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BIOACTIVE SAPONINS AND GLYCOSIDES. XXVI.[1] NEW TRITERPENE SAPONINS, THEASAPONINS E_{10}, E_{11}, E_{12}, E_{13}, AND G_2, FROM THE SEEDS OF TEA PLANT (*CAMELLIA SINENSIS*)

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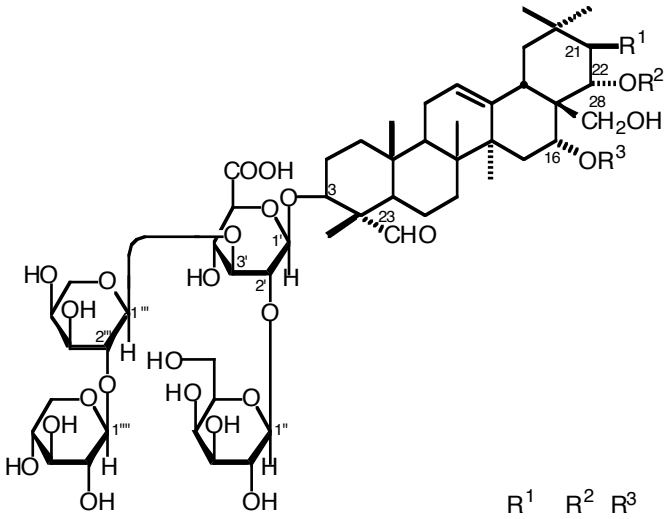
Abstract — New triterpene saponins, theasaponins E_{10}, E_{11}, E_{12}, E_{13}, and G_2, were isolated from the saponin fraction of the seeds of *Camellia sinesis*. Their stereostructures were elucidated on the basis of chemical and physicochemical evidence.

During the course of our characterization studies on the bioactive saponin constituents from *Camellia* species (Theaceae),[1-10] we have reported the isolation and structure elucidation of 29 saponins such as theasaponins A_1–A_5, C_1, E_1–E_9, F_1–F_3, H_1, and G_1, assamsaponins A–D, F, and I, camelliasaponins B_1 and C_1, and floratheasaponin A from the seeds of *C. sinensis* (L.) O. KUNTZE.[1–4] Furthermore, theasaponins A_2, E_1, E_2, and E_5, and assamsaponins A, C, and D, which were isolated from the seeds of *C. sinensis* and *C. sinensis* L. var. *assamica* PIERRE,[6,7] were found to show protective effects on ethanol-induced gastric lesions in rats.[3,4] Recently, floratheasaponins A–C with anti-hyperlipidemic activity were isolated from the flowers of *C. sinensis*.[5] As a continuing study on the seeds of *C. sinensis*, we have isolated five new triterpene saponins, named theasaponins E_{10} (**1**), E_{11} (**2**), E_{12} (**3**), E_{13} (**4**), and G_2 (**5**). This paper deals with the structure elucidation of these new saponins (**1**–**5**).

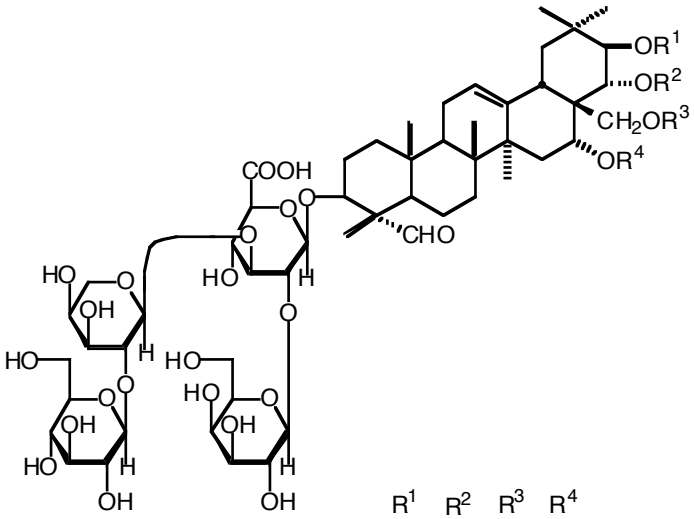
The saponin fraction of the methanolic extract of tea seeds (cultivated in Shizuoka prefecture, Japan), which was described previously,[3] was purified by HPLC to give **1** (0.0080%), **2** (0.0080%), **3** (0.0090%), **4** (0.018%), and **5** (0.0080%).

Structures of Theasaponins E_{10} (1), E_{11} (2), E_{12} (3), E_{13} (4), and G_2 (5)

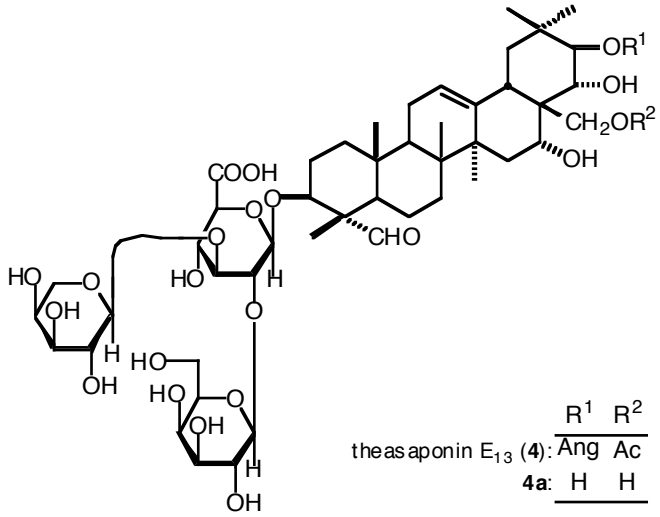
Theasaponin E_{10} (**1**), ($[\alpha]_D^{27}$ +1.7° in MeOH), was isolated as colorless fine crystals of mp 234.1–235.8 °C from $CHCl_3$–MeOH. The IR spectrum of **1** showed absorption bands at



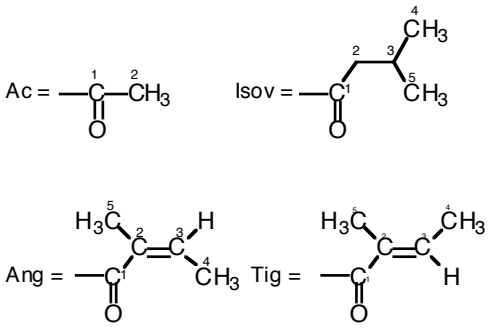
	R^1	R^2	R^3
theasaponin E_{10} (**1**):	Olsov	Ac	Ac
desacyl-theasaponin E (**1a**):	OH	H	H
theasaponin G_2 (**5**):	H	H	Ang
desacyl-assamsaponin A (**5a**):	H	H	H



	R^1	R^2	R^3	R^4
theasaponin E_{11} (**2**):	Ang	H	Ac	Ac
desacyl-assamsaponin F (**2a**):	H	H	H	H
theasaponin E_{12} (**3**):	Tig	Ac	H	H



	R^1	R^2
theasaponin E_{13} (**4**):	Ang	Ac
4a:	H	H



Ac: acetyl; Isov: isovaleryl; Ang: angeloyl; Tig: tigloyl

3453, 1734, and 1076 cm^{-1} ascribable to hydroxyl, carbonyl, and ether functions. In the positive and negative-ion fast atom bombardment (FAB)-MS of **1**, quasimolecular ion peaks were observed at *m/z* 1297 $(M+Na)^+$ and *m/z* 1273 $(M-H)^-$, respectively. High-resolution MS analysis of a quasimolecular ion peak $(M+Na)^+$ in the positive-ion FAB-MS revealed the molecular formula of **1** to be $C_{61}H_{94}O_{28}$. The fragmentation patterns in the negative-ion FAB-MS of **1** indicated the loss of mono-pentose [*m/z* 1141 $(M-C_5H_9O_4)^-$], mono-hexose [*m/z* 1111 $(M-C_6H_{11}O_5)^-$], di-pentoses [*m/z* 1009 $(M-C_{10}H_{17}O_8)^-$], and di-pentoses and mono-hexose [*m/z* 847 $(M-C_{16}H_{27}O_{13})^-$] units. On alkaline hydrolysis of **1** with 10% aqueous potassium hydroxide (KOH)–50% aqueous 1,4-dioxane (1:1, v/v), desacyl-theasaponin E (**1a**)[2] was obtained together with two organic acids, acetic acid and isovaleric acid, which were identified by HPLC analysis of their *p*-nitrobenzyl derivatives.[1,3–7] The ^1H- (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **1**, which were assigned by various NMR experiments,[11] showed signals assignable to six methyls [δ 0.70, 0.76, 1.07, 1.26, 1.42, 1.48 (3H each, all s, 26, 25, 29, 30, 27, 24-H_3), a methylene and four methines bearing an oxygen function [δ 3.45, 3.56 (1H each, both d, J = 10.4 Hz, 28-H_2), 3.94 (1H, dd-like, 3-H), 5.60 (1H, br s, 16-H), 5.77 (1H, d, J = 10.4 Hz, 21-H), 6.12 (1H, d, J = 10.4 Hz, 22-H)], an

olefin [δ 5.37 (1H, br s, 12-H)], an aldehyde [δ 9.95 (1H, s, 23-H)], and four glycopyranosyl moieties [δ 4.81 (1H, d, J = 6.8 Hz, 1'-H), 5.01 (1H, d, J = 7.3 Hz, 1''''-H), 5.77 (1H, d, J = 7.4 Hz, 1''-H), 5.79 (1H, d, J = 5.8 Hz, 1'''-H)] together with two acetyl groups [δ 2.11, 2.49 (3H each, both s, 22, 16-OAc)] and an isovaleryl moiety [δ 0.93 (6H, d, J = 6.8 Hz, Isov-4, 5-H_3), 2.19 (1H, m, Isov-3-H), 2.29 (2H, t-like, Isov-2-H_2)]. The positions of the acyl groups in **1** were clarified on the basis of the HMBC experiment. Thus, long-range correlations were observed between the 16-proton and acetyl carbonyl carbon (δ_C 169.8), the 21-proton and isovaleryl carbonyl carbon (δ_C 172.9), and the 22-proton and acetyl carbonyl carbon (δ_C 170.5). On the basis of the above-mentioned evidence, the structure of theasaponin E_{10} was determined to be 16,22-di-*O*-acetyl-21-*O*-isovaleryltheasapogenol E 3-*O*-β-D-galactopyranosyl(1→2)[β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**1**).

Theasaponin E_{11} (**2**) was obtained as colorless fine crystals from $CHCl_3$–MeOH with mp 224.1–225.5 °C, and exhibited a positive optical rotation ($[\alpha]_D^{27}$ +19.3° in MeOH). The IR spectrum of **2** showed absorption bands at 1734 and 1647 cm^{-1} ascribable to carbonyl and α,β-unsaturated ester functions, and broad bands at 3453 and 1078 cm^{-1}, suggestive of an oligoglycoside structure. In the positive- and negative-ion FAB-MS of **2**, quasimolecular ion peaks were observed at *m/z* 1325 $(M+Na)^+$ and *m/z* 1301 $(M–H)^–$, and high-resolution positive-ion FAB-MS analysis revealed the molecular formula of **2** to be $C_{62}H_{94}O_{29}$. On alkaline hydrolysis of **2** with 10% aqueous KOH–50% aqueous 1,4-dioxane (1:1, v/v), desacyl-assamsaponin F (**2a**)[7] was obtained together with two organic acids, acetic acid and angelic acid, which were identified by HPLC analysis of their *p*-nitrobenzyl derivatives.[1,3–7] The ^{1}H- (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[11] of **2** showed signals assignable to six methyls [δ 0.77, 0.87, 1.13, 1.26, 1.44, 1.46 (3H each, all s, 25, 26, 29, 30, 27, 24-H_3)], a methylene and four methines bearing an oxygen function [δ 3.96 (1H, dd-like, 3-H), 4.23 (2H, m, 28-H_2), 4.42 (1H, d, J = 10.1 Hz, 22-H), 5.84 (1H, br s, 16-H), 5.95 (1H, d, J = 10.1 Hz, 21-H)], an olefin [δ 5.42 (1H, br s, 12-H)], an aldehyde [δ 9.97 (1H, s, 23-H)], and four glycopyranosyl moieties [δ 4.83 (1H, d, J = 7.7 Hz, 1'-H), 5.11 (1H, d, J = 7.2, 1''''-H), 5.68 (1H, d, J = 7.4 Hz, 1''-H), 5.86 (1H, d, J = 6.2 Hz, 1'''-H)] together with two acetyl groups [δ 1.98, 2.54 (3H each, both s, 28, 16-OAc)] and an angeloyl moiety [δ 1.92 (3H, s, Ang-5-H_3), 2.01 (3H, d, J = 7.1 Hz, Ang-4-H_3), 5.91 (1H, dq-like, Ang-3-H)]. The HMBC experiment on **2** showed long-range correlations between the 16-proton and acetyl carbonyl carbon (δ_C 169.8), the 21-proton and angeloyl carbonyl carbon (δ_C 168.2), and the 28-protons and acetyl carbonyl carbon (δ_C 170.5). Consequently, the structure of theasaponin E_{11} was determined to be 16,28-di-*O*-acetyl-21-*O*-angeloyltheasapogenol E 3-*O*-β-D-galactopyranosyl(1→2)[β-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**2**).

Theasaponin E_{12} (**3**) with positive optical rotation ($[\alpha]_D^{27}$ +20.9° in MeOH) was also isolated as colorless fine crystals of mp 203.4–204.5 °C from $CHCl_3$–MeOH. The molecular formula $C_{60}H_{92}O_{28}$ of **3** was also determined from the positive- and negative-ion FAB-MS [*m/z* 1283 $(M+Na)^+$, *m/z* 1259 $(M–H)^–$] and by high-resolution positive-ion MS measurement. Furthermore, fragment ion peaks at *m/z* 1097 $(M–C_6H_{11}O_5)^–$, *m/z* 965 $(M–C_{11}H_{19}O_9)^–$, and *m/z* 803 $(M–C_{17}H_{29}O_{14})^–$, which were presumed to be derived by cleavage of the glycoside linkages at the 1'''', 1''', and 1''- and 1'''-protons, were observed in the negative-ion FAB-MS. Alkaline hydrolysis of **3** with 10% aqueous KOH–50% aqueous 1,4-dioxane (1:1,

Table 1. ^{13}C-NMR Data for Theasaponins E_{10} (**1**), E_{11} (**2**), E_{12} (**3**), E_{13} (**4**), and G_2 (**5**) and **4a** (125 MHz, pyridine-d_5)

	1	**2**	**3**	**4**	**4a**	**5**		**1**	**2**	**3**	**4**	**4a**	**5**
1	38.1	38.1	38.2	38.2	38.2	38.1	GlcA-1'	104.3	104.2	104.1	103.9	103.9	104.3
2	25.3	25.3	25.2	25.3	25.2	25.2	2'	78.4	78.5	78.5	78.7	78.6	78.3
3	84.7	84.6	84.4	84.5	84.4	84.7	3'	84.0	84.2	84.4	86.1	86.1	84.1
4	55.0	55.0	55.1	55.1	55.1	55.0	4'	70.8	71.1	71.1	71.1	71.3	70.8
5	48.4	48.5	48.4	48.5	48.4	48.5	5'	77.4	77.3	77.3	77.3	77.2	77.4
6	20.3	20.3	20.4	20.4	20.4	20.3	6'	171.9	171.9	172.0	171.8	171.9	171.9
7	32.3	32.3	32.4	32.4	32.4	32.3	Gal-1''	103.3	103.6	103.6	105.2	105.2	103.3
8	40.2	40.1	40.3	40.5	40.3	40.2	2''	73.7	73.7	73.7	73.7	73.8	73.7
9	46.7	46.7	46.8	46.9	46.9	46.8	3''	75.4	75.2	75.2	75.5	75.6	75.4
10	36.0	35.9	36.0	36.0	36.1	36.0	4''	70.5	70.2	70.3	70.2	70.2	70.5
11	23.7	23.8	23.8	23.8	23.8	23.6	5''	76.6	76.6	76.6	76.7	76.8	76.6
12	124.8	124.9	123.1	123.1	123.1	123.1	6''	62.1	62.2	62.1	61.8	61.8	62.1
13	141.0	141.0	142.9	142.8	144.0	142.5	Ara-1'''	101.7	101.7	101.7	104.3	104.3	101.7
14	41.1	41.4	41.7	41.8	42.0	41.6	2'''	82.3	81.2	81.2	72.9	72.9	82.3
15	30.9	30.9	34.6	34.6	34.3	31.6	3'''	73.4	72.3	72.3	74.8	74.8	73.3
16	71.2	70.8	67.9	67.5	67.7	71.0	4'''	68.3	67.6	67.5	69.6	69.7	68.3
17	46.9	46.2	48.0	47.2	47.3	44.2	5'''	66.0	64.8	64.7	67.8	67.7	66.0
18	39.5	40.3	40.1	40.3	41.2	44.8	Xyl or Glc-1''''	107.1	106.0	106.0			107.1
19	47.1	47.1	47.2	47.0	48.2	47.3	2''''	75.9	75.9	75.9			75.9
20	36.0	36.0	36.5	36.1	36.4	31.7	3''''	78.2	78.4	78.5			78.3
21	78.4	80.0	79.4	81.2	78.7	41.5	4''''	70.8	71.5	71.5			70.8
22	73.3	69.8	74.3	71.3	77.4	72.4	5''''	67.5	78.5	78.4			67.5
23	210.2	210.3	210.0	210.0	209.8	210.3	6''''		62.6	62.7			
24	11.2	11.1	11.1	11.1	11.0	11.2	16-*O*-acyl-1	169.8	169.8				167.2
25	15.7	15.8	15.8	15.8	15.8	15.8	2	21.9	22.1				128.7
26	16.7	16.8	16.8	17.0	16.8	16.6	3						138.0
27	26.9	27.0	27.4	27.4	27.4	27.0	4						15.9
28	63.7	65.9	63.7	66.4	68.3	69.3	5						21.3
29	29.5	29.9	29.5	29.7	30.6	33.4	21-*O*-acyl-1	172.9	168.2	168.0	168.5		
30	19.6	19.9	20.2	20.2	19.5	25.2	2	43.6	129.1	129.5	129.5		
							3	25.7	137.1	136.9	136.1		
							4	22.5	16.0	14.2	15.9		
							5	22.5	21.0	12.4	21.0		
							22-*O*-Ac	170.5		171.0			
								21.0		20.9			
							28-*O*-Ac		170.5		170.7		
									20.6		20.7		

GlcA: β-D-glucopyranosiduronic acid; Gal: β-D-galactopyranosyl; Ara: α-L-arabinopyranosyl; Xyl: β-D-xylopyranosyl; Glc: β-D-glucopyranosyl

v/v) provided **2a** and two organic acids, acetic acid and tiglic acid, which were identified by HPLC analysis of their *p*-nitrobenzyl derivatives.[1,3–7] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[11] of **3** indicated the presence of the following functions: a theasapogenol E part {six methyls [δ 0.81, 0.81, 1.11, 1.34, 1.47, 1.79 (3H each, all s, 25, 26, 29, 30, 24, 27-H_3)], a methylene and four methines bearing an oxygen function [δ 3.39, 3.61(1H each, both d, J = 10.4 Hz, 28-H_2), 4.04 (1H, dd-like, 3-H), 4.42 (1H, br s, 16-H), 6.27 (1H, d, J = 10.1 Hz, 22-H), 6.61 (1H, d, J = 10.1 Hz, 21-H)], an olefin [δ 5.38 (1H, br s, 12-H)], and an aldehyde [δ 9.95 (1H, s, 23-H)]}, four glycopyranosyl moieties [δ 4.88 (1H,

d, J = 7.1 Hz, 1'-H), 5.12 (1H, d, J = 7.1 Hz, 1''''-H), 5.69 (1H, d, J = 8.0 Hz, 1''-H), 5.86 (1H, d, J = 5.8 Hz, 1'''-H)], and two acyl functions {an acetyl group [δ 1.91 (3H, s, 22-OAc)] and an tigloyl moiety [δ 1.66 (3H, d, J = 7.0 Hz, Tig-4-H_3), 1.97 (3H, s, Tig-5-H_3), 7.13 (1H, dq-like, Tig-3-H)]}. The positions of acyl groups in the aglycone moiety were characterized by HMBC experiments. Thus, long-range correlations were observed between the 21-proton and tigloyl carbonyl carbon (δ_C 168.0) and the 22-proton and acetyl carbonyl carbon (δ_C 171.0). On the basis of this evidence, the structure of theasaponin E_{12} was determined to be **3** as shown.

Theasaponin E_{13} (**4**) was obtained as colorless fine crystals from $CHCl_3$–MeOH with mp 217.1–218.9 °C, and exhibited a positive optical rotation ($[\alpha]_D^{27}$ +22.7° in MeOH). The IR spectrum of **4** showed absorption bands at 1732 and 1647 cm^{-1} ascribable to carbonyl and α,β-unsaturated ester functions, and broad bands at 3432 and 1080 cm^{-1}, suggestive of an oligoglycoside structure. In the positive- and negative-ion FAB-MS of **4**, quasimolecular ion peaks were observed at m/z 1121 $(M+Na)^+$, and 1097 $(M-H)^-$, and high-resolution FAB-MS analysis revealed the molecular formula of **4** to be $C_{54}H_{82}O_{23}$. On the alkaline hydrolysis of **4**, the desacyl derivative (**4a**) was obtained together with two organic acids, acetic acid and angelic acid, which were identified by HPLC analysis of their *p*-nitrobenzyl derivatives.[1,3–7] Acid hydrolysis of **4a** with 5% aqueous H_2SO_4–1,4-dioxane (1:1, v/v) yielded theasapogenol E[2] together with D-glucuronic acid, D-galactose, and L-arabinose, which were identified by GLC analysis of their trimethylsilyl thiazolidine derivatives.[1,3–7] The ^{1}H- and ^{13}C-NMR (Table 1) spectra[11] of **4** and **4a** showed signals assignable to a theasapogenol E moiety [δ **4**: 0.81, 0.94, 1.12, 1.31, 1.47, 1.78 (3H each, all s, 25, 26, 29, 30, 24, 27-H_3), 4.08 (1H, m, 3-H), 4.14 (2H, m, 28-H_2), 4.48 (1H, m, 22-H), 4.71 (1H, br s, 16-H), 5.43 (1H, br s, 12-H), 6.49 (1H, d, J = 9.8 Hz, 21-H), 9.95 (1H, s, 23-H); **4a**: 0.81, 0.84, 1.34, 1.39, 1.47, 1.81 (3H each, all s, 25, 26, 29, 30, 24, 27-H_3), 3.73, 3.99 (1H each, both d, J = 10.5 Hz, 28-H_2), 4.09 (1H, m, 3-H), 4.63 (1H, d, J = 10.1 Hz, 22-H), 4.79 (1H, d, J = 10.1 Hz, 21-H), 5.00 (1H, br s, 16-H), 5.37 (1H, br s, 12-H), 9.93 (1H, s, 23-H)] and three glycopyranosyl moieties [δ **4**: 4.91 (1H, d, J = 7.4 Hz, 1'-H), 5.25 (1H, d, J = 7.6 Hz, 1''-H), 5.52 (1H, d, J = 6.1 Hz, 1'''-H); **4a**: 4.91 (1H, d, J = 7.9 Hz, 1'-H), 5.26 (1H, d, J = 8.3 Hz, 1''-H), 5.53 (1H, d, J = 6.1 Hz, 1'''-H)] together with an acetyl and an angeloyl groups [δ **4**: 1.98 (3H, s, Ang-5-H_3), 2.00 (3H, s, 28-OAc), 2.06 (3H, d, J = 6.5 Hz, Ang-4-H_3), 5.91 (1H, dq-like, Ang-3-H)]. The oligoglycoside structure and the positions of oligosugar and acyl moieties to the aglycone were characterized by a HMBC experiment on **4**, which showed long-range correlations between the following proton and carbon pairs: 1'-H and 3-C; 1''-H and 2'-C; 1'''-H and 3'-C; 21-H and angeloyl carbonyl carbon (δ_C 168.5); 28-H_2 and acetyl carbonyl carbon (δ_C 170.7). Consequently, the structure of theasaponin E_{13} (**4**) was determined to be as shown.[12,13]

Theasaponin G_2 (**5**), $[\alpha]_D^{27}$ –1.3° (MeOH), was also obtained as colorless fine crystals from $CHCl_3$–MeOH with mp 224.7–225.9 °C. The positive- and negative-ion FAB-MS of **5** showed quasimolecular ion peaks at m/z 1195 $(M+Na)^+$ and m/z 1171 $(M-H)^-$, respectively. The High-resolution FAB-MS of **5** revealed the molecular formula to be $C_{57}H_{88}O_{25}$. The IR spectrum of **5** showed absorption bands at 3453, 1717, 1638, and 1080 cm^{-1}, ascribable to hydroxyl, carbonyl, α,β-unsaturated ester, and ether functions. Alkaline hydrolysis of **5** liberated desacyl-assamsaponin A (**5a**)[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 (Tables 1) spectra[11] of **5** indicated the presence of the following functions: an aglycone 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 each, both 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), 6.33 (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)]} and four glycopyranosyl moieties [δ 4.81 (1H, d, J = 6.7 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)] together with an angeloyl moiety [δ 2.13 (3H, s, Ang-5-H_3), 2.14 (3H, d, J = 6.5 Hz, Ang-4-H_3), 6.01 (1H, dq-like, Ang-3-H)]. The position of an angeloyl group in **5** was characterized by the HMBC experiment, in which a long-range correlation was observed between the 16-proton and angeloyl carbonyl carbon (δ_C 167.2). On the basis of this evidence, the structure of theasaponin G_2 was elucidated to be 16-*O*-angeloylcamelliagenin B 3-*O*-β-D-galactopyranosyl(1→2)[β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**5**).

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); IR spectra, Shimadzu FTIR-8100 spectrophotometer; FAB-MS and high-resolution FAB-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; HPLC detector, Shimadzu RID-6A refractive index and SPD-10A*vp* UV-VIS detectors; and HPLC column, YMC-Pack ODS-A (250 × 4.6 mm i.d.) and (250 × 20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

The following experimental conditions were used for chromatography: normal-phase column chromatography; Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150–350 mesh), reversed-phase column chromatography; Diaion HP-20 (Nippon Rensui): TLC, pre-coated TLC plates with Silica gel $60F_{254}$ (Merck, 0.25 mm) (normal-phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed-phase); HPTLC, pre-coated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm) (reversed-phase) and detection was achieved by spraying with 1% $Ce(SO_4)_2$-10% aqueous H_2SO_4, followed by heating.

Isolation of Theasaponins E_{10} (1), E_{11} (2), E_{12} (3), E_{13} (4), and G_2 (5)

Fractions 2 (0.43 g), 5-12 (85 mg), 6-7 (75 mg), and 8 (0.97 g) were obtained from the saponin fraction (= methanol-eluted fraction, 6.34% from the seeds) of the seeds of *C. sinensis* (1.0 kg, cultivated in Shizuoka prefecture, Japan) as reported previously.[2] Fraction 2 (0.43 g) was purified by HPLC [CH_3CN–1% aqueous AcOH (40 : 60, v/v)] to give five fractions { Fr. 2-1 (= theasaponin E_6, 34 mg), Fr. 2-2 [= theasaponin G_2 (**5**, 20 mg, 0.0080%)], Fr. 2-3 (= theasaponin E_8, 17 mg), Fr. 2-4 [=theasaponin E_{12} (**3**, 24 mg, 0.0090%)], and Fr. 2-5 (= theasaponin E_7, 95 mg)}. Fraction 5-12 (85 mg) was further purified by HPLC [CH_3CN–MeOH–1% aqueous AcOH (37 : 16 : 47, v/v/v)] to give two fractions {Fr. 5-12-1 [= theasaponin E_{10} (**1**, 20 mg, 0.0080%)] and Fr. 5-12-2 (= theasaponin E_9, 32 mg)}. Fraction 6-7 (75 mg) was further purified by HPLC [CH_3CN–MeOH–1% aqueous AcOH (39 : 16 : 45, v/v/v)] to give two

fractions {Fr. 6-7-1 (= theasaponin E_1, 25 mg) and Fr. 6-7-2 [= theasaponin E_{11} (**2**, 19 mg, 0.0080%)]}. Fraction 8 (0.97 g) was subjected to HPLC [CH_3CN–1% aqueous AcOH (43 : 57, v/v)] to give five fractions [Fr. 8-1 (= theasaponin A_2, 323 mg), Fr. 8-2 (= theasaponin F_3, 136 mg), Fr. 8-3 [= theasaponin E_{13} (**4**, 46 mg, 0.018%), Fr. 8-4 (84 mg), and Fr. 8-5 (82 mg)].

Theasaponin E_{10} (**1**): colorless fine crystals, mp 234.1–235.8 °C (from $CHCl_3$–MeOH), $[\alpha]_D^{27}$ +1.7° (*c* = 0.70, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{61}H_{94}O_{28}Na$ $(M+Na)^+$: 1297.5829. Found: 1297.5834. IR (KBr): 3453, 1734, 1076 cm^{-1}. ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 0.70, 0.76, 1.07, 1.26, 1.42, 1.48 (3H each, all s, 26, 25, 29, 30, 27, 24-H_3), 0.93 (6H, d, *J* = 6.8 Hz, Isov-4, 5-H_3), 2.11, 2.49 (3H each, both s, 22, 16-OAc), 2.19 (1H, m, Isov-3-H), 2.29 (2H, t-like, Isov-2-H_2), 2.98 (1H, dd-like, 18-H), 3.45, 3.56 (1H each, both d, *J* = 10.4 Hz, 28-H_2), 3.94 (1H, dd-like, 3-H), 4.81 (1H, d, *J* = 6.8 Hz, 1'-H), 5.01 (1H, d, *J* = 7.3 Hz, 1''''-H), 5.37 (1H, br s, 12-H), 5.60 (1H, br s, 16-H), 5.77 (1H, d, *J* = 7.4 Hz, 1''-H), 5.77 (1H, d, *J* = 10.4 Hz, 21-H), 5.79 (1H, d, *J* = 5.8 Hz, 1'''-H), 6.12 (1H, d, *J* = 10.4 Hz, 22-H), 9.95 (1H, s, 23-H). ^{13}C-NMR (pyridine-d_5, 125 MHz) δc: given in Table 1. Positive-ion FAB-MS: *m/z* 1297 $(M+Na)^+$. Negative-ion FAB-MS: *m/z* 1273 $(M-H)^-$, 1141 $(M-C_5H_9O_4)^-$, 1111 $(M-C_6H_{11}O_5)^-$, 1009 $(M-C_{10}H_{17}O_8)^-$, 847 $(M-C_{16}H_{27}O_{13})^-$.

Theasaponin E_{11} (**2**): colorless fine crystals, mp 224.1–225.5 °C (from $CHCl_3$–MeOH), $[\alpha]_D^{27}$ +19.3° (*c* = 1.00, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{62}H_{94}O_{29}Na$ $(M+Na)^+$: 1325.5778. Found: 1325.5774. IR (KBr): 3453, 1734, 1647, 1078 cm^{-1}. ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 0.77, 0.87, 1.13, 1.26, 1.44, 1.46 (3H each, all s, 25, 26, 29, 30, 27, 24-H_3), 1.92 (3H, s, Ang-5-H_3), 1.98, 2.54 (3H each, both s, 28, 16-OAc), 2.01 (3H, d, *J* = 7.1 Hz, Ang-4-H_3), 2.79 (1H, dd-like, 18-H), 3.96 (1H, dd-like, 3-H), 4.23 (2H, m, 28-H_2), 4.42 (1H, d, *J* = 10.1 Hz, 22-H), 4.83 (1H, d, *J* = 7.7 Hz, 1'-H), 5.11 (1H, d, *J* = 7.2 Hz, 1''''-H), 5.42 (1H, br s, 12-H), 5.68 (1H, d, *J* = 7.4 Hz, 1''-H), 5.84 (1H, br s, 16-H), 5.86 (1H, d, *J* = 6.2 Hz, 1'''-H), 5.91 (1H, dq-like, Ang-3-H), 5.95 (1H, d, *J* = 10.1 Hz, 21-H), 9.97 (1H, s, 23-H). ^{13}C-NMR (pyridine-d_5, 125 MHz) δc: given in Table 1. Positive-ion FAB-MS: *m/z* 1325 $(M+Na)^+$. Negative-ion FAB-MS: *m/z* 1301 $(M-H)^-$, 1139 $(M-C_6H_{11}O_5)^-$, 1007 $(M-C_{11}H_{19}O_9)^-$, 845 $(M-C_{17}H_{29}O_{14})^-$.

Theasaponin E_{12} (**3**): colorless fine crystals, mp 203.4–204.4 °C (from $CHCl_3$–MeOH), $[\alpha]_D^{27}$ +20.9° (*c* = 1.00, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{60}H_{92}O_{28}Na$ $(M+Na)^+$: 1283.5673. Found: 1283.5677. IR (KBr): 3453, 1739, 1076 cm^{-1}. ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 0.81, 0.81, 1.11, 1.34, 1.47, 1.79 (3H each, all s, 25, 26, 29, 30, 24, 27-H_3), 1.66 (3H, d, *J* = 7.0 Hz, Tig-4-H_3), 1.91 (3H, s, 22-OAc), 1.97 (3H, s, Tig-5-H_3), 3.08 (1H, dd-like, 18-H), 3.39, 3.61 (1H each, both d, *J* = 10.4 Hz, 28-H_2), 4.04 (1H, dd-like, 3-H), 4.42 (1H, br s, 16-H), 4.88 (1H, d, *J* = 7.1 Hz, 1'-H), 5.12 (1H, d, *J* = 7.1 Hz, 1''''-H), 5.38 (1H, br s, 12-H), 5.69 (1H, d, *J* = 8.0 Hz, 1''-H), 5.86 (1H, d, *J* = 5.8 Hz, 1'''-H), 6.27 (1H, d, *J* = 10.1 Hz, 22-H), 6.61 (1H, d, *J* = 10.1 Hz, 21-H), 7.13 (1H, dq-like, Tig-3-H), 9.95 (1H, s, 23-H). ^{13}C-NMR (pyridine-d_5, 125 MHz) δc: given in Table 1. Positive-ion FAB-MS: *m/z* 1283 $(M+Na)^+$. Negative-ion FAB-MS: *m/z* 1259 $(M-H)^-$, 1097 $(M-C_6H_{11}O_5)^-$, 965 $(M-C_{11}H_{19}O_9)^-$, 803 $(M-C_{17}H_{29}O_{14})^-$.

Theasaponin E_{13} (**4**): colorless fine crystals, mp 217.1–218.9 °C (from $CHCl_3$–MeOH), $[\alpha]_D^{27}$ +22.7° (*c* = 2.00, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{54}H_{82}O_{23}Na$ $(M+Na)^+$: 1121.5145. Found: 1121.5140. IR (KBr): 3432, 1732, 1647, 1080 cm^{-1}. ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 0.81, 0.94, 1.12, 1.31, 1.47, 1.78 (3H each, all s, 25, 26, 29, 30, 24, 27-H_3), 1.98 (3H, s, Ang-5-H_3), 2.00 (3H, s, 28-OAc), 2.06 (3H, d, *J* = 6.5 Hz, Ang-4-H_3), 2.82 (1H, dd-like, 18-H), 4.08 (1H, m, 3-H), 4.14 (2H, m, 28-H_2), 4.48 (1H, m, 22-H), 4.71 (1H, br s, 16-H), 4.91 (1H, d, *J* = 7.4 Hz, 1'-H), 5.25 (1H, d, *J* = 7.6 Hz, 1''-H), 5.43 (1H, br s, 12-H), 5.52 (1H, d, *J* = 6.1 Hz, 1'''-H), 5.91 (1H, dq-like, Ang-3-H), 6.49 (1H, d, *J* = 9.8 Hz, 21-H), 9.95 (1H, s, 23-H). ^{13}C-NMR (pyridine-d_5, 125 MHz) δc: given in Table 1. Positive-ion FAB-MS: *m/z* 1121 $(M+Na)^+$. Negative-ion FAB-MS: *m/z* 1097 $(M-H)^-$, 965 $(M-C_5H_9O_4)^-$, 935 $(M-C_6H_{11}O_5)^-$.

Theasaponin G_2 (**5**): colorless fine crystals, mp 224.7–225.9 °C (from $CHCl_3$–MeOH), $[\alpha]_D^{27}$ –1.3° (*c* = 1.00, 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, 1717, 1638, 1080 cm^{-1}. ^{1}H-NMR (pyridine-d_5, 500 MHz) 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), 2.09 (1H, m, 18-H), 2.13 (3H, s, Ang-5-H_3), 2.14 (3H, d, *J* = 6.5 Hz, Ang-4-H_3), 3.65, 4.03 (1H each, both 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, d, *J* = 6.7 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), 6.01 (1H, dq-like, Ang-3-H), 6.33 (1H, br s, 16-H), 9.96 (1H, s, 23-H). ^{13}C-NMR (pyridine-d_5, 125 MHz) δc: given in Table 1. Positive-ion FAB-MS: *m/z* 1195 $(M+Na)^+$. Negative-ion FAB-MS: *m/z* 1171 $(M-H)^-$, 1039 $(M-C_5H_9O_4)^-$, 907 $(M-C_{10}H_{17}O_8)^-$, 745 $(M-C_{16}H_{27}O_{13})^-$.

Alkaline Hydrolysis of 1—5

A solution of each theasaponins (**1**–**3** or **5**: 10 mg each; **4**: 14 mg) 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 **1**–**4**, tiglic acid (**b**, t_R 14.5 min) from **3**, angelic acid (**c**, t_R 16.0 min) from **2**, **4** and **5**, and isovaleric acid (**d**, t_R 19.4 min) from **1**. 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 normal-phase silica gel column chromatography [2.0 g, $CHCl_3$–MeOH–H_2O (6:4:1, v/v/v)] to give desacyl-theasaponin E (**1a**, 6 mg from **1**), desacyl-assamsaponin F (**2a**, 6 mg each from **2** and **3**), **4a** (11 mg from **4**), and desacyl-assamsaponin A (**5a**, 6 mg each from **5**).

4a: colorless fine crystals, mp 210.3–211.5 °C (from $CHCl_3$–MeOH), $[\alpha]_D^{27}$ +23.4° (*c* = 0.50, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{47}H_{74}O_{21}Na$ $(M+Na)^+$: 997.4620. Found: 997.4621.

IR (KBr): 3453, 1736, 1078 cm^{-1}. ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 0.81, 0.84, 1.34, 1.39, 1.47, 1.81 (3H each, all s, 25, 26, 29, 30, 24, 27-H_3), 3.73, 3.99 (1H each, both d, J = 10.5 Hz, 28-H_2), 4.09 (1H, m, 3-H), 4.63 (1H, d, J = 10.1 Hz, 22-H), 4.79 (1H, d, J = 10.1 Hz, 21-H), 4.91 (1H, d, J = 7.9 Hz, 1'-H), 5.00 (1H, br s, 16-H), 5.26 (1H, d, J = 8.3 Hz, 1''-H), 5.37 (1H, br s, 12-H), 5.53 (1H, d, J = 6.1 Hz, 1'''-H), 9.93 (1H, s, 23-H). ^{13}C-NMR (pyridine-d_5, 125 MHz) δc: given in Table 1. Positive-ion FAB-MS: *m/z* 997 $(M+Na)^+$. Negative-ion FAB-MS: *m/z* 973 $(M-H)^-$, 841 $(M-C_5H_9O_4)^-$, 811 $(M-C_6H_{11}O_5)^-$, 679 $(M-C_{11}H_{19}O_9)^-$.

Acid Hydrolysis of 4a

A solution of **4a** (5 mg) 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^{TM}-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). The MeOH eluate 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 E^2 (2 mg).

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REFERENCES AND NOTES

1 Part XXV: H. Matsuda, T. Morikawa, N. Li, S. Nakamura, X. Li, and M. Yoshikawa, *Phytochemistry* submitted.

2 I. Kitagawa, K. Hori, T. Motozawa, T. Murakami, and M. Yoshikawa, *Chem. Pharm. Bull.*, 1998, **46**, 1901.

3 M. Yoshikawa, T. Morikawa, N. Li, A. Nagatomo, X. Li, and H. Matsuda, *Chem. Pharm. Bull.*, 2005, **53**, 1559.

4 T. Morikawa, N. Li, A. Nagatomo, H. Matsuda, X. Li, and M. Yoshikawa, *J. Nat. Prod.*, 2006, **69**, 185.

5 M. Yoshikawa, T. Morikawa, K. Yamamoto, Y. Kato, A. Nagatomo, and H. Matsuda. *J. Nat. Prod.*, 2005, **68**, 1360.

6 T. Murakami, J. Nakamura, H. Matsuda, and M. Yoshikawa, *Chem. Pharm. Bull.*, 1999, **47**, 1759.

7 T. Murakami, J. Nakamura, T. Kageura, H. Matsuda, and M. Yoshikawa, *Chem. Pharm. Bull.*, 2000, **48**, 1720.

8 M. Yoshikawa, E. Harada, T. Murakami, H. Matsuda, J. Yamahara, and N. Murakami, *Chem. Pharm. Bull.*, 1994, **42**, 742.

9 M. Yoshikawa, T. Murakami, S. Yoshizumi, N. Murakami, J. Yamahara, and H. Matsuda, *Chem. Pharm. Bull.*, 1996, **44**, 1899.

10 M. Yoshikawa, T. Morikawa, E. Fujiwara, T. Ohgushi, Y. Asao, and H. Matsuda, *Heterocycles*, 2001, **55**, 1653.

11 The ^{1}H- and ^{13}C-NMR spectra of **1**–**5** and **4a** were assigned with the aid of distortionless enhancement by polarization transfer (DEPT), double quantum filter correlation spectroscopy (DQF COSY), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond connectivity (HMBC), and homo- and heteronuclear Hartmann-Hahn spectroscopy (^{1}H–^{1}H, ^{13}C–^{1}H HOHAHA) experiments.

12 Although a mixture of the geometrical isomer in the 21-*O*-acyl part of **4** was reported in ref 13, the isolation and physical data of **4** were not reported. This is the first report of the isolation and structure elucidation of **4**.

13 G. Reznicek, H. Schröder, M. Schubert-Zsilavecz, T. Schöpke, S. Lehrkinder, E. Haslinger, K. Hiller, J. Jurenitsch, and W. Kubelka, *Pharmazie*, 1994, **49**, 58.