Bioactive Saponins and Glycosides

Part 29[1])

Acylated Oleanane-Type Triterpene Saponins: Theasaponins A_6, A_7, and B_5 from the Seeds of *Camellia sinensis*

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Three new acylated oleanane-type triterpene saponins, theasaponins A_6 (**1**), A_7 (**2**), and B_5 (**3**), were isolated from the saponin fraction of the seeds of the Japanese tea plant *Camellia sinensis* together with the known constituent foliatheasaponin III (**4**). The structures of the glycosides **1**–**3** were elucidated on the basis of spectroscopic, chemical, and physico-chemical evidence.

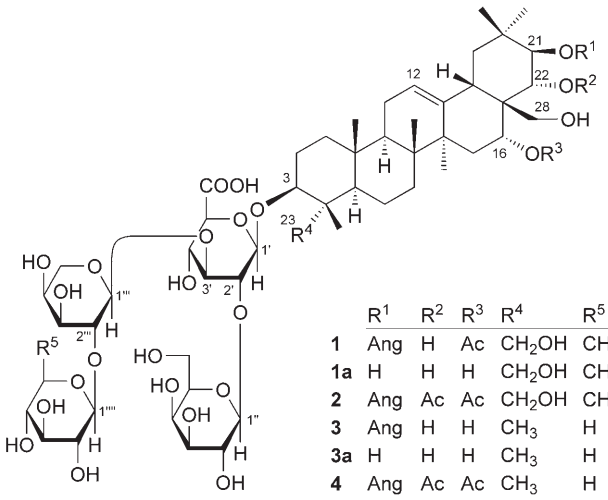
Introduction. – As part of our research on saponin constituents from the seeds of the Japanese tea plant, *Camellia sinensis* (L.) O. KUNTZE (*C. sinensis* L. var. *sinensis*), Theaceae, we have reported over the years the isolation and structure elucidation of theasaponins A_1–A_5, B_1, C_1, E_1–E_{13}, F_1–F_3, H_1, G_1, and G_2 [2–6], as well as those of four flavanol oligoglycosides, theaflavanosides I–IV [7]. Among the isolates, theasaponins A_2, E_1, E_2, and E_5, as well as assamsaponins A, C, and D, were found to show protective effects on EtOH-induced gastric lesions in rats [3][4], hepatoprotective activity [7], and anti-hyperlipidemic activity [8].

Our continuing search now led to the isolation of three new acylated oleanane-type triterpene saponins, theasaponins A_6 (**1**), A_7 (**2**), and B_5 (**3**), which were obtained from the seeds of *C. sinensis*, together with a known saponin, foliatheasaponin III (**4**) [1], which was isolated for the first time from this plant. Herein, we report the structure elucidation of the new compounds.

Results and Discussion. – The saponin fraction of the MeOH extract of tea seeds, cultivated in Shizuoka Prefecture, Japan, as described previously [3], was purified by HPLC to afford compounds **1**, **2**, **3**, and **4** in yields of 60, 100, 60, and 330 ppm, respectively.

Compound **1**, obtained as colorless fine crystals from $CHCl_3$/MeOH (m.p. 226.1–227.4°) exhibited a positive optical rotation ($[\alpha]_D^{23} = +8.4$ (MeOH)). The IR spectrum

[1]) For Part 28, see [1].



	R1	R2	R3	R4	R5
1	Ang	H	Ac	CH_2OH	CH_2OH
1a	H	H	H	CH_2OH	CH_2OH
2	Ang	Ac	Ac	CH_2OH	CH_2OH
3	Ang	H	H	CH_3	H
3a	H	H	H	CH_3	H
4	Ang	Ac	Ac	CH_3	H

Ang = Angeloyl (= (*Z*)-2-methylbut-2-enoyl); Ac = acetyl

of **1** showed absorption bands at 1716 and 1651 cm^{-1} ascribable to COOH and olefin moieties, and broad bands at 3431 and 1084 cm^{-1}, suggestive of an oligoglycoside structure. Positive- and negative-ion FAB-MS analysis of **1** showed the quasimolecular ions at *m*/*z* 1285 ($[M+Na]^+$) and 1261 ($[M-H]^-$), respectively, and positive HR-FAB-MS analysis revealed the molecular formula of **1** as $C_{60}H_{94}O_{28}$.

Alkaline hydrolysis of **1** with 10% aqueous KOH in H_2O/1,4-dioxane 1:1 gave desacyl-theasaponin A (**1a**) [5], together with two organic acids, acetic acid (AcOH) and angelic acid (=(*Z*)-2-methylbut-2-enoic acid; AngOH), which were identified by HPLC analysis of the corresponding 4-nitrobenzyl derivatives [3–5]. Previously, we reported that hydrolysis of **1a** with 5% aqueous H_2SO_4/1,4-dioxane 1:1 gave D-glucuronic acid (D-GlcA), D-galactose (D-Gal), L-arabinose (L-Ara), and D-glucose (D-Glc), as identified by GLC analysis of their trimethylsilyl thiazolidine derivatives [5].

The ^1H- and ^{13}C-NMR spectra of **1** (*Tables 1* and *2*, resp.), which were assigned by various NMR experiments (including DEPT, DQF, HMQC, and HMBC) showed signals assignable to six Me *singlet*s at δ(H) 0.78, 0.85, 1.04, 1.12, 1.29, and 1.42 (Me(26), Me(25), Me(24), Me(29), Me(30), and Me(27)); two oxygenated CH_2 groups at δ(H) 3.65, 3.92 (2*d*, $J = 10.7$ Hz each, CH_2(28)), and at 3.74–3.80 and 4.35–4.42 (2*m*, CH_2(23)); four oxygenated CH groups at δ(H) 4.11–4.17 (*m*, H–C(3)), 4.78 and 5.97 (2*d*, $J = 9.8$ Hz each, H–C(22), H–C(21)), and 5.92 (br. *s*, H–C(16)); an olefinic H-atom at δ(H) 5.34 (br. *s*, H–C(12)); and four glycosyl moieties at δ(H) 5.08 (*d*, $J =$ 6.9 Hz, H–C(1′) of GlcA), 5.14 (*d*, $J = 7.4$ Hz, H–C(1′′′′) of Glc), 5.79 (*d*, $J =$ 7.6 Hz, H–C(1′′) of Gal), and 5.84 (*d*, $J = 5.8$ Hz, H–C(1′′′) of Ara), together with an acetyl (Ac) group at δ(H) 2.51 (*s*) and an angeloyl (Ang) group at δ(H) 1.94 (*s*, Me), 2.02 (*d*, $J = 7.3$ Hz, Me), and 5.91 (*qd*-like, =CH).

The positions of the AcO and AngO groups in **1** were clarified on the basis of an HMBC experiment, which showed $^1H \rightarrow {}^{13}C$ long-range correlations between the

Table 1. *^{1}H-NMR Data of* **1**–**3**. Recorded at 500 MHz and 40 °C in (D_5)pyridine; δ in ppm, J in Hz. Arbitrary atom numbering; for abbreviations, see text.

Atom	**1**	**2**	**3**
H–C(3)	4.11–4.17 (*m*)	4.12–4.16 (*m*)	3.28 (*dd*, *J* = 7.4, 12.5)
H–C(12)	5.34 (br. *s*)	5.38 (br. *s*)	5.37 (br. *s*)
H–C(16)	5.92 (br. *s*)	5.60 (br. *s*)	4.87 (br. *s*)
H–C(18)	2.95 (*dd*-like)	3.01 (*m*)	2.93 (*dd*-like)
H–C(21)	5.97 (*d*, *J* = 9.8)	5.87 (*d*, *J* = 10.4)	6.49 (*d*, *J* = 9.8)
H–C(22)	4.78 (*d*, *J* = 9.8)	6.15 (*d*, *J* = 10.4)	4.81 (*d*, *J* = 9.8)
CH_2(23)	3.74–3.80 (*m*), 4.35–4.42 (*m*)	3.75–3.79 (*m*), 4.36–4.41 (*m*)	–
Me(23)	–	–	1.29 (*s*)
Me(24)	1.04 (*s*)	1.04 (*s*)	1.13 (*s*)
Me(25)	0.85 (*s*)	0.86 (*s*)	0.81 (*s*)
Me(26)	0.78 (*s*)	0.77 (*s*)	0.86 (*s*)
Me(27)	1.42 (*s*)	1.41 (*s*)	1.85 (*s*)
CH_2(28)	3.65 (*d*, *J* = 10.7), 3.92 (*d*, *J* = 10.7)	3.46 (*d*, *J* = 10.4), 3.59 (*d*, *J* = 10.4)	3.69 (*d*, *J* = 10.4), 3.96 (*d*, *J* = 10.4)
Me(29)	1.12 (*s*)	1.06 (*s*)	1.14 (*s*)
Me(30)	1.29 (*s*)	1.27 (*s*)	1.33 (*s*)
H–C(1′) of GlcA	5.08 (*d*, *J* = 6.9)	5.08 (*d*, *J* = 6.9)	4.96 (*d*, *J* = 7.4)
H–C(1″) of Gal	5.79 (*d*, *J* = 7.6)	5.79 (*d*, *J* = 7.9)	5.78 (*d*, *J* = 7.3)
H–C(1‴) of Ara	5.84 (*d*, *J* = 5.8)	5.84 (*d*, *J* = 5.8)	5.81 (*d*, *J* = 5.8)
H–C(1⁗) of Glc	5.14 (*d*, *J* = 7.4)	5.14 (*d*, *J* = 6.4)	–
H–C(1⁗) of Xyl	–	–	5.04 (*d*, *J* = 7.6)
16-AcO	2.51 (*s*)	2.51 (*s*)	–
H–C(3) of Ang	5.91 (*qd*-like)	5.99 (*qd*-like)	5.90 (*qd*-like)
Me(4) of Ang	2.02 (*d*, *J* = 7.3)	2.06 (*d*, *J* = 7.3)	2.06 (*d*, *J* = 7.0)
Me(5) of Ang	1.94 (*s*)	1.98 (*s*)	1.99 (*s*)
22-AcO	–	2.04 (*s*)	–

following pairs: H–C(16) and δ(C) 170.0 (C=O of Ac), H–C(21) and δ(C) 168.4 (C(1) of Ang), H–C(1′) of GlcA and δ(C) 82.7 (C(3)), H–C(1″) of Gal and δ(C) 78.8 (C(2′) of GlcA), H–C(1‴) of Ara and δ(C) 84.7 (C(3′) of GlcA), and H–C(1⁗) of Glc and δ(C) 81.2 (C(2‴) of Ara. Furthermore, comparison of the ^{13}C-NMR data of **1** with those of **1a** revealed acylation shifts at the 16- and 21-positions of the desacyltheasaponin A moiety [**1a**: δ(H) 5.00 (br. *s*, H–C(16)), 4.77 (*d*, *J* = 9.8 Hz, H–C(21)); δ(C) 67.8 (C(16)), 78.7 (C(21))] [5].

On the basis of the above evidence, the structure of theasaponin A_6 (**1**) was determined as 16-*O*-acetyl-21-*O*-angeloyltheasapogenol A 3-*O*-β-D-galactopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 3)]-β-D-glucuronopyranoside[2]).

Compound **2**, obtained as colorless fine crystals from $CHCl_3$/MeOH (m.p. 224.8–225.7°), exhibited a positive optical rotation ($[\alpha]_D^{24} = +7.4$ (MeOH)). The IR spectrum of **2** showed absorption bands at 3453, 1725, 1645, and 1076 cm^{-1}, ascribable to OH,

[2]) For systematic names, see *Exper. Part*.

Table 2. *^{13}C-NMR Data of* **1**–**3**. Recorded at 125 MHz and 40 °C in (D_5)pyridine; δ in ppm. Arbitrary atom numbering; for abbreviations, see text.

Position	**1**	**2**	**3**	Position	**1**	**2**	**3**
1	38.7	38.7	38.8	22-AcO:			
2	25.6	25.6	26.6	1		170.4	
3	82.7	82.7	89.5	2		21.0	
4	43.5	43.5	39.6	β-D-GlcA:			
5	48.0	48.0	55.8	1′	104.3	104.3	105.7
6	18.0	18.0	18.4	2′	78.8	78.7	79.0
7	32.7	32.7	33.1	3′	84.7	84.8	83.9
8	40.0	40.0	40.0	4′	71.5	71.4	71.1
9	47.0	46.9	46.9	5′	77.3	77.3	77.3
10	36.6	36.6	36.7	6′	171.9	171.9	172.2
11	23.8	23.8	23.8	β-D-Gal:			
12	124.5	125.1	122.7	1″	103.2	103.5	103.5
13	141.9	141.0	143.5	2″	73.8	73.8	73.3
14	41.4	41.1	41.8	3″	75.1	75.1	75.1
15	30.9	30.9	34.4	4″	69.9	69.9	70.1
16	71.6	71.4	67.8	5″	76.6	76.6	76.4
17	47.6	46.9	47.8	6″	61.9	61.9	61.9
18	39.8	39.5	40.4	α-L-Ara:			
19	47.3	47.1	46.9	1‴	101.7	101.8	101.7
20	36.0	35.9	36.1	2‴	81.2	81.1	82.1
21	80.5	78.3	81.6	3‴	72.3	72.3	73.8
22	70.8	73.3	73.0	4‴	67.5	67.5	68.3
23	64.7	64.6	28.0	5‴	64.7	64.7	65.9
24	13.6	13.6	16.8	β-D-Glc:			
25	16.2	16.1	15.8	1⁗	105.9	105.9	
26	16.8	16.8	16.8	2⁗	75.8	75.9	
27	27.1	27.0	27.3	3⁗	78.4	78.5	
28	64.7	63.7	65.9	4⁗	71.6	71.5	
29	30.0	29.4	29.9	5⁗	78.4	78.5	
30	20.2	19.7	20.4	6⁗	62.6	62.6	
16-AcO:				β-D-Xyl:			
1	170.0	169.8		1⁗			106.8
2	22.3	22.0		2⁗			75.7
21-AngO:				3⁗			78.3
1	168.4	167.8	168.6	4⁗			70.8
2	129.2	128.4	129.5	5⁗			67.5
3	136.8	138.1	136.0				
4	16.0	16.0	15.9				
5	21.1	20.9	21.0				

COOH, olefin, and ether functions. The molecular formula $C_{62}H_{96}O_{29}$ was determined by positive- and negative-ion FAB-MS (*m*/*z* 1327 ($[M+Na]^+$) and 1303 ($[M-H]^-$), resp.), and by HR-FAB-MS (positive-ion mode). The fragmentation patterns in the negative-ion FAB mass spectrum of **2** indicated the loss of a terminal mono-hexose (*m*/*z* 1141 ($[M-C_6H_{11}O_5]^-$) and a disaccharide part (pentose and hexose units; *m*/*z* 1009 ($[M-C_{11}H_{19}O_9]^-$).

Treatment of **2** with 10% aqueous KOH in H_2O/1,4-dioxane 1:1 liberated **1a** and both AcOH and AngOH, as identified by HPLC analysis of their 4-nitrobenzyl derivatives.

The ^{1}H- and ^{13}C-NMR spectra of **2** (*Tables 1* and *2*, resp.) revealed two AcO groups at δ(H) 2.04, 2.51 (2*s*) and an AngO group at δ(H) 1.98 (*s*, Me), 2.06 (*d*, $J = 7.3$ Hz, Me), and 5.99 (*qd*-like, =CH). Their positions were clarified by an HMBC experiment. Thus, long-range correlations were observed between H–C(16) and δ(C) 169.8 (C=O of Ac), between H–C(21) and δ(C) 167.8 (C(1) of Ang), and between H–C(22) and δ(C) 170.4 (C=O of Ac). Furthermore, comparison of the ^{13}C-NMR data of **2** with those of **1** revealed an acetylation shift near the 22-position of the aglycone. Consequently, the structure of theasaponin A_7 (**2**) was determined as 16,22-di-*O*-acetyl-21-*O*-angeloyltheasapogenol A 3-*O*-β-D-galactopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 3)]-β-D-glucuronopyranoside.

Compound **3**, obtained as colorless fine crystals from $CHCl_3$/MeOH (m.p. 233.1–233.9°), exhibited a positive optical rotation ($[\alpha]_D^{23} = +4.0$ (MeOH)). Analysis of the positive- and negative-ion FAB mass spectra of **3** showed quasimolecular ion peaks at *m*/*z* 1197 ($[M+Na]^+$) and 1173 ($[M-H]^-$), respectively. HR-FAB-MS Analysis (positive-ion mode) revealed the molecular formula to be $C_{57}H_{90}O_{25}$.

Alkaline hydrolysis of **3** with 10% aqueous KOH in H_2O/1,4-dioxane 1:1 liberated desacyl-assamsaponin E (**3a**) [9] and AngOH, which was identified by HPLC analysis of its 4-nitrobenzyl derivative. Previously, we reported that hydrolysis of **3a** with 5% aqueous H_2SO_4/1,4-dioxane 1:1 gave D-GlcA, D-Gal, L-Ara, and D-xylose (D-Xyl), which were identified by GLC analysis of their trimethylsilyl thiazolidine derivatives [9].

The ^{1}H- and ^{13}C-NMR Spectra of **3** (*Tables 1* and *2*, resp.) indicated the presence of a theasapogenol-B (barringtogenol-C) moiety, with seven Me *singlet*s at δ(H) 0.81, 0.86, 1.13, 1.14, 1.29, 1.33, and 1.85 (Me(25), Me(26), Me(24), Me(29), Me(23), Me(30), and Me(27)), a CH_2 moiety and four oxygenated CH groups [δ(H) 3.28 (*dd*, $J = 7.4$, 12.5 Hz, H–C(3)); 3.69, 3.96 (2*d*, $J = 10.4$ Hz each, CH_2(28)); 4.81, 6.49 (2*d*, $J = 9.8$ Hz, H–C(22), H–C(21)); 4.87 (br. *s*, H–C(16))], an olefinic H-atom at δ(H) 5.37 (br. *s*, H–C(12)), and four glycosyl moieties at δ(H) 4.96 (*d*, $J = 7.4$ Hz, H–C(1′) of GlcA), 5.04 (*d*, $J = 7.6$ Hz, H–C(1‴) of Xyl), 5.78 (*d*, $J = 7.3$ Hz, H–C(1″) of Gal), and 5.81 (*d*, $J = 5.8$ Hz, H–C(1‴) of Ara), together with an Ang group at δ(H) 1.99 (*s*, Me), 2.06 (*d*, $J = 7.0$ Hz, Me), and 5.90 (*qd*-like, =CH). In the HMBC spectrum of **3**, a long-range $^1H \rightarrow {}^{13}C$ correlation was observed between H–C(21) and δ(C) 168.6 (C(1) of Ang).

From the above data, the structure of theasaponin B_5 (**3**) was determined as 21-*O*-angeloyltheasapogenol B 3-*O*-β-D-galactopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 3)]-β-D-glucuronopyranoside.

Experimental Part

General. Column chromatography (CC): silica gel *BW-200* (150–300 mesh; *Fuji Silysia Chemical, Ltd.*, Japan). HPLC: *Shimadzu RID-6A* refractive-index and *SPD-10A* UV/VIS detectors, *LC-6AD* pump, *CTO-10A* oven, and *Chromatopac C-R6A* column. M.p: *Yanagimoto MP-500D* micro-melting-point apparatus; uncorrected. Optical rotations: *Horiba SEPA-300* digital polarimeter ($l = 5$ cm). IR

Spectra: *Shimadzu FT-IR-8100* spectrometer; in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Jeol JNM-LA500* spectrometer; at 500/125 MHz, resp., δ in ppm rel. to Me_4Si, J in Hz. FAB- and HR-FAB-MS: *Jeol JMS-SX 102A* mass spectrometer; in m/z.

Plant material. The seeds of *Camellia sinensis* were cultivated in 2004 at Shizuoka Prefecture, Japan, as described previously [2].

Extraction and Isolation. Compounds **1**–**4** were isolated as described below from three previously reported [2] fractions, *Fr. 5* (2.20 g), *Fr. 6* (0.96 g), and *Fr. 8* (0.97 g), originally obtained from the saponin fraction (eluted with MeOH) of the seeds of *C. sinensis* (1.0 kg).

Fr. 5 (2.20 g) was separated by HPLC [*YMC-Pack ODS-A*, 250 × 20 mm i.d.; MeCN/1% aq. AcOH 40:60; 9.0 ml/min] to afford the following twelve subfractions: *Fr. 5.1* [13 mg (50 ppm), theasaponin A_4; t_R 15.5 min], *Fr. 5.2* [53 mg (210 ppm), theasaponin A_1; t_R 21.9 min], *Fr. 5.3* [23 mg (90 ppm), theasaponin F_1; t_R 25.3 min], *Fr. 5.4* [14 mg (60 ppm), theasaponin A_6 (**1**); t_R 27.0 min], *Fr. 5.5* (37 mg), *Fr. 5.6* (164 mg), *Fr. 5.7* (100 mg), *Fr. 5.8* (328 mg), *Fr. 5.9* (148 mg (59 ppm), theasaponin A_3; t_R 42.2 min]; *Fr. 5.10* (200 mg), *Fr. 5.11* (645 mg), and *Fr. 5.12* (85 mg). Then, *Fr. 5.8* (328 mg) was subjected to HPLC [*Develosil C30-UG-5*, 250 × 20 mm i.d.; MeCN/MeOH/1% aq. AcOH 35:16:49; 9.0 ml/min] to afford theasaponin A_7 [**2**; 26 mg (100 ppm); t_R 48.2 min], together with camelliasaponin C_1 [10 mg (40 ppm); t_R 41.9 min], theasaponin C_1 [77 mg (310 ppm); t_R 44.7 min], and theosaponin F_2 [54 mg (210 ppm); t_R 46.5 min].

Fr. 6 (960 mg) was subjected to HPLC [*YMC-pack ODS-A*, 250 × 20 mm i.d.; MeCN/1% aq. AcOH 43:57; 9.0 ml/min] to give the following nine fractions: *Fr. 6.1* [16 mg (60 ppm), theasaponin B_5 (**3**); t_R 20.7 min], *Fr. 6.2* [56 mg (220 ppm), assamsaponin I; t_R 25.1 min], *Fr. 6.3* [323 mg (1300 ppm), assamsaponin C; t_R 27.3 min], *Fr. 6.4* (65 mg), *Fr. 6.5* [39 mg (160 ppm), floratheasaponin A; t_R 29.3 min], *Fr. 6.6* (15 mg), *Fr. 6.7* (75 mg), *Fr. 6.8* (20 mg), and *Fr. 6.9* [126 mg (500 ppm), theasaponin E_5; t_R 37.1 min].

Fr. 8 (0.97 g) was subjected to HPLC [*YMC-Pack ODS-A*, 250 × 20 mm i.d., MeCN/1% aq. AcOH 43:57; 9.0 ml/min] to afford five fractions: *Fr. 8.1* [323 mg (1300 ppm), theasaponin A_2; t_R 28.0 min], *Fr. 8.2* [136 mg (540 ppm), theasaponin F_3; t_R 31.8 min], *Fr. 8.3* (46 mg), *Fr. 8.4* (84 mg), and *Fr. 8.5* [82 mg (330 ppm), foliatheasaponin III (**4**); t_R 39.3 min].

Theasaponin A_6 (=*(3β,16α,21β,22α)-16-Acetoxy-22,23,28-trihydroxy-21-{[(2Z)-2-methylbut-2-enoyl]oxy}olean-12-en-3-yl β-*D*-Galactopyranosyl-(1→2)-[β-*D*-glucopyranosyl-(1→2)-α-*L*-arabinopyranosyl-(1→3)]-β-*D*-glucopyranosiduronic Acid*; **1**). Colorless, fine crystals. M.p. 226.1–227.4° ($CHCl_3$/MeOH). $[\alpha]_D^{23} = +8.4$ ($c = 0.55$, MeOH). IR (KBr): 3431, 1716, 1651, 1084. ^1H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. FAB-MS (pos.): 1285 ($[M+Na]^+$). FAB-MS (neg.): 1261 ($[M-H]^-$), 1099 ($[M-C_6H_{11}O_5]^-$), 967 ($[M-C_{11}H_{19}O_9]^-$). HR-FAB-MS (pos.): 1285.5839 ($[M+Na]^+$, $C_{60}H_{94}NaO_{28}^+$; calc. 1285.5829).

Theasaponin A_7 (=*(3β,16α,21β,22α)-16,22-Diacetoxy-23,28-dihydroxy-21-{[(2Z)-2-methylbut-2-enoyl]oxy}olean-12-en-3-yl β-*D*-Galactopyranosyl-(1→2)-[β-*D*-glucopyranosyl-(1→2)-α-*L*-arabinopyranosyl-(1→3)]-β-*D*-glucopyranosiduronic Acid*; **2**). Colorless, fine crystals. M.p. 224.8–225.7° ($CHCl_3$/MeOH). $[\alpha]_D^{24} = +7.4$ ($c = 1.01$, MeOH). IR (KBr): 3453, 1725, 1645, 1076. ^1H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. FAB-MS (pos.): 1327 ($[M+Na]^+$). FAB-MS (neg.): 1303 ($[M-H]^-$), 1141 ($[M-C_6H_{11}O_5]^-$), 1009 ($[M-C_{11}H_{19}O_9]^-$). HR-FAB-MS (pos.): 1327.5929 ($[M+Na]^+$, $C_{62}H_{96}NaO_{29}^+$; calc. 1327.5935).

Theasaponin B_5 (=*(3β,16α,21β,22α)-16,22,28-Trihydroxy-21-{[(2Z)-2-methylbut-2-enoyl]oxy}-olean-12-en-3-yl β-*D*-Galactopyranosyl-(1→2)-[β-*D*-xylopyranosyl-(1→2)-α-*L*-arabinopyranosyl-(1→3)]-β-*D*-glucopyranosiduronic Acid*; **3**). Colorless, fine crystals. M.p. 233.1–233.9° ($CHCl_3$/MeOH). $[\alpha]_D^{23} = +4.0$ ($c = 0.88$, MeOH). IR (KBr): 3453, 1716, 1645, 1084. ^1H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. FAB-MS (pos.): 1197 ($[M+Na]^+$). FAB-MS (neg.): 1173 ($[M-H]^-$), 1011 ($[M-C_6H_{11}O_5]^-$). HR-FAB-MS (pos.): 1197.5657 ($[M+Na]^+$, $C_{57}H_{90}NaO_{25}^+$; calc. 1197.5669).

Alkaline Hydrolysis of **1**–**3**. A soln. of the appropriate theasaponin (10 mg) in 50% aq. 1,4-dioxane (1 ml) was treated with 10% aq. KOH (1 ml), and the mixture was stirred at 37° for 1 h. An aliquot (0.1 ml) of the reaction mixture was concentrated under reduced pressure, and the resulting residue was dissolved in 1,2-dichloroethane (2 ml). This soln. was treated with '*para*-nitrobenzyl-*N*,*N*′-diisopropylisourea' (10 mg) and stirred at 80° for 1 h. The reaction mixture was then subjected to HPLC analysis

[*YMC-Pack ODS-A*, 250 × 4.6 mm i.d.; MeOH/H_2O 70 : 30; 0.9 ml/min, UV detection at 254 nm], and the *para*-nitrobenzyl esters of AcOH (t_R 6.3 min) from **1** and **2**, as well as angelic acid (t_R 16.0 min) from **1**–**3** were detected. The rest of each reaction mixture was neutralized over *Dowex HCR W2* resin (H^+ form), which was then removed by filtration. The filtrate was concentrated under reduced pressure, and the resulting product was subjected to CC (2 g SiO_2; $CHCl_3$/MeOH/H_2O 6 : 4 : 1) to give desacyl-theasaponin A [5] (**1a**; 6 mg each from **1** and **2**) or desacyl-assamsaponin E [9] (**3a**; 6 mg, from **3**).

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REFERENCES

[1] T. Morikawa, S. Nakamura, Y. Kato, O. Muraoka, H. Matsuda, M. Yoshikawa, *Chem. Pharm. Bull.* **2007**, *55*, 293.
[2] I. Kitagawa, K. Hori, T. Motozawa, T. Murakami, M. Yoshikawa, *Chem. Pharm. Bull.* **1998**, *46*, 1901.
[3] M. Yoshikawa, T. Morikawa, N. Li, A. Nagatomo, X. Li, H. Matsuda, *Chem. Pharm. Bull.* **2005**, *53*, 1559.
[4] T. Morikawa, N. Li, A. Nagatomo, H. Matsuda, X. Li, M. Yoshikawa, *J. Nat. Prod.* **2006**, *69*, 185.
[5] M. Yoshikawa, T. Morikawa, S. Nakamura, N. Li, X. Li, H. Matsuda, *Chem. Pharm. Bull.* **2007**, *55*, 57.
[6] T. Morikawa, H. Matsuda, N. Li, S. Nakamura, X. Li, M. Yoshikawa, *Heterocycles* **2006**, *68*, 1139.
[7] N. Li, T. Morikawa, H. Matsuda, K. Ninomiya, X. Li, M. Yoshikawa, *Heterocycles* **2007**, *71*, 1193.
[8] M. Yoshikawa, T. Morikawa, K. Yamamoto, Y. Kato, A. Nagatomo, H. Matsuda, *J. Nat. Prod.* **2005**, *68*, 1360.
[9] T. Murakami, J. Nakamura, H. Matsuda, M. Yoshikawa, *Chem. Pharm. Bull.* **1999**, *47*, 1759.

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