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Isotheasaponins B_1–B_3 from *Camellia sinensis* var. *sinensis* tea leaves

Keiko Kobayashi, Toshiaki Teruya, Kiyotake Suenaga, Yoko Matsui, Hideki Masuda, Hideo Kigoshi *

Department of Chemistry, University of Tsukuba, Tsukuba, Ibaraki 305-8571, Japan
Ogawa & Co., Ltd., Urayasu, Chiba 279-0032, Japan

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Abstract

Three saponins, isotheasaponins B_1–B_3, were isolated from the leaves of the tea plant *Camellia sinensis* var. *sinensis*, and their structures were determined to be theasapogenol B [β-D-galactopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 2)-α-L-arabinopyranosyl(1 → 3)]-β-D-gulcopyranosiduronic acid with two acyl groups by spectroscopic analysis.

Keywords: Tea leaves; *Camellia sinensis* var. *sinensis*; Theaceae; Isotheasaponin; Theasapogenol B

1. Introduction

Tea is regarded as a kind of folk medicine, and its contents have been investigated. Several saponins were isolated from tea plants, such as theasaponins E_1 and E_2 (Kitagawa et al., 1998) and assamsaponins A-I (Murakami et al., 1999, 2000) from the seeds and TR-saponins A-C (Lu et al., 2000) from the roots. Very recently, floratheasaponins A-C were isolated from tea flowers (Yoshikawa et al., 2005). Due to the low content of the saponins in tea leaves, they have not been well investigated; however, several saponins in the leaves were found with the development of analytical methods: theasaponin B_1 (Kitagawa et al., 1995) was isolated from *Camellia sinensis* L. and assamsaponin J (Murakami et al., 2000) from *Camellia sinensis* L. var. *assamica* PIERRE. We now report the isolation and structure elucidation of three new saponins, isotheasaponins B_1–B_3 (**1**–**3**) from the tea leaves.

2. Results and discussion

2.1. Isolation

Camellia sinensis var. *sinensis* green tea leaves were extracted with 50% ethanol. The extract was treated with two kinds of resins (SEPABEADA SP-70 and Divergan) and separated by ODS silica gel and reversed phase HPLC to give isotheasaponins B_1 (**1**), B_2 (**2**), and B_3 (**3**) along with theasaponin B_1. The extract contains a number of the other minor saponins; however, we could not isolated them.

2.2. Structures

First, isolated theasaponin B_1 (**4**) was identified by comparison of its NMR spectroscopic data with the reported results (Kitagawa et al., 1995), and then the all NMR signals of theasaponin B_1 in CD_3OD were reassigned by the 2D NMR technique (Tables 1 and 2).

The molecular formula of isotheasaponin B_1 (**1**) was found to be $C_{63}H_{92}O_{26}$ by its ESIMS (*m*/*z* 1287.5760, calcd for $C_{63}H_{92}O_{26}Na$ $[M + Na]^+$ 1287.5774). The UV and IR spectra indicated the presence of cinnamoyl, hydroxyl, carboxyl, and conjugated carbonyl groups. The ^1H and ^{13}C

* Corresponding author. Address: Department of Chemistry, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8571, Japan. Tel/fax: +81 29 853 4313.
E-mail address: kigoshi@chem.tsukuba.ac.jp (H. Kigoshi).

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Table 1
^1H and ^{13}C NMR spectroscopic data for the aglycone part of isotheasaponins B_1–B_3 (**1**–**3**) and theasaponin B_1 (**4**) in CD_3OD

Comp. No.	Isotheasaponin B_1 (**1**)		Isotheasaponin B_2 (**2**)		Isotheasaponin B_3 (**3**)		Theasaponin B_1 (**4**)	
	^{13}C[a]	^1H[c]	^{13}C[a]	^1H[d]	^{13}C[a]	^1H[d]	^{13}C[b]	^1H[d]
1	40.3	1.64 *m*	40.3	1.64 *m*	40.3	1.64 *m*	40.3	1.65 *m*
		1.01 *m*		1.01 *m*		1.01 *m*		1.01 *m*
2	27.4	1.82 *m*	27.4	1.79 *m*	27.4	1.81 *m*	27.4	1.85 *m*
		1.73 *m*		1.71 *m*		1.73 *m*		1.73 *m*
3	92.6	3.19 *m*	92.6	3.18 *m*	92.6	3.18 *m*	92.5	3.17 *m*
4	40.8		40.8		40.8		40.8	
5	57.4	0.78 *m*	57.4	0.79 *m*	57.4	0.79 *m*	57.3	0.78 *m*
6	19.7	1.58 *m*	19.7	1.58 *m*	19.7	1.57 *m*	19.6	1.57 *m*
		1.42 *m*		1.40 *m*		1.41 *m*		1.40 *m*
7	34.4	1.62 *m*	34.3	1.64 *m*	34.3	1.60 *m*	34.3	1.56 *m*
		1.37 *m*		1.34 *m*		1.34 *m*		1.33 *m*
8	41.3		41.4		41.4		41.4	
9	48.4	1.66 *m*	48.4	1.68 *m*	48.4	1.68 *m*	48.4	1.67 *m*
10	38.2		38.2		38.2		38.2	
11	25.0	1.91 *m*	25.1	1.91 *m*	25.1	1.94 *m*	25.0	1.95 *m*
		1.91 *m*		1.91 *m*		1.94 *m*		1.95 *m*
12	125.7	5.32 *br s*	125.7	5.38 *br s*	125.7	5.39 *br s*	127.1	5.46 *br s*
13	143.4		143.4		143.4		141.7	
14	42.8		42.8		42.8		42.5	
15	35.3	1.79 *m*	35.2	1.70 *m*	35.2	1.71 *m*	32.0	1.83 *m*
		1.42 *m*		1.35 *m*		1.39 *m*		1.43 *m*
16	69.2	4.12 *br s*	69.9	4.02 *br s*	69.9	4.03 *br s*	72.8	5.19 *br s*
17	48.0		49.1		49.0		47.9	
18	41.6	2.52 *br d* (13.9)	41.2	2.64 *m*	41.2	2.67 *m*	41.0	2.65 dd (3.7, 14.3)
19	48.4	2.65 *dd* (13.5, 13.9)	48.2	2.67 *m*	48.2	2.68 *m*	48.5	2.45 *dd* (13.6, 14.3)
		1.18 *m*		1.19 *m*		1.23 *m*		1.35 *m*
20	37.1		37.1		37.2		37.2	
21	83.0	5.66 *d* (10.0)	81.0	5.89 *d* (10.1)	80.2	6.00 *d* (10.1)	80.2	5.51 *d* (10.3)
22	72.7	3.98 *d* (10.0)	75.5	5.54 *d* (10.1)	75.6	5.58 *d* (10.1)	74.8	5.55 *d* (10.3)
23	28.8	1.08 *s*	28.8	1.08 *s*	28.8	1.08 *s*	28.7	1.08 *s*
24	17.3	0.88 *s*	17.3	0.88 *s*	17.3	0.88 *s*	17.3	0.88 *s*
25	16.6	0.97 *s*	16.6	0.97 *s*	16.6	0.97 *s*	16.5	0.98 *s*
26	17.8	0.93 *s*	17.7	0.93 *s*	17.3	0.94 *s*	17.7	0.95 *s*
27	28.0	1.46 *s*	28.1	1.48 *s*	28.1	1.50 *s*	27.7	1.35 *s*
28	67.4	3.90 *m*	64.9	3.28[e]	64.9	3.29[e]	65.4	3.30[e]
		3.75 *m*		2.98 *d* (11.2)		2.99 *d* (11.2)		3.13 *d* (11.2)
29	30.2	0.88 *s*	30.0	0.87 *s*	30.0	0.89 *s*	30.0	0.93 *s*
30	20.5	1.08 *s*	20.3	1.06 *s*	20.6	1.09 *s*	19.9	1.13 *s*
Cin	21*Cin*		22*Cin*		22*Cin*		21*Cin*	
Cα	169.8		169.4		169.4		169.0	
Cβ	120.1	6.59 *d* (16.0)	119.2	6.50 *d* (16.0)	119.2	6.45 *d* (16.0)	119.0	6.47 *d* (16.0)
Cγ	146.3	7.70 *d* (16.0)	147.2	7.73 *d* (16.0)	147.2	7.69 *d* (16.0)	147.1	7.65 *d* (16.0)
C1′	136.3		136.2		136.2		136.2	
C2′, 6′	129.6	7.61 *m*	129.7	7.59 *m*	129.6	7.56 *m*	129.7	7.59 *m*
C3′, 5′	130.4	7.40 *m*	130.4	7.40 *m*	130.4	7.39 *m*	130.5	7.40 *m*
C4′	131.8	7.40 *m*	132.0	7.40 *m*	131.9	7.39 *m*	132.0	7.40 *m*
Ac	28*Ac*		21*Ac*				16*Ac*	
C=O	173.0		173.5				172.1	
CH_3	21.2	2.07 *s*	21.4	1.92 *s*			22.5	2.26 *s*
Ac							22*Ac*	
C=O							172.9	
CH_3							21.2	1.84 *s*
Ang					21*Ang*			
C1″					169.9			
C2″					129.8			
C3″					139.0	5.98 *qq* (1.4, 7.2)		
C4″					16.4	1.81 *dq* (7.2, 1.4)		
C5″					21.2	1.78 *br s*		

[a] Recorded on 100 MHz.
[b] Recorded on 125 MHz.
[c] Recorded on 600 MHz.
[d] Recorded on 500 MHz.
[e] overlapped with solvent signals.

Table 2
^1H and ^{13}C NMR spectroscopic data for sugar part of isotheasaponins B_1 (**1**) in CD_3OD

No.	^{13}C[a]	^1H[b]
GlcA		
C-1	106.4	4.54 *br d* (7.5)
C-2	79.0	3.91 *m*
C-3	84.0	3.82 *m*
C-4	71.7	3.63 *m*
C-5	76.6	3.90 *m*
C-6	171.4	
Gal		
C-1	103.4	5.03 *br d* (5.0)
C-2	73.8	3.53 *m*
C-3	75.2	3.53 *m*
C-4	70.9	3.81 *m*
C-5	77.3	3.59 *m*
C-6	63.2	3.77 *m*
		3.62 *m*
Ara		
C-1	102.4	4.92[c]
C-2	83.5	3.73 *m*
C-3	74.4	3.79 *m*
C-4	69.9	3.86 *m*
C-5	67.4	3.90 *m*
		3.56 *m*
Xyl		
C-1	107.7	4.50 *br d* (7.6)
C-2	76.4	3.29 *m*
C-3	78.3	3.34 *m*
C-4	71.4	3.50 *m*
C-5	67.7	3.97 *m*
		3.20 *m*

[a] Recorded on 100 MHz.
[b] Recorded on 600 MHz.
[c] Overlapped with solvent signals.

NMR spectroscopic data are listed in Tables 1 and 2. The data clearly suggested that isotheasaponin B_1 (**1**) is an analog of theasaponin B_1 (**4**). From a comparison of the NMR data (Table 2) with those of theasaponin B_1 (**4**), isotheasaponin B_1 (**1**) contained [β-D-galactopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 2)-α-L-arabinopyranosyl(1 → 3)]-β-D-gulcopyranosiduronic acid as same as in theasaponin B_1. The connectivities between the sugars were confirmed by analysis of the HMBC data, and the stereochemistry of glycosyl bonds was confirmed by the coupling constants if available. On the basis of HMBC and HMQC data, all the ^1H and ^{13}C signals were assigned as shown in Table 1, confirming the presence of theasapogenol B (Yoshioka et al., 1966). Especially, the stereochemistry of C-16, -21, and -22 was determined by a comparison of their coupling constants ($J_{15–16}$ and $J_{21–22}$) with those of theasaponin B_1 (**4**). The molecular formula and the NMR spectroscopic data showed that isotheasaponin B_1 (**1**) lacked an acetyl group. The positions of an acetyl and a cinnamoyl group were determined by its HMBC correlations (Cin Cα/H21, Ac C=O/H28) and the chemical shifts of its oxymethine and oxymethylene protons (δ_{H21} 5.66, δ_{H28} 3.90 and 3.75) to be the 28- and 21-positions, respectively. Thus, the structure of isotheasaponin B_1 was determined as shown in structural formula **1**.

The molecular formula of isotheasaponin B_2 (**2**) was found to be $C_{63}H_{92}O_{26}$ by its ESIMS (*m*/*z* 1287.5749, calcd for $C_{63}H_{92}O_{26}Na$ $[M + Na]^+$ 1287.5774), indicating that isotheasaponin B_2 (**2**) is an isomer of isotheasaponin B_1 (**1**). This result was confirmed by analysis of the NMR spectroscopic data (Table 1). The comparison of the ^1H NMR data (δ_{H21} 5.89, δ_{H22} 5.54) with those of theasaponin B_1 (**4**) and isotheasaponin B_2 (**2**) and the HMBC correlations (Ac C=O/H21, Cin Cα/H22) indicated that the acetyl and cinnamoyl groups are bound to the 21- and 22-positions, respectively. Thus, the structure of isotheasaponin B_2 was determined to be that depicted in structural formula **2**.

The molecular formula of isotheasaponin B_3 (**3**) was found to be $C_{66}H_{96}O_{26}$ by its ESIMS (*m*/*z* 1327.6096, calcd for $C_{66}H_{96}O_{26}Na$ $[M + Na]^+$ 1327.6088), indicating that isotheasaponin B_3 (**3**) possesses a C_5H_7O instead of an acetyl group. The detailed analysis of the NMR spectroscopic data (Table 1) suggested the presence of CH_3–CH=C(CH_3)CO group. That one was angeloyl was deduced by comparison of the NMR data (δ_H 5.98 and 1.81, δ_C 16.4 and 21.2) with those of natural products (Murakami et al., 1999, 2000) having angeloyl and tigloyl groups: δ_H 5.9–6.0 and 2.0–2.1, δ_C 15.5–16.0 and 20.5–21.0 for angeloyl; δ_H 7.0 and 1.6, δ_C 14.0–14.1 and 12.0–

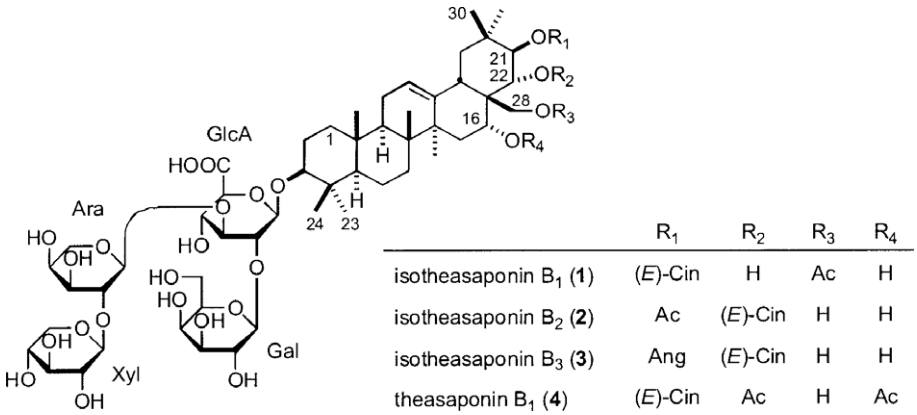

Fig. 1. Structures of isotheasaponins B_1–B_3 (**1**–**3**) and theasaponin B_1 (**4**).

12.5 for tigloyl. The positions of the acyl groups were determined by the HMBC correlations, establishing the structure of isotheasaponin B_3 as shown in Fig. 1.

2.3. Conclusions

Three saponins, isotheasaponins B1–B3, were isolated from the leaves of the tea plant Camellia sinensis var. sinensis, and their structures were determined by spectroscopic analysis. Structurally, isotheasaponins B_1–B_3 (**1**–**3**) are closely related to saponins isolated from the tea plant, such as theasaponin B_1 (Kitagawa et al., 1995) and E_1–E_2 (Kitagawa et al., 1998), assamsaponins A-J (Murakami et al., 1999, 2000), and floratheasaponins A-C (Yoshikawa et al., 2005). It is notable that the structurally related jegosaponina A-D (Yoshikawa et al., 2000; Tamura et al., 2005), berneuxia saponins A-C (Wang et al., 1998, 1999), and a triterpenoid saponin (saponin A) (Tuntiwachwuttikul et al., 1997) were isolated from *Styrax japonica*, *Berneuxia thibetica*, and *Maesa ramenyacea*, respectively. Investigation of the biological activities of isotheasaponins B_1–B_3 (**1**–**3**) are now in progress.

3. Experimental

3.1. General procedures

NMR spectra were recorded on BRUKER AVANCE 600 (600 MHz for ^{1}H), AVANCE 500 (500 MHz for ^{1}H, 125 MHz for ^{13}C), and AVANCE 400 (100 MHz for ^{13}C) instruments, respectively. The ^{1}H and ^{13}C chemical shifts are reported in parts per million (δ) relative to the solvent peaks (δ_H 3.30 and δ_C 49.0 ppm in methanol-d_4). Mass spectra and high-resolution mass spectra (HRMS) were measured using an Applied Biosystems QStar pulser *i* instrument in an ESI mode. Column chromatography was performed on ODS gel (Nacalai Tesque, Cosmosil 75 C18-OPN). Reversed-phase high-performance liquid chromatography (HPLC) was carried out on a Develosil ODS-HG-5 column (Nomura Chemical Co., Ltd).

3.2. Extraction and isolation

The leaves of *Camellia sinensis* var. *sinensis*, cultivated for green tea in Kagoshima, Japan, were steamed and dried to give green tea leaves. The latters (6 kg) were soaked overnight in EtOH–H_2O (1:1, 60 kg) at room temperature then filtered to produce the crude tea extract (TE). Distilled water was added to TE in order to adjust the ethanol concentration to 30% v/v, and TE was then passed through a column of SEPABEADS SP-70 (Mitsubishi Chemical Co., Ltd., Tokyo, Japan). The column was next washed with EtOH–H_2O (3:7, 9 L) and 95% EtOH (20 L). To the 95% EtOH fraction was added polyvinylpyrrolidone (Divergan, BASF Japan Ltd., Tokyo, Japan) in order to reduce the amount of tea catechins, and the resin was next removed by filtration. These operations were repeated two times. The catechin-free tea extract (CFTE) obtained from these operations was then concentrated in vacuo to give a green powdery residue (39 g). An aliquot (1.1 g) was applied to an ODS silica gel column [Cosmosil 75 C15-OPN 11 g, MeOH–H_2O (40:60 to 80:20), 110 mL each] to give the crude saponin (0.43 g) from the MeOH–H_2O (80:20) eluate.

The crude saponin (200 mg) was further purified by preparative HPLC (Develosil ODS-HG-5, 20 × 250 mm, MeCN-0.05% aqueous TFA 55:45, 5 mL/min) to give fraction A (t_R = 24–30 min, 97 mg) containing isotheasaponins B_1 and B_2 and fraction B (t_R = 49–55 min, 14 mg) containing isotheasaponin B_3.

Fraction A was further purified by preparative HPLC (Develosil ODS-HG-5, 20 × 250 mm, MeOH-0.05% aqueous TFA 75:25, 5 mL/min) to give fraction A-1 containing isotheasaponin B_1 and fraction A-2 containing isotheasaponin B_2. Fraction A-1 was further purified by preparative HPLC (Develosil ODS-HG-5, 20 × 250 mm, MeCN-0.05% aqueous TFA 46:54, 5 mL/min, t_R = 48–51 min) to give isotheasaponin B_1 (**1**, 2 mg) as a colorless powder. Fraction A-2 was further purified by preparative HPLC (Develosil ODS-HG-5, 20 × 250 mm, MeCN-0.05% aqueous TFA 45:55, 5 mL/min, t_R = 49–52 min) to give isotheasaponin B_2 (**2**, 4 mg) as a colorless powder.

Fraction B was further purified by repeated preparative HPLC (Develosil Ph-UG-5, 20 × 250 mm, MeOH-0.05% aqueous TFA 75:25, 5 mL/min; 2. Develosil ODS-HG-5, 20 × 250 mm, MeCN-0.05% aqueous TFA 55:45, 5 mL/min, t_R = 38–41 min) to give isotheasaponin B_3 (**3**, 2 mg) as a colorless powder.

Repetitive above mentioned HPLC separation of crude saponin provided isotheasaponin B_1 (**1**, 9.1 mg from crude saponin 1.0 g), isotheasaponin B_2 (**2**, 11.5 mg from crude saponin 0.5 g), and isotheasaponin B_3 (**3**, 10.3 mg from crude saponin 1.0 g).

Isoteasaponin B_1(1): $[\alpha]_D^{25}$=+9.0 (c = 0.35, MeOH); UV (MeOH) λ_{max} nm (log ε): 203 (4.3), 216 (sh), 276 (4.3); IR (neat) ν_{max} cm^{-1}: 3422, 2961, 1685, 1636, 1388, 1204, 1078, 1047; for ^{1}H and ^{13}C NMR spectra, see Tables 1 and 2; HRESIMS calcd for $C_{63}H_{92}NaO_{26}$ m/z 1287.5774 $(M + Na)^+$, found 1287.5760 (Δ−1.4 mmu).

Isotheasaponin B_2 (2): $[\alpha]_D^{25}$=−7.4 (c = 0.50, MeOH); UV (MeOH) λ_{max} nm (log ε): 203 (4.3), 216 (sh), 278 (4.3); IR (neat) ν_{max} cm^{-1}: 3419, 2951, 1458, 1717, 1635, 1374, 1257, 1078, 1046; ^{1}H NMR (600 MHz, CD_3OD) δ 5.03 (1H, *br d*, J = 5.3 Hz, Gal-1), 4.93 (1H, overlapped with water, Ara-1), 4.54 (1H, *br d*, J = 7.6 Hz, GlcA-1), 4.51 (1H, *br d*, J = 7.6 Hz, Xyl-1), 4.00–3.15 (sugars), see Table 1 for the aglycone part; ^{13}C NMR (100 MHz, CD_3OD) δ 171.4, 107.7, 106.4, 103.4, 102.4, 84.0, 83.6, 79.0, 78.3, 77.3, 76.6, 76.4, 75.2, 74.4, 73.8, 71.7, 71.4, 69.9, 67.6, 67.4, 63.2, see Table 1 for the aglycone part; HRESIMS calcd for $C_{63}H_{92}NaO_{26}$ m/z 1287.5774 $(M + Na)^+$, found 1287.5749 (Δ−2.5 mmu).

Isotheasaponin B_3 (3): $[\alpha]_D^{25}$=−9.1 (c=0.39, MeOH); UV (MeOH) λ_{max} nm (log ε): 203 (4.4), 216 (sh), 279 (4.3); IR

(neat) ν_{max} cm^{-1}: 3411, 2927, 1683, 1634, 1377, 1160, 1079, 1046; ^1H NMR (500 MHz, CD_3OD) δ 5.02 (1H, *br d*, $J = 5.3$ Hz, Gal-1), 4.92 (1H, overlapped with water, Ara-1), 4.54 (1H, *br d*, $J = 7.5$ Hz, GlcA-1), 4.51 (1H, *br d*, $J = 7.5$ Hz, Xyl-1), 4.00–3.15 (sugars), see Table 1 for the aglycone part; ^{13}C NMR (100 MHz, CD_3OD) δ 171.4, 107.8, 106.4, 103.4, 102.4, 84.2, 83.6, 78.9, 78.3, 77.3, 76.6, 76.4, 75.2, 74.4, 73.8, 71.8, 71.4, 69.9, 67.6, 67.4, 63.2, see Table 1 for the aglycone part; HRESIMS calcd for $C_{66}H_{96}NaO_{26}$ *m/z* 1327.6088 $(M + Na)^+$, found 1327.6096 ($\Delta + 0.8$ mmu).

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