Bioactive Saponins and Glycosides. XVII.[1] Inhibitory Effect on Gastric Emptying and Accelerating Effect on Gastrointestinal Transit of Tea Saponins: Structures of Assamsaponins F, G, H, I, and J from the Seeds and Leaves of the Tea Plant

Toshiyuki MURAKAMI, Junko NAKAMURA, Tadashi KAGEURA, Hisashi MATSUDA, and Masayuki YOSHIKAWA*

Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607–8414, Japan.
Received May 30, 2000; accpeted July 6, 2000

Following the investigation of assamsaponins A, B, C, D, and E, four new saponins termed assamsaponins F, G, H, and I were isolated from the seeds of the tea plant (*Camellia sinensis* L. var. *assamica* PIERRE), while assamsaponin J was isolated from its leaves. The structures of assamsaponins F—J were elucidated on the basis of chemical and physicochemical evidence and found to be 16,22-*O*-diacetyl-21-*O*-angeloyltheasapogenol E 3-*O*-[β-D-galactopyranosyl(1→2)][β-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid, 21-*O*-angeloyl-22-*O*-acetyltheasapogenol E 3-*O*-[β-D-galactopyranosyl(1→2)][β-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid, 21-*O*-angeloyl-28-*O*-acetyltheasapogenol E 3-*O*-[β-D-galactopyranosyl(1→2)][β-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid, 21-*O*-tigloyl-28-*O*-acetyltheasapogenol E 3-*O*-[β-D-galactopyranosyl(1→2)][β-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid, and 16,21-*O*-diacetyl-22-*O*-cinnamoyltheasapogenol B 3-*O*-[β-D-galactopyranosyl(1→2)][β-D-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid, respectively.

The saponin mixture from the seeds of the tea plant was found to exhibit an inhibitory effect on gastric emptying and an accelerating effect on gastrointestinal transit in mice. Theasaponin E_1, the principle saponin of the tea plant, showed potent activity, while theasaponin E_2 showed none, so that the position of the acyl groups in the sapogenin moiety is important from a pharmacological point of view.

Key words assamsaponin; *Camellia sinensis* var. *assamica*; tea plant; oleanene-type triterpene oligoglycoside; gastric emptying inhibitor; gastrointestinal transit accelerator

In the course of our studies of the bioactive saponins and glycosides from medicinal foodstuffs and natural medicines,[2] we isolated two triterpene saponins, theasaponins E_1 (**15**) and E_2 (**16**), from the seeds of the Japanese tea plant [*Camellia* (*C.*) *sinensis* or *Thea sinensis*] and characterized their structures and antisweet activity.[3] In a continuing study of the tea plant, the saponin mixture from the seeds of the tea plant (*C. sinensis* L. var. *assamica* PIERRE) cultivated in Sri Lanka was found to have a potent protective effect on gastric mucosal lesions induced by ethanol in rats. From the saponin mixture, we have isolated nine acylated polyhydroxyoleanene-type triterpene saponins called assamsaponins A (**8**), B (**11**), C (**12**), D (**13**), E (**14**), F (**1**), G (**2**), H (**3**), and I (**4**) together with three known saponins, camelliasaponin B_1 (**9**)[4] and theasaponins E_1 (**15**)[3] and E_2 (**16**).[3] In the preceding paper,[5] we reported the structures of assamsaponins A (**8**), B (**11**), C (**12**), D (**13**), and E (**14**) and the inhibitory effect of **15** and **16** on ethanol-induced gastric lesions in rats. We recently found that the saponin mixture from the seeds of *C. sinensis* var. *assamica* exhibited an inhibitory effect on gastric emptying and an accelerating effect on gastrointestinal transit in mice. In addition, we isolated a new saponin called assamsaponin J (**6**) from the leaves of *C. sinensis* var. *assamica*. This paper deals with the structural elucidation of five new acylated polyhydroxyoleanene-type triterpene saponins, assamsaponins F (**1**), G (**2**), H (**3**), I (**4**), and J (**6**) from the seeds and leaves of *C. sinensis* var. *assamica*. We also describe the effects of the saponin mixture and theasaponins E_1 (**15**) and E_2 (**16**), saponins common to both varieties of tea plant, on gastric emptying and gastrointestinal transit.

Structures of Assamsaponins F (1), G (2), H (3), and I (4) from the Seeds of *C. sinensis* var. *assamica* Assamsaponin F (**1**) was obtained as colorless fine crystals from $CHCl_3$–MeOH with a mp 209.8—210.3 °C. The IR spectrum of **1** showed absorption bands at 1721 and 1649 cm^{-1} assignable to carbonyl and α,β-unsaturated ester functions, and broad bands at 3432, 1078, and 1046 cm^{-1} suggestive of an oligoglycoside structure. In the negative- and positive-ion FAB-MS of **1**, quasimolecular ion peaks were observed at *m/z* 1301 $(M-H)^-$ and *m/z* 1325 $(M+Na)^+$, and high-resolution MS analysis revealed the molecular formula of **1** to be $C_{62}H_{94}O_{29}$. Furthermore, fragment ion peaks at *m/z* 1139 $(M-C_6H_{11}O_5)^-$ and *m/z* 1007 $(M-C_{11}H_{19}O_9)^-$, which were presumed to be derived by cleavage of the glycosidic linkages at the 2‴- and 3′-positions (*vide infra*), were observed in the negative-ion FAB-MS of **1**. Alkaline hydrolysis of 10% aqueous potassium hydroxide–50% aqueous dioxane (1 : 1, v/v) provided desacyl-assamsaponin F (**5**), angelic acid and acetic acid. The organic acids were converted to the *p*-nitrobenzyl ester derivatives,[6] which were identified by HPLC.

On acid hydrolysis with 5% aqueous sulfuric acid (H_2SO_4)–1,4-dioxane (1 : 1, v/v), desacyl-assamsaponin F (**5**) liberated D-glucuronic acid, D-galactose, L-arabinose, and D-glucose, which were identified by GLC[7] of their trimethylsilyl thiazolidine derivatives. The molecular formula of **5**, $C_{53}H_{84}O_{26}$, was also determined from negative- and positive-ion FAB-MS [*m/z* 1135 $(M-H)^-$, 973 $(M-C_6H_{11}O_5)^-$, 841

* To whom correspondence should be addressed. e-mail: shoyaku@mb.kyoto-phu.ac.jp

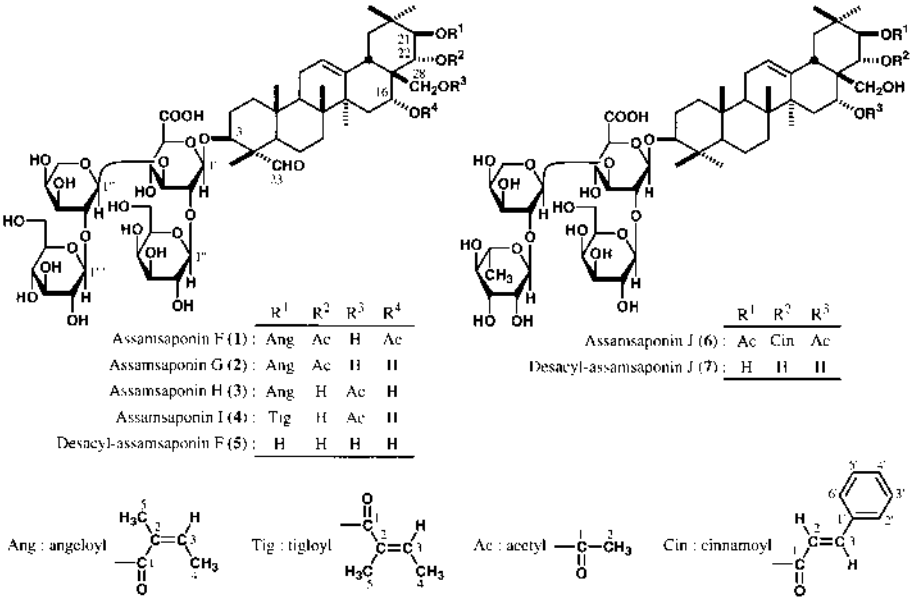

Chart 1

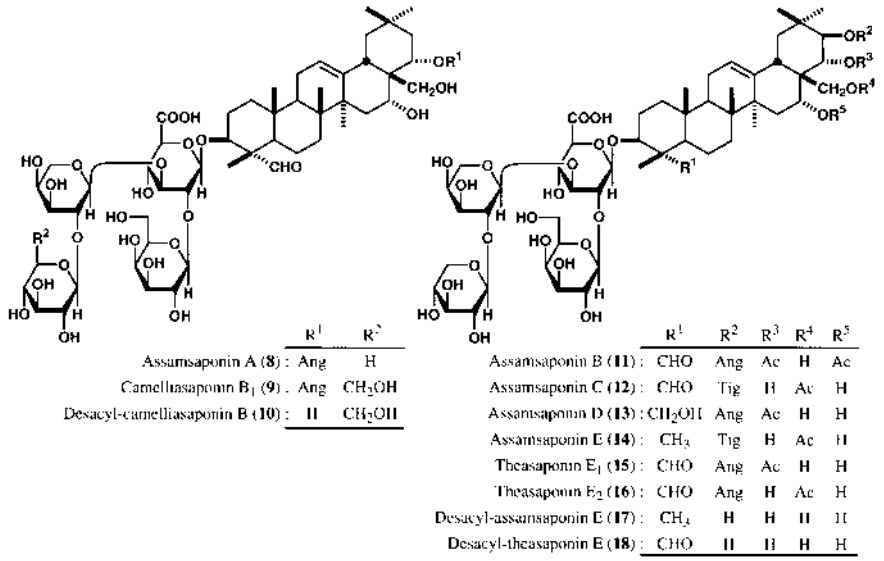

Chart 2

$(M-C_{11}H_{19}O_9)^-$; *m/z* 1159 $(M+Na)^+$] and by high-resolution MS measurement. The proton and carbon signals assignable to the sapogenin moiety in the ^{1}H-NMR and ^{13}C-NMR spectra of **5** were superimposable on those of desacyl-theasaponin E (**18**),[3] while the proton and carbon signals due to the oligoglycoside moiety in **5** were very similar to those of desacyl-camelliasaponin B (**10**).[4] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **5**, which were assigned by various NMR analytical methods,[8] showed signals due to a theasapogenol E moiety [δ 0.82, 0.85, 1.30, 1.34, 1.43, 1.78 (all s, 25, 26, 29, 30, 24, 27-H_3), 2.68 (dd-like, 18-H), 3.61, 3.87 (both m, 28-H_2), 4.03 (m, 3-H), 4.55 (m, 22-H), 4.72 (d, J=9.7 Hz, 21-H), 4.96 (br s, 16-H), 5.36 (br s, 12-H), 9.86 (s, 23-H)], a β-D-glucopyranosiduronic acid moiety [δ 4.85 (d, J=6.9 Hz, GlcA-1-H)], a β-D-galactopyranosyl moiety [δ 5.61 (d-like, Gal-1-H)], an α-L-arabinopyranosyl moiety [δ 5.75 (d, J=5.3 Hz, Ara-1-H)], and a β-D-glucopyranosyl moiety [δ 5.09 (d, J=5.3 Hz, Glc-1-H)]. The tetrasaccharide structure bonding to the 3-position of the theasapogenol E moiety was identified by HMBC experiments. Thus, long-range correlations were observed between the 1′-proton of the glucuronic acid moiety and the 3-carbon of the theasapogenol E 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 glucopyranosyl moiety and the 2‴-carbon of the arabinopyranosyl moiety. On the basis of this evidence, the structure of desacyl-assamsaponin F (**5**) was elucidated as shown.

The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[8] of **1** showed signals assignable to an angeloyl group [δ 1.98 (s, Ang-5-H_3), 2.05 (d, J=7.6 Hz, Ang-4-H_3), 5.98 (dq-like, Ang-3-H)] and two acetyl groups [δ 2.03, 2.51 (both s, Ac-2, Ac-2′-H_3),] together with the desacyl-assamsaponin F moiety [δ 5.58 (br s, 16-H), 5.85 (d, J=10.3 Hz, 21-H), 6.09

(d, J=10.3 Hz, 22-H)]. The positions of the angeloyl and acetyl groups of **1** were determined by an HMBC experiment on **1**, which showed long-range correlations between the 16- and 22-protons of the theasapogenol E moiety and two carbonyl carbons of acetyl groups and between the 21-proton of the theasapogenol E moiety and the carbonyl carbon of the angeloyl group. Finally, comparison of the ^{1}H- and ^{13}C-NMR data from **1** with those from **5** revealed acylation shifts around the 16-, 21-, and 22-positions of the theasapogenol E moiety. Consequently, the structure of assamsaponin F was determined to be 16,22-*O*-diacetyl-21-*O*-angeloyltheasapogenol E 3-*O*-[β-D-galactopyranosyl(1→2)][β-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**1**).

Assamsaponins G (**2**), H (**3**), and I (**4**) were isolated as colorless fine crystals from $CHCl_3$–MeOH. In the negative- and positive-ion FAB-MS of **2**, **3**, and **4**, quasimolecular ion peaks were observed at m/z 1259 $(M-H)^-$ and m/z 1283 $(M+Na)^+$ and high-resolution MS analysis revealed the common molecular formula of **2**, **3**, and **4** to be $C_{60}H_{92}O_{28}$. Furthermore, fragment ion peaks at m/z 1097 $(M-C_6H_{11}O_5)^-$ and 965 $(M-C_{11}H_{19}O_9)^-$, which were thought to be derived by cleavage of the glycosidic linkage at the 2‴- and 3′-positions (*vide infra*), were observed in the negative-ion FAB-MS of **2**, **3**, and **4**. Alkaline hydrolysis of 10% aqueous potassium hydroxide–50% aqueous dioxane (1 : 1, v/v) provided desacyl-assamsaponin F (**5**) and organic acids (acetic acid and angelic acid from **2** and **3**, and acetic acid and tiglic acid from **4**). The organic acids were converted to *p*-nitrobenzyl ester derivatives,[6] which were identified by HPLC.

The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[8] of **2** showed signals assignable to the angeloyl moiety [δ 2.02 (s, Ang-5-H_3), 2.07 (d, J=5.6 Hz, Ang-4-H_3), 6.00 (dq-like, Ang-3-H)] and the acetyl moiety [δ 1.94 (Ac-2-H_3)] together with the desacyl-assamsaponin F moiety [δ 3.03 (dd-like, 18-H), 3.37, 3.59 (both d-like, 28-H_2), 4.02 (m, 3-H), 4.43 (br s, 16-H), 5.39 (br s, 12-H), 6.14 (d, J=10.4 Hz, 22-H), 6.56 (d, J=10.4 Hz, 21-H), 9.90 (s, 23-H)]. The positions of the angeloyl and acetyl groups of **2** were determined by HMBC, which showed long-range correlations between the 21-protons of the theasapogenol E moiety and the carbonyl carbon of the angeloyl group and between the 22-proton of the theasapogenol E moiety and the carbonyl carbon of the acetyl group. Consequently, the structure of assamsaponin G was determined to be 21-*O*-angeloyl-22-*O*-acetyltheasapogenol E 3-*O*-[β-D-galactopyranosyl(1→2)][β-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**2**).

The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[8] of **3** and **4** showed signals due to two acyl groups (acetyl and angeloyl groups for **3**, acetyl and tigloyl groups for **4**) and the 21,28-di-*O*-acylated structure of the desacyl-assamsaponin F moiety [**3**: δ 4.21 (m, 28-H2), 6.42 (d, J=10.2 Hz, 21-H); **4**: δ 4.26 (m, 28-H_2), 6.38 (d, J=10.0 Hz, 21-H)]. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR data from **3** and **4** were very similar to those of assamsaponin G (**2**), except for the signals due to the acyl groups of **3** and **4**. Long-range correlations were observed between the 21-proton and the carbonyl carbon of the angeloyl or tigloyl group and between the 28-protons and the carbonyl carbon of the acetyl

Table 1. ^{13}C-NMR Data for Assamsaponins F (**1**), G (**2**), H (**3**), I (**4**), and J (**6**) and Desacyl-assamsaponins F (**5**) and J (**7**)

	1	**2**	**3**	**4**	**6**	**5**	**7**
C- 1	38.2	38.2	38.3	38.3	38.8	38.3	38.9
2	25.3	25.2	25.3	25.3	26.4	25.2	26.5
3	84.4	84.2	84.3	84.3	89.9	84.2	89.9
4	55.0	55.1	55.1	55.1	39.5	55.1	39.7
5	48.5	48.4	48.5	48.5	55.8	48.5	55.9
6	20.5	20.4	20.4	20.4	18.3	20.4	18.5
7	32.4	32.5	32.5	32.5	33.1	32.5	33.3
8	40.3	40.3	40.4	40.4	40.1	40.3	40.1
9	46.8	46.8	46.8	46.9	46.9	46.9	47.1
10	36.0	36.1	36.0	36.1	36.8	36.1	36.9
11	23.7	23.8	23.9	23.9	23.8	23.8	23.9
12	124.9	124.0	122.7	122.7	125.3	122.7	122.9
13	141.0	142.9	142.8	142.8	140.9	144.0	144.0
14	41.2	41.7	41.9	41.9	41.2	42.1	42.1
15	31.0	34.5	34.6	34.6	31.2	34.2	34.4
16	71.3	68.1	67.7	67.7	71.7	68.4	67.9
17	46.9	48.0	47.1	47.1	47.2	47.3	47.3
18	39.6	40.2	40.6	40.6	39.7	41.2	41.3
19	47.2	47.2	47.4	47.4	47.3	48.2	48.3
20	36.1	36.3	36.1	36.3	35.9	36.3	36.4
21	78.5	78.9	81.4	81.7	78.8	78.7	78.8
22	73.4	74.5	71.4	71.5	73.3	77.4	77.4
23	210.1	209.9	209.8	209.9	28.0	209.7	28.2
24	11.1	11.0	11.1	11.1	16.7	11.0	16.9
25	15.8	15.8	15.8	15.8	15.6	15.8	15.7
26	16.8	16.9	17.0	17.0	16.8	16.9	17.0
27	27.0	27.4	27.4	27.4	27.0	27.4	27.4
28	63.9	64.0	66.5	66.5	63.4	68.5	68.6
29	29.4	29.5	29.8	29.8	29.3	30.5	30.6
30	19.7	20.2	20.2	20.1	19.4	19.4	19.4
GlcA-1′	104.1	104.0	104.0	104.0	105.5	101.7	105.4
2′	78.4	78.4	78.5	78.4	79.3	78.4	79.4
3′	84.4	84.4	84.5	84.5	82.8	84.5	83.0
4′	71.0	71.0	71.0	71.0	71.2	71.0	71.3
5′	77.2	77.2	77.3	77.2	77.3	77.2	77.6
6′	171.8	171.9	171.8	171.8	172.1	171.8	172.2
Gal-1″	103.6	103.5	103.6	103.6	103.7	103.6	103.8
2″	73.7	73.7	73.7	73.7	73.5	73.7	73.4
3″	75.1	75.1	75.2	75.1	75.2	75.2	75.2
4″	70.3	70.3	70.3	70.3	70.0	70.5	70.0
5″	76.6	76.6	76.6	76.6	76.8	76.6	76.8
6″	62.2	62.1	62.2	62.1	62.5	62.1	62.5
Ara-1‴	101.7	101.6	101.7	101.7	101.1	104.0	101.2
2‴	81.3	81.3	81.3	81.2	76.9	81.3	77.0
3‴	72.5	72.5	72.4	72.4	73.6	72.4	73.6
4‴	67.7	67.7	67.6	67.6	69.0	67.6	68.8
5‴	64.9	64.9	64.9	64.9	65.6	64.9	65.7
Glc or Rha-1⁗	106.0	105.9	106.0	106.0	102.3	106.0	102.3
2⁗	75.9	75.8	75.9	75.8	72.5	75.9	72.5
3⁗	78.3	78.4	78.4	78.4	72.6	78.4	72.7
4⁗	71.6	71.6	71.6	71.6	74.0	71.6	74.0
5⁗	78.4	78.4	78.4	78.4	70.1	78.5	70.2
6⁗	62.7	62.7	62.8	62.7	18.2	62.7	18.3
16-Ac-1	169.8				170.3		
2	22.0				22.1		
21-Ang, Tig, or Ac-1	167.8	167.9	168.7	168.6	171.1		
2	128.5	129.0	129.6	129.9	20.8		
3	138.0	137.1	136.0	136.3			
4	15.9	15.9	15.9	14.1			
5	20.9	20.9	21.0	12.5			
22-Ac or Cin-1	170.5	171.1			166.4		
2	20.8	20.9			118.6		
3					145.5		
1′					135.1		
2′, 6′					128.6		
3′, 5′					129.2		
4′					130.6		
28-Ac-1			170.6	170.7			
2			20.9	20.7			

500 MHz, pyridine-d_5.

group in the HMBC experiment on **3** and **4**. In addition, acylation shifts were observed around the 21- and 28-positions in the ^{13}C-NMR data from **3** and **4**. Consequently, the structures of assamsaponins H and I were determined to be 21-*O*-angeloyl-28-*O*-acetyltheasapogenol E 3-*O*-[β-D-galactopyranosyl(1→2)][β-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**3**) and 21-*O*-tigloyl-28-*O*-acetyltheasapogenol E 3-*O*-[β-D-galactopyranosyl(1→2)][β-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**4**), respectively.

Isolation and Structural Elucidation of Assamsaponin J (6) from the Leaves The methanolic extract obtained from the leaves of the tea plant, *C. sinensis* var. *assamica* cultivated in Sri Lanka, was partitioned between an ethyl acetate and water mixture. In order to remove the polyphenols, the aqueous phase was treated with polyvinylpyrrolidone and filtered. The filtrate was extracted with 1-butanol and the 1-butanol extract was separated by normal-phase and reversed-phase silica gel column chromatography and finally HPLC to give assamsaponin J (**6**, 0.0012%). Assamsaponin J (**6**) was also isolated as colorless fine crystals of mp 230.2—232.0 °C. In the IR spectrum of **6**, absorption bands were observed at 3453, 1718, and 1653 cm^{-1} due to hydroxyl and carbonyl functions. The negative- and positive-ion FAB-MS of **6** showed quasimolecular ion peaks at *m/z* 1319 $(M-H)^-$ and *m/z* 1343 $(M+Na)^+$ and the molecular formula $C_{53}H_{86}O_{24}$ was determined by high-resolution MS measurement. The alkaline hydrolysis of **6** liberated desacyl-assamsaponin J (**7**) and two organic acids, cinnamic acid and acetic acid, which were identified by HPLC of their *p*-nitrobenzyl esters.[6] Acid hydrolysis of **7** liberated D-glucuronic acid, D-galactose, L-arabinose, and L-rhamnose, which were identified by GLC analysis. The proton and carbon signals in the ^{1}H-NMR and ^{13}C-NMR spectra of **7** resembled those of desacyl-assamsaponin E (**17**)[5] except for the signals due to the terminal α-L-rhamnopyranosyl moiety of **7**. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[8] of **7** showed signals due to a theasapogenol B moiety [δ 0.83, 0.89, 1.14, 1.26, 1.31, 1.35, 1.83 (all s, 25, 26, 24, 23, 29, 30, 27-H$_3$), 1.52 (d, J=5.8 Hz, Rha-6-H$_3$), 2.72 (dd-like, 18-H), 3.27 (dd-like, 3-H), 3.68, 3.99 (both d, J=10.7 Hz, 28-H$_2$), 4.55 (m, 22-H), 4.72 (d, J=9.8 Hz, 21-H), 5.37 (br s, 12-H)] and a tetrasaccharide moiety [δ 1.52 (d, J=5.8 Hz, Rha-6-H$_3$), 4.96 (d, J=7.3 Hz, GlcA-1-H), 5.62 (d, J=7.6 Hz, Gal-1-H), 5.98 (br s, Rha-1-H), 6.04 (d, J=5.5 Hz, Ara-1-H)]. Furthermore, the HMBC of **7** showed long-range correlations between the following protons and carbons: the 1′-proton of the glucopyranosiduronic acid moiety and the 3-carbon of theasapogenol B; the 1″-proton of the galactopyranosyl moiety and the 2′-carbon of the glucopyranosiduronic acid moiety; the 1‴-proton of the arabinopyranosyl moiety and the 3′-carbon of the glucopyranosiduronic acid moiety; the 1⁗-proton of the rhamnopyranosyl moiety and the 2‴-carbon of the arabinopyranosyl moiety. On the basis of these findings, the structure of desacyl-assamsaponin J (**7**) was determined as shown.

The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[8] of **6** showed the presence of a cinnamoyl group [δ 6.73 (d, J=16.2 Hz, Cin-2-H), 7.32 (m, Cin-3′, 4′, 5′-H), 7.64 (m, Cin-2′, 6′-H), 8.04 (d, J=16.2 Hz, Cin-3-H)] and two acetyl groups [δ 2.04, 2.56 (both s, Ac-2, Ac-2′-H$_3$)] together with a desacyl-assamsaponin J moiety [δ 3.07 (dd, J=4.0, 14.4 Hz, 18-H), 3.24 (dd, J=5.2, 11.6 Hz, 3-H), 3.48, 3.62 (both d, J=9.2 Hz, 28-H$_2$), 45.43 (br s, 16-H), 5.87 (d, J=10.4 Hz, 21-H), 6.27 (d, J=10.4 Hz, 22-H)]. The positions of the cinnamoyl and acetyl groups in **6** were determined by HMBC, which showed long-range correlations between the 16,21-protons of the theasapogenol B moiety and the two carbonyl carbons of the acetyl groups and between the 22-proton of the theasapogenol B moiety and the carbonyl carbon of the cinnamoyl group. Finally, comparison of the ^{1}H- and ^{13}C-NMR data from **6** with those from **7** revealed acylation shifts around the 16, 21, and 22-positions of the theasapogenol B moiety of **7**. Consequently, the structure of assamsaponin J was determined to be 16,21-di-*O*-acetyl-22-*O*-cinnamoyltheasapogenol B 3-*O*-[β-D-galactopyranosyl(1→2)]-[α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)]-β-D-glucopyranosiduronic acid (**6**).

Table 2. Effects of the Saponin Mixture and Theasaponins E$_1$ (**15**) and E$_2$ (**16**) from *C. sinensis* var. *assamica* on Gastric Emptying (GE) and Gastrointestinal Transit (GIT)

Treatment	Dose (mg/kg, *p.o.*)	N	GE (%)	GIT (%)
Control	—	10	88.5±1.8	45.2±2.7
Saponin Mixture	50	10	83.2±3.2	50.2±2.4
	100	10	75.0±3.7**	73.5±1.0**
	200	10	53.3±2.0**	75.1±1.8**
Control	—	7	90.9±1.4	44.8±1.6
Theasaponin E$_1$ (**15**)	25	7	89.5±3.0	46.4±1.5
	50	7	76.5±2.8**	49.8±4.3
	100	7	59.2±4.0**	60.0±3.4*
Theasaponin E$_2$ (**16**)	25	7	95.2±0.6	52.0±5.0
	50	7	90.7±1.9	53.1±5.1
	100	7	94.7±1.8	47.6±3.9

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

As shown in Table 2, the saponin mixture obtained from the seeds of *C. sinensis* var. *assamica* was found to exhibit an inhibitory effect on gastric emptying and an accelerating effect on gastrointestinal transit in mice. It is noteworthy that no drug having both gastric emptying inhibitory activity and gastrointestinal transit accelerating activity has been identified. These activities of the saponin fraction may be beneficial for the prevention and treatment of the inhibition of gastrointestinal transit such as affects the ileus in clinical situations. Next, the effects of theasaponins E$_1$ (**15**) and E$_2$ (**16**), isolated as common constituents from the seeds of *C. sinensis* var. *assamica* as well as Japanese *C. sinensis*, on gastric emptying and gastrointestinal transit were examined. Theasaponin E$_1$ (**15**) was found to inhibit gastric emptying and accelerate gastrointestinal transit. On the other hand, **16** showed no such activities. These findings indicate that the position of the acyl groups is important for pharmacological activity.

Experimental

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

Isolation of Assamsaponins F (1), G (2), H (3), and I (4) from the Seeds of *C. sinensis* var. *assamica* Assamsaponins F (**1**), G (**2**), H (**3**), and I (**4**) were isolated from the fresh seeds of *C. sinensis* var. *assamica* cultivated in Nuwara Eliya, Sri Lanka, as described earlier.[5]

Assamsaponin F (**1**): Colorless fine crystals from $CHCl_3$–MeOH, mp 209.8—210.3 °C, $[\alpha]_D^{26}$ +8.8° (c=0.1, MeOH). IR (KBr): 3432, 1721, 1649, 1078 cm^{-1}. High-resolution positive-ion FAB-MS: Calcd for $C_{62}H_{94}O_{29}Na$

$(M+Na)^+$: 1325.5778. Found: 1325.5798. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.74, 0.78, 1.07, 1.26, 1.43, 1.45, 1.98, 2.03, 2.51 (3H each, all s, 26, 25, 30, 29, 27, 24, Ang-5, Ac-2, Ac-2′-H_3), 2.05 (3H, d, J=7.6 Hz, Ang-4-H_3), 2.98 (1H, dd-like, 18-H), 3.45, 3.57 (1H each, both d, J=13.4 Hz, 28-H_2), 3.99 (1H, dd, J=2.8, 9.8 Hz, 3-H), 4.82 (1H, d, J=7.3 Hz, GlcA-1-H), 5.10 (1H, d, J=7.4 Hz, Glc-1-H), 5.39 (1H, br s, 12-H), 5.58 (1H, br s, 16-H), 5.61 (1H, d, J=7.7 Hz, Gal-1-H), 5.77 (1H, d, J=5.1 Hz, Ara-1-H), 5.85 (1H, d, J=10.3 Hz, 21-H), 5.98 (1H, dq-like, Ang-3-H), 6.09 (1H, d, J=10.3 Hz, 22-H), 9.94 (1H, s, 23-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: m/z 1301 $(M-H)^-$, 1139 $(M-C_6H_{11}O_5)^-$, 1007 $(M-C_{11}H_{19}O_9)^-$. Positive-ion FAB-MS: m/z 1325 $(M+Na)^+$.

Assamsaponin G (**2**): Colorless fine crystals from $CHCl_3$–MeOH, mp 187.7—188.5 °C, $[\alpha]_D^{26}$ +14.9° (c=0.1, MeOH). IR (KBr): 3454, 1719, 1655, 1076 cm^{-1}. High-resolution positive-ion FAB-MS: Calcd for $C_{60}H_{92}O_{28}Na$ $(M+Na)^+$: 1283.5673. Found: 1283.5686. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.82, 0.84, 1.08, 1.30, 1.44, 1.77, 1.94, 2.02 (3H each, all s, 25, 26, 29, 30, 24, 27, Ac-2, Ang-5-H_3), 2.07 (3H, d, J=5.6 Hz, And-4-H_3), 3.03 (1H, dd-like, 18-H), 3.37, 3.59 (1H each, both d-like, 28-H_2), 4.02 (1H, m, 3-H), 4.43 (1H, br s, 16-H), 4.86 (1H, d, J=7.3 Hz, GlcA-1-H), 5.11 (1H, d, J=7.0 Hz, Glc-1-H), 5.39 (1H, br s, 12-H), 5.61 (1H, d-like, Gal-1-H), 5.75 (1H, d, J=5.2 Hz, Ara-1-H), 6.00 (1H, dq-like, Ang-3-H), 6.14 (1H, d, J=10.4 Hz, 22-H), 6.56 (1H, d, J=10.4 Hz, 21-H), 9.90 (1H, s, 23-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: m/z 1259 $(M-H)^-$, 1097 $(M-C_6H_{11}O_5)^-$, 965 $(M-C_{11}H_{19}O_9)^-$. Positive-ion FAB-MS: m/z 1283 $(M+Na)^+$.

Assamsaponin H (**3**): Colorless fine crystals from $CHCl_3$–MeOH, mp 206.3—207.6 °C, $[\alpha]_D^{26}$ +41.0° (c=0.1, MeOH). IR (KBr): 3432, 1719, 1655, 1078 cm^{-1}. High-resolution positive-ion FAB-MS: Calcd for $C_{60}H_{92}O_{28}Na$ $(M+Na)^+$: 1283.5673. Found: 1283.5685. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.83, 0.95, 1.11, 1.29, 1.44, 1.76, 1.98, 1.99 (3H each, all s, 25, 26, 29, 30, 24, 27, Ang-5, Ac-2-H_3), 2.04 (3H, d, J=7.4 Hz, Ang-4-H_3), 2.81 (1H, dd-like, 18-H), 4.04 (1H, dd, J=4.6, 11.6 Hz, 3-H), 4.21 (2H, m, 28-H_2), 4.44 (1H, d-like, 22-H), 4.69 (1H, br s, 16-H), 4.85 (1H, d, J=7.3 Hz, GlcA-1-H), 5.09 (1H, d, J=7.3 Hz, Glc-1-H), 5.43 (1H, br s, 12-H), 5.62 (1H, d, J=7.6 Hz, Gal-1-H), 5.77 (1H, d, J=5.3 Hz, Ara-1-H), 5.90 (1H, dq-like, Ang-3-H), 6.42 (1H, d, J=10.2 Hz, 21-H), 9.90 (1H, s, 23-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: m/z 1259 $(M-H)^-$, 1097 $(M-C_6H_{11}O_5)^-$, 965 $(M-C_{11}H_{19}O_9)^-$. Positive-ion FAB-MS: m/z 1283 $(M+Na)^+$.

Assamsaponin I (**4**): Colorless fine crystals from $CHCl_3$–MeOH, mp 208.2—209.5 °C, $[\alpha]_D^{26}$ +35.6° (c=0.1, MeOH). IR (KBr): 3432, 1719, 1655, 1078 cm^{-1}. High-resolution positive-ion FAB-MS: Calcd for $C_{60}H_{92}O_{28}Na$ $(M+Na)^+$: 1283.5673. Found: 1283.5653. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.83, 0.95, 1.10, 1.31, 1.43, 1.76, 1.86, 2.03 (3H each, all s, 25, 26, 29, 30, 24, 27, Tig-5, Ac-2-H_3), 1.61 (3H, d, J=7.2 Hz, Tig-4-H_3), 2.83 (1H, dd-like, 18-H), 4.04 (1H, m, 3-H), 4.26 (2H, m, 28-H_2), 4.47 (1H, m, 22-H), 4.70 (1H, br s, 16-H), 4.85 (1H, d, J=7.2 Hz, GlcA-1-H), 5.09 (1H, d, J=6.5 Hz, Glc-1-H), 5.50 (1H, br s, 12-H), 5.60 (1H, d, J=7.2 Hz, Gal-1-H), 5.76 (1H, d, J=4.5 Hz, Ara-1-H), 6.38 (1H, d, J=10.0 Hz, 21-H), 7.00 (1H, dq-like, Tig-3-H), 9.89 (1H, s, 23-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: m/z 1259 $(M-H)^-$, 1097 $(M-C_6H_{11}O_5)^-$, 965 $(M-C_{11}H_{19}O_9)^-$. Positive-ion FAB-MS: m/z 1283 $(M+Na)^+$.

Isolation of Assamsaponins J (6) from the Leaves of *C. sinensis* var. *assamica* The leaves of *C. sinensis* L. var. *assamica* Pierre (4.1 kg, cultivated in Nuwara Eliya, Sri Lanka) were cut and extracted three times with MeOH under reflux. Evaporation of the solvent under reduced pressure provided the MeOH extract (1316 g, 32.1%). Part of it (418 g) was partitioned in an AcOEt–H_2O (1 : 1, v/v) mixture. The aqueous layer was suspended in water and treated with polyvinylpyrrolidone (Nacalai Tesque) to remove the polyphenols. After filtration, the filtrate was extracted with *n*-BuOH. Removal of the solvent from the AcOEt-soluble, *n*-BuOH-soluble, and H_2O-soluble fractions under reduced pressure yielded an AcOEt extract (235 g, 5.7%), an *n*-BuOH extract (73 g, 1.8%), and an H_2O phase. The *n*-BuOH extract (62 g) was subjected to normal-phase silica gel column chromatography [1 kg, $CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower layer→7 : 3 : 0.5, v/v)→MeOH] to give eight fractions [Fr. 1 (9.4 g), 2 (20.5 g), 3 (3.8 g), 4 (4.5 g), 5 (2.7 g), 6 (5.8 g), 7 (1.7 g), 8 (7.5 g)]. Reversed-phase silica gel column chromatography [114 g, MeOH–H_2O (50 : 50→80 : 20, v/v)→MeOH] of fraction 3 (3.8 g) gave three fractions [Fr. 3-1 (1.8 g), 2 (494 mg), 3 (1.4 g)]. Fraction 3-2 (480 mg) was purified by HPLC [CH_3CN–1% aq. AcOH (45 : 55, v/v)] to give assamsaponin J (**6**, 49 mg, 0.0012%).

Assamsaponin J (**6**): Colorless fine crystals from $CHCl_3$–MeOH, mp 230.2—232.0 °C, $[\alpha]_D^{25}$ −32.2° (c=0.1, MeOH). IR (KBr): 3453, 1718, 1653, 1075 cm^{-1}. High-resolution positive-ion FAB-MS: Calcd for $C_{66}H_{96}O_{27}Na$ $(M+Na)^+$: 1343.6037. Found: 1343.6047. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.77, 0.78, 1.07, 1.15, 1.27, 1.28, 1.50, 2.04, 2.56 (3H each, all s, 26, 25, 29, 24, 23, 30, 27, Ac-2, Ac-2′-H_3), 1.51 (3H, d, J=6.4 Hz, Rha-6-H_3), 3.07 (1H, dd, J=4.0, 14.4 Hz, 18-H), 3.24 (1H, dd, J=5.2, 11.6 Hz, 3-H), 3.48, 3.62 (1H each, both d, J=9.2 Hz, 28-H_2), 4.93 (1H, d, J=7.6 Hz, GlcA-1-H), 5.43 (1H, br s, 16-H), 5.99 (1H, br s, Rha-1-H), 5.63 (1H, d, J=7.6 Hz, Gal-1-H), 5.71 (1H, br s, 12-H), 5.87 (1H, d, J=10.4 Hz, 21-H), 6.04 (1H, d, J=5.8 Hz, Ara-1-H), 6.27 (1H, d, J=10.4 Hz, 22-H), 6.73 (1H, d, J=16.2 Hz, Cin-2-H), 7.32 (3H, m, Cin-3′, 4′, 5′-H), 7.64 (2H, m, Cin-2′, 6′-H), 8.04 (1H, d, J=16.2 Hz, Cin-3-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: m/z 1319 $(M-H)^-$. Positive-ion FAB-MS: m/z 1343 $(M+Na)^+$, 1365 $(M+2Na-H)^+$.

Alkaline Hydrolysis of Assamsaponins F (1), G (2), H (3), and I (4) A solution of assamsaponins (**1**, **2**: 5 mg; **3**: 30 mg; **4**: 20 mg) in 50% aqueous dioxane (2 ml) was treated with 10% aqueous KOH (2 ml) and stirred at 37 °C for 1 h. After removal of the solvent from an aliquot (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 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**, **2**, and **3**, tiglic acid (b) from **4**, and acetic acid (c) from **1**, **2**, **3**, and **4**. 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; t_R, 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, v/v)] to give desacyl-assamsaponin F (**5**, 3 mg from **1**; 16 mg from **2**; 17 mg from **3**; 4 mg from **4**).

Desacyl-assamsaponin F (**5**): Colorless fine crystals from $CHCl_3$–MeOH, mp 200.2—201.0 °C, $[\alpha]_D^{26}$ +36.0° (c=0.1, MeOH). IR (KBr): 3453, 1719, 1647, 1078 cm^{-1}. High-resolution positive-ion FAB-MS: Calcd for $C_{53}H_{84}O_{26}Na$ $(M+Na)^+$: 1159.5149. Found: 1159.5192. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.82, 0.85, 1.30, 1.34, 1.43, 1.78 (3H each, all s, 25, 26, 29, 30, 24, 27-H_3), 2.68 (1H, dd-like, 18-H), 3.61, 3.87 (1H each, both m, 28-H_2), 4.03 (1H, m, 3-H), 4.55 (1H, m, 22-H), 4.72 (1H, d, J=9.7 Hz, 21-H), 4.85 (1H, d, J=6.9 Hz, GlcA-1-H), 4.96 (1H, br s, 16-H), 5.09 (1H, d, J=5.3 Hz, Glc-1-H), 5.36 (1H, br s, 12-H), 5.61 (1H, d-like, Gal-1-H), 5.75 (1H, d, J=5.3 Hz, Ara-1-H), 9.86 (1H, s, 23-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: m/z 1135 $(M-H)^-$, 973 $(M-C_6H_{11}O_5)^-$, 841 $(M-C_{11}H_{19}O_9)^-$. Positive-ion FAB-MS: m/z 1159 $(M+Na)^+$.

Alkaline Hydrolysis of Assamsaponins J (6) A solution of assamsaponins (**6**, 11 mg) in 50% aqueous dioxane (2 ml) was treated with 10% aqueous KOH (2 ml) and stirred at 37 °C for 1 h. After removal of the solvent from an aliquot (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 stirred at 80 °C for 1 h. The reaction solution was subjected to HPLC analysis to identify the *p*-nitrobenzyl ester of acetic acid from **6**. 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; t_R: 8.3 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, v/v)] to give desacyl-assamsaponin J (**7**, 6 mg) and cinnamic acid (1 mg). Cinnamic acid was found to be identical to an authentic sample following TLC and ^{1}H-NMR ($CDCl_3$) comparisons.

Desacyl-assamsaponin J (**7**): Colorless fine crystals from $CHCl_3$–MeOH, mp 228.3—239.7 °C, $[\alpha]_D^{26}$ −6.8° (c=0.1, MeOH). IR (KBr): 3453, 1638, 1078 cm^{-1}. High-resolution positive-ion FAB-MS: Calcd for $C_{53}H_{86}O_{24}Na$ $(M+Na)^+$: 1129.5407. Found: 1129.5422. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.83, 0.89, 1.14, 1.26, 1.31, 1.35, 1.83 (3H each, all s, 25, 26, 24, 23, 29, 30, 27-H_3), 1.52 (3H, d, J=5.8 Hz, Rha-6-H_3), 2.72 (1H, dd-like, 18-H), 3.27 (1H, dd-like, 3-H), 3.68, 3.99 (1H each, both d, J=10.7 Hz, 28-H_2), 4.55 (1H, m, 22-H), 4.72 (1H, d, J=9.8 Hz, 21-H), 4.96 (1H, d, J=7.3 Hz, GlcA-1-H), 5.00 (1H, br s, 16-H), 5.37 (1H, br s, 12-H), 5.62 (1H, d, J=7.6 Hz, Gal-1-H), 5.98 (1H, br s, Rha-1-H), 6.04 (1H, d, J=5.5 Hz, Ara-1-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS:

m/z 1105 $(M-H)^-$, 943 $(M-C_6H_{11}O_5)^-$. Positive-ion FAB-MS: *m/z* 1129 $(M+Na)^+$.

Acid Hydrolysis of Desacyl-assamsaponins F (5) and J (7) A solution of desacyl-assamsaponins (**5**, **7**, 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, the residue was passed through a Sep-Pak C18 cartridge using 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 to identify the derivatives of D-glucuronic acid (i), D-galactose (ii), and L-arabinose (iii) from **5** and **7**; and D-glucose (iv) from **5**; L-rhamnose from **7**. 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: 24.4 min; v 12.2 min.

Measurement of Gastric Emptying Gastric emptying was determined by a modification of the phenol red method.[9] A solution of 1.5% carboxymethyl cellulose sodium salt (CMC-Na), 40% glucose, milk [milk powder : water (w/w)=1 : 3], or 60% ethanol containing 0.05% phenol red as a marker was given intragastrically (0.5 ml/mouse) to conscious mice. Thirty minutes later, mice were sacrificed by cervical dislocation. The abdominal cavity was opened, and the gastroesophageal junction and pylous were clamped, then the stomach was removed, weighed and placed in 14 ml 0.1 N of NaOH and homogenized. The suspension was allowed to settle for 1 h at room temperature and 5 ml of the supernatant was added to 0.5 ml of 20% trichloroacetic acid (w/v) and then centrifuged at 3000 rpm for 20 min. The supernatant was mixed with 4 ml of 0.5 N NaOH, and the amount of phenol red was determined from the absorbance at 560 nm (Beckman, DU530). Phenol red recovered from animals sacrificed immediately after the administration of a test meal was used as a standard (0% emptying). The gastric emptying (%) during the 30 min period was calculated from the following equation: Gastric emptying (%)=(1−amount of test sample/amount of standard)×100. The test sample was given orally by means of a metal orogastric tube 30 min prior to the administration of the test meal.

Measurement of Gastrointestinal Transit A charcoal meal containing a solution of 1.5% CMC-Na and 5% charcoal as a marker was given intragastrically (0.2 ml/mouse) to conscious mice. Twenty minutes later, mice were sacrificed by cervical dislocation. The abdominal cavity was opened, and the gastrointestinal tract was removed. The distance traveled by the marker was the length of the small intestine from the pylous to the caecum. Test samples were given orally by means of a metal orogastric tube 60 min prior to the administration of the charcoal meal.

Statistics Values are expressed as means±S.E.M. One-way analysis of variance following Dunnett's test[10] for multiple comparison analysis were used for statistical analysis. Probability (*p*) values less than 0.05 were considered significant.

Acknowledgments This research was supported by Grant-in-Aid for Scientific Research on Priority Areas (A) from the Ministry of Education, Science, Sports and Culture, Japan.

References and Notes

1) Part XVI: Yoshikawa M., Murakami T., Ohminami H., Matsuda H., *Chem. Pharm. Bull.*, **48**, 1045—1050 (2000).
2) *a*) Matsuda H., Li Y., Murakami T., Yamahara J., Yoshikawa M., *Eur. J. Pharmacol.*, **368**, 237—243 (1999); *b*) Matsuda H., Li Y., Yoshikawa M., *ibid.*, **373**, 63—70 (1999); *c*) Matsuda H., Li Y., Murakami T., Yamahara J., Yoshikawa M., *Bioorg. Med. Chem.*, **7**, 323—327 (1999); *d*) Matsuda H., Li Y., Yamahara J., Yoshikawa M., *J. Pharmacol. Exp. Ther.*, **289**, 729—734 (1999); *e*) Yoshikawa M., Murakami T., Hirano K., Matsuda H., Yamahara J., Ohtani K., Kasai R., Yamasaki K., *Heterocycles*, **49**, 93—96 (1999); *f*) Matsuda H., Li Y., Yoshikawa M., *Life Sci.*, **65**, PL27—PL32 (1999); *g*) Murakami T., Matsuda H., Inadzuki M., Hirano K., Yoshikawa M., *Chem. Pharm. Bull.*, **47**, 1717—1724 (1999); *h*) Matsuda H., Murakami T., Ikebata A., Yamahara J., Yoshikawa M., *ibid.*, **47**, 1744—1748 (1999); i) Li Y., Matsuda H., Wen S., Yamahara J., Yoshikawa M., *Eur. J. Pharmacol.*, **387**, 337—342 (2000); j) Li Y., Mtsuda H., Yamahara J., Yoshikawa M., *ibid.*, **329**, 71—77 (2000); *k*) Matsuda H., Li Y., Yoshikawa M., *Life Sci.*, **66**, 2233—2238 (2000).
3) Kitagawa I., Hori K., Motozawa T., Murakami T., Yoshikawa M., *Chem. Pharm. Bull.*, **46**, 1901—1906 (1998).
4) Yoshikawa M., Murakami T., Yoshizumi S., Murakami N., Yamahara J., Matsuda H., *Chem. Pharm. Bull.*, **44**, 1899—1907 (1996).
5) Murakami T., Nakamura J., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **47**, 1759—1764 (1999).
6) Yoshikawa K., Nakagawa M., Yamamoto R., Arihara S., Matsuura K., *Chem. Pharm. Bull.*, **40**, 1779—1782 (1992).
7) Hara S., Okabe H., Mihashi K., *Chem. Pharm. Bull.*, **34**, 1843—1845 (1986).
8) The ^{1}H- and ^{13}C-NMR spectra of **1**, **2**, **3**, **4**, **5**, **6**, and **7** were assigned on the basis of homo- and hetero-correlation spectroscopy (^{1}H–^{1}H, ^{1}H–^{13}C COSY), homo- and heteronuclear Hartmann-Hahn spectroscopy (^{1}H–^{1}H, ^{1}H–^{13}C HOHAHA) and heteronuclear multiple bond correlation (HMBC) experiments.
9) a) Taché Y., Maeda M., Hagiwara M., Turkelson C. M., *Am. J. Physiol.*, **253**, G241—G245 (1986); *b*) Barquist E., Bonaz B., Martinez V., Rivier J., Ziner M. J., Taché Y., *ibid.*, **270**, R888—R894 (1996).
10) *a*) Dunnett C. W., *J. Am. Statist. Assoc.*, **75**, 789—795 (1980); *b*) *Idem*, *ibid.*, **75**, 796—800 (1980).