Structures of New Acylated Oleanene-Type Triterpene Oligoglycosides, Theasaponins E_1 and E_2, from the Seeds of Tea Plant, *Camellia sinensis* (L.) O. KUNTZE[1)]

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Two new acylated oleanene-type triterpene oligoglycosides, theasaponins E_1 and E_2, were isolated from the seeds of tea plant [*Camellia sinensis* (L.) O. KUNTZE]. The structures of theasaponins E_1 and E_2 were elucidated on the basis of chemical and physicochemical evidence to be expressed as 21-*O*-angeloyl-22-*O*-acetyltheasapogenol E 3-*O*-[β-D-galactopyranosyl(1→2)][β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl (1→3)]-β-D-glucopyranosiduronic acid and 21-*O*-angeloyl-28-*O*-acetyltheasapogenol E 3-*O*-[β-D-galactopyranosyl(1→2)][β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl (1→3)]-β-D-glucopyranosiduronic acid, respectively. Theasaponin E_1 was found to show antisweet activity.

Key words theasaponin E_1; theasaponin E_2; *Camellia sinensis*; tea plant; oleanene-type triterpene oligoglycoside; antisweet activity

The seeds of tea plant [*Camellia sinensis* (L.) O. KUNTZE, Theaceae] are known to contain numerous saponin constituents and the crude saponin has been used as a surface-active agent. In regard to chemical study on the saponins from the seeds of tea plant, we first reported the isolation and structure elucidation of five triterpene sapogenols, theasapogenols A (**1**), B (**2**, barringtogenol C), C (**3**, camelliagenin C), D (**4**, dihydropriverogenin A), and E (**5**), which were obtained by alkaline hydrolysis followed by acid hydrolysis of the crude saponin from the seeds of Japanese tea plant.[3)] Since then, the isolation and structure determination of the saponin constituents have been the target of many investigations and the structure of "theasaponin" was hitherto presumed on the basis of chemical evidence obtained using the saponin mixture and biogenetical speculation concerning other acylated triterpene saponins.[4)]

As part of our chemical studies on the saponin constituents of tea plant, we isolated two new saponins called theasaponins E_1 (**6**) and E_2 (**7**) from the seeds of this plant. In this paper, we describe the isolation and structure elucidation of those theasaponins (**6**, **7**) and the antisweet activity.[5)]

The methanolic extract obtained from the seeds of tea plants cultivated in the medicinal plant garden of Kyoto Pharmaceutical University was subjected to octadecyl silica gel (ODS) column chromatography (Chromatorex DM1020T) to remove the sugar and lipid components. The methanol-eluted fraction was separated by silica gel column chromatography to give the saponin mixture, which was purified by HPLC (YMC-Pack R&D ODS-5) to afford theasaponins E_1 (**6**, 0.47%) and E_2 (**7**, 0.06%).

Theasaponin E_1 (**6**) was isolated as colorless fine crystals of mp 246—248 °C. The IR spectrum of **6** showed absorption bands due to hydroxyl and carbonyl functions at 3433, 1724, and 1040 cm^{-1}. In the liquid secondary ion mass spectrometry (SI-MS) of **6**, quasimolecular ion peaks were observed at *m/z* 1253 $(M+Na)^+$ and *m/z* 1269 $(M+K)^+$ and their high-resolution MS analysis revealed the molecular formula of **6** to be $C_{59}H_{90}O_{27}$. Alkaline hydrolysis of **6** with 5% aqueous potassium hydroxide liberated desacyl-theasaponin E (**8**) together with acetic acid and angelic acid, which were identified by GLC analysis.

Methanolysis of **8** with 9% hydrogen chloride in dry methanol liberated methyl glycosides of glucuronic acid, galactose, arabinose, and xylose in a 1 : 1 : 1 : 1 ratio.[6)] Methylation of **8** with diazomethane-etherate in methanol furnished the methyl ester (**8a**), which was treated with sodium borohydride ($NaBH_4$) to give **8b**. Methanolysis of **8b** liberated theasapogenol A (**1**) together with methyl glucosides, methyl galactosides, methyl arabinosides, and methyl xylosides, which were subjected to acid hydrolysis with 5% aqueous hydrochloric acid to afford D-glucose ($[\alpha]_D$ +43.5°), D-galactose ($[\alpha]_D$ +57.1°), L-arabinose ($[\alpha]_D$ +100.9°), and D-xylose ($[\alpha]_D$ +20.5°). The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **8**, which were assigned with the aid of various NMR experiments,[7)] showed the presence of a β-D-glucuronic acid moiety [δ 4.83 (d, *J*=7.7 Hz, 1′-H)], a β-D-galactopyranosyl moiety [δ 5.67 (d, *J*=7.3 Hz, 1″-H)], an α-L-arabinopyranosyl moiety [δ 5.63 (d, *J*=6.4 Hz, 1‴-H)], and a β-D-xylopyranosyl moiety [δ 5.00 (d, *J*=7.3 Hz, 1⁗-H)] together with a theasapogenol E moiety [δ 3.62, 3.92 (both d, *J*=10.5 Hz, 28-H_2), 4.52 (d, *J*=9.6 Hz, 22-H), 4.68 (d, *J*=9.6 Hz, 21-H), 9.83 (s, 23-H)]. The tetrasaccharide structure bonding to the 3-position of **8** was determined by a HMBC experiment. Namely, long-range correlations were observed between the 1⁗-proton of the D-xylose moiety and the 2‴-carbon of the L-arabinose moiety, between the 1‴-proton of the L-arabinose and the 3′-carbon of the D-glucuronic acid moiety, between the 1″-proton of the D-galactose and the 2′-carbon of the D-glucuronic acid moiety, and between the 1′-proton of the D-glucuronic acid moiety and the 3-carbon of the theasapogenol E moiety. The structure of desacyl-theasaponin E was thus characterized to be theasapogenol E 3-*O*-[β-D-galactopyranosyl(1→2)][β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**8**).

However, the oligosaccharide structure of "theasaponin"

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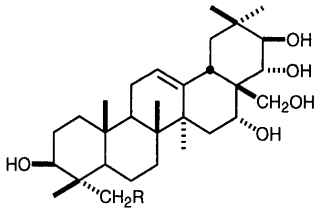
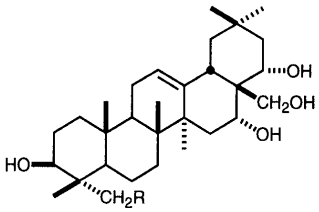
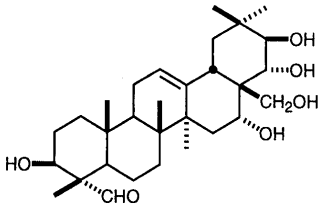


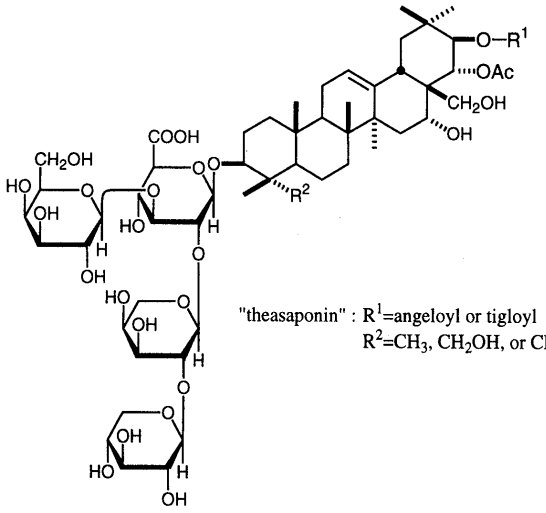
theasapogenol A (**1**) : R=OH
theasapogenol B (**2**) : R=H



theasapogenol C (**3**) : R=OH
theasapogenol D (**4**) : R=H

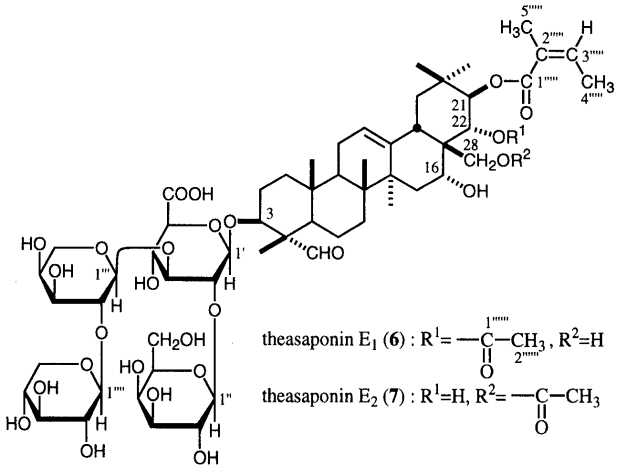


theasapogenol E (**5**)



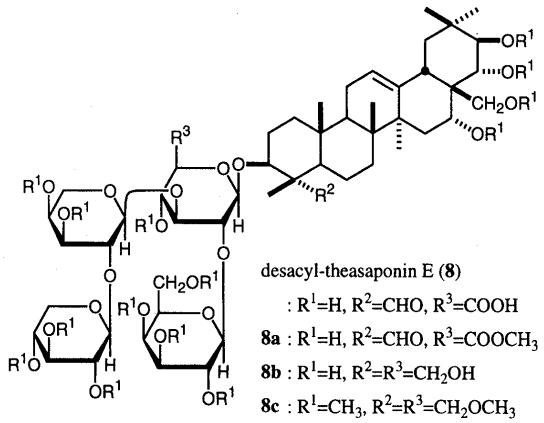
"theasaponin" : R^1=angeloyl or tigloyl
R^2=CH_3, CH_2OH, or CHO

Chart 1

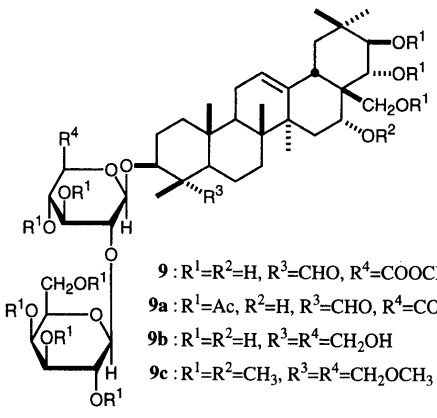


theasaponin E_1 (**6**) : R^1= $-\overset{\overset{1''''''}{}}{\underset{\underset{O}{\|}}{C}}-\underset{2''''''}{CH_3}$, R^2=H

theasaponin E_2 (**7**) : R^1=H, R^2= $-\underset{\underset{O}{\|}}{C}-CH_3$



desacyl-theasaponin E (**8**)
: R^1=H, R^2=CHO, R^3=COOH
8a : R^1=H, R^2=CHO, R^3=$COOCH_3$
8b : R^1=H, R^2=R^3=CH_2OH
8c : R^1=CH_3, R^2=R^3=CH_2OCH_3



9 : R^1=R^2=H, R^3=CHO, R^4=$COOCH_3$
9a : R^1=Ac, R^2=H, R^3=CHO, R^4=$COOCH_3$
9b : R^1=R^2=H, R^3=R^4=CH_2OH
9c : R^1=R^2=CH_3, R^3=R^4=CH_2OCH_3

Chart 2

was previously reported to be 3-*O*-[β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→2)][β-D-galactopyranosyl (1→3)]-β-D-glucopyranosiduronic acid,[4] so we carried out detailed chemical examination regarding the oligosaccharide structure of **8**.

Complete methylation of **8b** by Hakomori's method[8] furnished the hexadeca-*O*-methyl derivative (**8c**), which liberated methyl 4,6-di-*O*-methyl-D-glucopyranosides, methyl 2,3,4,6-tetra-*O*-methyl-D-galactopyranosides, methyl 3,4-di-*O*-methyl-L-arabinopyranosides, and methyl 2,3,4-tri-*O*-methyl-D-xylopyranosides by the methanolysis. Partial methanolysis of **8** with 2% hydrogen chloride in dry methanol at room temperature furnished the prosapogenol (**9**), which gave the nona-*O*-acetate (**9a**). The ^{1}H-NMR ($CDCl_3$) spectrum of **9a** showed signals due to a methyl(3,4,6-tri-*O*-acetyl-β-D-glucopyranosid)uronate moiety [δ 4.41 (d, *J*=7.3 Hz, 1′-H), 3.73 (dd, *J*=7.3, 8.8 Hz, 2′-H), 5.14 (dd, *J*=8.8, 9.2 Hz, 3′-H), 5.08 (dd, *J*=8.8, 9.4 Hz, 4′-H), 3.98 (d, *J*=9.4 Hz, 5′-H)] and a 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl moiety [δ 4.58 (d, *J*=8.1 Hz, 1″-H), 5.10 (dd, *J*=8.1, 10.3 Hz, 2″-H), 4.93 (dd, *J*=3.4, 10.3 Hz, 3″-H), 5.34 (br d, *J*=3.4 Hz, 4″-H), 3.87 (t-like, 5″-H), 4.15 (2H, m, 6″-H_2)] together with the 21,22,28-tri-*O*-acetyltheasapogenol E moiety. Treatment of **9** with $NaBH_4$ provided the reduction product (**9b**). Complete methylation of **9b** afforded the dodeca-*O*-methyl derivative (**9c**), which liberated methyl 3,4,6-tri-*O*-methyl-D-glucopyranosides and methyl 2,3,4,6-tetra-*O*-methyl-D-galactopyranosides by the methanolysis. On the basis of the above evidence and comparison of the ^{13}C-NMR data for **9** and **9b** with those for **5**, **8**, and **8b** (Table 2), the structure of prosapogenol (**9**) was determined to be theasapogenol E 3-*O*-β-D-galactopyranosyl(1→2)-methyl(β-D-glucopyranosid)uronate. Consequently, the oligosaccharide structure of desacyltheasaponin E (**8**) was confirmed to be [β-D-galactopyranosyl(1→2)][β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl (1→3)]-β-D-glucopyranosiduronic acid.

The proton and carbon signals in the ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[7] of theasaponin E_1 (**6**) were similar to those of desacyl-theasaponin E (**8**), except for the signals assignable to the acetyl [δ 1.94 (s, 2‴″-H_3) and 6.16 (d, *J*=10.1 Hz, 22-H)] and angeloyl [δ 2.02 (s, 5‴″-H_3), 2.10 (d, *J*=7.0 Hz, 4‴″-H), 5.99 (dq-like, 3‴″-H), and 6.57 (d, *J*=10.1 Hz, 21-H)]. The positions of the acetyl and angeloyl groups in **6** were determined by HMBC experiment, which showed long-range correlations between the 21-proton of the theasapogenol E moiety and the 1‴″-carbonyl carbon of the angeloyl group and between the 22-proton of the theasapogenol E moiety and the 1‴″-carbonyl carbon of the acetyl group together with the following protons and carbons of the oligoglycoside moiety : 1‴″-H and 2‴-C; 1‴-H and 3′-C; 1″-H and 2′-C; 1′-H and 3-C. Furthermore, comparison of the ^{13}C-NMR data for **6** with those for **8** revealed acetylation shifts around the 21- and 22-positions of the theasapogenol E moiety of **6**. These findings led us to characterize the structure of theasaponin E_1 to be 21-*O*-angeloyl-22-*O*-acetyltheasapogenol E 3-*O*-[β-D-galactopyranosyl(1→2)][β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**6**).

Theasaponin E_2 (**7**), also obtained as colorless fine crystals of mp 240—242 °C, liberated desacyl-theasaponin E (**8**) together with acetic acid and angelic acid. The molecular formula $C_{59}H_{90}O_{27}$, which was the same as that of theasaponin E_1 (**6**), was determined from the liquid SI-MS [*m/z* 1253 $(M+Na)^+$, 1269 $(M+K)^+$] and by high-resolution MS measurement. The ^{1}H-NMR and ^{13}C-NMR (Table 2) spectra[7] of **7** showed signals due to the 21,28-acylated structure of **8**. In the HMBC experiment of **8**, long-range correlations were ob-

Table 1. ^{13}C-NMR Data for Theasaponins E_1 (**6**) and E_2 (**7**) and Desacyltheasaponin E (**8**) (Pyridine-d_5)

	6	**7**	**8**
C-1	38.3	38.3	38.3
C-2	25.2	25.3	25.3
C-3	84.5	84.5	84.5
C-4	55.2	55.3	55.3
C-5	48.4	48.4	48.4
C-6	20.4	20.5	20.5
C-7	32.5	32.6	32.6
C-8	40.4	40.4	40.4
C-9	46.8	46.9	46.9
C-10	36.1	36.2	36.2
C-11	23.8	23.8	23.7
C-12	123.1	123.2	123.0
C-13	142.9	142.9	143.2
C-14	41.8	42.0	42.0
C-15	34.6	34.8	34.4
C-16	68.1	67.7	68.0
C-17	48.0	47.2	48.2
C-18	40.2	40.5	40.7
C-19	47.2	47.3	48.2
C-20	36.3	36.5	36.6
C-21	78.9	81.5	78.0
C-22	74.5	71.4	75.5
C-23	209.8	209.8	209.7
C-24	11.0	11.1	11.1
C-25	15.8	15.8	15.8
C-26	16.9	17.0	17.0
C-27	27.4	27.2	27.2
C-28	64.0	66.5	66.5
C-29	29.5	29.5	30.2
C-30	20.3	20.3	19.5
GlcA-1′	104.1	104.2	104.2
2′	78.4	78.3	78.3
3′	84.2	84.4	84.4
4′	70.8	70.9	70.8
5′	77.3	77.3	77.3
6′	171.8	171.8	171.8
Gal-1″	103.2	103.3	103.2
2″	73.7	73.7	73.7
3″	75.3	75.3	75.3
4″	70.5	70.4	70.5
5″	76.5	76.5	76.5
6″	62.1	62.3	62.3
Ara-1‴	101.7	101.7	101.7
2‴	82.3	82.3	82.4
3‴	73.4	73.5	73.4
4‴	68.4	68.4	68.4
5‴	66.1	66.2	66.1
Xyl-1⁗	107.0	107.0	107.1
2⁗	75.9	75.9	75.9
3⁗	78.2	78.1	78.2
4⁗	70.8	70.9	70.9
5⁗	67.5	67.5	67.5
Ang-1⁗′	167.9	167.9	
2⁗′	129.0	129.0	
3⁗′	137.0	137.0	
4⁗′	15.9	15.9	
5⁗′	21.0	21.0	
Ac-1⁗″	171.1	170.5	
2⁗″	20.9	20.6	

Table 2. ^{13}C-NMR Data for **5**, **8**, **8b**, **9**, and **9b** (Pyridine-d_5+D_2O)

		5	8	8b	9	9b
Sapogenol moiety	C-3	71.6	83.9[a]	83.0[a]	83.2	83.2
	12	122.3	122.4	122.7	123.0	122.7
	13	143.7	143.7	143.6	143.8	143.6
	16	68.2	68.1[b]	68.2	68.1	67.9
	21	78.5	78.5	78.4	78.5	78.4
	22	77.2	77.0[c]	77.2[b]	77.2[a]	77.3[a]
	23	207.0	209.4	65.0	209.1	64.4
	28	67.7	67.5	67.6	67.6	67.5
3-*O*-β-Glucuronopyranosyl or Glucopyranosyl moiety	C-1′		103.7	103.3	103.0	103.3
	2′		78.0	78.1	81.9	82.1
	3′		84.1[a]	85.5[a]	76.5	76.4
	4′		70.5[d]	69.7	74.1[b]	73.8[b]
	5′		77.8[c]	77.7[b]	77.1[a]	77.9[a]
	6′		171.5	61.5	170.0	61.2
2′-*O*-β-Galactopyranosyl moiety	C-1″		106.6	106.7	105.9	105.9
	2″		73.1[e]	73.3	72.3[b]	71.0[b]
	3″		75.0[d]	74.7	74.7	74.5
	4″		70.2	69.0	69.9	69.3
	5″		76.9	76.9[b]	76.8	76.9
	6″		61.8	62.2	62.0	62.3
3′-*O*-α-Arabinopyranosyl moiety	C-1‴		101.4	101.4		
	2‴		82.0	82.3		
	3‴		70.7	70.4		
	4‴		68.1[b]	67.6[c]		
	5‴		65.9	66.2		
2‴-*O*-β-Xylopyranosyl moiety	C-1⁗		102.9	102.4		
	2⁗		73.4[e]	73.3		
	3⁗		75.5	75.4		
	4⁗		68.0[b]	67.8[c]		
	5⁗		67.1	67.0		
OMe					51.9	

a—e): Assignments may be interchangeable within the same column.

served between the 21-proton and the 1⁗-carbonyl carbon of the angeloyl group and the between the 28-protons and the 1⁗′-carbonyl carbon of the acetyl group. Finally, comparison of the ^{13}C-NMR data for **7** with those for **6** and **8** led us to confirm the structure of theasaponin E_2 as 21-*O*-angeloyl-28-*O*-acetyltheasapogenol E 3-*O*-[β-D-galactopyranosyl(1→2)]-[β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**7**).

On application of 0.5 mM solutions of **6** and **7**, **7** was found to suppress the sweet taste of 0.1 M sucrose, while **6** showed no activity. These results suggested that the position of acyl group related to the antisweet activity of saponin.

Experimental

The instruments used for obtaining physical data and experimental conditions for chromatography were the same as described in our previous paper.[1]

Isolation of Theasaponins E_1 (6) and E_2 (7) from the Seeds of Tea Plant The fresh seeds of tea plants (2 kg, cultivated in the medicinal plant garden of Kyoto Pharmaceutical University, Kyoto Prefecture, Japan) were crushed and extracted with MeOH under reflux three times. After removal of the solvent from the MeOH solution under reduced pressure, the extract was subjected to ODS column chromatography [Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd.), H_2O–MeOH] to give the MeOH eluted fraction (73 g). The MeOH eluted fraction was separated by silica gel column chromatography [BW-200 (Fuji Silysia Chemical Ltd.), $CHCl_3$–MeOH–H_2O] to furnish the saponin mixture (31 g). HPLC [YMC-Pack R&D ODS-5 (20×250 mm, i.d.), MeOH-1% aqueous AcOH (7 : 3, v/v)] of the saponin mixture (500 mg) yielded **6** (150 mg) and **7** (20 mg).

Theasaponin E_1 (**6**): Colorless fine crystals from $CHCl_3$–MeOH–H_2O, mp 246—248 °C, $[\alpha]_D^{25}$ +8.9° (c=1.1, MeOH), High-resolution SI-MS: Calcd for $C_{59}H_{90}NaO_{27}$ $(M+Na)^+$: 1253.5562. Found: 1253.5564. Calcd for $C_{59}H_{90}KO_{27}$ $(M+K)^+$: 1269.5300. Found: 1269.5304. IR (KBr): 3433, 2923, 1724, 1616, 1361, 1261, 1040 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ: 0.83, 0.84, 1.08, 1.31, 1.46, 1.78 (3H each, all s, 25, 26, 29, 30, 24, 27-H_3), 1.94 (3H, s, 2⁗′-H_3), 2.02 (3H, s, 5⁗′-H_3), 2.10 (3H, d, J=7.0 Hz, 4⁗′-H_3), 4.84 (1H, d, J=7.3 Hz, 1′-H), 5.00 (1H, d, J=7.6 Hz, 1⁗-H), 5.39 (1H, br s, 12-H), 5.70 (1H, d, J=6.5 Hz, 1‴-H), 5.72 (1H, d, J=7.6 Hz, 1″-H), 5.99 (1H, dq-like, 3⁗′-H), 6.16 (1H, d, J=10.1 Hz, 22-H), 6.57 (1H, d, J=10.1 Hz, 21-H), 9.89 (1H, s, 23-H). ^{13}C-NMR (pyridine-d_5) δc: given in Table 1. Liquid SI-MS: m/z 1253 $(M+Na)^+$, 1269 $(M+K)^+$.

Theasaponin E_2 (**7**): Colorless fine crystals from $CHCl_3$–MeOH–H_2O, mp 240—242 °C, $[\alpha]_D^{25}$ +22.2° (c=1.2, MeOH), High-resolution SI-MS: Calcd for $C_{59}H_{90}NaO_{27}$ $(M+Na)^+$: 1253.5562. Found: 1253.5562. Calcd for $C_{59}H_{90}KO_{27}$ $(M+K)^+$: 1269.5300. Found: 1269.5300. IR (KBr): 3420, 2920, 1716, 1614, 1384 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ: 0.86, 0.91, 1.07, 1.28, 1.44, 1.69 (3H each, all s, 25, 26, 29, 30, 24, 27-H_3), 2.01 (3H, s, 5⁗′-H_3), 2.07 (3H, d, J=6.8 Hz, 4⁗′-H_3), 2.11 (3H, s, 2⁗′-H_3), 4.75 (1H, d, J=6.8 Hz, 1′-H), 5.06 (1H, d, J=6.8 Hz, 1⁗-H), 5.46 (1H, br s, 12-H), 5.53 (1H, d, J=7.3 Hz, 1″-H), 5.61 (1H, d, J=5.6 Hz, 1‴-H), 6.03 (1H, dq-like, 3⁗′-H), 6.28 (1H, d, J=8.8 Hz, 21-H), 9.75 (1H, s, 23-H). ^{13}C-NMR (pyridine-d_5) δc: given in Table 1. Liquid SI-MS: m/z 1253 $(M+Na)^+$, 1269 $(M+K)^+$.

Alkaline Hydrolysis of Theasaponins E_1 (6) and E_2 (7) A solution of **6** (30 mg) in 5% aqueous KOH (5 ml) was stirred at room temperature for 3 h. The reaction solution was made acidic with 5% HCl and extracted with ether. The ether extract was subjected to GLC analysis to identify acetic acid and angelic acid. GLC conditions: condition-1) column: 15% free fatty acid polyester (FFAP) on Chromosorb GAW DMCS (100—120 mesh), 3 mm×2 m glass column; column temp.: 200 °C; N_2 flow rate: 35 ml/min; t_R (min): acetic acid 5.96, angelic acid 17.27. The aqueous layer was extracted with *n*-BuOH and the *n*-BuOH extract was washed with aqueous saturated $NaHCO_3$ and H_2O. After removal of the solvent from the extract under reduced pressure, the residue was purified by silica gel column chromatography ($CHCl_3$–MeOH–H_2O) to give desacyl-theasaponin E (**8**, 20 mg).

A solution of **7** (5 mg) in 5% aqueous KOH (0.5 ml) was stirred at room temperature for 3 h. By work-up of the reaction mixture as described above, acetic acid and angelic acid were identified by GLC analysis from the ether extract, whereas **8** (3 mg) was obtained from the aqueous layer by the above

procedure.

Desacyl-theasaponin E (**8**): Colorless fine crystals from $CHCl_3$–MeOH–H_2O, mp 231—233 °C, $[\alpha]_D^{25}$ +13.7° (c=1.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{52}H_{82}NaO_{25}$ $(M+Na)^+$: 1129.5041. Found: 1129.5025. ^{1}H-NMR (pyridine-d_5) δ: 0.83, 0.86, 1.28, 1.32, 1.43, 1.76 (3H each, all s, *tert*-CH_3×6), 3.62, 3.92 (1H each, both d, J=10.5 Hz, 28-H_2), 4.52 (1H, d, J=9.6 Hz, 22-H), 4.68 (1H, d, J=9.6 Hz, 21-H), 4.83 (1H, d, J=7.7 Hz, 1′-H), 4.91 (1H, br s, 16-H), 5.00 (1H, d, J=7.3 Hz, 1‴-H), 5.38 (1H, br s, 12-H), 5.63 (1H, d, J=6.4 Hz, 1‴-H), 5.67 (1H, d, J=7.3 Hz, 1″-H), 9.83 (1H, s, 23-H). ^{13}C-NMR (pyridine-d_5) δc: given in Table 1. Positive-ion FAB-MS: m/z 1129 $(M+Na)^+$.

Methanolysis of Desacyl-theasaponin E (8) A solution of **8** (10 mg) in 9% HCl–dry MeOH (1.5 ml) was heated under reflux for 3 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and the insoluble portion was removed by filtration. After evaporation of the solvent from a part of the filtrate under reduced pressure, the residue was dissolved in pyridine (0.01 ml) and this solution was treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 0.02 ml) for 1 h. The reaction solution was subjected to GLC analysis to identify the trimethylsilyl (TMS) derivatives of methyl glucuronides, methyl galactosides, methyl arabinosides, and methyl xylosides. GLC conditions: condition-2) column: 3% silicone SE-30 on Chromosorb WAW DMCS (80—100 mesh), 3 mm×1 m glass column; column temp. 140 °C; N_2 flow rate 35 ml/min; t_R (min): TMS-methyl glucuronides 16.9, 41.1 (main peak), TMS-methyl galactosides 25.8, 29.6 (main peak), 34.5, TMS-methyl arabinosides 5.9, 7.0 (main peak), 8.7, TMS-methyl xylosides 11.5 (main peak), 12.7; condition-3) column: 5% silicone SE-52 on Uniport HP (80—100 mesh), 3 mm×1 m glass column; column temp. 170 °C; N_2 flow rate 35 ml/min; t_R (min): TMS-methyl glucuronides 11.6, 22.8 (main peak), TMS-methyl galactosides 13.3, 15.4 (main peak), 17.7, TMS-methyl arabinosides 4.7 (main peak), 5.6, TMS-methyl xylosides 7.3 (main peak), 7.9.

Diazomethane Methylation of Desacyl-theasaponin E (8) A solution of **8** (100 mg) in MeOH (20 ml) was treated with ethereal diazomethane until the yellow color persisted. The solution was left standing for 1 h, then the solvent was removed under reduced pressure to furnish **8a** (100 mg).

Desacyl-theasaponin E Methyl Ester (**8a**): Colorless fine crystals from MeOH, mp 237—238 °C, $[\alpha]_D^{25}$ +19.2° (c=1.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{53}H_{84}NaO_{25}$ $(M+Na)^+$: 1143.5199. Found: 1143.5150. IR (KBr): 3330, 2918, 1714, 1619, 1070 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ: 0.84, 0.86, 1.29, 1.33, 1.42, 1.76 (3H each, all s, *tert*.-CH_3×6), 3.75 (3H, s, $COOCH_3$), 4.50 (1H, d, J=8.8 Hz, 22-H), 4.70 (1H, d, J=8.8 Hz, 21-H), 4.82 (1H, d, J=7.3 Hz, 1′-H), 4.92 (1H, br s, 16-H), 5.01 (1H, d, J=7.3 Hz, 1‴-H), 5.38 (1H, br s, 12-H), 5.60 (1H, d, J=6.4 Hz, 1‴-H), 5.66 (1H, d, J=7.7 Hz, 1″-H), 9.83 (1H, s, 23-H). ^{13}C-NMR (pyridine-d_5) δc: given in Table 1. Positive-ion FAB-MS: m/z 1143 $(M+Na)^+$.

Reduction of Desacyl-theasaponin E Methyl Ester (8a) with $NaBH_4$ A solution of **8a** (120 mg) in MeOH (20 ml) was treated with $NaBH_4$ (180 mg) and the mixture was stirred at room temperature for 4 h. Excess $NaBH_4$ was quenched with acetone, then the solution was neutralized with Dowex 50 W×8 (H^+ form) and filtered. Removal of the solvent from the filtrate yielded **8b** (110 mg).

8b: Colorless fine crystals from MeOH, mp 232—233 °C, $[\alpha]_D^{25}$ +21.1° (c=0.9, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{52}H_{88}LiO_{24}$ $(M+Li)^+$: 1103.5826; Found: 1103.5900. IR (KBr) 3330, 2951, 1592, 1373 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ: 0.93, 0.94, 1.05, 1.27, 1.30, 1.78 (3H each, all s, *tert*.-CH_3×6), 4.53 (1H, d, J=9.4 Hz, 22-H), 4.68 (1H, d, J=9.4 Hz, 21-H), 4.85 (1H, br s, 16-H), 4.96 (1H, d, J=7.7 Hz, 1′-H), 5.02 (1H, d, J=7.6 Hz, 1‴-H), 5.41 (1H, br s, 12-H), 5.56 (1H, d, J=6.7 Hz, 1‴-H), 5.84 (1H, d, J=7.9 Hz, 1″-H). ^{13}C-NMR (pyridine-d_5+D_2O): given in Table 2. Positive-ion FAB-MS: m/z 1119 $(M+Na)^+$; (matrix+LiCl): m/z 1103 $(M+Li)^+$.

Methanolysis of 8b Followed by Acid Hydrolysis A solution of **8b** (200 mg) in 9% HCl-dry MeOH (5 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and then filtered. Removal of the solvent under reduced pressure provided a residue, which was separated by silica gel column chromatography ($CHCl_3$–MeOH) to give **1** (35 mg), methyl glucoside (8.4 mg), methyl arabinoside (10.0 mg), methyl galactoside (9.1 mg), and methyl xyloside (8.4 mg). Theasapogenol A (**1**), thus obtained, was identified with an authentic sample by TLC, IR (KBr), and ^{1}H-NMR spectra comparisons and methyl glycosides were also identified with authentic samples by TLC. Each methyl glycoside was dissolved in 5% HCl (1 ml) and the whole mixture was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and then filtered. Evaporation of the solvent from the filtrate under reduced pressure gave D-glucose {$[\alpha]_D^{26}$ +43.5° (c=0.10, H_2O)}, D-galactose {$[\alpha]_D^{26}$ +57.1° (c=0.10, H_2O)}, L-arabinose {$[\alpha]_D^{26}$ +100.9° (c=0.10, H_2O)}, and D-xylose {$[\alpha]_D^{26}$ +20.5° (c=0.10, H_2O)}.

Complete Methylation of 8b A solution of **8b** (30 mg) in dimethyl sulfoxide (DMSO, 1 ml) was treated with a dimsyl carbanion solution (3 ml, prepared with NaH and DMSO). The mixture was stirred in the dark at room temperature (15 °C) for 2 h, then treated with CH_3I (6 ml), and the whole was stirred for a further 12 h. The reaction mixture was poured into ice-water and the whole was extracted with $CHCl_3$. The $CHCl_3$ extract was washed with saturated saline and then dried over $MgSO_4$ and filtered. Evaporation of the filtrate under reduced pressure afforded a residue, which was purified by silica gel column chromatography (benzene–acetone) to give **8c**.

8c: Colorless needles from MeOH, mp 98—99 °C, $[\alpha]_D^{25}$ −2.6° (c=0.6, $CHCl_3$). High-resolution positive-ion FAB-MS: Calcd for $C_{68}H_{118}LiO_{24}$ $(M+Li)^+$: 1325.8173. Found: 1325.8110. IR ($CHCl_3$): 2937, 1597, 1373, 1045 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.71, 0.87, 0.91, 0.95, 1.31 (3H each, all s, *tert*.-CH_3×6), 3.25, 3.27, 3.31, 3.37, 3.38, 3.42, 3.51, 3.52, 3.55, 3.57 (3H each), 3.45, 3.50, 3.58 (6H each) (all s, OCH_3×16), 4.20 (1H, dd-like, 16-H), 4.26 (1H, d, J=7.6 Hz), 4.58 (1H, d, J=7.0 Hz), 4.84 (1H, d, J=7.3 Hz), 5.03 (1H, d, J=5.5 Hz) (1′, 1″, 1‴, 1⁗-H), 5.27 (1H, br s, 12-H). Positive-ion FAB-MS: m/z 1325 $(M+Li)^+$.

Methanolysis of 8c A solution of **8c** (3 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 1 h, then neutralized with Ag_2CO_3 and filtered. Work-up of the filtrate yielded a residue, which was subjected to GLC analysis. GLC conditions: condition-4) column: 15% polyneopentyl glycol succinate (NPGS) on Chromosorb WAW (80—100 mesh), 3 mm×2 m glass column; column temp. 170 °C; N_2 flow rate 35 ml/min; t_R (min): methyl 4,6-di-*O*-methyl-D-glucopyranoside (**a**) 18.5, 21.5, methyl 2,3,4,6-tetra-*O*-methyl-D-galactopyranoside (**b**) 18.5, 21.5, methyl 3,4-di-*O*-methyl-L-arabinopyranoside (**c**) 21.5, methyl 2,3,4-tri-*O*-methyl-D-xylopyranoside (**d**) 12.5, 16.7, condition-5) column: 15% polyethylene glycol succinate (PEGS) on Chromosorb WAW (80—100 mesh), 3 mm×2 m glass column, column temp. 170 °C, N_2 flow rate 35 ml/min, t_R (min): **b** 39.9, 42.5, **c** 24.0, 32.8, **d**, 10.9, 13.8. condition-6) column: 15% diethylene glycol succinate (DEGS) on Uniport B (80—100 mesh), 3 mm×2 m glass column; column temp. 170 °C; N_2 flow rate 35 ml/min; t_R (min): **a** 56.6, **b** 31.4, 33.3, **c** 25.6, **d** 8.6, 10.5.

Partial Methanolysis of 8 A solution of **8** (100 mg) in 2% HCl–dry MeOH (10 ml) was stirred at 40 °C for 9 h. The reaction solution was neutralized with Amberlite IRA-400 (OH^- form) and then filtered. After removal of the solvent from the filtrate under reduced pressure, the residue was separated by silica gel column chromatography ($CHCl_3$–MeOH–H_2O) to give **9** (28 mg).

9: Colorless fine crystals from $CHCl_3$–MeOH–H_2O, mp 222—223 °C, $[\alpha]_D^{25}$ +13.4° (c=1.0, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{43}H_{68}NaO_{17}$ $(M+Na)^+$: 879.4347. Found: 879.4379. IR (KBr): 3419, 2954, 1729, 1635, 1080 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ: 0.86, 0.87, 1.29, 1.33, 1.40, 1.77 (3H each, all s, *tert*.-CH_3×6), 3.73 (3H, s, $COOCH_3$), 4.53 (1H, d, J=10.0 Hz, 22-H), 4.69 (1H, d, J=10.0 Hz, 21-H), 4.86 (1H, d, J=9.7 Hz, 1′-H), 5.18 (1H, d, J=7.7 Hz, 1″-H), 5.38 (1H, br s, 12-H), 9.87 (1H, s, 23-H). ^{13}C-NMR (pyridine-d_5+D_2O): given in Table 2. Positive-ion FAB-MS: m/z 879 $(M+Na)^+$.

Methanolysis of 9 A solution of **9** (3 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 3 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3. Following removal of the unsoluble part by filtration, the filtrate was dissolved in pyridine (0.1 ml) and the solution was treated with BSTFA (0.1 ml) for 1 h. The reaction solution was subjected to GLC analysis [condition-2) t_R (min) ; TMS-methyl glucuronide 16.9, 41.1 (main peak), TMS-methyl galactoside 25.8, 29.6 (main peak), 34.5, condition-3) t_R (min): TMS-methyl glucuronide 11.6, 22.8 (main peak), TMS-methyl galactoside 13.3, 15.4 (main peak), 17.7.

Acetylation of 9 A solution of **9** (100 mg) in Ac_2O (2 ml) and pyridine (2 ml) was stirred at 40 °C for 5 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract furnished a residue which was purified by silica gel column chromatography (benzene-acetone) to give **9a** (35 mg).

9a: A white powder, $[\alpha]_D^{25}$ −0.5° (c=2.5, $CHCl_3$). High-resolution positive-ion FAB-MS: Calcd for $C_{61}H_{86}LiO_{26}$ $(M+Li)^+$: 1241.5567. Found: 1241.5550. IR ($CHCl_3$): 3681, 3491, 2956, 1739, 1600, 1368, 1235 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88, 0.89, 0.98, 1.05, 1.28, 1.43 (3H each, all s, *tert*.-CH_3×6), 1.96, 1.97, 2.00, 2.01, 2.02, 2.06, 2.07, 2.08, 2.09 (3H each, all s, OAc×9), 3.65 (2H, br s, 28-H_2), 3.73 (1H, dd, J=7.3, 8.8 Hz, 2′-H), 3.73 (3H, s, $COOCH_3$), 3.80 (1H, m, 3-H), 3.87 (1H, t-like, 5″-H), 3.98 (1H, d, J=9.4 Hz, 5′-H), 4.15 (2H, d-like, 6″-H_2), 4.16 (1H, m, 16-H), 4.41 (1H, d,

J=7.3 Hz, 1′-H), 4.58 (1H, d, J=8.1 Hz, 1″-H), 4.93 (1H, dd, J=3.4, 10.3 Hz, 3″-H), 5.08 (1H, dd, J=8.8, 9.4 Hz, 4′-H), 5.10 (1H, dd, J=8.1, 10.3 Hz, 2″-H), 5.14 (1H, dd, J=8.8, 9.2 Hz, 3′-H), 5.34 (1H, d, J=3.4 Hz, 4″-H), 5.36 (1H, br s, 12-H), 5.40 (1H, d, J=10.3 Hz, 22-H), 5.53 (1H, d, J=10.3 Hz, 21-H), 9.64 (1H, s, 23-H). Positive-ion FAB-MS (matrix+Li): m/z 1241 $(M+Li)^+$.

Reduction of 9 with $NaBH_4$ A solution of **9** (25 mg) in MeOH (1 ml) was treated with $NaBH_4$ (22 mg) and the reaction mixture was stirred at room temperature for 2 h. The mixture was neutralized with Dowex 50 W×8 (H^+ form) and then filtered. Work-up of the filtrate gave a residue, which was purified by silica gel column chromatography ($CHCl_3$–MeOH–H_2O) to furnish **9b** (12 mg).

9b: Colorless fine crystals from $CHCl_3$–MeOH–H_2O, mp 218—220 °C, $[\alpha]_D^{25}$ +15.1° (c=1.2, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{42}H_{70}NaO_{16}$ $(M+Na)^+$: 853.4561. Found: 853.4555. IR (KBr): 3433, 2940, 1635, 1385, 1075 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ: 0.93, 0.94, 1.04, 1.23, 1.30, 1.75 (3H each, all s, *tert*-CH_3×6), 4.51 (1H, d, J=9.8 Hz, 22-H), 4.65 (1H, d, J=9.8 Hz, 21-H), 4.87 (1H, br s, 16-H), 5.01 (1H, d, J=7.3 Hz, 1′-H), 5.24 (1H, d, J=7.7 Hz, 1″-H), 5.41 (1H, br s, 12-H). ^{13}C-NMR (pyridine-d_5+D_2O): given in Table 2. Positive-ion FAB-MS: m/z 853 $(M+Na)^+$.

Complete Methylation of 9b A solution of **9b** (15 mg) in DMSO (1 ml) was treated with a dimsyl carbanion solution (3 ml). The mixture was stirred in the dark at room temperature for 1.5 h, then treated with CH_3I (6 ml) and the whole was stirred for a further 12 h. The reaction mixture was poured into ice-water and the whole was extracted with $CHCl_3$. Work-up of the $CHCl_3$ extract furnished a residue, which was purified by silica gel column chromatography (benzene-acetone) to give **9c** (13 mg).

9c: Colorless needles from MeOH, mp 87—88 °C, $[\alpha]_D^{25}$ +5.6° (c=1.3, $CHCl_3$). High-resolution positive-ion FAB-MS: Calcd for $C_{54}H_{94}LiO_{16}$ $(M+Li)^+$: 1005.6702. Found: 1005.6720. IR ($CHCl_3$): 2937, 1600, 1090 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.71, 0.87, 0.98, 1.31 (3H each), 0.91 (6H) (all s, *tert*.-CH_3×6), 3.25, 3.27, 3.33, 3.35, 3.38, 3.50, 3.55, 3.60, 3.65 (3H each), 3.52, 3.53 (6H each) (all s, OCH_3×12), 4.24 (1H, d, J=7.9 Hz), 4.61 (1H, d, J=7.6 Hz) (1′, 1″-H), 5.27 (1H, br s, 12-H). Positive-ion FAB-MS: m/z 1021 $(M+Na)^+$; (matrix+LiCl): m/z 1005 $(M+Li)^+$.

Methanolysis of 9c A solution of **9c** (3 mg) in 9% HCl–dry MeOH (0.8 ml) was heated under reflux for 1 h. The reaction mixture was neutralized by Ag_2CO_3 and then filtered. The filtrate was subjected to GLC analysis [condition-4) t_R (min): methyl 3,4,6-tri-*O*-methyl-D-glucopyranoside (**e**) 13.5, 15.3, methyl 2,3,4,6-tetra-*O*-methyl-D-galactopyranoside (**b**) 9.0; condition-5) t_R (min): **e** 14.9, 17.6, **b** 7.5, 9.5; condition-6) t_R (min): **e** 10.7, 12.8, **b** 6.0.

Bioassay of Antisweet Activity Treatment of the antisweet activity of 0.5 mM solutions of **6** and **7** was made to five volunteers. Each participant held the theasaponin solution in the mouth for 3 min and spit the solution. After rinsing out their mouths with distilled water, the taste of a sucrose solution remained.

References and Notes

1) This paper constituted Part LI in the series of "Saponin and Sapogenol". Part L: Kitagawa I., Hori K., Uchida E., Chen W. Z., Yoshikawa M., Ren J., *Chem. Pharm. Bull.*, **41**, 1567—1572 (1993).
2) Present address: *Faculty of Pharmaceutical Sciences, Kinki University, 3–4–1, Kowakae, Higashiosaka, Osaka 577–0818, Japan.*
3) *a*) Yosioka I., Nishimura T., Matsuda A., Kitagawa I., *Chem. Pharm. Bull.*, **18**, 1610—1620 (1970); *b*) *Idem, ibid.*, **18**, 1621—1632 (1970); *c*) *Idem, ibid.*, **19**, 1186—1199 (1971).
4) Tschesche R., Weber A., Wulff G., *Justus Liebigs Ann. Chem.*, **721**, 209—219 (1969).
5) Yoshikawa M., Uchida E., Okaichi Y., Kitagawa I., presented at the 109th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April 1989, Abstract of Paper Vol. III, p.181.
6) The proportions of carbohydrates were determined from the peak areas in GLC analysis.
7) The ^{1}H- and ^{13}C-NMR data of **6**, **7**, and **8** were assigned on the basis of homo- and hetero-correlation spectroscopy (^{1}H–^{1}H, ^{1}H–^{13}C COSY), homo- and hetero-nuclear Hartmann-Hahn spectroscopy (^{1}H–^{1}H, ^{1}H–^{13}C HOHAHA), distortionlesss enhancement polarization transfer (DEPT) and heteronuclear multiple bond connectivity (HMBC) experiments.
8) Hakomori S., *J. Biochem.*, **55**, 205—208 (1964).