), 5.38 (1H, br s, 12-H), 5.77 (1H, d, J=6.5 Hz, 1‴-H), 5.88 (1H, d, J=7.4 Hz, 1″-H), 5.92 (1H, dq-like, Ang-3-H), 6.21 (1H, dd, J=5.5, 12.6 Hz, 22-H). ^{13}C-NMR data (125 MHz, pyridine-d_5) δ_C: given in Tables 1, 2. Positive-ion FAB-MS m/z: 1197 $(M+Na)^+$. Negative-ion FAB-MS m/z: 1173 $(M-H)^-$, 1041 $(M-C_5H_9O_4)^-$.

Theasaponin E_8 (**4**): Colorless fine crystals (from $CHCl_3$–MeOH), mp 216.4—217.1 °C, $[\alpha]_D^{27}$ +23.6° (c=0.80, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{59}H_{90}O_{27}Na$ $(M+Na)^+$: 1253.5567. Found: 1253.5559. IR (KBr): 3453, 1736, 1078 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5): δ 0.71, 0.76, 1.13, 1.31, 1.46, 1.48 (3H each, all s, 26, 25, 29, 30, 27, 24-H_3), 1.60 (3H, d, J=7.0 Hz, Tig-4-H_3), 1.84 (3H, s, Tig-5-H_3), 2.55 (3H, s, Ac-H_3), 3.00 (1H, dd-like, 18-H), 3.65, 3.94 (1H each, both d, J=10.4 Hz, 28-H_2), 3.97 (1H, dd-like, 3-H), 4.82 (1H, d, J=7.4 Hz, 1′-H), 4.84 (1H, d, J=9.5 Hz, 22-H), 5.02 (1H, d, J=7.2 Hz, 1‴-H), 5.35 (1H, br s, 12-H), 5.78 (1H, d, J=7.3 Hz, 1″-H), 5.81 (1H, d, J=6.7 Hz, 1‴-H), 5.94 (1H, br s, 16-H), 5.97 (1H, d, J=9.5 Hz, 21-H), 6.97 (1H, dq-like, Tig-3-H), 9.96 (1H, s, 23-H). ^{13}C-NMR data (125 MHz, pyridine-d_5) δ_C: given in Tables 1, 2. Positive-ion FAB-MS m/z: 1253 $(M+Na)^+$. Negative-ion FAB-MS m/z: 1229 $(M-H)^-$.

Theasaponin E_9 (**5**): Colorless fine crystals (from $CHCl_3$–MeOH), mp 192.0—193.4 °C, $[\alpha]_D^{27}$ +26.5° (c=1.30, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{61}H_{92}O_{28}Na$ $(M+Na)^+$: 1295.5673. Found: 1253.5573. IR (KBr): 3453, 1736, 1078 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5): δ 0.78, 0.87, 1.12, 1.27, 1.45, 1.48 (3H each, all s, 25, 26, 29, 30, 27, 24-H_3), 1.58 (3H, d, J=7.0 Hz, Tig-4-H_3), 1.82 (3H, s, Tig-5-H_3), 2.02, 2.54 (3H each, both s, Ac-H_3), 2.80 (1H, dd-like, 18-H), 3.94 (1H, dd-like, 3-H), 4.28 (2H, m, 28-H_2), 4.45 (1H, d, J=10.1 Hz, 22-H), 4.82 (1H, d, J=7.4 Hz, 1′-H), 5.01 (1H, d, J=7.3 Hz, 1‴-H), 5.43 (1H, br s, 12-H), 5.77 (1H, d, J=8.0 Hz, 1″-H), 5.78 (1H, d, J=6.1 Hz, 1‴-H), 5.86 (1H, br s, 16-H), 5.93 (1H, d, J=10.1 Hz, 21-H), 6.96 (1H, dq-like, Tig-3-H), 9.96 (1H, s, 23-H). ^{13}C-NMR data (125 MHz, pyridine-d_5) δ_C: given in Tables 1, 2. Positive-ion FAB-MS m/z: 1295 $(M+Na)^+$. Negative-ion FAB-MS m/z: 1271 $(M-H)^-$.

Theasaponin G_1 (**6**): Colorless fine crystals (from $CHCl_3$–MeOH), mp 227.1—228.2 °C, $[\alpha]_D^{27}$ +36.3° (c=0.70, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{57}H_{88}O_{25}Na$ $(M+Na)^+$: 1195.5512. Found: 1195.5520. IR (KBr): 3453, 1743, 1645, 1086 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5): δ 0.74, 0.77, 1.06, 1.11, 1.43, 1.47 (3H each, all s, 26, 25, 29, 30, 27, 24-H_3), 2.13 (3H, s, Ang-5-H_3), 2.14 (3H, d, J=6.5 Hz, Ang-4-H_3), 2.04, 2.49 (1H each, both m, 21-H_2), 2.80 (1H, m, 18-H), 3.65, 4.03 (1H, d, J=10.4 Hz, 28-H_2), 3.96 (1H, dd, J=3.7, 10.7 Hz, 3-H), 4.58 (1H, m, 22-H), 4.81 (1H, br s, 16-H), 4.83 (1H, d, J=7.3 Hz, 1′-H), 5.01 (1H, d, J=7.4 Hz, 1⁗-H), 5.31 (1H, br s, 12-H), 5.77 (1H, d, J=7.7 Hz, 1″-H), 5.79 (1H, d, J=6.1 Hz, 1‴-H), 5.95 (1H, dq-like, Ang-3-H), 9.96 (1H, s, 23-H). ^{13}C-NMR data (125 MHz, pyridine-d_5) δ_C: given in Tables 1, 2. Positive-ion FAB-MS m/z: 1195 $(M+Na)^+$. Negative-ion FAB-MS m/z: 1171 $(M-H)^-$, 1039 $(M-C_5H_9O_4)^-$, 1009 $(M-C_6H_{11}O_5)^-$, 907 $(M-C_{10}H_{17}O_8)^-$, 745 $(M-C_{16}H_{27}O_{13})^-$.

Theasaponin H_1 (**7**): Colorless fine crystals (from $CHCl_3$–MeOH), mp 218.0—219.4 °C, $[\alpha]_D^{27}$ +10.0° (c=0.90, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{58}H_{90}O_{26}Na$ $(M+Na)^+$: 1225.5618. Found: 1225.5613. IR (KBr): 3453, 1717, 1638, 1080 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5): δ 0.80, 0.83, 1.04, 1.29, 1.52, 1.79 (3H each, all s, 25, 26, 29, 30, 24, 27-H_3), 1.95 (3H, s, Ang-5-H_3), 2.09 (3H, d, J=7.3 Hz, Ang-4-H_3), 2.04, 2.82 (1H each, both m, 21-H_2), 3.03 (1H, m, 18-H), 3.55, 3.72 (1H, d, J=10.4 Hz, 28-H_2), 3.68 (3H, s, $COOCH_3$), 4.36 (1H, m, 3-H), 4.61 (1H, br s, 16-H), 4.97 (1H, d, J=7.4 Hz, 1′-H), 4.99 (1H, d, J=7.6 Hz, 1⁗-H), 5.35 (1H, br s, 12-H), 5.76 (1H, d, J=7.6 Hz, 1″-H), 5.79 (1H, d, J=6.1 Hz, 1‴-H), 5.92 (1H, dq-like, Ang-3-H), 6.21 (1H, dd, J=5.5, 11.9 Hz, 22-H). ^{13}C-NMR data (125 MHz, pyridine-d_5) δ_C: given in Tables 1, 2. Positive-ion FAB-MS m/z: 1225 $(M+Na)^+$. Negative-ion FAB-MS m/z: 1201 $(M-H)^-$, 1069 $(M-C_5H_9O_4)^-$, 937 $(M-C_{10}H_{17}O_8)^-$, 907 $(M-C_{11}H_{19}O_9)^-$.

Alkaline Hydrolysis of Theaponins A_4 (1), A_5 (2), C_1 (3), E_8 (4), E_9 (5), G_1 (6), and H_1 (7) A solution of each theasaponins (**1**—**7**: 10 mg each) in 50% aqueous 1,4-dioxane (1.0 ml) was treated with 10% aqueous KOH (1.0 ml) and the whole was stirred at 37 °C for 1 h. After removal of the solvent from a part (0.1 ml) of the reaction mixture under reduced pressure, the residue was dissolved in $(CH_2)_2Cl_2$ (2.0 ml) and the solution was treated with *p*-nitrobenzyl-*N*-*N*′-diisopyopylisourea (10 mg), then the whole was stirred at 80 °C for 1 h. The reaction mixture was subjected to HPLC analysis [column: YMC-Pack ODS-A, 250×4.6 mm i.d.; mobile phase: MeOH–H_2O (70 : 30, v/v); detection: UV (254 nm); flow rate: 0.9 ml/min] to identify the *p*-nitrobenzyl esters of acetic acid (**a**, t_R 6.3 min) from **2**, **4**, and **5**, tiglic acid (**b**, t_R 14.5 min) from **4** and **5**, and angelic acid (**c**, t_R 16.0 min) from **1**—**3**, **6**, and **7**. The rest of each reaction mixture was neutralized with Dowex HCR W2 (H^+ form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure yielded a product, which was subjected to ordinary-phase silica gel column chromatography [2.0 g, $CHCl_3$–MeOH–H_2O (6 : 4 : 1, v/v/v)] to give desacyl-theasaponin A (**1a**, 6 mg each from **1** and **2**), desacyl-theasaponin C (**3a**, 6 mg from **3**), desacyl-theasaponin E (**4a**,[2] 6 mg each from **4** and **5**), desacyl-assamsaponin A (**6a**,[6] 6 mg from **6**), and desacyl-theasaponin H (**7a**, 6 mg from **7**).

Desacyl-theasaponin A (**1a**): Colorless fine crystals (from $CHCl_3$–MeOH), mp 224.8—225.6 °C, $[\alpha]_D^{27}$ +11.8° (c=0.50, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{53}H_{86}O_{26}Na$ $(M+Na)^+$: 1161.5305. Found: 1161.5308. IR (KBr): 3453, 1719, 1076 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5): δ 0.90, 0.92, 1.04, 1.31, 1.37, 1.81 (3H each, all s, 25, 26, 24, 29, 30, 27-H_3), 4.14 (1H, m, 3-H), 4.61 (1H, d, J=9.8 Hz, 22-H), 4.77 (1H, d, J=9.8 Hz, 21-H), 5.00 (1H, br s, 16-H), 5.07 (1H, d, J=7.6 Hz, 1′-H), 5.13 (1H, d, J=7.6 Hz, 1⁗-H), 5.38 (1H, br s, 12-H), 5.79 (1H, d, J=7.6 Hz, 1″-H), 5.82 (1H, d, J=5.9 Hz, 1‴-H). ^{13}C-NMR data (125 MHz, pyridine-d_5) δ_C: given in Tables 1, 2. Positive-ion FAB-MS m/z: 1161 $(M+Na)^+$. Negative-ion FAB-MS m/z: 1137 $(M-H)^-$, 975 $(M-C_6H_{11}O_5)^-$, 843 $(M-C_{11}H_{19}O_9)^-$.

Desacyl-theasaponin C (**3a**): Colorless fine crystals (from $CHCl_3$–MeOH), mp 210.7—211.5 °C, $[\alpha]_D^{27}$ +17.4° (c=0.50, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{52}H_{84}O_{24}Na$ $(M+Na)^+$: 1115.5250. Found: 1115.5254. IR (KBr): 3453, 1742, 1081 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5): δ 0.91, 0.92, 1.04, 1.07, 1.14, 1.83 (3H each, all s, 25, 26, 29, 24, 30, 27-H_3), 4.15 (1H, m, 3-H), 5.03 (1H, d, J=8.0 Hz, 1⁗-H), 5.05 (1H, d, J=7.6 Hz, 1′-H), 5.15 (1H, br s, 16-H), 5.35 (1H, br s, 12-H), 5.78 (1H, d, J=5.8 Hz, 1‴-H), 5.89 (1H, d, J=7.7 Hz, 1″-H). ^{13}C-NMR data (125 MHz, pyridine-d_5) δ_C: given in Tables 1, 2. Positive-ion FAB-MS m/z: 1115 $(M+Na)^+$. Negative-ion FAB-MS m/z: 1091 $(M-H)^-$.

Desacyl-theasaponin H (**7a**): Colorless fine crystals (from $CHCl_3$–MeOH), mp 216.4—217.9 °C, $[\alpha]_D^{27}$ +12.9° (c=0.50, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{53}H_{84}O_{25}Na$ $(M+Na)^+$: 1143.5199. Found: 1143.5208. IR (KBr): 3453, 1744, 1078 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5): δ 0.80, 0.84, 1.04, 1.14, 1.51, 1.83 (3H each, all s, 25, 26, 29, 30, 24, 27-H_3), 3.67 (3H, s, $COOCH_3$), 4.36 (1H, m, 3-H), 4.98 (1H, d, J=7.9 Hz, 1′-H), 5.00 (1H, d, J=7.9 Hz, 1⁗-H), 5.14 (1H, br s, 16-H), 5.31 (1H, br s, 12-H), 5.78 (1H, d, J=7.6 Hz, 1″-H), 5.80 (1H, d, J=6.4 Hz, 1‴-H). ^{13}C-NMR data (125 MHz, pyridine-d_5) δ_C: given in Tables 1, 2. Positive-ion FAB-MS m/z: 1143 $(M+Na)^+$. Negative-ion FAB-MS m/z: 1119 $(M-H)^-$.

Acid Hydrolysis of 1a and 3a A solution of **1a** and **3a** (5 mg each) in 5% aqueous H_2SO_4–1,4-dioxane (1 : 1, v/v, 1.0 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and the resin was filtered. On removal of the solvent from the filtrate under reduced pressure, the residue was passed through a Sep-Pack C_{18} cartridge by elution with H_2O and then MeOH. The H_2O eluate was concentrated and the residue was treated with L-cysteine methyl ester hydrochloride (0.01 ml) in pyridine (0.02 ml) at 60 °C for 1 h. After this reaction, the solution was treated with *N*,*O*-bis(trimethyl silyl)trifluoroacetamide (0.01 ml) at 60 °C for 1 h. The supernatant was then subjected to GLC analysis [column: Supelco™-1, 0.25 mm i.d.×30 m; column temperature: 230 °C; detector temperature: 230 °C; injector temperature: 230 °C; He gas flow rate: 15 ml/min] to identify the derivatives of D-glucuronic acid (i, t_R 26.5 min), D-galactose (ii, t_R 25.6 min), and L-arabinose (iii, t_R 15.1 min) from **1a** and **3a**, D-glucose (iv, t_R 24.4 min) from **1a**, and D-xylose (v, t_R 19.3 min) from **3a**. The MeOH eluate from **1a** was purified by normal-phase silica gel column chromatography [200 mg, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer, v/v/v)] to give theasapogenol A (**1b**,[8] 2 mg). Through a similar procedure, theasapogenol C (**3b**,[10] 2 mg) was obtained from the MeOH eluate of **3a**.

$NaBH_4$ Reduction of 7a A solution of **7a** (4.0 mg) in EtOH (2.0 ml) was treated with $NaBH_4$ (4.0 mg) and the mixture was stirred at room temperature for 24 h. The reaction mixture was quenched in acetone, and then removal of the solvent under reduced pressure yielded a reduction mixture. The reduction mixture was purified by normal-phase silica gel column chromatography [200 mg, $CHCl_3$–MeOH–H_2O (5 : 4 : 1, v/v/v)] to give desacyl-theasaponin C (**3a**, 2.0 mg).

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References and Notes

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