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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 polyhydroxylolane-12-ene oligoglycosides called assamsaponins A—I were isolated from the active saponin fraction together with three known saponins, theasaponin E1 and E2 and camelliasaponin B1. The structures of assamsaponins A—E were elucidated on the basis of chemical and physicochemical evidence. Theasaponin E1 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 and medicinal foodstuffs, we reported the isolation and structure elucidation of six acylated polyhydroxylolane-12-ene oligoglycosides, camelliasaponins A1, A2, B1 (2), B2, C1, and C2, from the seeds of camellia (*Camellia japonica* L., Theaceae). 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. We recently isolated two acylated polyhydroxylolane-12-ene oligoglycosides, theasaponins E1 (6) and E2 (7), from the seeds of Japanese tea plant [*Camellia sinensis* (L.) O. Kuntze, *Thea sinensis* L.] and elucidated their structures and antisweet activities. 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 polyhydroxylolane-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 E1 (6) and E2 (7) and camelliasaponin B1 (2). This paper deals with the isolation of assamsaponins and the structure elucidations of assamsaponins A—I, B (4), C (5), D (9), and E (11). We also describe the gastroprotective effect of theasaponin E1 (6), which is isolated from the seeds of both varieties of tea plant.

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 assamsaponin A (1), B (4), C (5), D (9), E (11), F, G, H, and I together with theasaponins E1 (6) and E2 (7) and camelliasaponin B1 (2). Theasaponin E1 (6) was also isolated from the seeds of Japanese tea plant as the principle constituent, and the gastroprotective effect was examined as a representative of tea saponins. Theasaponin E1 (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 C₅₂H₈₂O₂₄. Furthermore, fragment ion peaks at m/z 1039 (M⁻C₄H₆O₅)⁻ and m/z 907 (M⁻C₁₀H₁₉O₆)⁻, 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 desacetyl-assamsaponin A (3) and angelic acid. The angelic acid was converted to the p-nitrobenzyl ester, which was identified by HPLC analysis.

Desacetyl-assamsaponin A (3), whose molecular formula C₂₉H₃₈O₁₃ was determined by the negative- and positive-ion FAB-MS [m/z 1089 (M⁻H)⁻, 957 (M⁻C₄H₆O₅)⁻, 825 (M⁻C₉H₁₀O₇)⁻; m/z 1113 (M⁺Na)⁺] and high-resolution MS measurement, liberated δ-glucuronic acid, δ-galactose, l-arabinose, and D-xylose by acid hydrolysis with 5% aqueous H₂SO₄–1,4-dioxane (1:1), which were identified by GLC analysis of their trimethylsilyl thiazolidine derivatives. The ¹H-NMR (pyridine-d₅) and ¹³C-NMR (Table 1) spectra of 3, which were assigned by various NMR analytical methods, showed signals due to a camellagenin B moiety [δ 0.82,
acid and angelic acid, which were identified by HPLC analyses.

The tetrasaccharide structure bonding to the 3-position of camelliagenin B moiety, between the 1-proton of the galactopyranosyl moiety and the 2-carbon of the glucuronic acid moiety, and between the 1-proton of the arabinopyranosyl moiety and the 2-carbon of the 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 assamsaponin A was elucidated as shown.

The $^1$H-NMR (pyridine-$d_5$) and $^{13}$C-NMR (Table 1) spectra of assamsaponin A revealed an acylation shift around the 22-position of the camelliagenin B moiety. Consequently, the structure of assamsaponin A was determined to be 22-angeloylcamelliagenin B 3-$O$-[β-D-galactopyranosyl(1→2)]-[β-D-xylpyranosyl(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, desacetyl-theasaponin E (8) 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-)$^+$, 1139 (M-C$_2$H$_4$O$_2$)$^+$, 1109 (M-C$_6$H$_7$O$_3$)$^+$, 1007 (M-C$_{12}$H$_{17}$O$_3$)$^+$; 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 of assamsaponin B showed the presence of an angelic acid moiety [δ 1.98 (s, 5$^\text{m}$-H$_3$)], 2.06 (d, $J=7.3$ Hz, 4$^\text{m}$-H$_3$), 5.90 (dq-like, 3$^\text{m}$-H$_3$)] and two acetic acid moieties [δ 2.03, 2.50 (both s, 2$^\text{m}$, 2$^\text{m}$-H$_2$)] together with a β-D-glucuronopyranosyl moiety [δ 4.81 (d, $J=7.3$ Hz, 1$^\text{m}$-H$_3$)], a β-D-galactopyranosyl moiety [δ 5.71 (d-like, 1$^\text{m}$-H$_3$)], a α-L-arabinopyranosyl moiety [δ 5.71 (d-like, 1$^\text{m}$-H$_3$)], a β-D-xylpyranosyl moiety [δ 5.00 (d, $J=7.6$ Hz, 1$^\text{m}$-H$_3$)], 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 (brs, 12-H), 5.58 (brs, 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 angelyl 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-angelyltheasapogenol 3-O-[β-D-galactopyranosyl(1→2)]-[β-D-xylpyranosyl(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-)$^+$, 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 superimpor-
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 [$\delta$ 1.86 (s, 5-H$_3$), 1.61 (d, $J$=6.3 Hz, 4-H$_2$), 7.00 (dq-like, 3-H$_2$)] and the acetyl group [$\delta$ 2.02 (s, 2-H$_3$)] bonding to the 21, 28-hydroxyl groups of the theasapogenol E moiety [$\delta$ 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 $\alpha$,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 quasi-molec-

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

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

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Chart 2
nose, and D-xylose, which were identified by GLC analysis. The alkaline hydrolysis of desacyl-assamsaponin E (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-xylene, which were identified by GLC analysis. The 1H-NMR (pyridine-d$_5$) and 13C-NMR (Table 1) spectra of 10 showed signals due to a theasapogenol A moiety [δ 7.27 (dd, 1H), 3.76, 4.35 (both m, 23-H$_3$), 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, 17'-H), 5.05 (d, 17-H), 5.70 (d, J=6.4 Hz, 18-H), 5.83 (d, J=7.9 Hz, 19'-H)], which were very similar to those of 3 and 8, except for the signals due to the 23-hydroxymethyl group. Furthermore, the HMBCC 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 1H-NMR (pyridine-d$_5$) and 13C-NMR (Table 1) spectra 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$_3$)] and an acetyl group [δ 1.95 (s, 2'''-H$_2$)] 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 1H- and 13C-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-ᴅ-ᴅ-xylpyranosyl(1→2)-α-ᴅ-ᴅ-arabinopyranosyl(1→3)]-β-ᴅ-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, quasi-molecular 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$_{28}$H$_{43}$O$_{25}$. Fragment ion peaks were observed at m/z 1083 (M–C$_6$H$_5$O$_7$)$_-$, m/z 1053 (M–C$_6$H$_5$O$_5$)$_-$, and m/z 951 (M–C$_6$H$_5$O$_3$)$_-$, 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-xylene 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 1H-NMR (pyridine-d$_6$) and 13C-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 1H-NMR (pyridine-d$_6$) and 13C-NMR (Table 1) spectra$^9$ of 11 showed signals due to the 21,28-acetylated 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 13C-NMR data for 11 with those of 12 led us to confirm the structure of asassamaponin E as 21-O-angeloyl-28-O-acetylthapsagatol 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.$^3$

**Isolation of Assasamaponins 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* var. assamica Pierre (13.4 kg, cultivated in the northeastern part of Elypa, Sri Lanka) were extracted with MeOH 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 Siliysa Chemical, Ltd., 2 kg), H$_2$O–MeOH–CHCl$_3$–MeOH–H$_2$O (6:4:1) to give three fractions [fr. 1 (33.1 g), fr. 2 (21.9 g), fr. 3 (3.3 g)]. Normal-phase silica gel column chromatography [BW-200 (Fuji Siliysa Chemical Ltd., 1 kg), CHCl$_3$–MeOH–H$_2$O (7:3:1, lower layer: 15 mm i.d., 1 mm), and CHCl$_3$–MeOH–H$_2$O (60:40, v/v)] to give asassamaponin A (4, 38 mg, 0.0028%) and thespasimon B (7, 110 mg, 0.0082%). Fraction 2-3 (5.7 g) was separated by reversed-phase silica gel column chromatography [200 g, MeOH–H$_2$O (60:40:80:20, v/v) to give four fractions, fr. 2-3-1 (11 mg), fr. 3-2-1 (1.4 g), fr. 2-3-3 (2.3 g), fr. 3-2-4 (74 mg)], Fraction 2-3-2 (1.4 g) was purified by HPLC (1 CH$_3$CN–1%aq. AcOH (60:40, v/v), 2 MeOH–1%aq. AcOH (AcOH:70:30, v/v), 3 YMCPack Ph (250×20 mm i.d.), CH$_3$CN–1%aq. AcOH (AcOH:60:40, v/v)] to give 1 (134 mg, 0.010%), 2 (35 mg, 0.0026%), asassamaponin F (19 mg, 0.0014%), and 12 (10 mg, 0.0009%) and camellasaponin A (2, 19.4 mg, 0.0014%). Fraction 2-3-3 (3.6 g) was purified by HPLC (1 MeOH–1%aq. AcOH (AcOH:70:30, v/v), 2 CH$_3$CN–1%aq. AcOH (AcOH:60:40, v/v) to give 5 (49 mg, 0.0036%), 11 (15 mg, 0.0011%), asassamaponin G (106 mg, 0.0079%), and H (18 mg, 0.0014%) and thespasimon E (6, 63 mg, 0.0047%).

**Acknowledgment**

The known compounds (2, 6, 7) were identified by comparison of their physical data (δ$_{2H}$, IR, 1H-NMR, 13C-NMR) with reported values.$^2$,

**Alkaloid Hydrolysis of 1, 4, 5, 9, and 11**

A solution of asassamaponin As (1: 30 mg, 4, 11: 5 mg, 5: 9 mg, 10: 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 CHCl$_3$ (2 mL) and the solution was treated with p-nitrobenzoyl-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, 9, and 11, tigliic acid (b) from 5, and acetic acid (c) from 4, 5, and 9, and HPLC conditions: column, YMCPack ODS-A (YM Co., Ltd.), 250×4.6 mm (i.d.); solvent, MeOH–H$_2$O (70:30, v/v); flow rate, 1.0 mL/min; at 18.6 min; b 17.1 min; e 8.2 min. 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 normal-phase silica gel column chromatography [3 g, CHCl$_3$–MeOH–H$_2$O (6:4:1) to give desacyl-saponins (desacyl-assassamaponins A (3, 18 mg from 1, D (10, 7 mg from 9), and E (12, 4 mg from 11, and desacyl-thapsagatol E (8, 4 mg from 4, 8 mg from 5)]. It was identified by comparison of its physical data (δ$_{2H}$, IR, 1H-NMR, 13C-NMR) with an authentic sample. Desacyl-assassamaponin A (3): Colorless fine crystals from CHCl$_3$–MeOH, 185.7–189.0°C, [α]$_{20}$$^{20}$ +34.7° (c = 0.1, MeOH). High-resolution posi-
Acid Hydrolysis of 3, 10, and 12
A solution of desacyl-asasampasomons (3, 10, 12, 2 mg each) in 5% aq. H2SO4, 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 C8 cartridge with H2O and MeOH. The H2O 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 r-xylene (iv) from 3, 10, and 12. GLC conditions: column, SupelcoTM-1, 0.25 mm (i.d.);×30 m; column temperature, 230 °C; tR, 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 oragastic 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

References and Notes
9) The 1H- and 13C-NMR spectra were assigned on the basis of homo- and hetero-correlation spectroscopy (1H—1H, 1H—13C COSY), homonuclear and heteronuclear Hartmann-Hahn spectroscopy (1H—13C, 1H—13C HO-HAHA) and HMBC experiments.