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 A₄ (1), A₅ (2), C₁ (3), E₈ (4), E₉ (5), G₁ (6), and H₁ (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 (H₂O \rightarrow MeOH \rightarrow CHCl₃) 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 CHCl₃–MeOH with mp 236.1—236.9 °C, and exhibited a positive optical rotation ($[\alpha]_D^{27} + 25.8^\circ$ 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 highresolution 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*-





Chart 1

nitrobenzyl derivative.^{1,3-7)} Acid hydrolysis of 1a with 5% aqueous H₂SO₄-1,4-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 ¹H- (pyridine- d_5) and ¹³C-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-H₃)], two methylenes and four methines bearing an oxygen function { δ 3.69, 3.96 (1H each, both d, $J=10.0 \text{ Hz}, 28 \text{-H}_2$), [3.77 (1H, d, J=10.1 Hz), 4.42 (1H, m), 23-H₂], 4.14 (1H, m, 3-H), 4.80 (1H, d, J=10.1 Hz, 22-H), 4.84 (1H, brs, 16-H), 6.47 (1H, d, J=10.1 Hz, 21-H)}, an olefin [δ 5.37 (1H, brs, 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 ($\delta_{\rm C}$ 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 ¹³C-NMR data for 1 with those for 1a revealed an acylation shift around the 21-position of the aglycon moiety [1a: $\delta_{\rm C}$ 78.7 (21-C), 36.4 (20-C), 77.3 (22-C); 1: $\delta_{\rm C}$ 81.7 (21-C), 36.1 (20-C), 73.1 (22-C)]. On the basis of the above-mentioned evidence, the structure of theasaponin A4 was determined to be 21-O-angeloyltheasapogenol A 3-O- β -D-galactopyranosyl(1 \rightarrow 2)[β -Dglucopyranosyl(1 \rightarrow 2)- α -L-arabinopyranosyl(1 \rightarrow 3)]- β -D-glucuronopyranoside (1).

Theasaponin A_5 (2) was also obtained as colorless fine crystals from CHCl₃-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 ¹H- (pyridine- d_5) and ¹³C-NMR⁹ (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-H₃)], two methylenes and four methines bearing an oxygen function { δ [3.77 (1H, d, J=10.1 Hz), 4.40 (1H, m), 23-H₂], 4.14 (1H, m, 3-H), 4.25 (2H, m, 28-H₂), 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, brs, 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-H₃), 1.99 (3H, s, Ac-H₃), 2.05 (3H, d, J=7.1 Hz, Ang-4-H₃), 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 ($\delta_{\rm C}$ 168.6) and between the 28-protons and the acetyl carbonyl carbon ($\delta_{\rm C}$ 170.1). Furthermore, comparison of the ¹³C-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 A₅ was determined to be 21-*O*-angeloyl-28-*O*-acetyltheasapogenol A 3-*O*- β -D-galactopyranosyl(1 \rightarrow 2)[β -D-glucopyranosyl(1 \rightarrow 2)- α -L-arabinopyranosyl(1 \rightarrow 3)]- β -D-glucuronopyranoside (**2**).

Theasaponin C₁ (3), $[\alpha]_D^{27}$ +7.9° (MeOH), was also obtained as colorless fine crystals from CHCl₃-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, Dgalactose, 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 ¹H- (pyridine- d_5) and ¹³C-NMR⁹ (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-H₂), [3.76 (1H, d, J=10.4 Hz), 4.39 (1H, m), 23-H₂], 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 angelov moiety [δ 1.96 (3H, s, Ang-5-H₂), 2.09 (3H, d, J=7.0 Hz, Ang-4-H₃), 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 ($\delta_{\rm C}$ 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 C₁ was elucidated to be 22-O-angeloyltheasapogenol C 3-O- β -D-galactopyranosyl(1 \rightarrow 2)[β -D-xylopyranosyl(1 \rightarrow 2)- α -L-arabinopyranosyl($1 \rightarrow 3$)]- 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 CHCl₃–MeOH) with positive optical rotation ($[\alpha]_D^{27} + 23.6^\circ$ 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 $C_{59}H_{90}O_{27}$. Alka-line hydrolysis of 4 with 10% aqueous potassium hydroxide (KOH)–50% aqueous 1,4-dioxane (1:1, v/v) provided de-sacyl-theasaponin E (4a)² together with two organic acids,

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Table 1.	¹³ C-NMR (125 MHz)	Data for the Aglycon and	Acyl Moieties in 1-	–7, 1a, 3a, and 7a	(Pyridine- d_5)
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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- <i>O</i> -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	21-0-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	21-0-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	22-0-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- <i>O</i> -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	28-0-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 Mojeties in 1-7 19 39 and 79 (Pyridine-d)
	C-NMR (125 MHZ) Data for the retragiycoside Moleties in $1-7$, $1a$, $5a$, and $7a$ (Fyridine- a_5)

C-	1	1a	2	3	3a	4	5	6	7	7a
β -D-Glucopyranos	iduronic 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-Galactopyrand	osyl									
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-Arabinopyran	osyl									
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-Glucopyranos	yl									
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-Xylopyranosy	1									
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

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acetic acid and tiglic acid, which were identified by HPLC analysis of those p-nitrobenzyl derivatives.^{1,3-7)} The ¹H-(pyridine- d_5) and ¹³C-NMR⁹ (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-H₃)], a methylene and four methines bearing an oxygen function [δ 3.65, 3.94 (1H each, both d, J=10.4 Hz, 28-H₂), 3.97 (1H, dd-like, 3-H), 4.84 (1H, d, J=9.5 Hz, 22-H), 5.94 (1H, brs, 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, $J=6.7 \text{ Hz},1^{\prime\prime\prime}\text{-H}$]}, together with an acetyl and a tigloyl groups [δ 1.60 (3H, d, J=7.0 Hz, Tig-4-H₂), 1.84 (3H, s, Tig-5-H₃), 2.55 (3H, s, Ac-H₃), 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 ($\delta_{\rm C}$ 170.1) and between the 21-proton and the carbonyl carbon of the tigloyl part ($\delta_{\rm C}$ 168.3). On the basis of this evidence, the structure of theasaponin E8 was determined to be 16-O-acetyl-21-O-tigloyltheasapogenol E 3-O- β -Dgalactopyranosyl(1 \rightarrow 2)[β -D-xylopyranosyl(1 \rightarrow 2)- α -L-arabinopyranosyl($1 \rightarrow 3$)]- β -D-glucuronopyranoside (4).

Theasaponin E_0 (5) was also obtained as colorless fine crystals from CHCl₃-MeOH with mp 192.0-193.4 °C and positive optical rotation ($[\alpha]_D^{27} + 26.5^\circ$ 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 ¹H-(pyridine- d_5) and ¹³C-NMR⁹ (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-H₃), 3.94 (1H, dd-like, 3-H), 4.28 $(2H, m, 28-H_2), 4.45 (1H, d, J=10.1 Hz, 22-H), 5.43 (1H, d)$ 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-H₃), 1.82 (3H, s, Tig-5-H₃), 2.02, 2.54 (3H each, both s, Ac-H₃), 6.96 (1H, dq-like, Tig-3-H)]. The proton and carbon signals in the ¹H- and ¹³C-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 ¹³C-NMR data for 5 with those for 4 revealed an acetylation shift around the 28position of the theasapogenol E moiety. This evidence was supported by the HMBC experiment of 5, in which longrange correlations were observed between the 16-proton and the acetyl carbonyl carbon ($\delta_{\rm C}$ 169.8), between the 21-proton and the tigloyl carbonyl carbon ($\delta_{\rm C}$ 168.1), and between the 28-protons and the acetyl carbonyl carbon ($\delta_{\rm C}$ 170.5). Consequently, the structure of the asaponin E_{0} was determined to be 16,28-di-O-acetyl-21-O-tigloyltheasapogenol E 3-O- β -Dgalactopyranosyl(1 \rightarrow 2)[β -D-xylopyranosyl(1 \rightarrow 2)- α -L-arabinopyranosyl($1 \rightarrow 3$)]- β -D-glucuronopyranoside (5).

Theasaponin G₁ (6), $[\alpha]_D^{27}$ +36.3° (MeOH), was also obtained as colorless fine crystals from CHCl₃-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 ¹H- (pyridine- d_5) and ¹³C-NMR⁸) (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-H₃)], a methylene and three methines bearing an oxygen function [δ 3.65, 4.03 (1H, d, J=10.4 Hz, 28-H₂), 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-H₃), 2.14 (3H, d, J=6.5 Hz, Ang-4-H₃), 5.95 (1H, dq-like, Ang-3-H)]. The proton and carbon signals of the aglycon part in the ¹H- and ¹³C-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 ($\delta_{\rm C}$ 167.2). On the basis of this evidence, the structure of theasaponin G₁ was elucidated to be 28-Oangeloylcamelliagenin B 3-O- β -D-galactopyranosyl(1 \rightarrow 2)[β -D-xylopyranosyl(1 \rightarrow 2)- α -L-arabinopyranosyl(1 \rightarrow 3)]- β -Dglucuronopyranoside (6).

Theasaponin H_1 (7) was obtained as colorless fine crystals from CHCl₃-MeOH with mp 218.0-219.4 °C, and exhibited a positive optical rotation ($[\alpha]_{D}^{27}$ +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 highresolution 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/z937 $(M-C_{10}H_{17}O_8)^{-}]$, 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 ¹H- (pyridine- d_5) and ¹³C-NMR⁹ (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-H₃)], a methylene and three me-



Fig. 1. Selected HMBC Correlations of 7

thines bearing an oxygen function [δ 3.55, 3.72 (1H, d, J=10.4 Hz, 28-H₂), 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-H₃), 2.09 (3H, d, J=7.3 Hz, Ang-4-H₃), 5.92 (1H, dq-like, Ang-3-H)]. The proton and carbon signals in the ¹H- and ¹³C-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-H₃, 1'-H and 3-C; 24-H₃, 23-methoxycarbonyl methyl proton and 4-C; 16-H, 22-H, 28-H₂ and 17-C; 29-H₃, 30-H₃ and 20-C, 21-C; 21-H₂, 28-H₂ and 22-C; 22-H and 21-C, angeloyl carbonyl carbon ($\delta_{\rm C}$ 168.0); 24-H₃ 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 (NaBH₄) 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; ¹H-NMR spectra, JNM-LA500 (500 MHz) spectrometer; ¹³C-NMR spectra, JNM-LA500 (125 MHz) spectrometer with tetramethylsilane as an internal standard; and HPLC detector, Shimadzu RID-6A refractive index and SPD-10Avp 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(SO₄)₂–10% aqueous H₂SO₄ 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., CH₃CN-1% aqueous AcOH (40:60, v/v)] to give five fractions {Fr. 2-1 (=theasaponin E₆, 34 mg, 0.013%), Fr. 2-2 (20 mg), Fr. 2-3 [=theasaponin E₈ (4, 17 mg, 0.007%)], Fr. 2-4 (24 mg), and Fr. 2-5 (=theasaponin E₇, 95 mg, 0.037%)}. Fraction 5 (2.20 g) was separated by HPLC [YMC-Pack ODS-A, 250×20 mm i.d., CH₃CN-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 A1, 53 mg, 0.021%), Fr. 5-3 (=theasaponin F1, 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 A₃, 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., CH₃CN-MeOH-1% aqueous AcOH (35:16:49, v/v/v] to give four fractions {Fr. 5-8-1 (=camelliasaponin C₁, 10 mg, 0.004%), Fr. 5-8-2 [=theasaponin C₁ (3, 77 mg, 0.031%)], Fr. 5-8-3 (=theasaponin F2, 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., CH₃CN-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, $250 \times 20 \text{ mm i.d.}$, CH₃CN-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 E₅, 126 mg, 0.050%)]. Fraction 6-7 (75 mg) was further purified by HPLC [YMC-Pack ODS-A, 250×20 mm i.d., CH₃CN-MeOH-1% aqueous AcOH (39:16:45, v/v/v)] to give two fractions {Fr. 6-7-1 [=theasaponin H₁ (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., CH₃CN-1% aqueous AcOH (43:57, v/v)] to give five fractions [Fr. 8-1 (=theasaponin A2, 323 mg, 0.13%), Fr. 8-2 (=theasaponin F₃, 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., CH₃CN-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 A₅ (2, 19 mg, 0.010%)]}.

Theasaponin A₄ (1): Colorless fine crystals (from CHCl₃-MeOH), mp 236.1—236.9 °C, $[\alpha]_{D}^{27}$ +25.8° (c=0.50, MeOH). High-resolution positiveion 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. ¹H-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-H₃), 1.98 (3H, s, Ang-5-H₃), 2.05 (3H, d, J=7.0 Hz, Ang-4-H₃), 2.92 (1H, dd-like, 18-H), 3.69, 3.96 (1H each, both d, J=10.0 Hz, 28-H₂), [3.77 (1H, d, J=10.1 Hz), 4.42 (1H, m), 23-H₂], 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). ¹³C-NMR data (125 MHz, pyridine- d_5) δ_C : given in Tables 1, 2. Positive-ion FAB-MS m/z: 1243 (M+Na)⁺. Negativeion FAB-MS m/z: 1219 (M-H)⁻, 1057 (M-C₆H₁₁O₅)⁻, 925 $(M - C_{11}H_{19}O_9)^-$

Theasaponin A₅ (2): Colorless fine crystals (from CHCl₃–MeOH), mp 233.0–234.2 °C, $[\alpha]_D^{27} + 34.5^{\circ} (c=0.30, MeOH)$. High-resolution positiveion FAB-MS: Calcd for C₆₀H₉₄O₂₈Na (M+Na)⁺: 1285.5829. Found: 1285.5837. IR (KBr): 3432, 1719, 1647, 1078 cm⁻¹. ¹H-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-H₃), 1.97 (3H, s, Ang-5-H₃), 1.99 (3H, s, Ac-H₃), 2.05 (3H, d, J=7.1 Hz, Ang-4-H₃), 2.83 (1H, dd-like, 18-H), [3.77 (1H, d, J=10.1 Hz), 4.40 (1H, m), 23-H₂], 4.14 (1H, m, 3-H), 4.25 (2H, m, 28-H₂), 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). ¹³C-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-C₆H₁₁O₅)⁻, 967 (M-C₁₁H₁₉O₉)⁻.

Theasaponin C₁ (**3**): Colorless fine crystals (from CHCl₃–MeOH), mp 223.1—224.3 °C, $[\alpha]_D^{27}$ +7.9° (*c*=2.00, MeOH). High-resolution positiveion FAB-MS: Calcd for C₅₇H₉₀O₂₅Na (M+Na)⁺: 1197.5669. Found: 1197.5657. IR (KBr): 3453, 1742, 1085 cm⁻¹. ¹H-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-H₃), 1.96 (3H, s, Ang-5-H₃), 2.09 (3H, d, *J*=7.0 Hz, Ang-4-H₃), 2.80, 2.85 (1H each, both m, 21-H₂), 3.04 (1H, dd-like, 18-H), 3.55, 3.70 (1H each, both d, J=11.0 Hz, 28-H₂), [3.76 (1H, d, J=10.4 Hz), 4.39 (1H, m), 23-H₂], 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). ¹³C-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-C₅H₉O₄)⁻.

Theasaponin E₈ (4): Colorless fine crystals (from CHCl₃–MeOH), mp 216.4—217.1 °C, $[\alpha]_D^{27} + 23.6^{\circ} (c=0.80, MeOH)$. High-resolution positiveion FAB-MS: Calcd for C₅₉H₉₀O₂₇Na (M+Na)⁺: 1253.5567. Found: 1253.5559. IR (KBr): 3453, 1736, 1078 cm⁻¹. ¹H-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-H₃), 1.60 (3H, d, J=7.0 Hz, Tig-4-H₃), 1.84 (3H, s, Tig-5-H₃), 2.55 (3H, s, Ac-H₃), 3.00 (1H, dd-like, 18-H), 3.65, 3.94 (1H each, both d, J=10.4 Hz, 28-H₂), 3.97 (1H, dd-like, 3-H), 4.82 (1H, d, J=7.4 Hz, 1'-H), 4.84 (1H, d, J=7.5 Hz, 22-H), 5.02 (1H, d, J=7.2 Hz, 1""-H), 5.35 (1H, brs, 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, brs, 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). ¹³C-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 E₉ (**5**): Colorless fine crystals (from CHCl₃–MeOH), mp 192.0—193.4 °C, $[\alpha]_D^{27} + 26.5^{\circ} (c=1.30, MeOH)$. High-resolution positiveion FAB-MS: Calcd for C₆₁H₉₂O₂₈Na (M+Na)⁺: 1295.5673. Found: 1253.5573. IR (KBr): 3453, 1736, 1078 cm⁻¹. ¹H-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-H₃), 1.58 (3H, d, J=7.0 Hz, Tig-4-H₃), 1.82 (3H, s, Tig-5-H₃), 2.02, 2.54 (3H each, both s, Ac-H₃), 2.80 (1H, dd-like, 18-H), 3.94 (1H, dd-like, 3-H), 4.28 (2H, m, 28-H₂), 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). ¹³C-NMR data (125 MHz, pyridine- d_3) 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 G₁ (**6**): Colorless fine crystals (from CHCl₃–MeOH), mp 227.1–228.2 °C, $[\alpha]_D^{27} + 36.3^{\circ} (c=0.70, MeOH)$. High-resolution positive-ion FAB-MS: Calcd for C₅₇H₈₈O₂₅Na (M+Na)⁺: 1195.5512. Found: 1195.5520. IR (KBr): 3453, 1743, 1645, 1086 cm⁻¹. ¹H-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-H₃), 2.13 (3H, s, Ang-5-H₃), 2.14 (3H, d, J=6.5 Hz, Ang-4-H₃), 2.04, 2.49 (1H each, both m, 21-H₂), 2.80 (1H, m, 18-H), 3.65 (4.03 (1H, d, J=10.4 Hz, 28-H₂), 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), 5.95 (1H, dq-like, Ang-3-H), 9.96 (1H, s, 23-H). ¹³C-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-C₅H₉Q₄)⁻, 1009 (M-C₆H₁₁O₅)⁻, 907 (M-C₁₀H₁₇O₈)⁻, 745 (M-C₁₆H₂₇O₁₃)⁻.

Theasaponin H₁ (7): Colorless fine crystals (from CHCl₃–MeOH), mp 218.0—219.4 °C, $[\alpha]_D^{27}$ +10.0° (c=0.90, MeOH). High-resolution positiveion FAB-MS: Calcd for C₅₈H₉₀O₂₆Na (M+Na)⁺: 1225.5618. Found: 1225.5613. IR (KBr): 3453, 1717, 1638, 1080 cm⁻¹. ¹H-NMR (500 MHz, pyridine- d_3): δ 0.80, 0.83, 1.04, 1.29, 1.52, 1.79 (3H each, all s, 25, 26, 29, 30, 24, 27-H₃), 1.95 (3H, s, Ang-5-H₃), 2.09 (3H, d, J=7.3 Hz, Ang-4-H₃), 2.04, 2.82 (1H each, both m, 21-H₂), 3.03 (1H, m, 18-H), 3.55, 3.72 (1H, d, J=10.4 Hz, 28-H₂), 3.68 (3H, s, COOCH₃), 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). ¹³C-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-C₅H₉O₄)⁻, 937 (M-C₁₀H₁₇O₈)⁻, 907 (M-C₁₁H₁₉O₉)⁻.

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 (CH₂)₂Cl₂ (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–H₂O (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, CHCl₃–MeOH–H₂O (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 A (**6a**,⁶⁾ 6 mg from **6**), and desacyl-theasaponin H (**7a**, 6 mg from **7**).

Desacyl-theasaponin A (1a): Colorless fine crystals (from CHCl₃–MeOH), mp 224.8—225.6 °C, $[\alpha]_D^{27} + 11.8^{\circ} (c=0.50, MeOH)$. 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. ¹H-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-H₃), 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), 5.38 (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). ¹³C-NMR data (125 MHz, pyridine- d_5) δ_{C} : given in Tables 1, 2. Positive-ion FAB-MS m/z: 1137 (M-H)⁻, 975 (M-C₆H₁₁O₅)⁻, 843 (M-C₁₁H₁₉O₉)⁻.

Desacyl-theasaponin C (**3a**): Colorless fine crystals (from CHCl₃– MeOH), mp 210.7—211.5 °C, $[\alpha]_D^{27} + 17.4^{\circ}$ (c=0.50, MeOH). High-resolution positive-ion FAB-MS: Calcd for C₅₂H₈₄O₂₄Na (M+Na)⁺: 1115.5250. Found: 1115.5254. IR (KBr): 3453, 1742, 1081 cm⁻¹. ¹H-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-H₃), 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). ¹³C-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 CHCl₃–MeOH), mp 216.4—217.9 °C, $[\alpha]_D^{27} + 12.9^{\circ} (c=0.50, MeOH)$. 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. ¹H-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-H₃), 3.67 (3H, s, COOCH₃), 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, brs, 16-H), 5.31 (1H, brs, 12-H), 5.78 (1H, d, J=7.6 Hz, 1"'-H), 5.80 (1H, d, J=6.4 Hz, 1"'-H). ¹³C-NMR data (125 MHz, pyridine- d_5) δ_C : given in Tables 1, 2. Positive-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 C₁₈ cartridge by elution with H₂O and then MeOH. The H₂O 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: SupelcoTM-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, $t_{\rm R}$ 26.5 min), D-galactose (ii, $t_{\rm R}$ 25.6 min), and L-arabinose (iii, $t_{\rm R}$ 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 $[200 \text{ mg}, \text{CHCl}_3-\text{MeOH}-\text{H}_2\text{O} (10:3:1, \text{MeOH}-\text{H}_2\text{O})]$ lower layer, v/v/v] to give the asapogenol A (1b,⁸⁾ 2 mg). Through a similar procedure, theasapogenol C (3b, 10, 2 mg) was obtained from the MeOH eluate of 3a.

NaBH₄ **Reduction of 7a** A solution of 7a (4.0 mg) in EtOH (2.0 ml) was treated with NaBH₄ (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, CHCl₃–MeOH–H₂O (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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