Bioactive Saponins and Glycosides. XV.[1] Saponin Constituents with Gastroprotective Effect from the Seeds of Tea Plant, *Camellia sinensis* L. var. *assamica* PIERRE, Cultivated in Sri Lanka: Structures of Assamsaponins A, B, C, D, and E

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The saponin fraction from the seeds of the tea plant, *Camellia sinensis* L. var. *assamica* PIERRE cultivated in Sri Lanka, was found to show a potent protective effect on gastric mucosal lesions induced by ethanol in rats. Nine new acylated polyhydroxyoleanene-type triterpene oligoglycosides called assamsaponins A—I were isolated from the active saponin fraction together with three known saponins, theasaponin E_1 and E_2 and camelliasaponin B_1. The structures of assamsaponins A—E were elucidated on the basis of chemical and physicochemical evidence. Theasaponin E_1 exhibited potent gastroprotective activity.

Key words assamsaponin; *Camellia sinensis* var. *assamica*; gastroprotective effect; tea plant; oleanane-type triterpene oligoglycoside

During the course of our chemical and pharmacological studies on the saponin constituents of natural medicines[1,2] and medicinal foodstuffs,[3] we reported the isolation and structure elucidation of six acylated polyhydroxyolean-12-ene oligoglycosides, camelliasaponins A_1, A_2, B_1 (**2**), B_2, C_1, and C_2, from the seeds of camellia (*Camellia japonica* L., Theaceae).[4] Camelliasaponins were found to show potent inhibitory activity on ethanol absorption in rats and the structure requirement of camelliasaponin for the inhibitory activity was characterized by comparison of the activities of the related compounds.[4] We recently isolated two acylated polyhydroxyolean-12-ene oligoglycosides, theasaponins E_1 (**6**) and E_2 (**7**), from the seeds of Japanese tea plant [*Camellia sinensis* (L.) O. KUNTZE, *Thea sinensis* L.] and elucidated their structures and antisweet activities.[5] In a continuing study of the tea plant, we have examined the saponin constituents from the seeds of *Camellia sinensis* L. var. *assamica* PIERRE, which is widely cultivated in Sri Lanka, India, and Indonesia and processed to black tea. As shown in Table 1, the saponin fraction from the seeds of *Assamica* variety showed a remarkable protective effect against gastric lesions induced by ethanol in rats. From the saponin fraction, nine new acylated polyhydroxyolean-12-ene oligoglycosides called assamsaponins A (**1**), B (**4**), C (**5**), D (**9**), E (**11**), F, G, H, and I, have been isolated together with three known saponins, theasaponins E_1 (**6**) and E_2 (**7**) and camelliasaponin B_1 (**2**). This paper deals with the isolation of assamsaponins and the structure elucidations of assamsaponins A (**1**), B (**4**), C (**5**), D (**9**), and E (**11**). We also describe the gastroprotective effect of theasaponin E_1 (**6**), which is isolated from the seeds of both varieties of tea plant.[6]

The methanolic extract obtained from the seeds of tea plant, which was cultivated in Nuwara Eliya, Sri Lanka, was subjected to reversed-phase silica gel column chromatography to remove the sugar and lipid components. The methanol-eluted fraction was separated by normal-phase silica gel column chromatography to give the saponin fraction (fraction 2). As is apparent from Table 1, the saponin fraction was found to exhibit a potent protective effect against ethanol-induced gastric lesions in rats, and to dose-dependently reduce the scores of lesions and to improve the pathogenic changes (data not shown). The saponin fraction was purified by HPLC to afford assamsaponins A (**1**), B (**4**), C (**5**), D (**9**), E (**11**), F, G, H, and I together with theasaponins E_1[5] (**6**) and E_2[5] (**7**) and camelliasaponin B_1[4] (**2**). Theasaponin E_1 (**6**) was also isolated from the seeds of Japanese tea plant as the principle constituent,[4] and the gastroprotective effect was examined as a representative of tea saponins. Theasaponin E_1 (**6**) was found to much more strongly reduce the lengths and scores than the reference drug omeprazole, which showed significant inhibition at a dose of 10 mg/kg.

Structures of 1, 4, 5, 9, and 11 Assamsaponin A (**1**) was isolated as colorless fine crystals of mp 211.7—212.2 °C. The IR spectrum of **1** showed absorption bands at 1721 and 1655 cm^{-1} assignable to carbonyl and α,β-unsaturated esters, and broad bands at 3432, 1078, and 1048 cm^{-1} suggestive of a glycosidic structure. In the negative- and positive-ion FAB-MS of **1**, quasimolecular ion peaks were observed at *m/z* 1171 $(M-H)^-$ and *m/z* 1195 $(M+Na)^+$, and high-resolution MS analysis revealed the molecular formula of **1** to be $C_{57}H_{88}O_{25}$. Furthermore, fragment ion peaks at *m/z* 1039 $(M-C_5H_9O_4)^-$ and *m/z* 907 $(M-C_{10}H_{17}O_8)^-$, which were derived by cleavage of the glycosidic linkage at the 2‴ and 3′-positions, were observed in the negative-ion FAB-MS of **1**. Alkaline hydrolysis of **1** with 10% aqueous potassium hydroxide–50% aqueous dioxane (1 : 1) provided desacyl-assamsaponin A (**3**) and angelic acid. The angelic acid was converted to the *p*-nitrobenzyl ester,[7] which was identified by HPLC analysis.

Desacyl-assamsaponin A (**3**), whose molecular formula $C_{52}H_{82}O_{24}$ was determined by the negative- and positive-ion FAB-MS [*m/z* 1089 $(M-H)^-$, 957 $(M-C_5H_9O_4)^-$, 825 $(M-C_{10}H_{17}O_8)^-$; *m/z* 1113 $(M+Na)^+$] and high-resolution MS measurement, liberated D-glucuronic acid, D-galactose, L-arabinose, and D-xylose by acid hydrolysis with 5% aqueous H_2SO_4–1,4-dioxane (1 : 1), which were identified by GLC analysis[8] of their trimethylsilyl thiazolidine derivatives. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **3**, which were assigned by various NMR analytical methods,[9] showed signals due to a camelliagenin B[4] moiety [δ 0.82,

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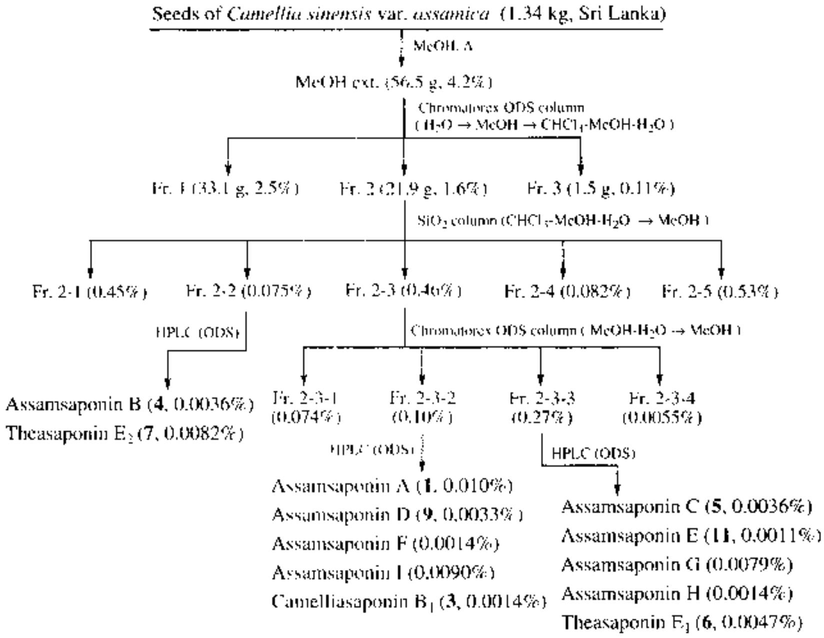

Chart 1

0.85, 1.05, 1.27, 1.43, 1.79 (all s, 25, 26, 29, 30, 24, 27-H_3), 2.43 (dd-like, 18-H), 3.65, 4.05 (both m, 28-H_2), 4.01 (m, 3-H), 4.60 (dd-like, 22-H), 5.10 (br s, 16-H), 5.32 (br s, 12-H), 9.84 (s, 23-H)], a *β*-D-glucuronopyranosyl moiety [*δ* 4.83 (d, *J*=7.3 Hz, 1′-H)], a *β*-D-galactopyranosyl moiety [*δ* 5.68 (d-like, 1″-H)], an *α*-L-arabinopyranosyl moiety [*δ* 5.65 (d-like, 1‴-H)], and a *β*-D-xylopyranosyl moiety [*δ* 5.00 (d, *J*=7.6 Hz, 1⁗-H)]. The tetrasaccharide structure bonding to the 3-position of camelliagenin B was identified by heteronuclear multiple bond connectivity (HMBC) experiment. Thus, long-range correlations were observed between the 1-proton of glucuronic acid moiety and the 3-carbon of camelliagenin B moiety, between the 1-proton of the galactopyranosyl moiety and the 2-carbon of the glucuronic acid moiety, between the 1-proton of the arabinopyranosyl moiety and 3-carbon of glucuronic acid moiety, and between the 1-proton of the xylopyranosyl moiety and the 2-carbon of the arabinopyranosyl moiety. On the basis of this evidence, the structure of **3** was elucidated as shown.

The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[9] of **1** showed signals assignable to an angeloyl group at *δ* 1.96 (s, 5‴″-H_3), 2.08 (d, *J*=6.9 Hz, 4‴″-H_3), 6.00 (dq-like, 3‴″-H) together with the desacyl-assamsaponin A moiety [*δ* 6.12 (dd-like, 22-H)]. The position of the angeloyl group of **1** was determined by HMBC, which showed a long-range correlation between the 22-proton of the camelliagenin B moiety and the 1-carbonyl carbon of the angeloyl moiety. Finally, comparison of the NMR data for **1** with those for **3** revealed an acylation shift around the 22-position of the camelliagenin B moiety. Consequently, the structure of assamsaponin A was determined to be 22-*O*-angeloylcamelliagenin B 3-*O*-[*β*-D-galactopyranosyl(1→2)][*β*-D-xylopyranosyl(1→2)-*α*-L-arabinopyranosyl(1→3)]-*β*-D-glucopyranosiduronic acid (**1**).

Assamsaponin B (**4**) was isolated as colorless fine crystals of mp 199.0—200.4 °C. Upon alkaline hydrolysis of **4**, desacyl-theasaponin E (**8**)[5] was obtained together with acetic acid and angelic acid, which were identified by HPLC analysis of their *p*-nitrobenzyl derivatives. The molecular formula $C_{61}H_{92}O_{28}$ was determined from negative- and positive-ion FAB-MS [*m/z* 1271 $(M-H)^-$, 1139 $(M-C_5H_9O_4)^-$, 1109 $(M-C_6H_{11}O_5)^-$, 1007 $(M-C_{10}H_{17}O_8)^-$; *m/z* 1295 $(M+Na)^+$] and by high-resolution MS measurement. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[9] of **4** showed the presence of an angelic acid moiety [*δ* 1.98 (s, 5‴″-H_3), 2.06 (d, *J*=7.3 Hz, 4‴″-H_3), 5.90 (dq-like, 3‴″-H)] and two acetic acid moieties [*δ* 2.03, 2.50 (both s, 2‴‴, 2‴‴′-H_3)] together with a *β*-D-glucuronopyranosyl moiety [*δ* 4.81 (d, *J*=7.3 Hz, 1′-H)], a *β*-D-galactopyranosyl moiety [*δ* 5.71 (d-like, 1″-H)], an *α*-L-arabinopyranosyl moiety [*δ* 5.71 (d-like, 1‴-H)], a *β*-D-xylopyranosyl moiety [*δ* 5.00 (d, *J*=7.6 Hz, 1⁗-H)], and a theasapogenol E moiety [*δ* 2.98 (dd, *J*=4.9, 13.6 Hz, 18-H), 3.45, 3.46 (both d, *J*=11.0 Hz, 28-H_2), 3.98 (dd-like, 3-H), 5.38 (br s, 12-H), 5.58 (br s, 16-H), 5.85 (d, *J*=10.4 Hz, 21-H), 6.09 (d, *J*=10.4 Hz, 22-H), 9.93 (s, 23-H)]. Furthermore, comparison of the ^{1}H-NMR and ^{13}C-NMR spectra of **4** with those of **8** revealed an acylation shift around the 16, 21, and 22-positions of **4**. In the HMBC of **4**, long-range correlations were observed between the 21-proton and 1-carbonyl carbon of the angeloyl group and between 16, 22-protons and 1-carbonyl carbons of two acetyl groups. Consequently, the structure of assamsaponin B was confirmed to be 16,22-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 (**4**).

Assamsaponin C (**5**), obtained as colorless fine crystals of mp 201.0—202.0 °C, liberated **8**, tiglic acid, and acetic acid upon alkaline hydrolysis. The organic acids were identified by HPLC analysis of their *p*-nitrobenzyl derivatives. The molecular formula $C_{61}H_{92}O_{28}$, which was the same as that of **6** and **7**, was determined from the negative- and positive-ion FAB-MS [*m/z* 1271 $(M-H)^-$, 1295 $(M+Na)^+$] and by high-resolution MS measurement. The carbon signals in the ^{13}C-NMR (Table 1) spectrum of **5** were shown to be superimpos-

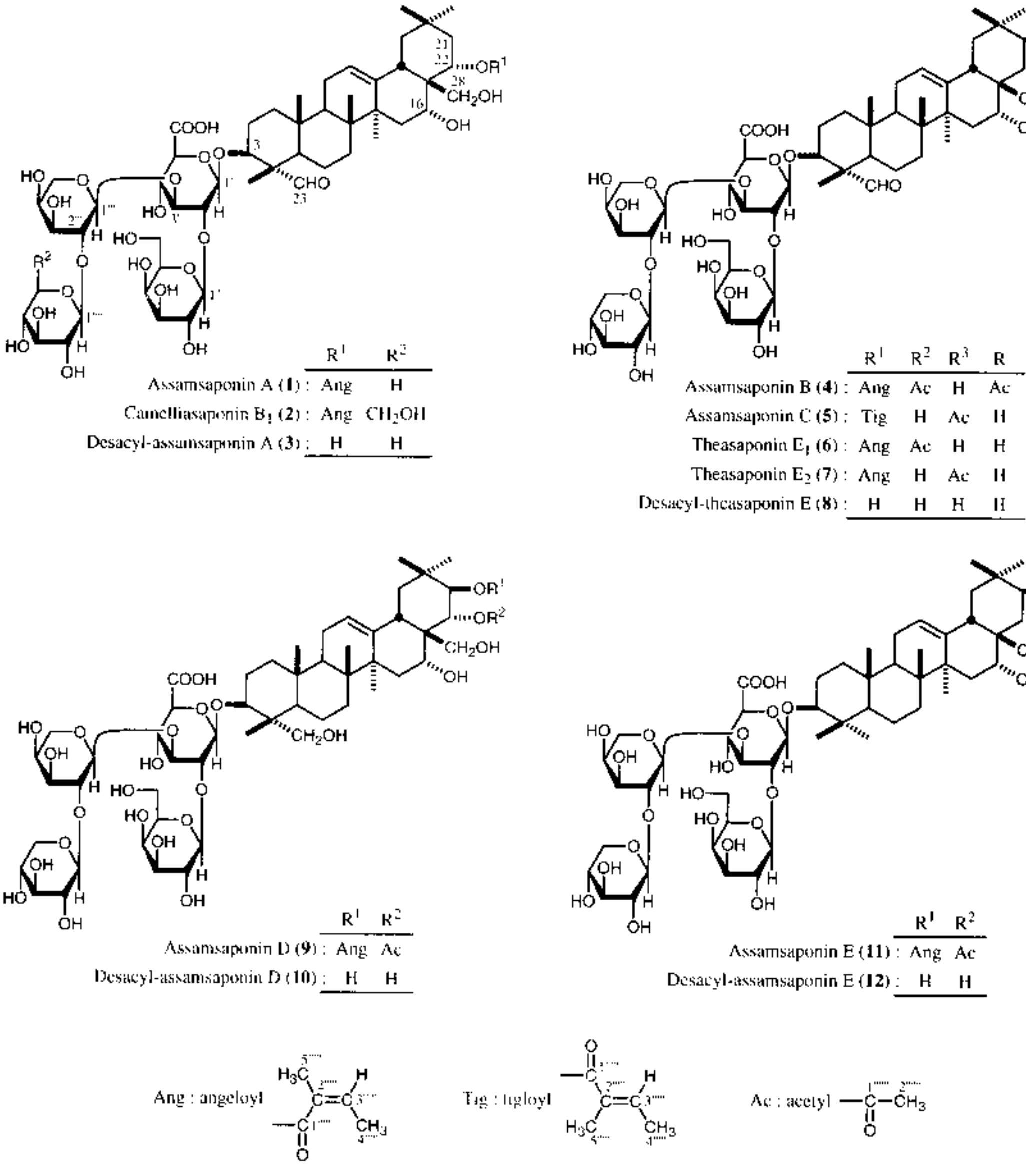

Chart 2

able on those of **7**, except for some signals assignable to a tigloyl group. The ^{1}H-NMR (pyridine-d_5) spectrum of **5** showed signals due to the tigloyl group [δ 1.86 (s, 5‴‴-H_3), 1.61 (d, J=6.3 Hz, 4‴‴-H_3), 7.00 (dq-like, 3‴‴-H)] and the acetyl group [δ 2.02 (s, 2‴‴-H_3)] bonding to the 21, 28-hydroxyl groups of the theasapogenol E moiety [δ 4.25 (m, 28-H_2), 6.38 (d, J=9.6 Hz, 21-H)]. In the HMBC of **5**, long-range correlations were observed between the 21-proton and the 1-carbonyl carbon of the tigloyl group and between the 28-proton and the 1-carbonyl carbon of the acetyl group. Finally, comparison of the ^{13}C-NMR data of **5** with those of **7** and **8** led us to confirm the structure of assamsaponin C as 21-*O*-tigloyl-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 (**5**).

Assamsaponin D (**9**) was isolated as colorless fine crystals of mp 190.6—191.2 °C, and it showed absorption bands due to hydroxyl, carbonyl, and α,β-unsaturated ester functions at 3432, 1719, 1649, 1078, and 1046 cm^{-1} in IR spectrum. The negative- and positive-ion FAB-MS of **9** showed quasimolec-

Table 1. Effects of Saponin Fraction from *Camellia sinensis* var. *assamica* and **6** on Gastric Mucosal Lesions Induced by Ethanol in Rats

Treatment	Dose (mg/kg, *p.o.*)	*n*	Gastric mucosal lesions Length (mm)	Inhibition (%)	Score
Control (5% Acacia)	—	8	156.2±11.1	—	7.4±0.2
Saponin fraction	5	6	124.1±11.1	20.6	5.0±0.7
	10	6	72.8±11.1*	53.4	2.5±0.2*
	20	6	39.7±8.7*	74.6	2.3±0.3*
	50	6	3.3±1.6*	97.9	0.7±0.3*
	100	6	1.5±0.8*	99.0	0.5±0.2*
Omeplazole	30	6	57.1±15.8*	63.4	4.5±1.0*
Control (PBS)	—	4	154.0±27.4	—	7.5±0.3
6	10	4	8.8±6.6*	94.3	1.3±0.3*

Each value represents the mean±S.E. Significantly different from the control group, $*p<0.01$.

Table 2. ^{13}C-NMR Data of **1**, **4**, **5**, **9**, **11**, **3**, **10**, and **12**

	1	**4**	**5**	**9**	**11**	**3**	**10**	**12**
C-1	38.1	38.2	38.2	38.8	39.1	39.2	38.8	38.9
2	25.0	25.2	25.2	25.7	26.7	25.2	25.3	26.6
3	84.2	84.5	84.4	83.3	89.9	84.2	83.3	89.7
4	55.0	55.1	55.0	43.5	39.8	55.1	43.5	39.7
5	48.2	48.5	48.4	48.3	56.1	48.4	48.3	55.9
6	20.2	20.3	20.3	18.3	18.6	20.4	18.3	18.5
7	32.3	32.4	32.3	32.9	33.4	32.4	32.9	33.3
8	40.2	40.3	40.2	40.2	40.3	40.4	48.3	40.1
9	46.7	46.8	46.8	47.2	47.2	46.9	47.3	47.1
10	35.9	36.0	36.0	36.8	37.0	36.1	36.8	36.9
11	23.6	23.7	23.8	23.9	24.1	23.8	23.9	23.9
12	124.1	124.8	124.2	122.7	124.1	122.7	122.7	122.7
13	143.6	141.1	142.6	142.9	142.9	144.3	143.9	144.0
14	41.6	41.2	41.7	41.7	42.0	42.2	42.0	42.1
15	34.9	31.0	34.4	34.6	34.9	34.6	34.3	34.4
16	70.0	71.3	67.6	68.2	68.0	68.3	67.9	67.9
17	44.7	46.9	47.0	48.0	47.3	44.8	47.1	47.3
18	40.8	39.6	40.5	40.2	40.8	42.5	41.3	41.3
19	47.3	47.2	47.2	47.0	47.6	47.8	48.2	48.3
20	31.9	36.1	36.2	36.3	36.3	31.7	36.3	36.4
21	41.6	78.4	81.6	79.0	81.6	46.0	78.8	78.8
22	72.9	73.4	71.4	74.6	71.7	74.4	77.5	77.3
23	209.6	210.0	209.7	65.0	28.3	209.6	65.0	28.1
24	10.8	11.1	10.9	13.5	17.0	11.0	13.5	16.8
25	15.6	15.8	15.7	16.2	16.0	15.8	16.2	15.9
26	16.7	16.8	16.9	17.0	17.3	16.9	17.0	17.0
27	27.4	26.9	27.2	27.5	27.6	27.4	27.4	27.3
28	63.7	63.8	66.4	64.0	66.8	70.0	68.4	68.6
29	33.3	29.4	29.6	29.4	30.0	25.4	19.4	19.4
30	25.0	19.7	20.0	20.3	20.4	33.7	30.5	30.6

	1	**4**	**5**	**9**	**11**	**3**	**10**	**12**
C-1′	103.9	104.2	104.0	104.2	105.8	104.1	104.1	105.6
2′	78.1	78.3	78.1	78.5	79.2	78.2	78.5	79.1
3′	84.0	84.2	84.0	84.6	84.3	84.3	84.7	84.2
4′	70.6	70.8	70.7	71.0	71.2	70.8	71.0	71.0
5′	77.0	77.3	77.2	77.3	77.5	77.3	77.3	77.6
6′	171.6	171.8	171.7	171.9	172.1	171.8	171.8	172.0
C-1″	103.0	103.3	103.1	103.1	103.7	103.2	103.1	103.5
2″	73.5	73.7	73.6	73.8	74.0	73.7	73.8	73.8
3″	75.1	75.4	75.2	75.2	75.3	75.4	75.2	75.2
4″	70.3	70.5	70.4	70.2	70.3	70.5	70.2	70.2
5″	76.3	76.5	76.4	76.5	76.6	76.5	76.5	76.4
6″	61.9	62.2	62.0	62.0	62.2	62.1	62.0	62.1
C-1‴	101.4	101.7	101.5	101.7	101.9	101.6	101.7	101.8
2‴	82.1	82.3	82.2	82.3	82.2	82.3	82.3	82.0
3‴	73.2	73.3	73.3	73.4	73.6	73.3	73.4	73.4
4‴	68.2	68.4	68.3	68.4	68.5	68.3	68.5	68.3
5‴	65.9	66.1	66.0	66.2	66.2	66.1	66.1	66.0
C-1⁗	106.8	107.0	106.9	107.0	107.1	107.0	107.1	106.9
2⁗	75.6	75.9	75.8	75.9	75.9	75.8	75.8	75.7
3⁗	78.0	78.2	78.0	78.2	78.4	78.2	78.2	78.3
4⁗	70.6	70.7	70.6	70.8	71.0	70.7	70.8	70.8
5⁗	67.2	67.5	67.3	67.4	67.7	67.5	67.5	67.5
C-1⁗′	167.9	167.8	168.5	167.9	168.8			
2⁗′	129.4	128.5	129.7	129.0	129.8			
3⁗′	136.3	138.0	136.2	137.0	136.0			
4⁗′	15.6	15.9	14.0	15.6	15.9			
5⁗′	20.7	20.9	12.3	21.0	21.1			
C-1⁗″		170.5	170.6	171.1	170.9			
2⁗″		20.8	20.6	20.9	20.9			
C-1⁗‴		169.8						
2⁗‴		22.0						

500 MHz, pyridine-d_5.

ular ion peaks at m/z 1231 $(M-H)^-$ and m/z 1255 $(M+Na)^+$ together with fragment ion peaks at m/z 1099 $(M-C_5H_9O_4)^-$ and m/z 967 $(M-C_{10}H_{17}O_8)^-$ and the molecular formula $C_{59}H_{92}O_{27}$ was determined by high-resolution MS measurement. The alkaline hydrolysis of **9** liberated desacyl-assamsaponin D (**10**) and two organic acids, angelic acid and acetic acid, which were identified by HPLC analysis. Acid hydrolysis of **10** liberated D-glucuronic acid, D-galactose, L-arabinose, and D-xylose, which were identified by GLC analysis. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[9] of **10** showed signals due to a theasapogenol A[5] moiety [δ 2.72 (dd-like, 18-H), 3.76, 4.35 (both m, 23-H$_2$), 3.61, 3.87 (both m, 28-H$_2$), 4.12 (m, 3-H), 4.54 (m, 22-H), 4.70 (d, J=9.7 Hz, 21-H), 4.97 (br s, 16-H)] and a tetrasaccharide moiety [δ 5.00 (d-like, 1⁗-H), 5.05 (d-like, 1′-H), 5.70 (d, J=6.4 Hz, 1‴-H), 5.83 (d, J=7.9 Hz, 1″-H)], which were very similar to those of **3** and **8**, except for the signals due to the 23-hydroxymethyl group. Furthermore, the HMBC of **10** showed long-range correlations between the following protons and carbons: 1′-H and 3-C; 1″-H and 2′-C; 1‴-H and 3′-C; 1⁗-H and 2‴-C. On the basis of these findings, the structure of desacyl-assamsaponin D (**10**) was determined as shown.

The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[9] of **9** showed the presence of an angeloyl group [δ 2.01 (s, 5⁗′-H$_3$), 2.09 (d, J=7.0 Hz, 4⁗′-H$_3$), 5.98 (dq-like, 3⁗′-H)] and an acetyl group [δ 1.95 (s, 2⁗″-H$_3$)] together with a desacyl-assamsaponin D moiety [δ 6.15 (d, J=10.1 Hz, 22-H), 6.55 (d, J=10.1 Hz, 21-H)]. The positions of the angeloyl and acetyl groups in **9** were determined by HMBC, which showed long-range correlations between the 21-proton of the theasapogenol A moiety and the 1-carbonyl carbon of the angeloyl group and between the 22-proton of the theasapogenol A moiety and the 1-carbonyl carbon of the acetyl group. Finally, comparison of the ^{1}H- and ^{13}C-NMR data for **9** with those of **10** revealed acylation shifts around the 21, 22-positions of the theasapogenol A moiety of **9**. Consequently, the structure of assamsaponin D was determined to be 21-*O*-angeloyl-22-*O*-acetyltheasapogenol A 3-*O*-[β-D-galactopyranosyl(1→2)][β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**9**)

Assamsaponin E (**11**) was also isolated as colorless fine crystals of mp 189.4—190.4 °C. In the negative- and positive-ion FAB-MS of **11**, quasimolecular ion peaks were observed at m/z 1215 $(M-H)^-$ and m/z 1239 $(M+Na)^+$, and high-resolution MS analysis revealed the molecular formula of **11** to be $C_{57}H_{88}O_{25}$. Fragment ion peaks were observed at m/z 1083 $(M-C_5H_9O_4)^-$, m/z 1053 $(M-C_6H_{11}O_5)^-$, and m/z 951 $(M-C_{10}H_{17}O_8)^-$, which were derived by cleavage of the glycosidic linkage at the 2‴, 2′, and 3′-positions. Alkaline hydrolysis of **11** with 10% aqueous potassium hydroxide-50% aqueous dioxane furnished desacyl-assamsaponin E (**12**) together with angelic acid and acetic acid, which were identified by HPLC analysis.

Desacyl-assamsaponin E (**12**) liberated D-glucuronic acid, D-galactose, L-arabinose, and D-xylose by heating with 5% aqueous sulfuric acid-1,4-dioxane, which were identified by GLC analysis. The proton and carbon signals due to the tetrasaccharide moiety in the ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[9] of **12** were superimposable on those of **8** and **10**. The HMBC experiment of **12** showed long-range correlations between the following protons and carbons: 1′-H and 3-C; 1″-H and 2′-C; 1‴-H and 3′-C; 1⁗-H and 2‴-C. Consequently, the structure of **12** was characterized as shown.

The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[9] of **11** showed signals due to the 21,28-acylated structure of **12**. In the HMBC experiment of **11**, long-range correlations were observed between the 21-proton and the 1-carbonyl carbon of the angeloyl group and between the 28-protons and the 1-carbonyl carbon of the acetyl group. Finally, comparison of the ^{13}C-NMR data for **11** with those of **12** led us to confirm the structure of assamsaponin E as 21-*O*-angeloyl-28-*O*-acetyltheasapogenol B 3-*O*-[β-D-galactopyranosyl(1→2)][β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**11**).

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper.[2,3]

Isolation of Assamsaponins A (1), B (4), C (5), D (9), E (11), F, G, H, and I and Known Compounds (2, 6, 7) from the Seeds of *Camellia sinensis* var. *assamica* The seeds of *Camellia sinensis* L. var. *assamica* PIERRE (1.34 kg, cultivated in Nuwara Eliya, Sri Lanka) were crushed and extracted three times with MeOH under reflux. Evaporation of the solvent under reduced pressure provided the MeOH extract (56.5 g, 4.2%). The MeOH extract (56.5 g) was subjected to reversed-phase silica gel column chromatography [Chromatorex DM1020T (Fuji Silysia Chemical, Ltd., 2 kg), H_2O→MeOH→$CHCl_3$–MeOH–H_2O (6 : 4 : 1)] to give three fractions [fr. 1 (33.1 g), fr. 2 (21.9 g), fr. 3 (1.3 g)]. Normal-phase silica gel column chromatography [BW-200 (Fuji Silysia Chemical Ltd., 1 kg,), $CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower layer→65 : 35 : 10, lower layer→6 : 4 : 1)→MeOH] of fr. 2 gave five fractions [fr. 2-1 (6.0 g), fr. 2-2 (1.0 g), fr. 2-3 (6.1 g), fr. 2-4 (1.1 g), fr. 2-5 (7.0 g)]. Fraction 2-2 (1.0 g) was purified by HPLC [YMC-Pack ODS-A (250×20 mm i.d.), 1) MeOH–H_2O (75 : 25, v/v); 2) CH_3CN–H_2O (40 : 60, v/v)] to give assamsaponin B (**4**, 38 mg, 0.0028%) and theasaponin E_2 (**7**, 110 mg, 0.0082%). Fraction 2-3 (5.7 g) was separated by reversed-phase silica gel column chromatography [200 g, MeOH–H_2O (60 : 40→80 : 20, v/v)→MeOH] to give four fractions [fr. 2-3-1 (1.0 g), fr. 2-3-2 (1.4 g), fr. 2-3-3 (3.6 g), fr. 2-3-4 (74 mg)]. Fraction 2-3-2 (1.4 g) was purified by HPLC [1) CH_3CN–1% aq. AcOH (40 : 60, v/v), 2) MeOH–1% aq. AcOH (70 : 30, v/v), 3) YMC-Pack Ph (250×20 mm i.d.), CH_3CN–1% aq. AcOH (40 : 60, v/v)] to give **1** (134 mg, 0.010%), **9** (35 mg, 0.0026%), assamsaponins F (19 mg, 0.0014%), and I (120 mg, 0.0090%) and camelliasaponin B_1 (**2**, 19 mg, 0.0014%). Fraction 2-3-3 (3.6 g) was purified by HPLC [1) MeOH-1% aq. AcOH (70 : 30, v/v), 2) CH_3CN–1% aq. AcOH (40 : 60, v/v)] to give **5** (49 mg, 0.0036%), **11** (15 mg, 0.0011%), assamsaponins G (106 mg, 0.0079%), and H (18 mg, 0.0014%) and theasaponin E_1 (**6**, 63 mg, 0.0047%). The known compounds (**2**, **6**, **7**) were identified by comparison of their physical data ([α]$_D$, IR, ^{1}H-NMR, ^{13}C-NMR) with reported values.[4,5]

Assamsaponin A (**1**): Colorless fine crystals from $CHCl_3$–MeOH, mp 211.7—212.2 °C, $[\alpha]^{26}_{D}$ +19.6° (c=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{57}H_{88}O_{25}Na$ $(M+Na)^+$: 1195.5513. Found: 1195.5518. IR (KBr): 3432, 1721, 1655, 1078, 1048 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.82, 0.85, 1.04, 1.27, 1.43, 1.80, 1.96 (3H each, all s, 25, 26, 29, 30, 24, 27, 5⁗′-H$_3$), 2.08 (3H, d, J=6.9 Hz, 4⁗′-H$_3$), 3.00 (1H, m, 18-H), 3.15, 3.66 (1H each, both d-like, 28-H$_2$), 4.00 (1H, m, 3-H), 4.59 (1H, br s, 16-H), 4.82 (1H, d, J=7.3 Hz, 1′-H), 4.98 (1H, d, J=7.2 Hz, 1⁗-H), 5.37 (1H, br s, 12-H), 5.65 (1H, d, J=5.2 Hz, 1‴-H), 5.68 (1H, d, J=7.3 Hz, 1″-H), 6.00 (1H, dq-like, 3⁗′-H), 6.12 (1H, dd-like, 22-H), 9.85 (1H, s, 23-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: m/z 1171 $(M-H)^-$, 1039 $(M-C_5H_9O_4)^-$, 907 $(M-C_{10}H_{17}O_8)^-$. Positive-ion FAB-MS: m/z 1195 $(M+Na)^+$.

Assamsaponin B (**4**): Colorless fine crystals from $CHCl_3$–MeOH, mp 199.0—200.4 °C, $[\alpha]^{26}_{D}$ +21.6° (c=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{61}H_{92}O_{28}Na$ $(M+Na)^+$: 1295.5673. Found: 1295.5681. IR (KBr): 3432, 1725, 1655, 1078, 1048 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.74, 0.79, 1.07, 1.26, 1.44, 1.46, 1.98, 2.03, 2.50 (3H each, all s, 26, 25, 29, 30, 27, 24, 5⁗′, 2⁗″, 2⁗‴-H$_3$), 2.06 (3H, d, J=7.3 Hz, 4⁗′-H$_3$), 2.98 (1H, dd, J=4.9, 13.6 Hz, 18-H), 3.45, 3.46 (1H each, both d, J=11.0 Hz, 28-H$_2$), 3.98 (1H, dd-like, 3-H), 4.81 (1H, d, J=7.3 Hz, 1′-H), 5.00 (1H, d, J=7.6 Hz, 1⁗-H), 5.38 (1H, br s, 12-H), 5.58 (1H, br s, 16-H), 5.71 (2H, d-like, 1″, 1‴-H), 5.85 (1H, d, J=10.4 Hz, 21-H), 5.90 (1H, dq-like, 3⁗′-H), 6.09 (1H, d, J=10.4 Hz, 22-H), 9.93 (1H, s, 23-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: m/z 1271 $(M-H)^-$, 1139 $(M-C_5H_9O_4)^-$, 1109 $(M-C_6H_{11}O_5)^-$, 1007 $(M-C_{10}H_{17}O_8)^-$. Positive-ion FAB-MS: m/z 1295 $(M+Na)^+$.

Assamsaponin C (**5**): Colorless fine crystals from $CHCl_3$–MeOH, mp 201.0—202.0 °C, $[\alpha]^{28}_{D}$ +21.0° (c=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{59}H_{90}O_{27}Na$ $(M+Na)^+$: 1253.5567. Found: 1253.5553. IR (KBr): 3453, 1721, 1656, 1078, 1048 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.83, 0.95, 1.09, 1.31, 1.44, 1.76, 1.86, 2.02 (3H each, all s, 25, 26, 29, 30, 24, 27, 5⁗′, 2⁗″-H$_3$), 1.61 (3H, d, J=6.3 Hz, 4⁗′-H$_3$), 2.82 (1H, dd-like, 18-H), 4.00 (1H, dd-like, 3-H), 4.25 (2H, m, 28-H$_2$), 4.46 (1H, d-like, 22-H), 4.70 (1H, br s, 16-H), 4.83 (1H, d, J=6.9 Hz, 1′-H), 4.99 (1H, d, J=6.9 Hz, 1⁗-H), 5.44 (1H, br s, 12-H), 5.65 (1H, d-like, 1‴-H), 5.68 (1H, d-like, 1″-H), 6.38 (1H, d, J=9.6 Hz, 21-H), 7.00 (1H, dq-like, 3⁗′-H), 9.88 (1H, s, 23-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: m/z 1229 $(M-H)^-$, 1097 $(M-C_5H_9O_4)^-$, 965 $(M-C_{10}H_{17}O_8)^-$. Positive-ion FAB-MS: m/z 1253 $(M+Na)^+$.

Assamsaponin D (**9**): Colorless fine crystals from $CHCl_3$–MeOH, mp 190.6—191.2 °C, $[\alpha]^{26}_{D}$ +15.6° (c=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{59}H_{92}O_{27}Na$ $(M+Na)^+$: 1255.5724. Found: 1255.5717. IR (KBr): 3432, 1719, 1649, 1078, 1046 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.90, 0.91, 1.06, 1.07, 1.30, 1.79, 1.95, 2.01 (3H each, all s, 26, 25, 29, 24, 30, 27, 2⁗″, 5⁗′-H$_3$), 2.09 (3H, d, J=7.0 Hz, 4⁗′-H$_3$), 3.02 (1H, dd-like, 18-H), 3.38, 3.62 (1H each, both d, J=11.0 Hz, 28-H$_2$), 3.77, 4.35 (1H each, both d-like, 23-H$_2$), 4.12 (1H, m, 3-H), 4.43 (1H, br s, 16-H), 5.01 (1H, d, J=7.6 Hz, 1⁗-H), 5.06 (1H, d, J=8.2 Hz, 1′-H), 5.35 (1H, br s, 12-H), 5.69 (1H, d, J=6.5 Hz, 1‴-H), 5.83 (1H, d, J=7.6 Hz, 1″-H), 5.98 (1H, dq-like, 3⁗′-H), 6.15 (1H, d, J=10.1 Hz, 22-H), 6.55 (1H, d, J=10.1 Hz, 21-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: m/z 1231 $(M-H)^-$, 1099 $(M-C_5H_9O_4)^-$, 967 $(M-C_{10}H_{17}O_8)^-$. Positive-ion FAB-MS: m/z 1255 $(M+Na)^+$.

Assamsaponin E (**11**): Colorless fine crystals from $CHCl_3$–MeOH, mp 189.4—190.4 °C, $[\alpha]^{27}_{D}$ +23.8° (c=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{59}H_{92}O_{26}Na$ $(M+Na)^+$: 1239.5775. Found: 1239.5763. IR (KBr): 3453, 1718, 1649, 1080, 1048 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.84, 0.87, 1.10, 1.12, 1.81, 1.98, 1.99 (3H each, all s, 25, 26, 29, 24, 27, 5⁗′, 2⁗″-H$_3$), 1.30 (6H, s, 23, 30-H$_3$), 2.05 (3H, d, J=7.0 Hz, 4⁗′-H$_3$), 2.80 (1H, dd-like, 18-H), 3.29 (1H, dd, J=4.6, 11.9 Hz, 3-H), 4.25 (2H, m, 28-H$_2$), 4.44 (1H, d-like, 22-H), 4.72 (1H, br s, 16-H), 4.94 (1H, d, J=7.4 Hz, 1′-H), 5.02 (1H, d, J=7.6 Hz, 1⁗-H), 5.45 (1H, br s, 12-H), 5.73 (1H, d, J=6.0 Hz, 1‴-H), 5.74 (1H, d, J=7.6 Hz, 1″-H), 5.90 (1H, dq-like, 3⁗′-H), 6.43 (1H, d, J=10.1 Hz, 21-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: m/z 1215 $(M-H)^-$, 1083 $(M-C_5H_9O_4)^-$, 1053 $(M-C_6H_{11}O_5)^-$, 951 $(M-C_{10}H_{17}O_8)^-$. Positive-ion FAB-MS: m/z 1239 $(M+Na)^+$.

Alkaline Hydrolysis of 1, 4, 5, 9, and 11 A solution of assamsaponins (**1**: 30 mg; **4**, **11**: 5 mg; **5**, **9**: 10 mg) in 50% aqueous dioxane (2 ml) was treated with 10% aqueous KOH (2 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 $(CH_2)Cl_2$ (2 ml) and the solution was treated with p-nitrobenzyl-N,N'-diisopropylisourea (10 mg), then the whole was stirred at 80 °C for 1 h. The reaction solution was subjected to HPLC analysis to identify the p-nitrobenzyl esters of angelic acid (**a**) from **1**, **4**, **9**, and **11**, tiglic acid (**b**) from **5**, and acetic acid (**c**) from **4**, **5**, **9**, and **11**. HPLC conditions: column, YMC-Pack ODS-A (YMC Co., Ltd.), 250×4.6 mm (i.d.); solvent, MeOH–H_2O (70 : 30, v/v); flow rate, 1.0 ml/min; **a**: 18.6 min; **b**: 17.1 min; **c**: 8.2 min.

The rest of the reaction mixture was neutralized with Dowex HCR W2 (H^+ from) 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 [3 g, $CHCl_3$–MeOH–H_2O (6 : 4 : 1)] to give desacyl-saponins [desacyl-assamsaponins A (**3**, 18 mg from **1**), D (**10**, 7 mg from **9**), and E (**12**, 4 mg from **11**), and desacyl-theasaponin E (**8**, 4 mg from **4**; 8 mg from **5**)]. **8** was identified by comparison of its physical data ([α]$_D$, IR, ^{1}H-NMR, ^{13}C-NMR) with an authentic sample.

Desacyl-assamsaponin A (**3**): Colorless fine crystals from $CHCl_3$–MeOH, mp 187.5—189.0 °C, $[\alpha]^{26}_{D}$ +34.7° (c=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{52}H_{82}O_{24}Na$ $(M+Na)^+$: 1113.5094. Found: 1113.5114. IR (KBr): 3453, 1718, 1647, 1078, 1048 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.82, 0.85, 1.05, 1.27, 1.43, 1.79 (3H each, all s, 25, 26, 29, 30, 24, 27-H_3), 2.43 (1H, dd-like, 18-H), 3.65, 4.05 (1H each, both m, 28-H_2), 4.01 (1H, m, 3-H), 4.60 (1H, dd-like, 22-H), 4.83 (1H, d, J=7.3 Hz, 1′-H), 5.00 (1H, d, J=7.6 Hz, 1⁗-H), 5.10 (1H, br s, 16-H), 5.32 (1H, br s, 12-H), 5.65 (1H, d-like, 1‴-H), 5.68 (1H, d-like, 1″-H), 9.84 (1H, s, 23-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: m/z 1089 $(M-H)^-$, 957 $(M-C_5H_9O_4)^-$, 825 $(M-C_{10}H_{17}O_8)^-$. Positive-ion FAB-MS: m/z 1113 $(M+Na)^+$.

Desacyl-assamsaponin D (**10**): Colorless fine crystals from $CHCl_3$–MeOH, mp 187.5—189.0 °C, $[\alpha]_D^{26}$ +26.2° (c=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{52}H_{84}O_{25}Na$ $(M+Na)^+$: 1131.5200. Found: 1131.5214. IR (KBr): 3453, 1655, 1078, 1044 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.92, 0.93, 1.06, 1.28, 1.34, 1.79 (3H each, all s, 26, 25, 24, 29, 30, 27-H_3), 2.72 (1H, dd-like, 18-H), 3.76, 4.35 (1H each, both m, 23-H_2), 3.61, 3.87 (1H each, both m, 28-H_2), 4.12 (1H, m, 3-H), 4.54 (1H, m, 22-H), 4.70 (1H, d, J=9.7 Hz, 21-H), 4.97 (1H, br s, 16-H), 5.00 (1H, d-like, 1⁗-H), 5.05 (1H, d-like, 1′-H), 5.38 (1H, br s, 12-H), 5.70 (1H, d, J=6.4 Hz, 1‴-H), 5.83 (1H, d, J=7.9 Hz, 1″-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: m/z 1107 $(M-H)^-$, 943 $(M-C_{10}H_{17}O_8)^-$. Positive-ion FAB-MS: m/z 1131 $(M+Na)^+$.

Desacyl-assamsaponin E (**12**): Colorless fine crystals from $CHCl_3$–MeOH, mp 186.0—187.2 °C, $[\alpha]_D^{26}$ +11.7° (c=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{52}H_{84}O_{24}Na$ $(M+Na)^+$: 1115.5250. Found: 1115.5240. IR (KBr): 3432, 1647, 1078, 1046 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.84, 0.90, 1.13, 1.28, 1.31, 1.34, 1.84 (3H each, all s, 25, 26, 24, 23, 29, 30, 27-H_3), 2.70 (1H, dd-like, 18-H), 3.30 (1H, dd-like, 3-H), 3.60, 3.88 (1H each, both m, 28-H_2), 4.56 (1H, m, 22-H), 4.71 (1H, d-like, 21-H), 4.95 (1H, d, J=7.2 Hz, 1′-H), 4.96 (1H, br s, 16-H), 5.02 (1H, d, J=7.6 Hz, 1⁗-H), 5.38 (1H, br s, 12-H), 5.71 (1H, d, J=6.0 Hz, 1‴-H), 5.73 (1H, d, J=7.6 Hz, 1″-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: m/z 1091 $(M-H)^-$. Positive-ion FAB-MS: m/z 1115 $(M+Na)^+$.

Acid Hydrolysis of 3, 10, and 12 A solution of desacyl-assamsaponins (**3**, **10**, **12**, 2 mg each) in 5% aq. H_2SO_4–1,4-dioxane (1 : 1, v/v, 1 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and the resin was filtered. After removal of the solvent under reduced pressure from the filtrate, the residue was passed through a Sep-Pak C_{18} cartridge with H_2O and MeOH. The H_2O eluate was concentrated and the residue was treated with L-cysteine methyl ester hydrochloride (0.01 ml) in pyridine (0.02 ml) at 60 °C for 1 h. After reaction, the solution was treated with N,O-bis(trimethylsilyl)trifluoroacetamide (0.01 ml) at 60 °C for 1 h. The supernatant was then subjected to GLC analysis to identify the derivatives of D-glucuronic acid (i), D-galactose (ii), L-arabinose (iii), and D-xylose (iv) from **3**, **10**, and **12**. GLC conditions: column, Supelco™-1, 0.25 mm (i.d.)×30 m; column temperature, 230 °C; t_R, i: 26.5 min; ii: 25.6 min; iii: 15.1 min; iv: 19.3 min.

Ethanol-Induced Gastric Mucosal Lesions in Rats The acute gastric lesions were induced by intragastric application of ethanol. Briefly, 99.5% ethanol (1.5 ml) was orally administered to 24—26 h fasted rats (about 250 g) by means of a metal orogastric tube. One hour later, the animals were sacrificed by cervical dislocation under ether anesthesia, and the stomach was dissected out 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 the necrotizing lesions were examined at 10× magnification by 2 or 3 observers unaware of the treatment. The lesions were scored with arbitrary scales in which the severity rating and number of lesions were considered according to a modification of the scoring system of Martin *et al.*[10]: 0: no lesion; 1: less than 5 slight lesions <5 mm in length and <2 mm in width; 2: more than 5 slight lesions <5 mm in length and <2 mm in width; 3: less than 5 medium lesions >5 mm in length and <2 mm in width; 4: more than 5 medium lesions >5 mm in length and <2 mm in width; 5: from 1—3 hemorrhagic bands of moderate lesions in length <5 mm and width >2 mm; 6: more than 4 hemorrhagic bands of moderate lesions in length <5 mm and width >2 mm; 7: from 1—3 hemorrhagic bands of severe lesions in length >5 mm and width >2 mm; 8: from 4—6 hemorrhagic bands of severe lesions in length >5 mm and width >2 mm; 9: more than 6 hemorrhagic bands of severe lesions in length >5 mm and width >2 mm. Mean scores for each group were calculated. The samples were given orally 1 h prior to the application of ethanol. Omeprazole was used as a reference drug in this experiment.

References and Notes

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