Bioactive Saponins and Glycosides. XXIII.[1] Triterpene Saponins with Gastroprotective Effect from the Seeds of *Camellia sinensis* —Theasaponins E_3, E_4, E_5, E_6, and E_7—

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The saponin fraction from the seeds of the tea plant [*Camellia sinensis* (L.) O. KUNTZE (Theaceae)] was found to exhibit potent protective effects on ethanol- and indomethacin-induced gastric mucosal lesions in rats. Five new triterpene saponins, theasaponins E_3 (1), E_4 (2), E_5 (3), E_6 (4), and E_7 (5), were isolated together with 11 known saponins from the saponin fraction. The chemical structures of 1—5 were elucidated on the basis of chemical and physicochemical evidence. Among the isolated saponins, theasaponins E_1 (6), E_2 (7), and E_5 (3) and assamsaponin C (10) showed an inhibitory effect on ethanol-induced gastric mucosal lesions at a dose of 5.0 mg/kg, *p.o.* and their activities were stronger than that of omeplazole. With regard to the structure–activity relationships of theasaponins, the following structural requirements for a protective effect on ethanol-induced gastric lesions were suggested; 1) the 21- and/or 22-acyl groups are essential for the activity, 2) acetylation of the 16-hydroxyl group reduce the activity.

Key words *Camellia sinensis*; gastroprotective activity; theasaponin; triterpene saponin; tea plant; structure–activity relationship

The seeds of the tea plant [*Camellia sinensis* (L.) O. KUNTZE (Theaceae)] are known to contain saponin constituents with an insectifuge activity and its crude saponin fraction has been used as a surface-active agent.[2] Previously, we reported the structure elucidation and anti-sweet activity of two acylated polyhydroxyoleane-12-ene oligoglycosides, theasaponins E_1 (**6**) and E_2 (**7**), from the seeds of *C. sinensis* cultivated in Japan.[2] In addition, from the seeds and leaves of *C. sinensis* var. *assamica* PIERRE cultivated in Sri Lanka, we isolated assamsaponins A (**8**), B (**9**), C (**10**), D (**11**), E, F (**12**), G, H, and I (**13**) with gastric emptying activity and an accelerating effect on gastrointestinal transit.[3,4] Recently, we characterized floratheasaponins A (**14**), B, and C with anti-hyperlipidemic activity from the flowers of *C. sinensis*.[1] As a continuing study on the bioactivity of saponin constituents from the tea plant, we found that the saponin fraction of the methanolic extract from the seeds of *C. sinensis* showed protective effects on ethanol- and indomethacin-induced gastric

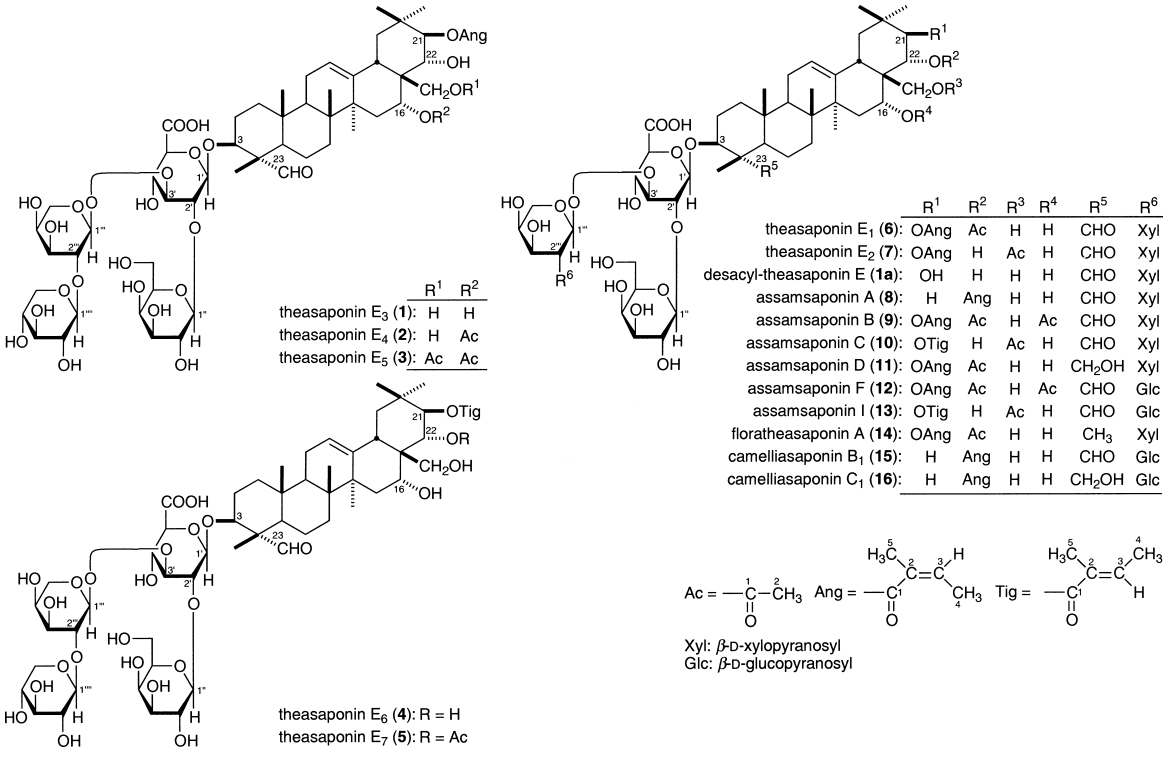

Chart 1

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mucosal lesions in rats. From the saponin fraction, five new acylated triterpene saponins, theasaponins E_3 (**1**), E_4 (**2**), E_5 (**3**), E_6 (**4**), and E_7 (**5**), were isolated together with 11 known saponins (**6**—**16**). This paper deals with the structure elucidation of the five new saponins (**1**—**5**) as well as the gastroprotective effects of the principal saponins (**3**, **6**, **7**, **9**, **10**).

Seeds of the 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 diethylether and the precipitation was subjected to Diaion HP-20 column chromatography ($H_2O \rightarrow MeOH \rightarrow CHCl_3$) to give the saponin fraction (=methanol-eluted fraction, 6.3%). As shown in Table 1, the saponin fraction significantly inhibited ethanol- and indomethacin-induced gastric mucosal lesions in rats (ED_{50}=4.0, 65 mg/kg, *p.o.*, respectively).

The saponin fraction was then subjected to HPLC to give five new saponins, theasaponins E_3 (**1**, 0.036%), E_4 (**2**, 0.008%), E_5 (**3**, 0.050%), E_6 (**4**, 0.013%), and E_7 (**5**, 0.037%), together with 11 known saponins, theasaponins E_1 (**6**, 1.02%)[2] and E_2 (**7**, 1.24%),[2] assamsaponins A (**8**,[3] 0.086%), B (**9**,[3] 0.056%), C (**10**,[3] 0.13%), D (**11**,[3] 0.039%), F (**12**,[4] 0.014%), and I (**13**,[4] 0.022%), floratheasaponin A (**14**,[1] 0.016%), and camelliasaponins B_1 (**15**,[5,6] 0.024%) and C_1 (**16**,[5,6] 0.004%).

Structures of Theasaponins E_3 (1), E_4 (2), E_5 (3), E_6 (4), and E_7 (5) Theasaponin E_3 (**1**) was isolated as colorless fine crystals of mp 214.4—215.5 °C (from $CHCl_3$–MeOH) with positive optical rotation ($[\alpha]_D^{27}$ +17.0° in MeOH). The IR spectrum of **1** showed absorption bands due to hydroxyl, carbonyl, α,β-unsaturated ester, and ether functions at 3453, 1719, 1650, and 1078 cm^{-1}. In the positive- and negative-ion FAB-MS, quasimolecular ion peaks were observed at *m/z* 1211 $(M+Na)^+$ and *m/z* 1187 $(M-H)^-$, respectively, and high-resolution positive-ion FAB-MS revealed the molecular formula of **1** to be $C_{57}H_{88}O_{26}$. Alkaline hydrolysis of **1** with 10% aqueous potassium hydroxide (KOH)–50% aqueous 1,4-dioxane (1 : 1, v/v) provided desacyl-theasaponin E (**1a**)[2] and angelic acid. The angelic acid was identified by HPLC analysis of its *p*-nitrobenzyl derivative.[1,3—6] The ^{1}H- (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra of **1**, which were assigned by various NMR experiments,[7] showed signals assignable to six methyls [δ 0.81, 0.82, 1.12, 1.33, 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 3.65, 3.94 (1H each, both d, J=9.8 Hz, 28-H_2), 4.04 (1H, m, H-3), 4.79 (1H, d, J=10.1 Hz, 22-H), 4.84 (1H, br s, 16-H), 6.48 (1H, d, J=10.1 Hz, 21-H)], an olefin [δ 5.36 (1H, br s, 12-H)], an aldehyde [δ 9.90 (1H, s, 23-H)], and four glycopyranosyl moieties {a β-D-glucuronopyranosyl [δ 4.84 (1H, d, J=7.4 Hz, 1′-H)], a β-D-xylopyranosyl [δ 5.01 (1H, d, J=7.7 Hz, 1‴-H)], a β-D-galactopyranosyl [δ 5.77 (1H, d, J=7.6 Hz, 1″-H)], an α-L-arabinopyranosyl [δ 5.77 (1H, d, J=6.1 Hz, 1⁗-H)]} together with an angeloyl moiety [δ 2.00 (3H, s, 21-*O*-Ang-5-H_3), 2.06 (3H, d, J=7.4 Hz, 21-*O*-Ang-4-H_3), 5.91 (1H, dq-like, 21-*O*-Ang-3-H)]. The position of an angeloyl group in **1** was clarified on the basis of an HMBC experiment. Thus, a long-range correlation was observed between the 21-proton and the carbonyl carbon of the angeloyl part (δ_C 168.7). On the basis of this evidence, the structure of theasaponin E_3 was determined to be 21-*O*-angeloyltheasapogenol E 3-*O*-β-D-galactopyranosyl(1→2)[β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**1**).

Table 1. Inhibitory Effects of the Saponin Fraction from the Seeds of *Camellia sinensis* on Ethanol- or Indomethacin-Induced Gastric Mucosal Lesions in Rats

Treatment	Dose (mg/kg, *p.o.*)	*n*	Gastric lesions	
			Length (mm)	Inhibition (%)
Ethanol-induced				
Control	—	10	122.3±12.9	—
Saponin fraction	1.25	8	106.2±15.4	13.1
	2.5	8	60.4±10.2**	50.6
	5.0	8	50.7±11.4**	58.5
	10	8	44.5±10.5**	63.6
	20	8	10.2±4.2**	91.6
Indomethacin-induced				
Control	—	8	67.2±10.6	—
Saponin fraction	50	7	35.9±3.4**	46.6
	100	7	31.7±6.2**	52.9
	200	7	5.9±2.3**	91.2

Values represent the means±S.E.M. Significantly different from the control group, ** $p<0.01$.

Theasaponin E_4 (**2**) was also obtained as colorless fine crystals from MeOH with mp 223.8—224.3 °C with positive optical rotation ($[\alpha]_D^{27}$ +17.4° in $CHCl_3$–MeOH). The IR spectrum of **2** showed absorption bands at 3453, 1719, 1638, 1078 cm^{-1} ascribable to hydroxyl, carbonyl, α,β-unsaturated ester, and ether functions. The molecular formula, $C_{59}H_{90}O_{27}$, of **2** was determined from the positive- and negative-ion FAB-MS [*m/z* 1253 $(M+Na)^+$ and *m/z* 1229 $(M-H)^-$] and by high-resolution positive-ion FAB-MS. 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—6] The ^{1}H- (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[7] of **2** indicated the presence of the following functions: a theasapogenol E part [δ 0.71, 0.76, 1.14, 1.29, 1.45, 1.47 (3H each, all s, 25, 26, 29, 30, 24, 27-H_3), 3.94 (1H, dd-like, 3-H), 4.28 (2H, m, 28-H_2), 4.77 (1H, d, J=10.4 Hz, 22-H), 5.33 (1H, br s, 12-H), 5.91 (1H, br s, 16-H), 5.97 (1H, d, J=10.4 Hz, 21-H), 9.94 (1H, s, 23-H)], a tetrasaccharide moiety [δ 4.81 (1H, d, J=7.0 Hz, 1′-H), 5.00 (1H, d, J=7.0 Hz, 1⁗-H), 5.75 (1H, d, J=7.6 Hz, 1″-H), 5.79 (1H, d, J=5.5 Hz, 1‴-H)], and an acetyl and an angeloyl moiety [δ 2.53 (3H, s, 16-O-Ac), 1.94 (3H, s, 21-*O*-Ang-5-H_3), 2.02 (3H, d, J=7.3 Hz, 21-*O*-Ang-4-H_3), 5.91 (1H, dq-like, 21-*O*-Ang-3-H)]. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra of **2** were shown to be superimposable on those of **1**, except for the signals due to an acetyl group. Comparison of the ^{13}C-NMR data for **2** with those for **1** revealed an acetylation shift around the 16-position of the theasapogenol E moiety. This evidence was supported by the HMBC experiment of **2**, in which a long-range correlation was observed between the 16-proton and the acetyl carbonyl carbon (δ_C 170.0). Consequently, the structure of theasaponin E_4 was determined to be 16-*O*-acetyl-21-*O*-angeloyltheasapogenol E 3-*O*-β-D-galactopyranosyl(1→2)[β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**2**).

Table 2. ^{13}C-NMR (125 MHz) Data of Theasaponins E_3 (**1**), E_4 (**2**), E_5 (**3**), E_6 (**4**), and E_7 (**5**)

C-	1	2	3	4	5	C-	1	2	3	4	5
1	38.2	38.1	38.1	38.2	38.2	GlcA					
2	25.3	25.3	25.3	25.3	25.3	1′	104.2	104.3	104.3	104.2	104.2
3	84.1	84.6	84.7	84.5	84.5	2′	78.3	78.3	78.2	78.3	78.2
4	55.2	55.1	55.0	55.1	55.1	3′	84.5	84.0	84.0	84.1	84.1
5	48.4	48.4	48.4	48.4	48.3	4′	70.8	70.8	70.8	70.8	70.8
6	20.4	20.3	20.3	20.3	20.4	5′	77.3	77.4	77.3	77.4	77.4
7	32.4	32.3	32.3	32.4	32.4	6′	172.0	171.9	171.9	171.9	171.9
8	40.3	40.3	40.1	40.3	40.3	Gal					
9	46.9	46.7	46.7	46.8	46.8	1″	103.3	103.3	103.2	103.3	103.3
10	36.2	36.0	35.9	36.4	36.0	2″	73.7	73.7	73.7	73.7	73.7
11	23.8	23.7	23.7	23.8	23.8	3″	75.4	75.4	75.4	75.4	75.4
12	123.1	124.4	124.8	123.1	123.1	4″	70.5	70.5	70.4	70.5	70.5
13	143.6	141.9	141.0	143.6	142.9	5″	76.5	76.6	76.5	76.5	76.5
14	41.9	41.4	41.3	41.9	41.7	6″	62.1	62.1	62.1	62.0	62.0
15	34.4	30.9	30.9	34.4	34.6	Ara					
16	67.8	71.5	70.8	67.8	67.9	1‴	101.7	101.7	101.6	101.7	101.7
17	47.8	47.6	46.2	48.2	47.9	2‴	82.4	82.3	82.3	82.3	82.3
18	40.5	39.8	40.2	40.4	40.1	3‴	73.4	73.3	73.4	73.4	73.3
19	47.0	47.3	47.1	47.8	47.2	4‴	68.3	68.3	68.3	68.3	68.3
20	36.1	36.0	36.0	36.0	36.5	5‴	66.0	66.0	66.1	66.0	66.0
21	81.7	80.4	80.0	82.0	79.3	Xyl					
22	73.1	70.8	69.8	72.9	74.3	1⁗	107.1	107.1	107.1	107.1	107.1
23	209.9	210.1	210.3	209.9	210.0	2⁗	75.9	75.9	75.9	76.4	75.9
24	11.1	11.1	11.1	11.1	11.1	3⁗	78.3	78.3	78.2	78.3	78.2
25	15.8	15.8	15.8	15.8	15.8	4⁗	70.8	70.8	70.8	70.8	70.8
26	16.9	16.7	16.8	16.8	16.8	5⁗	67.5	67.5	67.5	67.5	67.5
27	27.4	27.0	27.0	27.4	27.4						
28	66.0	64.8	65.9	65.9	63.7						
29	29.9	30.0	29.9	29.9	29.5						
30	20.4	20.1	19.9	20.3	20.2						
16-*O*-Ac											
1		170.0	169.8								
2		22.2	22.1								
21-*O*-Ang											
1	168.7	168.3	168.1								
2	129.6	129.2	129.0								
3	136.0	136.8	137.0								
4	15.9	16.0	16.0								
5	21.1	21.1	21.0								
21-*O*-Tig											
1				168.6	168.0						
2				129.9	129.5						
3				136.1	136.9						
4				14.1	14.2						
5				12.4	12.4						
22-*O*-Ac											
1			170.5		171.0						
2			20.6		20.9						

Measured in pyridine-d_5.

Theasaponin E_5 (**3**), $[\alpha]_D^{25}$ +21.5° (MeOH), was also obtained as colorless fine crystals from $CHCl_3$–MeOH with mp 216.2—216.4 °C. The positive- and negative-ion FAB-MS of **3** showed quasimolecular ion peaks at *m/z* 1295 $(M+Na)^+$ and *m/z* 1271 $(M-H)^-$, respectively. The high-resolution positive-ion FAB-MS of **3** revealed the molecular formula to be $C_{61}H_{92}O_{28}$. The IR spectrum of **3** showed absorption bands at 3453, 1731, 1647, 1078 cm^{-1}, ascribable to hydroxyl, carbonyl, α,β-unsaturated ester, and ether functions. Alkaline hydrolysis of **3** 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—6] The proton and carbon signals in the ^{1}H- (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[7] of **3** were similar to those of **2**, except for the signals due to the 28-acetyl moiety {six methyls [δ 0.78, 0.87, 1.12, 1.26, 1.44, 1.47 (3H each, all s, 25, 26, 29, 30, 27, 24-H_3)], a methylene, and four methines bearing an oxygen function [δ 3.93 (1H, dd-like, H-3), 4.23 (2H, m, 28-H_2), 4.42 (1H, d, J=11.2 Hz, 22-H), 5.83 (1H, br s, 16-H), 5.94 (1H, d, J=11.2 Hz, 21-H)], an olefin [δ 5.42 (1H, br s, 12-H)], an aldehyde [δ 9.95 (1H, s, 23-H)], and four glycopyranosyl moieties [δ 4.81 (1H, d, J=7.3 Hz, 1′-H), 5.00 (1H, d, J=7.3 Hz, 1⁗-H), 5.75 (1H, d, J=7.4 Hz, 1″-H), 5.77 (1H, d, J=5.5 Hz, 1‴-H)]} together with two acetyl groups [δ 1.98, 2.53 (3H each, both s, 28-*O*- and 16-*O*-Ac)] and an angeloyl moiety [δ 1.93 (3H, s, 21-*O*-Ang-5-H_3), 2.01 (3H, d, J=7.0 Hz, 21-*O*-Ang-4-H_3), 5.92 (1H, dq-like, 21-*O*-Ang-3-H)]. The positions of three acyl groups in **3** were characterized by the HMBC experiments, in which long-range correla-

tions were observed between the following proton and carbon pairs: the 16-proton, the acetyl methyl [δ 2.53 (3H, s)] and the acetyl carbonyl carbon (δ_C 169.8); the 28-protons, the acetyl methyl [δ 1.98 (3H, s)] and the acetyl carbonyl carbon (δ_C 170.5); the 21-proton and the angeloyl carbonyl carbon (δ_C 168.1). On the basis of this evidence, the structure of theasaponin E_5 was elucidated to be 16,28-di-*O*-acetyl-21-*O*-angeloyltheasapogenol E 3-*O*-β-D-galactopyranosyl(1→2)[β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**3**).

Theasaponin E_6 (**4**), colorless fine crystals from $CHCl_3$–MeOH (mp 209.1—210.0 °C), was obtained with positive optical rotation ($[\alpha]_D^{27}$ +18.2° in MeOH). The molecular formula of **4** was determined to be $C_{57}H_{88}O_{26}$ by positive- and negative-ion FAB-MS [*m/z* 1211 $(M+Na)^+$ and *m/z* 1187 $(M-H)^-$] and by high-resolution positive-ion FAB-MS. Treatment of **4** with 10% aqueous KOH–50% aqueous 1,4-dioxane (1 : 1, v/v) liberated **1a** and tiglic acid, which was identified by HPLC analysis of its *p*-nitrobenzyl derivative.[1,3—6] The proton and carbon signals in the ^{1}H- (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[7] of **4** resembled those of **1**, except for the signals due to the acyl groups: [δ 1.61 (3H, d, *J*=7.3 Hz, 21-*O*-Tig-4-H_3), 1.86 (3H, s, 21-*O*-Tig-5-H_3), 7.02 (1H, dq-like, 21-*O*-Tig-3-H)]. The position of the tigloyl group in **4** was characterized by the HMBC experiments, which showed a long-range correlation between the 21-proton [δ 6.46 (1H, d, *J*=9.8 Hz)] and the tigloyl carbonyl carbon (δ_C 168.6). Consequently, the structure of theasaponin E_6 was determined to be 21-*O*-tigloyltheasapogenol E 3-*O*-β-D-galactopyranosyl(1→2)[β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**4**).

Theasaponin E_7 (**5**) was also obtained as colorless fine crystals from $CHCl_3$–MeOH with mp 196.4—198.0 °C with positive optical rotation ($[\alpha]_D^{27}$ +10.9° in MeOH). The molecular formula, $C_{59}H_{90}O_{27}$, of **5** was determined from the positive- and negative-ion FAB-MS [*m/z* 1253 $(M+Na)^+$ and *m/z* 1229 $(M-H)^-$] 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 **1a** and two organic acids, acetic acid and tiglic acid, which were identified by HPLC analysis of those *p*-nitrobenzyl derivatives.[1,3—6] The proton and carbon signals in the ^{1}H- (pyridine-d_5) and ^{13}C-NMR (Table 2) spectra[7] of **5** were superimposable on those of **4**, except for the signals due to an acetyl group: [δ 1.91 (3H, s, 22-*O*-Ac)]. Comparison of the ^{13}C-NMR data for **5** with those for **4** revealed an acetylation shift around the 22-position of the theasapogenol E moiety. The positions of an acetyl and a tigloyl group in **5** were characterized by the HMBC experiments, which showed long-range correlations between the 21-proton [δ 6.60 (1H, d, *J*=10.1 Hz)] and the tigloyl carbonyl carbon (δ_C 168.0) and between the 22-proton [δ 6.26 (1H, d, *J*=10.1 Hz)] and the acetyl carbonyl carbon (δ_C 171.0). Thus the structure of theasaponin E_7 was determined to be 21-*O*-tigloyl-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 (**5**).

Protective Effects of Saponin Constituents (3, 6, 7, 9, 10) from Tea Seeds and 1a on Ethanol-Induced Gastric Lesions in Rats In the course of our characterization studies of gastroprotective constituents from natural resources, we previously reported that several triterpene[8—11] and steroid[12] saponins, sesquiterpenes,[13,14] phenylpropanoids,[15] and amide[16] constituents showed protective effects on ethanol- and/or indomethacin-induced gastric lesions in rats. Since the saponin fraction from the seeds of *C. sinensis* was found to show a potent protective effect on ethanol-induced gastric lesions in rats, the principal isolated constituents (**3**, **6**, **7**, **9**, **10**) and **1a** were also examined. Among them, the major saponin constituents from tea seeds, theasaponins E_1 (**6**) and E_2 (**7**), were found to show potent protective effects [inhibition (%) at 5.0 mg/kg, *p.o.*=71.4 and 77.6, respectively] and their activities were stronger than those of reference compounds, omeprazole and cetraxate hydrochloride (Table 3). However, desacyl-theasaponin E (**1a**) markedly reduced the activity. On the other hand, assamsaponin B (**9**) and theasaponin E_5 (**3**) having the 16-acetyl moiety showed a weaker effect than **6** and **7** with the 16-hydroxyl group on ethanol-induced gastric lesions. With regard to structure–activity relationships of theasaponins for the protective effects on ethanol-induced gastric lesions, the following structural requirements were suggested; 1) the 21- and/or 22-acyl groups were essential for the activity, and 2) acetylation of the 16-hydroxyl group reduced the activity.

Table 3. Inhibitory Effects of the Saponin Constituents from the Seeds of *Camellia sinensis* on Ethanol-Induced Gastric Mucosal Lesions in Rats

Treatment	Dose (mg/kg, *p.o.*)	*n*	Gastric lesions	
			Length (mm)	Inhibition (%)
Control	—	6	162.6±16.4	—
Theasaponin E_5 (**3**)	5.0	5	88.8±24.9*	45.4
Theasaponin E_1 (**6**)	5.0	5	46.4±16.7**	71.4
Theasaponin E_2 (**7**)	5.0	4	36.4±11.8**	77.6
Desacyl-theasaponin E (**1a**)	5.0	5	112.4±21.2	30.9
Assamsaponin B (**9**)	5.0	5	98.1±18.7	39.7
Assamsaponin C (**10**)	5.0	5	57.8±21.7**	64.4
Control	—	6	159.2±21.0	—
Omeprazole	10	6	90.6±21.2**	43.1
	15	6	28.6±13.4**	82.0
	20	6	16.9±6.1**	89.4
Control	—	6	148.4±9.8	—
Cetraxate hydrochloride	75	6	87.2±7.4**	41.2
	150	6	51.0±4.0**	65.5
	300	6	30.5±8.3**	79.4

Values represent the means±S.E.M. Significantly different from the control group, * $p<0.05$, ** $p<0.01$.

Experimental

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

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

followed by heating.

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

Extraction and Isolation The dried seeds of *C. sinensis* (1.0 kg, cultivated in Shizuoka prefecture, Japan) were powdered and defatted with hexane under reflux. After removal of the solvent, the residue was further extracted three times with methanol under reflux for 3 h. Concentration of the extract under reduced pressure and exhaustive deposition with diethylether (Et_2O) gave a deposition (=crude saponin fraction of 100.0 g, 10.0% from the dried seeds). The crude saponin fraction was subjected to Diaion HP-20 column chromatography [2.0 kg, $H_2O \rightarrow MeOH \rightarrow CHCl_3$] to give H_2O-, MeOH-, and $CHCl_3$-eluted fractions (32.1, 63.4, 1.4 g, respectively). The MeOH-eluted fraction (=saponin fraction, 16 g) was subjected to HPLC [YMC-pack ODS-A, 250×20 mm i.d., MeOH–1% aqueous AcOH (70 : 30, v/v)] to give eight fractions {Fr. 1 (3.50 g), Fr. 2 (0.43 g), Fr. 3 (0.17 g), Fr. 4 [=theasaponin E_1 (**6**, 2.60 g, 1.02%)], Fr. 5 (2.20 g), Fr. 6 (0.96 g), Fr. 7 [=theasaponin E_2 (**7**, 3.10 g, 1.24%)], Fr. 8 (0.97 g)}. Fraction 2 (0.43 g) was purified by HPLC [YMC-pack ODS-A, 250×20 mm i.d., CH_3CN–1% aqueous AcOH (40 : 60, v/v)] to give five fractions {Fr. 2-1 [=theasaponin E_6 (**4**, 34 mg, 0.013%)], Fr. 2-2 (20 mg), Fr. 2-3 (17 mg), Fr. 2-4 (24 mg), and Fr. 2-5 [=theasaponin E_7 (**5**, 95 mg, 0.037%)]}. Fraction 3 (0.17 g) was further separated by HPLC [YMC-pack ODS-A, 250×20 mm i.d., CH_3CN–1% aqueous AcOH (44 : 56, v/v)] to give theasaponin E_3 (**1**, 91 mg, 0.036%). Fraction 5 (2.20 g) was separated by HPLC [YMC-pack ODS-A, 250×20 mm i.d., CH_3CN–1% aqueous AcOH (40 : 60, v/v)] to give 12 fractions [Fr. 5-1 (13 mg), Fr. 5-2 (53 mg), Fr. 5-3 (23 mg), 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 (148 mg), Fr. 5-10 (200 mg), Fr. 5-11 (645 mg), and Fr. 5-12 (85 mg)]. Fraction 5-6 (164 mg) was purified by HPLC [YMC-pack ODS-A, 250×20 mm i.d., CH_3CN–MeOH–1% aqueous AcOH (36 : 16 : 48, v/v/v)] to furnish theasaponin E_4 (**2**, 20 mg, 0.008%) and assamsaponin D (**11**, 99 mg, 0.039%). Fraction 5-8 (328 mg) was subjected to HPLC [Develosil C30-UG-5, 250×20 mm i.d., CH_3CN–MeOH–1% aqueous AcOH (35 : 16 : 49, v/v/v)] to give four fractions {Fr. 5-8-1 [=camelliasaponin C_1 (**16**, 10 mg, 0.004%)], Fr. 5-8-2 (77 mg), Fr. 5-8-3 (54 mg), Fr. 5-8-4 (26 mg)}. Fraction 5-10 (200 mg) was purified by HPLC [YMC-pack ODS-A, 250×20 mm i.d., CH_3CN–MeOH–1% aqueous AcOH (35 : 16 : 49, v/v/v)] to give assamsaponin F (**12**, 36 mg, 0.014%) and camelliasaponin B_1 (**15**, 61 mg, 0.024%). Fraction 5-11 (645 mg) was purified by HPLC [YMC-pack ODS-A, 250×20 mm i.d., CH_3CN–MeOH–1% aqueous AcOH (36 : 16 : 48, v/v/v)] to furnish assamsaponins A (**8**, 218 mg, 0.086%) and B (**9**, 140 mg, 0.056%). Fraction 6 (960 mg) was subjected to HPLC [YMC-pack ODS-A, 250×20 mm i.d., CH_3CN–1% aqueous AcOH (43 : 57, v/v)] to give nine fractions {Fr. 6-1 (16 mg), Fr. 6-2 [=assamsaponin I (**13**, 56 mg, 0.022%)], Fr. 6-3 [=assamsaponin C (**10**, 323 mg, 0.13%)], Fr. 6-4 (65 mg), Fr. 6-5 [=floratheasaponin A (**14**, 39 mg, 0.016%)], Fr. 6-7 (75 mg), Fr. 6-8 (20 mg), Fr. 6-9 [=theasaponin E_5 (**3**, 126 mg, 0.050%)]}.

These known constituents (**6**—**16**) were identified by comparison of their physical data with those of authentic samples ($[\alpha]_D$, IR, MS, and ^{1}H- and ^{13}C-NMR).[1—6]

Theasaponin E_3 (**1**): Colorless fine crystals (from $CHCl_3$–MeOH), mp 214.4—215.5 °C, $[\alpha]_D^{27}$ +17.0° (c=0.95, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{57}H_{88}O_{26}Na$ $(M+Na)^+$: 1211.5462. Found: 1211.5474. IR (KBr): 3453, 1719, 1650, 1078 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.81, 0.82, 1.12, 1.33, 1.47, 1.79 (3H each, all s, 25, 26, 29, 30, 24, 27-H_3), 2.00 (3H, s, 21-*O*-Ang-5-H_3), 2.06 (3H, d, J=7.4 Hz, 21-*O*-Ang-4-H_3), 2.92 (1H, dd-like, 18-H), 3.65, 3.94 (1H each, both d, J=9.8 Hz, 28-H_2), 4.04 (1H, m, 3-H), 4.79 (1H, d, J=10.1 Hz, 22-H), 4.84 (1H, br s, 16-H), 4.84 (1H, d, J=7.4 Hz, 1′-H), 5.01 (1H, d, J=7.7 Hz, 1‴-H), 5.36 (1H, br s, 12-H), 5.77 (1H, d, J=7.6 Hz, 1″-H), 5.77 (1H, d, J=6.1 Hz, 1‴-H), 5.91 (1H, dq-like, 21-*O*-Ang-3-H), 6.48 (1H, d, J=10.1 Hz, 21-H), 9.90 (1H, s, 23-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 2. Positive-ion FAB-MS: m/z 1233 $(M+2Na-H)^+$, 1211 $(M+Na)^+$. Negative-ion FAB-MS: m/z 1187 $(M-H)^-$, 1025 $(M-C_6H_{11}O_5)^-$, 923 $(M-C_{10}H_{17}O_8)^-$, 761 $(M-C_{16}H_{27}O_{13})^-$.

Theasaponin E_4 (**2**): Colorless fine crystals (from $CHCl_3$–MeOH), mp 223.8—224.3 °C, $[\alpha]_D^{27}$ +17.4° (c=1.00, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{59}H_{90}O_{27}Na$ $(M+Na)^+$: 1253.5567. Found: 1253.5573. IR (KBr): 3453, 1719, 1638, 1078 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.71, 0.76, 1.14, 1.29, 1.45, 1.47 (3H each, all s, 25, 26, 29, 30, 24, 27-H_3), 1.94 (3H, s, 21-*O*-Ang-5-H_3), 2.02 (3H, d, J=7.3 Hz, 21-*O*-Ang-4-H_3), 2.53 (3H, s, 16-*O*-Ac), 2.94 (1H, dd-like, 18-H), 3.94 (1H, dd-like, 3-H), 4.28 (2H, m, 28-H_2), 4.77 (1H, d, J=10.4 Hz, 22-H), 4.81 (1H, d, J=7.0 Hz, 1′-H), 5.00 (1H, d, J=7.0 Hz, 1⁗-H), 5.33 (1H, br s, 12-H), 5.75 (1H, d, J=7.6 Hz, 1″-H), 5.79 (1H, d, J=5.5 Hz, 1‴-H), 5.91 (1H, br s, 16-H), 5.91 (1H, dq-like, 21-*O*-Ang-3-H), 5.97 (1H, d, J=10.4 Hz, 21-H), 9.94 (1H, s, 23-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 2. Positive-ion FAB-MS: m/z 1253 $(M+Na)^+$. Negative-ion FAB-MS: m/z 1229 $(M-H)^-$, 1097 $(M-C_5H_9O_4)^-$, 1067 $(M-C_6H_{11}O_5)^-$, 965 $(M-C_{10}H_{17}O_8)^-$, 935 $(M-C_{11}H_{19}O_9)^-$.

Theasaponin E_5 (**3**): Colorless fine crystals (from $CHCl_3$–MeOH), mp 216.2—216.4 °C, $[\alpha]_D^{27}$ +21.5° (c=1.00, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{61}H_{92}O_{28}Na$ $(M+Na)^+$: 1295.5673. Found: 1295.5682. IR (KBr): 3453, 1731, 1647, 1078 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.78, 0.87, 1.12, 1.26, 1.44, 1.47 (3H each, all s, 25, 26, 29, 30, 27, 24-H_3), 1.93 (3H, s, 21-*O*-Ang-5-H_3), 1.98, 2.53 (3H each, both s, 28-*O*-, 16-*O*-Ac), 2.01 (3H, d, J=7.0 Hz, 21-*O*-Ang-4-H_3), 2.77 (1H, dd-like, 18-H), 3.93 (1H, dd-like, 3-H), 4.23 (2H, m, 28-H_2), 4.42 (1H, d, J=11.2 Hz, 22-H), 4.81 (1H, d, J=7.3 Hz, 1′-H), 5.00 (1H, d, J=7.3 Hz, 1⁗-H), 5.42 (1H, br s, 12-H), 5.75 (1H, d, J=7.4 Hz, 1″-H), 5.77 (1H, d, J=5.5 Hz, 1‴-H), 5.83 (1H, br s, 16-H), 5.92 (1H, dq-like, 21-*O*-Ang-3-H), 5.94 (1H, d, J=11.2 Hz, 21-H), 9.95 (1H, s, 23-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 2. Positive-ion FAB-MS: m/z 1317 $(M+2Na-H)^+$, 1295 $(M+Na)^+$. Negative-ion FAB-MS: m/z 1271 $(M-H)^-$, 1139 $(M-C_5H_9O_4)^-$, 1109 $(M-C_6H_{11}O_5)^-$, 1007 $(M-C_{10}H_{17}O_8)^-$, 845 $(M-C_{16}H_{27}O_{13})^-$.

Theasaponin E_6 (**4**): Colorless fine crystals (from $CHCl_3$–MeOH), mp 209.1—210.0 °C, $[\alpha]_D^{27}$ +18.2° (c=1.50, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{57}H_{88}O_{26}Na$ $(M+Na)^+$: 1211.5462. Found: 1211.5472. IR (KBr): 3453, 1744, 1046 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.74, 0.76, 1.12, 1.35, 1.47, 1.78 (3H each, all s, 25, 26, 29, 30, 24, 27-H_3), 1.61 (3H, d, J=7.3 Hz, 21-*O*-Tig-4-H_3), 1.86 (3H, s, 21-*O*-Tig-5-H_3), 2.96 (1H, dd-like, 18-H), 3.67, 3.97 (1H each, both d, J=10.4 Hz, 28-H_2), 4.04 (1H, m, 3-H), 4.84 (1H, br s, 16-H), 4.85 (1H, d, J=9.8 Hz, 22-H), 4.86 (1H, d, J=7.3 Hz, 1′-H), 5.01 (1H, d, J=7.7 Hz, 1⁗-H), 5.37 (1H, br s, 12-H), 5.78 (1H, d, J=7.7 Hz, 1″-H), 5.79 (1H, d, J=6.1 Hz, 1‴-H), 6.46 (1H, d, J=9.8 Hz, 21-H), 7.02 (1H, dq-like, 21-*O*-Tig-3-H), 9.91 (1H, s, 23-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 2. Positive-ion FAB-MS: m/z 1233 $(M+2Na-H)^+$, 1211 $(M+Na)^+$. Negative-ion FAB-MS: m/z 1187 $(M-H)^-$, 1055 $(M-C_5H_9O_4)^-$, 1025 $(M-C_6H_{11}O_5)^-$, 923 $(M-C_{10}H_{17}O_8)^-$, 761 $(M-C_{16}H_{27}O_{13})^-$.

Theasaponin E_7 (**5**): Colorless fine crystals (from $CHCl_3$–MeOH), mp 196.4—198.0 °C, $[\alpha]_D^{27}$ +10.9° (c=3.00, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{59}H_{90}O_{27}Na$ $(M+Na)^+$: 1253.5567. Found: 1253.5573. IR (KBr): 3453, 1743, 1085 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.81, 0.81, 1.11, 1.34, 1.48, 1.79 (3H each, all s, 25, 26, 29, 30, 24, 27-H_3), 1.66 (3H, d, J=7.0 Hz, 21-*O*-Tig-4-H_3), 1.91 (3H, s, 22-*O*-Ac), 1.97 (3H, s, 21-*O*-Tig-5-H_3), 3.07 (1H, m, 18-H), 3.38, 3.61 (1H each, both d, J=10.7 Hz, 28-H_2), 4.04 (1H, m, 3-H), 4.42 (1H, br s, 16-H), 4.86 (1H, d, J=7.3 Hz, 1′-H), 5.01 (1H, d, J=7.7 Hz, 1⁗-H), 5.38 (1H, br s, 12-H), 5.77 (1H, d, J=7.7 Hz, 1″-H), 5.78 (1H, d, J=5.5 Hz, 1‴-H), 6.26 (1H, d, J=10.1 Hz, 22-H), 6.60 (1H, d, J=10.1 Hz, 21-H), 7.12 (1H, dq-like, 21-*O*-Tig-3-H), 9.93 (1H, s, 23-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 2. Positive-ion FAB-MS: m/z 1253 $(M+Na)^+$. Negative-ion FAB-MS: m/z 1229 $(M-H)^-$, 1097 $(M-C_5H_9O_4)^-$, 1067 $(M-C_6H_{11}O_5)^-$, 965 $(M-C_{10}H_{17}O_8)^-$, 803 $(M-C_{16}H_{27}O_{13})^-$.

Alkaline Hydrolysis of Theaponins E_3—E_7 (1—5) A solution of theasaponins E_3—E_7 (**1**—**5**, 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. Removal of the solvent under reduced pressure gave a reaction mixture. A part of the reaction mixture 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 solution was subjected to HPLC analysis [column: YMC-Pack ODS-A, 250×4.6 mm i.d.; mobile phase: MeOH–H_2O (70 : 30, v/v); detection: UV (254 nm); flow rate: 0.9 ml/min] to identify the *p*-nitrobenzyl esters of acetic acid (**a**, t_R 6.3 min) from **2**, **3**, 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**. The rest of the reaction mixture was neutralized with Dowex HCR W2 (H^+ form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure yielded a product, which was subjected to ordinary-phase silica gel column chromatography [500 mg, $CHCl_3$–MeOH–H_2O (6 : 4 : 1, v/v/v)] to give desacyl-theasaponin E (**1a**,[2] 6 mg each).

Bioassay. Animals Male Sprague-Dawley rats weighing about 230—250 g were purchased from Kiwa Laboratory Animal Co., Ltd., Wakayama, Japan. The animals were housed at a constant temperature of 23±2 °C and were fed a standard laboratory chow (MF, Oriental Yeast Co., Ltd., Tokyo,

Japan). The animals were fasted for 24—26 h prior to the beginning of the experiment, but were allowed free access to tap water. All of experiments were performed with conscious rats unless otherwise noted. The experimental protocols were approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

Effect of Ethanol- or Indomethacin-Induced Gastric Mucosal Lesions in Rats The acute gastric lesions were induced by oral administration of ethanol and indomethacin according to the method described previously.[10,14—16] Briefly, 99.5% ethanol and indomethacin (20 mg/kg, dissolved in 5% sodium bicarbonate, and then diluted in water and neutralized with 0.2 M HCl and adjusted to 1.5 ml/rat) were administered to 24—26 h fasted rats using a metal orogastric tube. One hour after administration of ethanol or 4 h after administration of indomethacin, the animals were killed by cervical dislocation under ether anesthesia and the stomach was removed and inflated by injection of 10 ml 1.5% formalin to fix the inner and outer layers of the gastric walls. Subsequently, the stomach was incised along the greater curvature, the lengths of gastric lesions were measured as previously described, and the total length (mm) was expressed as a lesion index.

The saponin fraction of tea seeds, compounds **3**, **6**, **7**, **9**, **10**, and **1a**, and cetraxate hydrochloride were suspended in 5% acacia solution. Omeprazole was suspended in 0.5% CMC-Na. Test samples in vehicle and vehicle only (control group) were administered orally at a dose of 5.0 ml/kg 1 h prior to the application of ethanol and indomethacin.

Statistics Values were expressed as means±S.E.M. For statistical analysis, one-way analysis of variance followed by Dunnett's test was used. Probability (p) values less than 0.05 were considered significant. ED_{50} values were estimated based on linear regressions of probit-transformed values of inhibition (%).

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