Triterpene Saponins with Gastroprotective Effects from Tea Seed (the Seeds of *Camellia sinensis*)[1]

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Six new triterpene saponins, theasaponins A_1 (**1**), A_2 (**2**), A_3 (**3**), F_1 (**4**), F_2 (**5**), and F_3 (**6**), were isolated from the saponin fraction of the seeds of *Camellia sinensis*. The stereostructures of **1**−**6** were elucidated on the basis of chemical and physicochemical evidence. Theasaponin A_2 (**2**) showed an inhibitory effect on ethanol-induced gastric mucosal lesions in rats at a dose of 5.0 mg/kg, p.o., and its activity was more potent than that of omeplazole. Structure−activity relationships for theasaponins on ethanol-induced gastroprotective activities may be suggested as follows: (1) the 28-acetyl moiety enhances activity; (2) theasaponins having a 23-aldehyde group exhibit more potent activities than those with a 23-hydroxymethyl group or a 23-methoxycarbonyl group.

The cultivation of the tea plant has a long history in Asian countries, and the seeds and fruits of this plant have been used as an antitussive and expectorant in Chinese traditional medicine.[1−6] During the course of our characterization studies on the bioactive saponin constituents from *Camellia* species,[1−8] we have reported the isolation and structure elucidation of theasaponins E_1 (**7**), E_2 (**8**), and E_3−E_7 together with nine saponins from the seeds of *Camellia sinensis* (L.) O. Kuntze (*C. sinensis* L. var. *sinensis*).[1,2,4] In addition, we isolated assamsaponins A−I with gastric emptying activity and an accelating effect on gastrointestinal transit from the seeds and leaves of *C. sinensis* L. var. *assamica* Pierre.[5,6] Recently, floratheasaponins A−C with antihyperlipidemic activities were also isolated from the flower part of *C. sinensis*.[8] As a continuing study on the seeds of *C. sinensis*, we have isolated six new triterpene saponins, named theasaponins A_1 (**1**), A_2 (**2**), A_3 (**3**), F_1 (**4**), F_2 (**5**), and F_3 (**6**). This paper deals with the structure elucidation of these six new saponins as well as the gastroprotective effects of several saponin constituents on ethanol-induced gastric mucosal lesions in rats.

Results and Discussion

The seeds of the tea plant cultivated in Shizuoka Prefecture, Japan, were defatted with *n*-hexane and then extracted with methanol. The methanolic extracted solution was concentrated under reduced pressure and then deposited with diethyl ether to give a precipitate (10.0%). The precipitate was subjected to Diaion HP-20 column chromatography ($H_2O \rightarrow MeOH \rightarrow CHCl_3$) to give a methanol-eluted fraction (=saponin fraction, 6.3%). The saponin fraction was subjected to HPLC to give 16 saponins including **9** and **10**.[1] By continued isolation of the saponin constituents using HPLC, six new triterpene saponins, theasaponins A_1 (**1**, 0.021%), A_2 (**2**, 0.13%), A_3 (**3**, 0.059%), F_1 (**4**, 0.009%), F_2 (**5**, 0.021%), and F_3 (**6**, 0.054%), were isolated.

Theasaponin A_1 (**1**) was obtained as colorless fine crystals from $CHCl_3$−MeOH with mp 219.3−220.4 °C and exhibited a positive optical rotation ($[\alpha]_D^{27}$ +6.5 in MeOH). The IR spectrum of **1** showed absorption bands at 1719 and 1650 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 FABMS of **1**, quasimolecular ion peaks were observed at *m/z* 1213 $[M + Na]^+$ and 1189 $[M - H]^-$, and HRFABMS analysis revealed the molecular formula of **1** to be $C_{57}H_{90}O_{26}$. On alkaline hydrolysis of **1** with 10% aqueous KOH−50% aqueous 1,4-dioxane (1:1), desacyl-assamsaponin D (**1a**)[5] was obtained together with angelic acid, which was identified by HPLC analysis of its *p*-nitrobenzyl derivative.[1,5−8] The ^1H (pyridine-d_5) and ^{13}C NMR (Table 1) spectra of **1**, which were assigned by various NMR experiments,[9] showed signals assignable to six methyls [δ 0.89, 0.90, 1.07, 1.10, 1.32, 1.80 (3H each, all s, H_3-26, 25, 24, 29, 30, 27)], two methylenes and four methines bearing an oxygen function {δ 3.68, 3.96 (1H each, both d, *J* = 10.4 Hz, H_2-28), [3.77 (1H, d, *J* = 10.4 Hz), 4.42 (1H, m), H_2-23], 4.15 (1H, m, H-3), 4.80 (1H, d, *J* = 10.1 Hz, H-22), 4.84 (1H, br s, H-16), 6.46 (1H, d, *J* = 10.1 Hz, H-21)}, an olefin [δ 5.37 (1H, br s, H-12)], and four glycopyranosyl moieties [δ 5.02 (1H, d, *J* = 7.7 Hz, H-1′′′′), 5.06 (1H, d, *J* = 7.7 Hz, H-1′), 5.78 (1H, d, *J* = 6.1 Hz, H-1′′′), 5.88 (1H, d, *J* = 7.9 Hz, H-1′′)], together with an angeloyl group [δ 1.98 (3H, s, H_3-Ang-5), 2.06 (3H, d, *J* = 7.3 Hz, H_3-Ang-4), 5.90 (1H, dq-like, H-Ang-3)]. The position of the angeloyl group in **1** was clarified on the basis of a HMBC experiment. Thus, a long-range correlation was observed between the 21-proton and the angeloyl carbonyl carbon (δ_C 168.7). Furthermore, comparison of the ^{13}C NMR data for **1** with those for **1a** revealed an acylation shift around the 21-position of the sapogenol moiety. On the basis of the above-mentioned evidence, the structure of theasaponin A_1 was determined to be 21-*O*-angeloyltheasapogenol A 3-*O*-β-D-galactopyranosyl(1→2)[β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**1**).

Theasaponin A_2 (**2**) was also obtained as colorless fine crystals from $CHCl_3$−MeOH with mp 219.6−221.1 °C and a positive optical rotation ($[\alpha]_D^{27}$ +23.2 in MeOH). The IR spectrum of **2** showed absorption bands at 3453, 1721, 1650, and 1080 cm^{-1}, ascribable to hydroxyl, carbonyl, α,β-unsaturated ester, and ether functions. The molecular formula, $C_{59}H_{92}O_{27}$, of **2** was determined from the positive- and negative-ion FABMS (*m/z* 1255 $[M + Na]^+$ and 1231 $[M - H]^-$) and by HRFABMS. The fragmentation patterns in the negative-ion FABMS of **2** indicated the loss of monopentose (*m/z* 1099 $[M - C_5H_9O_4]^-$), mono-hexose (*m/z* 1069 $[M - C_6H_{11}O_5]^-$), and di-pentose (*m/z* 967 $[M - C_{10}H_{17}O_8]^-$) units. In addition, theasaponin A_3 (**3**), $[\alpha]_D^{25}$ −8.9 (MeOH), was also obtained as colorless fine crystals from $CHCl_3$−MeOH, with mp 228.0−229.2 °C. The positive- and negative-ion FABMS of **3** showed quasimolecular ion peaks at *m/z* 1297 $[M + Na]^+$ and *m/z* 1273 $[M - H]^-$, respectively. The HRFABMS of **3** revealed the molecular formula to be $C_{61}H_{94}O_{28}$. Treatment of **2** and **3** with 10%

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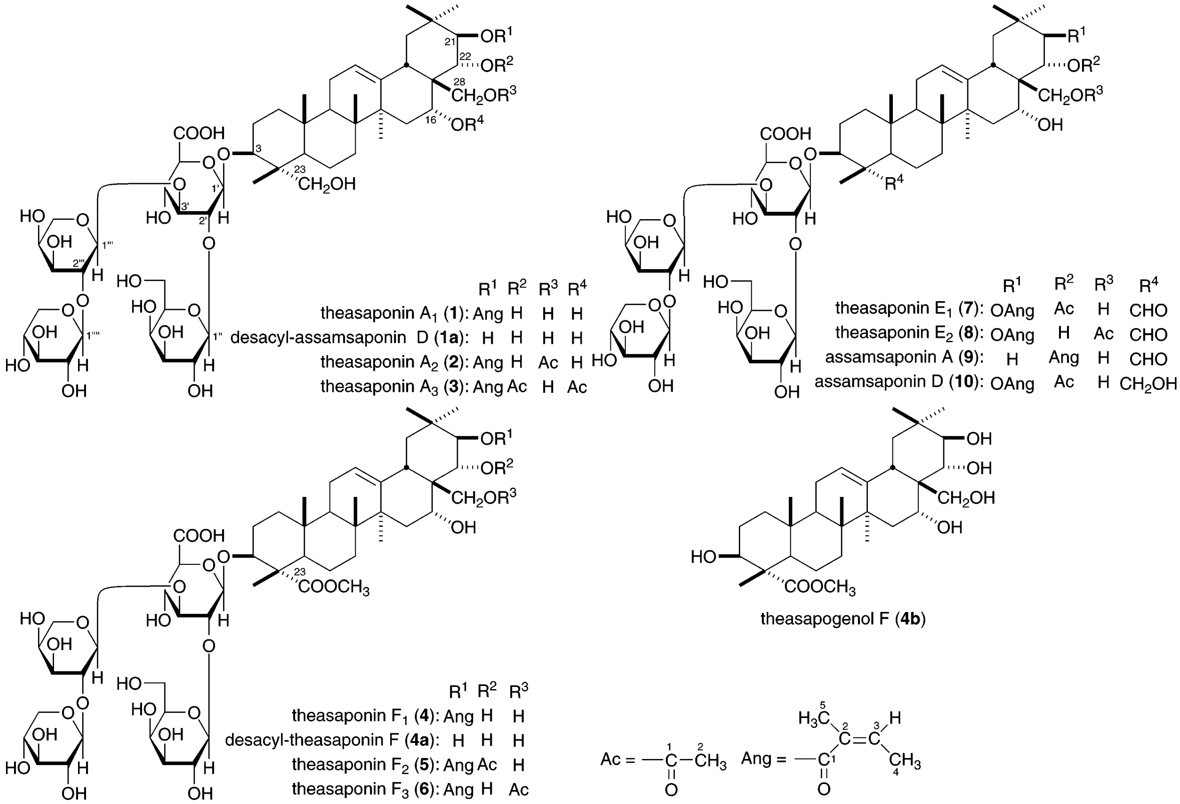
Chart 1



aqueous KOH−50% aqueous 1,4-dioxane (1:1) liberated **1a**[5] and two organic acids, acetic acid and angelic acid, which were identified by HPLC analysis of their *p*-nitrobenzyl derivatives, respectively.[1,5−8] The ^{1}H (pyridine-d_5) and ^{13}C NMR (Table 1) spectra[9] of **2** indicated the presence of the following functions: a theasapogenol A part {six methyls [δ 0.91, 1.01, 1.05, 1.09, 1.29, 1.78 (3H each, all s, H_3-25, 26, 24, 29, 30, 27)], two methylenes and four methines bearing an oxygen function (δ [3.76 (1H, d, J = 10.7 Hz), 4.37 (1H, m), H_2-23], 4.13 (1H, m, H-3), 4.38 (2H, m, H_2-28), 4.44 (1H, d, J = 10.1 Hz, H-22), 4.71 (1H, br s, H-16), 6.46 (1H, d, J = 10.1 Hz, H-21)), an olefin [δ 5.44 (1H, br s, H-12)], four glycopyranosyl moieties [δ 5.00 (1H, d, J = 7.7 Hz, H-1′′′′), 5.04 (1H, d, J = 7.3 Hz, H-1′), 5.75 (1H, d, J = 6.1 Hz, H-1′′′), 5.85 (1H, d, J = 7.6 Hz, H-1′′)]}, an acetyl unit, and an angeloyl moiety [δ 1.97 (3H, s, H_3-Ang-5), 1.99 (3H, s, H_3-Ac), 2.04 (3H, d, J = 7.4 Hz, H_3-Ang-4), 5.90 (1H, dq-like, H-Ang-3)]. The HMBC experiment on **2** showed long-range correlations between the 21-proton and the angeloyl carbonyl carbon (δ_C 168.5) and the 28-protons and the acetyl carbonyl carbon (δ_C 170.7). Furthermore, comparison of the ^{13}C NMR data for **2** with those for **1** revealed an acetylation shift around the 28-position of the aglycon moiety. Consequently, the structure of theasaponin A_2 was determined to be 21-*O*-angeloyl-28-*O*-acetyltheasapogenol A 3-*O*-β-D-galactopyranosyl(1→2)[β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**2**). In turn, the proton and carbon signals of the acyl groups in **3** showed signals assignable to two acetyl groups [δ 2.04, 2.50 (3H each, both s, H_3-22−, 16-Ac)] and an angeloyl moiety [δ 1.98 (3H, s, H_3-Ang-5), 2.06 (3H, d, J = 7.4 Hz, H_3-Ang-4), 6.00 (1H, dq-like, H-Ang-3)]. In a HMBC experiment on **3**, long-range correlations were observed between the following proton and carbon pairs: the 16-proton [δ 5.59 (1H, br s)] and the acetyl methyl [δ 2.50 (3H, s)] and the acetyl carbonyl carbon (δ_C 169.8); the 22-proton [δ 6.13 (1H, d, J = 10.4 Hz)] and the acetyl methyl [δ 2.04 (3H, s)] and the acetyl carbonyl carbon (δ_C 170.4); and the 21-proton [δ 5.86 (1H, d, J = 10.4 Hz)] and the angeloyl carbonyl carbon (δ_C 167.8). On the basis of the above-mentioned evidence, the structure of theasaponin A_3 was elucidated as 16,28-di-*O*-acetyl-21-*O*-angeloyltheasapogenol A 3-*O*-β-D-galactopyranosyl(1→2)[β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**3**).

Theasaponin F_1 (**4**) was obtained as colorless fine crystals from $CHCl_3$−MeOH with mp 230.4−231.1 °C and exhibited a positive optical rotation ($[\alpha]_D^{27}$ +29.8° in MeOH). The IR spectrum of **4** showed absorption bands at 1717 and 1647 cm^{-1}, ascribable to carbonyl and α,β-unsaturated ester functions, and broad bands at 3453 and 1080 cm^{-1}, suggestive of an oligoglycoside structure. In the positive- and negative-ion FABMS of **4**, quasimolecular ion peaks were observed at m/z 1241 $[M + Na]^+$ and 1217 $[M - H]^-$, and HRFABMS analysis revealed the molecular formula of **4** to be $C_{58}H_{90}O_{27}$. The fragmentation patterns in the negative-ion FABMS of **4** indicated the loss of mono-pentose (m/z 1085 $[M - C_5H_9O_4]^-$) and di-pentose (m/z 953 $[M - C_{10}H_{17}O_8]^-$) units. On alkaline hydrolysis of **4** with 10% aqueous KOH−50% aqueous 1,4-dioxane (1:1), desacyl-theasaponin F (**4a**) was obtained together with angelic acid, which was identified by HPLC analysis of its *p*-nitrobenzyl derivative.[1,5−8] Methanolysis of **4a** with 9% HCl−dry-MeOH gave a new triterpene aglycon, theasapogenol F (**4b**). The proton and carbon signals in the ^{1}H and ^{13}C NMR spectra of **4b** (in pyridine-d_5), which were assigned by various NMR experiments,[9] showed signals assignable to 3,16,21,22,28-pentahydroxyolean-12-en-23-oic acid methyl ester moiety {six methyls [δ 0.93, 0.96, 1.30, 1.37, 1.53, 1.80 (3H each, all s, H_3-25, 26, 29, 30, 24, 27)], a methylene and four methines bearing an oxygen function [δ 3.71, 3.83 (1H each, both d-like, H_2-28), 4.00 (1H, dd, J = 5.2, 10.4 Hz, H-3), 4.61 (1H, d, J = 9.5 Hz, H-21), 4.76 (1H, d, J = 9.5 Hz, H-22), 4.99 (1H, br s, H-16)], a methoxycarboxyl group [δ 3.61 (3H, s)], and an olefin [δ 5.41 (1H, br s, H-12)]}. The ^{1}H and ^{13}C NMR (Table 1) spectra[9] of **4** (in pyridine-d_5) and **4a** [in pyridine-d_5−D_2O (6:1)] showed signals for six methyls [δ **4**: 0.80, 0.81, 1.10, 1.31, 1.52, 1.80 (3H each, all s, H_3-25, 26, 29, 30, 24, 27); **4a**: 0.81, 0.81, 1.23, 1.31, 1.53, 1.82 (3H each, all s, H_3-25, 26, 29, 30, 24, 27)], a methylene and four methines bearing an oxygen function [δ **4**: 3.65, 3.93 (1H each, both d, J = 10.1 Hz,

Table 1. ^{13}C NMR (125 MHz) Data for **1**, **2**, **3**, **4**, **5**, and **6**

carbon	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**4a**[b]	**5**[a]	**6**[a]
1	38.8	38.8	38.7	38.6	38.7	38.6	38.6
2	25.5	25.5	25.6	26.1	25.8	26.1	26.1
3	83.1	83.1	82.9	85.7	86.8	85.7	85.7
4	43.5	43.5	43.5	53.8	53.8	53.8	53.8
5	48.1	48.2	48.0	52.2	52.2	52.2	52.2
6	18.2	18.2	18.0	20.9	20.8	20.9	20.9
7	32.8	32.8	32.7	32.7	32.5	32.7	32.7
8	40.1	40.5	40.0	40.4	40.5	40.2	40.4
9	47.0	47.1	46.9	47.1	47.0	47.0	47.0
10	36.8	36.7	36.6	36.5	36.3	36.4	36.4
11	23.9	23.9	23.8	23.8	23.6	23.8	23.8
12	123.1	123.8	125.1	123.1	122.9	123.1	123.8
13	143.5	142.7	140.9	143.4	143.3	142.8	142.7
14	41.8	41.8	41.1	41.8	41.5	41.6	41.7
15	34.5	34.7	30.9	34.4	33.9	34.6	34.6
16	67.9	67.7	71.4	67.8	67.7	67.9	67.5
17	48.2	47.1	46.9	48.1	47.3	48.0	47.0
18	40.4	40.1	39.5	40.2	40.1	40.0	40.2
19	47.8	47.3	47.1	47.8	48.0	47.1	47.1
20	36.1	36.1	35.9	36.1	36.2	36.2	36.1
21	81.6	81.2	78.3	81.6	78.7	78.9	81.2
22	73.1	71.2	73.3	73.1	75.9	74.3	71.1
23	64.8	64.8	64.7	178.1	178.7	178.1	178.1
24	13.6	13.6	13.5	12.3	12.2	12.2	12.2
25	16.2	16.2	16.0	16.1	16.0	16.0	16.0
26	16.9	17.1	16.8	17.0	16.5	16.6	16.8
27	27.4	27.4	27.0	27.3	27.2	27.3	27.3
28	66.0	66.4	63.7	65.9	66.7	63.8	66.0
29	29.8	29.7	29.4	29.8	30.2	29.4	29.7
30	20.4	20.2	19.7	20.4	19.3	20.3	20.2
COOCH_3				52.2	52.7	52.2	52.2
16-*O*-Ac							
1			169.8				
2			22.0				
21-*O*-Ang							
1	168.7	168.5	167.8	168.7		167.8	168.5
2	129.6	129.5	128.4	129.6		129.0	129.5
3	136.0	136.1	138.1	136.0		137.1	136.1
4	15.9	15.9	16.1	15.9		15.9	15.9
5	21.1	21.0	20.8	21.1		21.0	21.0
22-*O*-Ac							
1			170.4			170.9	
2			20.9			20.9	
28-*O*-Ac							
1		170.7					170.7
2		20.7					20.7

carbon	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**4a**[b]	**5**[a]	**6**[a]
GlcA							
1′	104.1	104.1	104.3	104.7	104.3	104.8	104.7
2′	78.5	78.5	78.6	78.4	77.5	78.4	78.4
3′	84.6	84.5	84.5	84.2	82.9	84.3	84.3
4′	71.0	71.0	71.0	70.8	70.8	70.9	70.9
5′	77.4	77.4	77.4	77.2	77.1	77.2	77.2
6′	171.9	171.9	171.9	171.9	171.8	172.0	172.0
Gal							
1″	103.1	103.1	103.2	103.3	102.6	103.3	103.3
2″	73.8	73.8	73.8	73.6	72.9	73.6	73.6
3″	75.3	75.2	75.3	75.7	74.7	75.7	75.6
4″	70.1	70.1	70.1	70.7	70.4	70.7	70.6
5″	76.4	76.4	76.5	76.4	76.6	76.4	76.4
6″	61.9	61.9	61.9	61.8	62.1	61.8	61.7
Ara							
1‴	101.7	101.7	101.7	101.6	101.0	101.6	101.6
2‴	82.3	82.3	82.3	82.4	81.8	82.5	82.4
3‴	73.3	73.4	73.3	73.4	73.0	73.3	73.3
4‴	68.3	68.3	68.3	68.3	68.4	68.3	68.3
5‴	66.0	66.0	66.0	66.1	65.7	66.0	66.4
Xyl							
1⁗	107.1	107.0	107.0	107.1	106.1	107.1	107.1
2⁗	75.9	75.9	75.9	76.0	77.1	76.0	76.0
3⁗	78.3	78.2	78.3	78.2	75.5	78.2	78.2
4⁗	70.8	70.8	70.8	70.8	70.4	70.8	70.8
5⁗	67.5	67.5	67.5	67.5	66.7	67.5	67.5

[a] Measured in pyridine-d_5. [b] Measured in pyridine-d_5−D_2O (6:1).

H_2-28), 4.30 (1H, m, H-3), 4.78 (1H, d, J = 10.1 Hz, H-22), 4.83 (1H, br s, H-16), 6.46 (1H, d, J = 10.4 Hz, H-21); **4a**: 3.67, 3.95 (1H each, both d-like, H_2-28), 4.28 (1H, m, H-3), 4.55 (1H, m, H-22), 4.68 (1H, d-like, H-21), 4.81 (1H, br s, H-16)], a methoxycarboxyl group [δ **4**: 3.69 (3H, s); **4a**: 3.87 (3H, s)], an olefin [δ **4**: 5.34 (1H, br s, H-12); **4a**: 5.40 (1H, br s, H-12)], and four glycopyranosyl moieties [δ **4**: 4.98 (1H, d, J = 7.4 Hz, H-1′), 4.99 (1H, d, J = 7.6 Hz, H-1⁗), 5.77 (1H, d, J = 7.6 Hz, H-1″), 5.79 (1H, d, J = 6.1 Hz, H-1‴); **4a**: 4.81 (1H, d-like, H-1′), 5.08 (1H, d-like, H-1⁗), 5.68 (1H, d-like, H-1″), 5.75 (1H, d-like, H-1‴)], together with an angeloyl group [δ **4**: 1.98 (3H, s, H_3-Ang-5), 2.05 (3H, d, J = 7.0 Hz, H_3-Ang-4), 5.90 (1H, dq-like, H-Ang-3)]. The oligoglycoside structure, the connectivities of oligoglycoside and angeloyl moieties to the aglycon, and the position of a methyl ester in the aglycon were characterized by a HMBC experiment on **4**. Thus, the HMBC experiment of **4** showed long-range correlations between the following proton and carbon pairs: H_3-24, H-1′ and C-3; H_3-24, the 23-methoxycarbonyl methyl proton and C-4; H-16, H-22, H_2-28 and C-17; H_3-29, H_3-30 and C-20, C-21; H_2-28 and C-22; H_3-24 and C-23; H-21 and the angeloyl carbonyl carbon (δ_C 168.7); H-1″ and C-2′; H-1‴ and C-3′; H-1⁗ and C-2‴ (Figure 1). Finally, reduction of **4a** with sodium borohydride ($NaBH_4$) in EtOH liberated **1a**,[11] so that the partial structures of the new aglycon

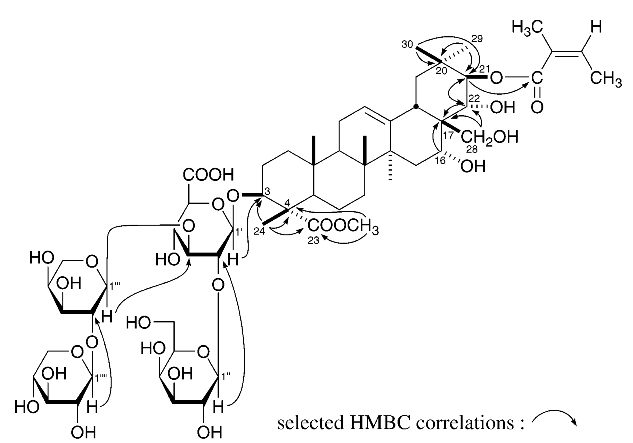


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Figure 1. Selected HMBC correlations of **4**.

part and the tetraglycoside moiety were confirmed. Consequently, the structure of theasaponin F_1 was determined as 21-*O*-angeloyltheasapogenol F 3-*O*-β-D-galactopyranosyl(1→2)[β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**4**).

Theasaponins F_2 (**5**) and F_3 (**6**) were obtained as colorless fine crystals from $CHCl_3$−MeOH (mp **5**: 211.3−212.8 °C; **6**: 216.9−217.7 °C) with positive optical rotation (**5**: $[\alpha]_D^{27}$ +8.5; **6**: $[\alpha]_D^{27}$ +25.1, MeOH). The same molecular formula, $C_{60}H_{92}O_{28}$, for both **5** and **6** was determined individually from the positive- and negative-ion FABMS (*m/z* 1283 $[M + Na]^+$ and 1259 $[M - H]^-$) and by HRFABMS. Treatment of **5** and **6** with 10% aqueous KOH−50% aqueous 1,4-dioxane (1:1) liberated **4a** and two organic acids, acetic acid and angelic acid, which were identified by HPLC analysis of their *p*-nitrobenzyl derivatives, respectively.[1,5−8] The ^1H (pyridine-d_5) and ^{13}C NMR (Table 1) spectra[9] of **5** indicated the presence of the following functions: a theasapogenol F part {six methyls [δ 0.80, 0.80, 1.07, 1.31, 1.53, 1.80 (3H each, all s, H_3-25, 26, 29, 30, 24, 27)], a methylene and four methines bearing an oxygen function [δ 3.37, 3.59 (1H each, both d, J = 10.4 Hz, H2−28), 4.45 (1H, m, H-3), 4.42 (1H, br s, H-16), 6.20 (1H, d, J = 9.8 Hz, H-22), 6.61 (1H, d, J = 9.8 Hz, H-21)], a methoxycarboxyl group [δ 3.73 (3H, s)], and an olefin [δ 5.36 (1H, br s, H-12)]} together with four glycopyranosyl moieties [δ 5.00 (1H, d, J = 7.5 Hz, H-1′), 5.00 (1H, d, J = 7.5 Hz, H-1′′′′), 5.79 (1H, d, J = 7.7 Hz, H-1′′), 5.80 (1H, d, J = 5.8 Hz, H-1′′′)], an acetyl group [δ 1.93 (3H, s, H_3-Ac)], and an angeloyl moiety [δ 2.03 (3H, s, H_3-Ang-5), 2.11 (3H, d, J = 7.0 Hz, H_3-Ang-4), 5.99 (1H, dq-like, H-Ang-3)]. The positions of two acyl groups in **5** were determined from a HMBC experiment, which showed long-range correlations between the 21-proton and the angeloyl carbonyl carbon (δ_C 167.8) and between the 22-proton and the acetyl carbonyl carbon (δ_C 170.9). Furthermore, comparison of the ^{13}C NMR data for **5** with those for **4** revealed an acylation shift around the 22-position of the theasapogenol F moiety. On the other hand, the positions of two acyl groups in **6** were also clarified from a HMBC experiment, which exhibited long-range correlations between the 21-proton [δ 6.47 (1H, d, J = 10.1 Hz)] and the angeloyl carbonyl carbon (δ_C 168.5) and between the 28-protons [δ 4.33 (2H, m)] and the acetyl carbonyl carbon (δ_C 170.7). Consequently, the structures of theasaponins F_2 and F_3 were elucidated as 21-*O*-angeloyl-22-*O*-acetyltheasapogenol F 3-*O*-β-D-galactopyranosyl-(1→2)[β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**5**) and 21-*O*-angeloyl-28-*O*-acetyltheasapogenol F 3-*O*-β-D-galactopyranosyl(1→2)[β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**6**), respectively. The 6-secondary carboxyl group with a 5-oxygen function in D-glucuronic acid is known to be partly derived from the methyl ester by methanol treatment such as extraction under heating or chromatography with silica gel. In contrast, the tertiary carboxyl groups in their triterpene units could not be esterified under similar conditions. Although the D-glucuronic acid part in the oligoglycoside of theasaponins F_1−F_3 (**4**−**6**) is not esterified, they have a 23-methyl ester group in the triterpene portion. On the basis of this evidence, theasaponins F_1−F_3 (**4**−**6**) appear to be novel genuine triterpene saponins having a methyl ester function.

The effects of the principal theasaponins (**1**, **2**, **6**, **9**, and **10**) on ethanol-induced gastric mucosal lesions in rats were examined. Previously, we reported that several triterpene[1,7,11−13] and steroid[14] saponin, sesquiterpene,[15,16] phanylpropanoid,[17] and amide[18] constituents showed protective effects on ethanol- and/or indomethacin-induced gastric lesions in rats. Recently, we described the saponin fraction from the seeds of *C. sinensis*, and its principal constituents, theasaponins E_1 (**7**) and E_2 (**8**), were found to show potent protective effects on ethanol-induced gastric lesions in rats [inhibition (%) at 5.0 mg/kg, p.o.; 71.4 and 77.6, respectively].[1] To clarify the structure−activity relationships of theasaponins for protective activity on ethanol-induced gastric lesions, we further examined several additional theasaponin constituents (**1**, **2**, **6**, **9**, and **10**). As shown in Table 2, theasaponin A_2 (**2**) and assamsaponins A (**9**) and D (**10**) significantly inhibited ethanol-induced gastric mucosal lesions at a dose of 5.0 mg/kg, p.o. [inhibition (%) = 54.7, 61.0, and 47.9, respectively]. However, the gastroprotective activities of **2**, **9**, and **10** tended to be weaker than those of theasaponins E_1 (**7**) and E_2 (**8**). On the basis of the present results, the following structure−activity relationships of theasaponins for gastroprotective activity may be suggested: (1) the 28-acetyl moiety enhances the activity; (2) theasaponins having a 23-aldehyde group exhibit more potent activities than those with a 23-hydroxymethyl group or a 23-methoxycarbonyl group.

Table 2. Inhibitory Effects of 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)[a]	gastric lesions: inhibition (%)
control		6	162.6 ± 16.4	
theasaponin A_1 (**1**)	5.0	5	102.4 ± 12.2	37.0
theasaponin A_2 (**2**)	5.0	4	73.6 ± 21.5[b]	54.7
theasaponin F_3 (**6**)	5.0	5	94.2 ± 24.1	42.1
assamsaponin A (**9**)	5.0	4	63.3 ± 20.1[c]	61.0
assamsaponin D (**10**)	5.0	5	84.8 ± 9.9[b]	47.9
control		6	159.2 ± 21.0	
omeprazole[d]	10	6	90.6 ± 21.2[c]	43.1
	15	6	28.6 ± 13.4[c]	82.0
	20	6	16.9 ± 6.1[c]	89.4
control		6	148.4 ± 9.8	
cetraxate hydrochloride[d]	75	6	87.2 ± 7.4[c]	41.2
	150	6	51.0 ± 4.0[c]	65.6
	300	6	30.5 ± 8.3[c]	79.4

[a] Values represent the means ± SEM. [b] Significantly different from the control group, $p < 0.05$. [c] Significantly different from the control group, $p < 0.01$. [d] Omeprazole and cetraxate hydrochloride were used as positive controls.

Experimental Section

General Experimental Procedures. 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; ^1H NMR spectra, JEOL JNM-LA500 (500 MHz) spectrometer; ^{13}C NMR spectra, JEOL JNM-LA500 (125 MHz) spectrometer with tetramethylsilane as an internal standard; FABMS and HRFABMS, JEOL JMS-SX 102A mass spectrometer; HPLC detector, Shimadzu RID-6A refractive index and SPD-10A UV−vis detectors; HPLC column, YMC-Pack ODS-A and Develosil C30-UG-5 (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 silica gel column chromatography, silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150−350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100−200 mesh); TLC, precoated TLC plates with silica gel 60F254 (Merck, 0.25 mm) (normal-phase) and silica gel RP-18 F254S (Merck, 0.25 mm) (reversed-phase); reversed-phase HPTLC, precoated TLC plates with silica gel RP-18 WF254S (Merck, 0.25 mm); detection was carried out spraying with 1% $Ce(SO_4)_2$−10% aqueous H_2SO_4, followed by heating.

Plant Material. As described in a previous report.[1]

Extraction and Isolation. Fractions 5 (2.20 g) and 8 (0.97 g) were obtained from the saponin fraction (=methanol-eluted fraction) of the seeds of *C. sinensis* (1.0 kg, cultivated in Shizuoka Prefecture, Japan) as reported previously.[1] 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)] to give 12 fractions {[Fr. 5-1 (13 mg), Fr. 5-2 [=theasaponin A_1 (**1**, 53 mg, 0.021%)], Fr. 5-3 [=theasaponin F_1 (**4**, 23 mg, 0.009%)], Fr. 5-4 (14 mg), Fr. 5-5 (37 mg), Fr. 5-6 (164 mg), Fr. 5-7 (100 mg), Fr. 5-8 (328 mg), Fr. 5-9 [=theasaponin A_3 (**3**, 148 mg, 0.059%)], Fr. 5-10 (200 mg), Fr. 5-11 (645 mg), and Fr. 5-12 (85 mg)]. Fraction 5-8 (328 mg) was subjected to HPLC [Develosil C30-UG-5, 250 × 20 mm i.d., CH_3CN−MeOH−1% aqueous AcOH (35:16:49)] to afford four fractions {Fr. 5-8-1 [=camelliasaponin C_1 (10 mg, 0.004%)], Fr.

5-8-2 (77 mg), Fr. 5-8-3 [=theasaponin F_2 (**5**, 54 mg, 0.021%)], and Fr. 5-8-4 (26 mg)}. Fraction 8 (0.97 g) was subjected to HPLC [YMC-Pack ODS-A, 250 × 20 mm i.d., CH_3CN−1% aqueous AcOH (43:57)] to produce five fractions {Fr. 8-1 [=theasaponin A_2 (**2**, 323 mg, 0.13%)], Fr. 8-2 [=theasaponin F_3 (**6**, 136 mg, 0.054%)], Fr. 8-3 (46 mg), Fr. 8-4 (84 mg), and Fr. 8-5 (82 mg)}.

Theasaponin A_1 (1): colorless fine crystals from $CHCl_3$−MeOH; mp 219.3−220.4 °C; $[\alpha]_D^{27}$ +6.5 (*c* 2.50, MeOH); IR (KBr) ν_{max} 3453, 1719, 1650, 1078 cm^{-1}; ^1H NMR (500 MHz, pyridine-d_5) δ 0.89, 0.90, 1.07, 1.10, 1.32, 1.80 (3H each, all s, H_3-26, 25, 24, 29, 30, 27), 1.98 (3H, s, H_3-Ang-5), 2.06 (3H, d, J = 7.3 Hz, H_3-Ang-4), 2.92 (1H, dd-like, H-18), 3.68, 3.96 (1H each, both d, J = 10.4 Hz, H_2-28), [3.77 (1H, d, J = 10.4 Hz), 4.42 (1H, m), H_2-23], 4.15 (1H, m, H-3), 4.80 (1H, d, J = 10.1 Hz, H-22), 4.84 (1H, br s, H-16), 5.02 (1H, d, J = 7.7 Hz, H-1′′′′), 5.06 (1H, d, J = 7.7 Hz, H-1′), 5.37 (1H, br s, H-12), 5.78 (1H, d, J = 6.1 Hz, H-1′′′), 5.88 (1H, d, J = 7.9 Hz, H-1′′), 5.90 (1H, dq-like, H-Ang-3), 6.46 (1H, d, J = 10.1 Hz, H-21); ^{13}C NMR data, see Table 1; positive-ion FABMS m/z 1213 $[M + Na]^+$; negative-ion FABMS m/z 1189 $[M - H]^-$, 1057 $[M - C_5H_9O_4]^-$, 1027 $[M - C_6H_{11}O_5]^-$, 925 $[M - C_{10}H_{17}O_8]^-$; HRFABMS m/z 1213.5627 (calcd for $C_{57}H_{90}O_{26}Na$ $[M + Na]^+$, 1213.5618).

Theasaponin A_2 (2): colorless fine crystals from $CHCl_3$−MeOH; mp 219.6−221.1 °C; $[\alpha]_D^{27}$ +23.2 (*c* 2.00, MeOH); IR (KBr) ν_{max} 3453, 1721, 1650, 1080 cm^{-1}; ^1H NMR (500 MHz, pyridine-d_5) δ 0.91, 1.01, 1.05, 1.09, 1.29, 1.78 (3H each, all s, H_3-25, 26, 24, 29, 30, 27), 1.97 (3H, s, H_3-Ang-5), 1.99 (3H, s, H_3-Ac), 2.04 (3H, d, J = 7.4 Hz, H_3-Ang-4), 3.05 (1H, dd-like, H-18), [3.76 (1H, d, J = 10.7 Hz), 4.37 (1H, m), H_2-23], 4.13 (1H, m, H-3), 4.38 (2H, m, H_2-28), 4.44 (1H, d, J = 10.1 Hz, H-22), 4.71 (1H, br s, H-16), 5.00 (1H, d, J = 7.7 Hz, H-1′′′′), 5.04 (1H, d, J = 7.3 Hz, H-1′), 5.44 (1H, br s, H-12), 5.75 (1H, d, J = 6.1 Hz, H-1′′′), 5.85 (1H, d, J = 7.6 Hz, H-1′′), 5.90 (1H, dq-like, H-Ang-3), 6.46 (1H, d, J = 10.1 Hz, H-21); ^{13}C NMR data, see Table 1; positive-ion FABMS m/z 1277 $[M + 2Na - H]^+$, 1255 $[M + Na]^+$; negative-ion FABMS m/z 1231 $[M - H]^-$, 1099 $[M - C_5H_9O_4]^-$, 1069 $[M - C_6H_{11}O_5]^-$, 967 $[M - C_{10}H_{17}O_8]^-$; HRFABMS m/z 1255.5729 (calcd for $C_{59}H_{92}O_{27}Na$ $[M + Na]^+$, 1255.5724).

Theasaponin A_3 (3): colorless fine crystals from $CHCl_3$−MeOH; mp 228.0−229.2 °C; $[\alpha]_D^{27}$ −8.9 (*c* 0.95, MeOH); IR (KBr) ν_{max} 3453, 1731, 1674, 1080 cm^{-1}; ^1H NMR (500 MHz, pyridine-d_5) δ 0.78, 0.86, 1.06, 1.06, 1.26, 1.41 (3H each, all s, H_3-26, 25, 24, 29, 30, 27), 1.98 (3H, s, H_3-Ang-5), 2.04, 2.50 (3H each, both s, H_3-22-, 16-Ac), 2.06 (3H, d, J = 7.4 Hz, H_3-Ang-4), 3.00 (1H, dd-like, H-18), 3.46, 3.59 (1H each, both d, J = 10.7 Hz, H_2-28), 3.76, 4.40 (1H each, both m, H_2-23), 4.12 (1H, m, H-3), 5.01 (1H, d, J = 7.6 Hz, H-1′′′′), 5.06 (1H, d, J = 7.6 Hz, H-1′), 5.38 (1H, br s, H-12), 5.59 (1H, br s, H-16), 5.76 (1H, d, J = 6.1 Hz, H-1′′′), 5.85 (1H, d, J = 7.6 Hz, H-1′′), 5.86 (1H, d, J = 10.4 Hz, H-21), 6.00 (1H, dq-like, H-Ang-3), 6.13 (1H, d, J = 10.4 Hz, H-22); ^{13}C NMR data, see Table 1; positive-ion FABMS m/z 1319 $[M + 2Na - H]^+$, 1297 $[M + Na]^+$; negative-ion FABMS 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 $[M - C_{16}H_{27}O_{13}]^-$; HRFABMS m/z 1297.5839 (calcd for $C_{61}H_{94}O_{28}Na$ $[M + Na]^+$, 1297.5829).

Theasaponin F_1 (4): colorless fine crystals from $CHCl_3$−MeOH; mp 230.4−231.1 °C; $[\alpha]_D^{27}$ +29.8 (*c* 0.70, MeOH); IR (KBr) ν_{max} 3453, 1717, 1647, 1080 cm^{-1}; ^1H NMR (500 MHz, pyridine-d_5) δ 0.80, 0.81, 1.10, 1.31, 1.52, 1.80 (3H each, all s, H_3-25, 26, 29, 30, 24, 27), 1.98 (3H, s, H_3-Ang-5), 2.05 (3H, d, J = 7.0 Hz, H_3-Ang-4), 3.04 (1H, dd-like, H-18), 3.65, 3.93 (1H each, both d, J = 10.1 Hz, H_2-28), 3.69 (3H, s, COOCH_3), 4.30 (1H, m, H-3), 4.78 (1H, d, J = 10.1 Hz, H-22), 4.83 (1H, br s, H-16), 4.98 (1H, d, J = 7.4 Hz, H-1′), 4.99 (1H, d, J = 7.6 Hz, H-1′′′′), 5.34 (1H, br s, H-12), 5.77 (1H, d, J = 7.6 Hz, H-1′′), 5.79 (1H, d, J = 6.1 Hz, H-1′′′), 5.90 (1H, dq-like, H-Ang-3), 6.46 (1H, d, J = 10.4 Hz, H-21); ^{13}C NMR data, see Table 1; positive-ion FABMS m/z 1263 $[M + 2Na - H]^+$, 1241 $[M + Na]^+$; negative-ion FABMS m/z 1217 $[M - H]^-$, 1085 $[M - C_5H_9O_4]^-$, 953 $[M - C_{10}H_{17}O_8]^-$; HRFABMS m/z 1241.5575 (calcd for $C_{58}H_{90}O_{27}Na$ $[M + Na]^+$, 1241.5567).

Theasaponin F_2 (5): colorless fine crystals from $CHCl_3$−MeOH; mp 211.3−212.8 °C; $[\alpha]_D^{27}$ +8.5 (*c* 2.00, MeOH); IR (KBr) ν_{max} 3453, 1743, 1645, 1078 cm^{-1}; ^1H NMR (500 MHz, pyridine-d_5) δ 0.80, 0.80, 1.07, 1.31, 1.53, 1.80 (3H each, all s, H_3-25, 26, 29, 30, 24, 27), 1.93 (3H, s, H_3-Ac), 2.03 (3H, s, H_3-Ang-5), 2.11 (3H, d, J = 7.0 Hz, H_3-Ang-4), 3.05 (1H, dd-like, H-18), 3.37, 3.59 (1H each, both d, J = 10.4 Hz, H_2-28), 3.73 (3H, s, COOCH_3), 4.45 (1H, m, H-3), 4.42 (1H, br s, H-16), 5.00 (1H, d, J = 7.5 Hz, H-1′), 5.00 (1H, d, J = 7.5 Hz, H-1′′′′), 5.36 (1H, br s, H-12), 5.79 (1H, d, J = 7.7 Hz, H-1′′), 5.80 (1H, d, J = 5.8 Hz, H-1′′′), 5.99 (1H, dq-like, H-Ang-3), 6.20 (1H, d, J = 9.8 Hz, H-22), 6.61 (1H, d, J = 9.8 Hz, H-21); ^{13}C NMR data, see Table 1; positive-ion FABMS m/z 1283 $[M + Na]^+$; negative-ion FABMS m/z 1259 $[M - H]^-$, 1097 $[M - C_6H_{11}O_5]^-$, 995 $[M - C_{10}H_{17}O_8]^-$; HRFABMS m/z 1283.5660 (calcd for $C_{60}H_{92}O_{28}Na$ $[M + Na]^+$, 1283.5673).

Theasaponin F_3 (6): colorless fine crystals from $CHCl_3$−MeOH; mp 216.9−217.7 °C; $[\alpha]_D^{27}$ +25.1 (*c* 0.95, MeOH); IR (KBr) ν_{max} 3453, 1718, 1650, 1080 cm^{-1}; ^1H NMR (500 MHz, pyridine-d_5) δ 0.80, 0.93, 1.09, 1.29, 1.54, 1.79 (3H each, all s, H_3-25, 26, 29, 30, 24, 27), 1.97 (3H, s, H_3-Ang-5), 1.99 (3H, s, H_3-Ac), 2.05 (3H, d, J = 7.3 Hz, H_3-Ang-4), 2.82 (1H, dd-like, H-18), 3.71 (3H, s, COOCH_3), 4.33 (2H, m, H_2-28), 4.35 (1H, m, H-3), 4.49 (1H, d, J = 10.1 Hz, H-22), 4.71 (1H, br s, H-16), 4.97 (1H, d, J = 7.4 Hz, H-1′), 4.98 (1H, d, J = 7.6 Hz, H-1′′′′), 5.41 (1H, br s, H-12), 5.76 (1H, d, J = 8.0 Hz, H-1′′), 5.77 (1H, d, J = 6.7 Hz, H-1′′′), 5.90 (1H, dq-like, H-Ang-3), 6.47 (1H, d, J = 10.1 Hz, H-21); ^{13}C NMR data, see Table 1; positive-ion FABMS m/z 1305 $[M + 2Na - H]^+$, 1283 $[M + Na]^+$; negative-ion FABMS m/z 1259 $[M - H]^-$, 1127 $[M - C_5H_9O_4]^-$, 1097 $[M - C_6H_{11}O_5]^-$, 995 $[M - C_{10}H_{17}O_8]^-$, 965 $[M - C_{11}H_{19}O_9]^-$; HRFABMS m/z 1283.5682 (calcd for $C_{60}H_{92}O_{28}Na$ $[M + Na]^+$, 1283.5673).

Alkaline Hydrolysis of Theaponins A_1 (1), A_2 (2), A_3 (3), F_1 (4), F_2 (5), and F_3 (6). A solution of each theasaponin (**1**−**5**: 10 mg each, **6**: 30 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 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); 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**, **5**, and **6** and angelic acid (**b**, t_R 16.0 min) from **1**−**6**. The remainder 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)] to give desacyl-assamsaponin D (**1a**,[5] 6 mg each, from **1**−**3**) and desacyl-theasaponin F (**4a**, 6 mg each, from **4** and **5**; 22 mg from **6**).

Desacyl-theasaponin F (4a): colorless fine crystals from $CHCl_3$−MeOH; mp 223.8−224.3 °C; $[\alpha]_D^{27}$ +16.5 [*c* 1.00, MeOH−H_2O (6:1)]; IR (KBr) ν_{max} 3410, 1719, 1655, 1078 cm^{-1}; ^1H NMR [500 MHz, pyridine-d_5−D_2O (6:1)] δ 0.81, 0.81, 1.23, 1.31, 1.53, 1.82 (3H each, all s, H_3-25, 26, 29, 30, 24, 27), 3.67, 3.95 (1H each, both d-like, H_2-28), 3.87 (3H, s, COOCH_3), 4.28 (1H, m, H-3), 4.55 (1H, m, H-22), 4.68 (1H, d-like, H-21), 4.81 (1H, br s, H-16), 4.81 (1H, d-like, H-1′), 5.08 (1H, d-like, H-1′′′′), 5.40 (1H, br s, H-12), 5.68 (1H, d-like, H-1′′), 5.75 (1H, d-like, H-1′′′); ^{13}C NMR data, see Table 1; positive-ion FABMS m/z 1159 $[M + Na]^+$; negative-ion FABMS m/z 1135 $[M - H]^-$, 1003 $[M - C_5H_9O_4]^-$, 871 $[M - C_{10}H_{17}O_8]^-$, 709 $[M - C_{16}H_{27}O_{13}]^-$; HRFABMS m/z 1159.5138 (calcd for $C_{58}H_{90}O_{27}Na$ $[M + Na]^+$, 1159.5149).

Methanolysis of 4a. A solution of **4a** (10 mg) in 9% HCl−dry-MeOH (1.0 mL) was heated under reflux for 2 h. After cooling, the reaction mixture was poured into ice−water and the whole was extracted with EtOAc. The EtOAc extract was successively washed with saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by normal-phase silica gel column chromatography [1.0 g, $CHCl_3$−MeOH−H_2O (10:3:1, lower layer)] to give theasapogenol F (**4b**, 3.0 mg).

Theasapogenol F (4b): colorless fine crystals from MeOH; mp 271.1−272.4 °C; $[\alpha]_D^{27}$ +10.9 (*c* 0.50, MeOH); IR (KBr) ν_{max} 3453, 1711, 1647 cm^{-1}; ^1H NMR (500 MHz, pyridine-d_5) δ 0.93, 0.96, 1.30, 1.37, 1.53, 1.80 (3H each, all s, H_3-25, 26, 29, 30, 24, 27), 2.79 (1H, dd, J = 3.8, 13.2 Hz, H-18), 3.61 (3H, s, COOCH_3), 3.71, 3.83 (1H each, both d-like, H_2-28), 4.00 (1H, dd, J = 5.2, 10.4 Hz, H-3), 4.61 (1H, d, J = 9.5 Hz, H-22), 4.76 (1H, d, J = 9.5 Hz, H-21), 4.99 (1H, br s, H-16), 5.41 (1H, br s, H-12); ^{13}C NMR (125 MHz, pyridine-d_5) δ_C 39.1 (C-1), 27.7 (C-2), 77.3 (C-3), 54.9 (C-4), 51.7 (C-5), 21.8 (C-6), 32.9 (C-7), 40.4 (C-8), 47.3 (C-9), 36.7 (C-10), 23.9 (C-11), 123.3 (C-12), 144.0 (C-13), 42.0 (C-14), 34.3 (C-15), 67.7 (C-16), 47.4 (C-

17), 41.2 (C-18), 48.2 (C-19), 36.4 (C-20), 78.6 (C-21), 75.2 (C-22), 176.2 (C-23), 11.9 (C-24), 16.2 (C-25), 16.8 (C-26), 27.3 (C-27), 68.4 (C-28), 30.5 (C-29), 19.5 (C-30), 52.2 (COO*C*H$_3$); positive-ion FABMS *m/z* 557 $[M + Na]^+$; negative-ion FABMS *m/z* 533 $[M - H]^-$; HRFABMS *m/z* 557.5022 (calcd for $C_{31}H_{50}O_7Na$ $[M + Na]^+$, 557.5014).

$NaBH_4$ Reduction of 4a. A solution of **4a** (4.0 mg) in EtOH (2.0 mL) was treated with $NaBH_4$ (4.0 mg), and the mixture was stirred at room temperature for 2 h. The reaction mixture was quenched in acetone, and then removal of the solvent under reduced pressure yielded a reduction mixture. The reduction mixture was purified by normal-phase silica gel column chromatography [0.5 g, $CHCl_3$−MeOH−H_2O (5:4:1)] to give desacyl-assamsaponin D^5 (**1a**, 2.4 mg).

Bioassay Procedure. Animals. Male ddY rats weighing about 25−30 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 the experiments were performed with conscious rats unless otherwise noted. The experimental protocol was approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

Effect of Ethanol-Induced Gastric Mucosal Lesions in Rats. Acute gastric lesions were induced by oral administration of ethanol according to the method described previously.[1,13,16−18] Briefly, 99.5% ethanol (1.5 mL/rat) was administered to 24−26 h fasted rats using a metal orogastric tube. One hour after administration of ethanol, the animals were killed by cervical dislocation under ether anesthesia and the stomach was removed and inflated by injection of 10 mL of 1.5% formalin to fix the inner and outer layers of the gastric walls. Subsequently, the stomach was incised along the greater curvature and the lengths of gastric lesions were measured as previously described; the total length (mm) was expressed as a lesion index. Compounds **1**, **2**, **9**, and **10** 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.

Statistics. Values are expressed as means ± SEM. For statistical analysis, one-way analysis of variance followed by Dunnett's test was used. Probability (*P*) values less than 0.05 are considered significant.

Note Added after ASAP Publication: In the version posted on Jan 13, 2006, there were errors in Chart 1 and Figure 1. The corrected graphics appear in the version posted on Feb 3, 2006. On Feb. 9, 2006, a further change was made in paragraph 1 of the second page to correct an NMR resonance value.

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References and Notes

(1) This paper is number 24 in the series "Bioactive Saponins and Glycosides". For paper number 23, see: Yoshikawa, M.; Morikawa, T.; Li, N.; Nagatomo, A.; Li, X.; Matsuda, H. *Chem. Pharm. Bull*. **2005**, *53*, 1559−1564.

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(9) The ^{1}H and ^{13}C NMR spectra of **1**−**6** 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.

(10) Previously, we found that the 30-ester glycoside (licorice-saponin A_3 dimethyl ester from *Glycyrrhiza uralensis*) was reduced to the 30-hydroxyl methyl group by $NaBH_4$ at room temperature. It seems that the methyl ester in **4a** was also reduced by $NaBH_4$ in a similar manner: Kitagawa, I.; Hori, K.; Taniyama, T.; Zhou, J.-L.; Yoshikawa, M. *Chem. Pharm. Bull.* **1993**, *41*, 43−49.

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