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Triterpenoid saponins from the roots of tea plant (*Camellia sinensis* var. *assamica*)

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Abstract

Three olean-12-ene type triterpenoid saponins, named TR-saponins A, B and C, were isolated as methyl esters from tea roots (*Camellia sinensis* var. *assamica*) after treatment with diazomethane. Their structures were established as the methyl esters of 3-*O*-α-L-arabinopyranosyl (1 → 3)-β-D-glucuronopyranosyl-21, 22-di-*O*-angeloyl-R_1-barrigenol-23-oic acid, 3-*O*-α-L-arabinopyranosyl (1 → 3)-β-D-glucuronopyranosyl-21-*O*-angeloyl-22-*O*-2-methylbutanoyl-R_1-barrigenol-23-oic acid and 3-*O*-α-L-arabinopyranosyl (1 → 3)-β-D-glucuronopyranosyl-16α-*O*-acetyl-21-*O*-angeloyl-22-*O*-2-methylbutanoyl-R_1-barrigenol-23-oic acid,[2] by extensive 1D and 2D-NMR as well as FABMS and HR-MS analyses.

Keywords: *Camellia sinensis* var. *assamica*; Tea root; Triterpenoid saponin; Olean-12-ene

1. Introduction

Saponins occur widely in the plant kingdom. Recently, their pharmacological effects, such as cancer-related activity and antiallergenic, antibacterial, anti-inflammatory and antihepatotoxic activities, etc., have been reviewed (Mahato & Nandy, 1991; Timbekova, Isaev & Abubakirov, 1996; Lacaille-Dubois & Wagner, 1996; Yoshiki, Kudou & Okubo, 1998). Saponins have also been recognized as bitter components in tea (Hashizume & Sakato, 1966). The structures of the major tea seed saponins, theasaponins E1 and E2 (Kitagawa, Hori, Motozawa, Murakami & Yoshikawa, 1998), and a major tea leaf saponin, 3-*O*-{β-D-galactopyranosyl (1 → 2)-[β-D-xylopyranosyl (1 → 2)-α-L-arabinopyranosyl (1 → 3)]-β-D-glucopyranosyl}-21-*O*-cinnamoyl-16,22-di-*O*-acetylbarringtogenol C (Sagesaka, Uemura, Watanabe, Sakata & Uzawa, 1994), have been recently determined. However, no investigation seems to have been carried out on the tea root saponins. The alcoholic extract of tea roots (*C. sinensis* var. *assamica*) has shown to exhibit inhibitory effects on neoplasm and solid tumors (Sur & Ganguly, 1994; Chaudhuri, Sur, Gomes, Das & Ganguly, 1998). We report here, for the first time, the isolation and structure elucidation of the methyl esters of three new saponins, TR-saponins A, B and C, from tea roots.

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[2] The absolute configurations of the arabinose and the glucuronic acid have not been determined, but deduced from the fact that those found in leaves of *C. sinensis* so far are L- and D-isomers, respectively.

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2. Results and discussion

A methanolic extract of the air-dried roots (1 kg) of *C. sinensis* var. *assamica* was partitioned between H_2O and EtOAc, and then between H_2O and BuOH. The EtOAc fraction contained 2-methyl anthraquinone, α-spinosterol and its glucoside (Chaudhuri et al., 1997) Following chromatography, esterification, and HPLC treatments (see Section 3), the BuOH layer yielded three main saponin methyl esters **1** (2.2 mg), **2** (5.9 mg) and **3** (2.8 mg).

The fragmentation patterns of the FABMS spectra of these three compounds were similar to each other (see Table 1), indicating the sequential loss of a pentose, a hexuronic acid methyl ester unit and C_5-acyl groups. The sugar components were confirmed to be arabinose and glucuronic acid by GLC analysis of the alditols derived from the saponin methyl ester mixture (see Section 3). The ^{13}C NMR spectral data (see Table 2) revealed sugar moieties (two anomeric signals (δ 105) and the presence of hydroxyl bearing carbons (δ 64–76)), ester carbonyl (δ 169–179) and double bond (δ 126–143) functionalities. The remaining unsaturation degrees of each compound indicated that the aglycone is pentacyclic.

Saponin methyl ester B (**2**) showed an $[M + H]^+$ ion at *m/z* 1039 and an $[M + Na]^+$ ion at *m/z* 1061 in the positive FABMS spectrum (see Table 1). HR-FABMS gave the molecular formula of $C_{53}H_{82}O_{20}$.

Full assignment of the ^1H and ^{13}C NMR signals of **2** was accomplished through analysis of the ^1H–^1H COSY, TOCSY, HSQC, DEPT and HMBC spectra. The olean-12-ene type skeleton was suggested by the presence of a trisubstituted double bond (δ 5.46, *t*, $J =$ 3.3 Hz), and six signals of tertiary methyl groups at δ 0.85, 0.99, 1.00, 1.07, 1.17 and 1.41 (3H each, *s*) in the ^1H NMR spectrum of **2** (Jia, Koike, Kudo, Li & Nikaido, 1998).

The skeleton was confirmed by detailed analysis of 2D-NMR spectra. A combination of COSY and TOCSY experiments (see Fig. 1) allowed us to assemble the following partial structural units of the aglycone moiety: C-1 to C-3 for ring A, C-5 to C-7 for ring B, C-9 to C-12 for ring C, C-15 to C-16 for ring D, C-18 to C-19 and C-21 to C-22 for ring E. The connectivity of these segments was established by HMBC data as shown by arrows in the Fig. 1. Evidence for the trisubstituted olefinic linkage at C-12/C-13 was provided by the correlation of H-11 (δ 1.95, *m*), H-18 (δ 2.62, *m*) and H-27 (δ 1.41, *s*) to one sp^2 carbon (δ 143.6, C-13), and from H-11 to another one (δ 126.7, C-12). The appearance of a carbonyl group instead of the methyl group at C-23 was evident from the relative downfield shift of the C-4 signal (δ 54.4) in comparison with methyl oleanate (δ 39.4–39.6) (Sagesaka et al., 1994; Wang, Cai, Peng, Ding, Wu & Chen, 1998; Spengel, 1996) and the correlation between the carbonyl carbon and H-24 observed in the HMBC experiment. The NMR chemical shifts of the aglycone were in close accordance with those of barringtogenol C (Sagesaka et al., 1994), a major sapogenin in tea leaves and seeds, except for rings A and D.

The HMBC experiment (see Fig. 1) clarified the position in the aglycone structure where C_5 acyl groups are attached. C-21 (δ 79.6) was shown to be further linked to one of the C_5 acyl groups via ester linkage, whose NMR data (see Tables 2 and 3) were in good accordance with those of an authentic angelic acid methyl ester. This acid has been reported as an acyl substituent of saponins from tea leaves and seeds (Hashizume, 1969). H-22 (δ 5.55, *d*, $J = 10.1$ Hz) had a cross peak with a carboxyl carbon (δ 178.5) of another C_5 acyl groups which was identified as 2-methylbutanoic acid with the help of ^1H–^1H COSY and TOCSY spectra.

The protons and carbons of the two sugar units

Table 1
Mass spectral fragmentations of **1**–**3** (*m/z*, relative intensity %)[a]

Fragments	1	2	3
$[M + Na]^+$	1059 (0.3)	1061 (4.0)	1103 (1.7)
$[M + H^+]^+$	1037 (0.2)	1039 (0.5)	1081 (0.1)
$[M + H^+ - Ara]^+$	905 (0.2)	907 (0.5)	949 (0.1)
$[M + H^+ - R_1 - H_2O]^+$	697 (0.3)	699 (0.5)	741 (1.0)
			699 (–$COCH_3$, 0.8)
$[M + H^+ - R_1 - 2H_2O]^+$	679 (0.3)	681 (0.5)	723 (0.5)
			681 (–$COCH_3$, 1.6)
$[M + 2H^+ - R_1 - R_2 - H_2O]^+$	615 (0.8)	617 (1.4)	617 (–$COCH_3$, 1.6)
$[M + 2H^+ - R_1 - R_2 - 2H_2O]^+$	597 (1.5)	599 (3.3)	599 (–$COCH_3$, 1.8)
$[M + 2H^+ - R_1 - R_3 - H_2O]^+$		615 (0.7)	615 (–$COCH_3$, 0.6)
$[M + 2H^+ - R_1 - R_3 - 2H_2O]^+$		597 (1.0)	597 (–$COCH_3$, 1.8)
$[R_1 + H_2O]^+$	341 (3.8)	341 (2.3)	341 (3.8)
$[R_1 + H^+ - OCH_3]^+$	307 (18.0)	307 (15.0)	307 (11.5)

[a] Ara, arabinosyl ($C_5H_8O_4$); R_1, sugar moiety ($C_{12}H_{18}O_{10}$); R_2, angeloyl (C_5H_7O); R_3, 2-methylbutanoyl (C_5H_9O).

Table 2
^{13}C NMR spectral data for compounds **1**–**3**

Carbon	**1**[a]	**2**[b]	**3**[a]
1	36.5[c]	36.9 (36.2)	36.7
2	26.1	26.4 (25.9)	26.1
3	86.2	87.2 (85.7)	86.1
4	53.8	54.4 (53.5)	53.8
5	52.3	52.7 (52.0)	52.3
6	21.6	22.1 (21.4)	21.7
7	39.4	39.9 (38.7)	39.4
8	42.0	42.5 (41.6)	42.2
9	47.7	48.3 (47.2)	48.0
10	37.1	37.5 (36.5)	37.2
11	24.3	24.7 (23.8)	24.3
12	126.0	126.7 (125.0)	127.1
13	143.5	143.6 (143.6)	142.3
14	47.9	48.4 (47.6)	na[d]
15	67.6	68.4 (67.4)	67.8
16	72.5	74.0 (72.9)	74.8
			$\underline{C}OCH_3$ 170.8
			$CO\underline{C}H_3$ 22.0
17	48.3	49.2 (48.3)	na[d]
18	41.0	41.5 (41.4)	41.0
19	47.2	47.5 (46.7)	47.2
20	36.3	36.7 (36.2)	36.4
21	79.2	79.6 (78.5)	77.9
22	78.4	73.8 (73.0)	72.5
23	177.9	179.7 (177.9)	177.9
$COO\underline{C}H_3$	52.3	52.9 (52.1)	52.4
24	12.1	12.2 (12.2)	12.2
25	17.5	17.7 (17.3)	16.8
26	15.8	16.6 (16.0)	16.1
27	20.8	21.0 (21.0)	20.2
28	63.4	62.7 (62.8)	64.1
29	29.6	29.6 (29.3)	29.6
30	20.1	20.2 (20.1)	19.6
1′	167.5	169.1 (167.6)	167.6
2′	128.9	129.1 (128.6)	128.5
3′	138.6	140.6 (138.6)	139.7
4′	16.5	16.2 (16.1)	16.5
5′	20.9	21.0 (21.1)	20.9
1″	168.7	178.5 (176.5)	176.7
2″	129.1	42.7 (40.7)	41.9
3″	137.6	27.8 (26.8)	26.9
4″	15.8	12.2 (11.8)	12.0
5″	21.1	17.0 (16.6)	17.5
Glucuronic acid methyl ester			
1‴	105.4	105.9 (105.8)	105.4
2‴	73.8	74.2 (74.4)	73.8
3‴	86.9	85.8 (85.2)	86.8
4‴	71.2	71.6 (71.0)	71.2
5‴	76.0	76.3 (76.6)	76.0
6‴	169.7	171.1 (170.2)	169.7
$COO\underline{C}H_3$	52.3	53.0 (52.1)	52.4
Arabinose			
1⁗	105.4	105.5 (105.7)	105.4
2⁗	73.6	72.9 (72.8)	73.5
3⁗	73.2	74.1 (74.0)	72.2
4⁗	69.0	69.6 (69.2)	69.0
5⁗	67.0	67.3 (67.0)	67.0

[a] Recorded in acetone-d_6.
[b] Recorded in methanol-d_4. The data in parentheses were recorded in pyridine-d_5.
[c] Chemical shifts are expressed as δ values using TMS as an internal standard.
[d] na: Not assigned yet (not observed).

were assigned by a combination of 2D-NMR starting from two anomeric protons (δ 4.31 (*d*, $J = 7.9$ Hz) and 4.50 (*d*, $J = 7.0$ Hz)). The hexuronic acid methyl ester was identified as glucuronic acid methyl ester from all vicinal coupling constants larger than 7 Hz (see Table 3). The pentose in the pyranose form was evident from the large vicinal coupling constants of three sequential *trans*-diaxial protons, H-1⁗ (δ 4.50, *d*, $J =$ 7.0 Hz), 2⁗ (δ 3.61, *dd*, $J = 7.0$, 9.1 Hz) and 3⁗ (δ 3.56, *q*, $J = 4.0$, 9.1 Hz). The coupling constants (J_{3}⁗, 4⁗ $= 4.0$ Hz, J_{4}⁗, 5⁗ $= 3.0$ Hz) clearly indicated an arabinopyranose. These were confirmed by GLC analysis of sugar components of the saponin mixture. The absolute configurations of the sugar components have not yet been determined, but are presumed to be the same as those found in the leaves of the tea plants (Sagesaka et al., 1994).

Key correlation peaks in the HMBC spectrum (see Fig. 1) between H-1‴ (δ 4.31, *d*) of the glucuronic acid methyl ester and C-3 (δ 87.2) of the aglycone and between H-1⁗ (δ 4.50, *d*) of the arabinose unit and C-3‴ (δ 85.8) of the glucuronic acid methyl ester allowed us to determine the sequence of the disaccharide chain at C-3 as α-L-arabinopyranosyl(1 → 3)-β-D-glucuronopyranosyl.

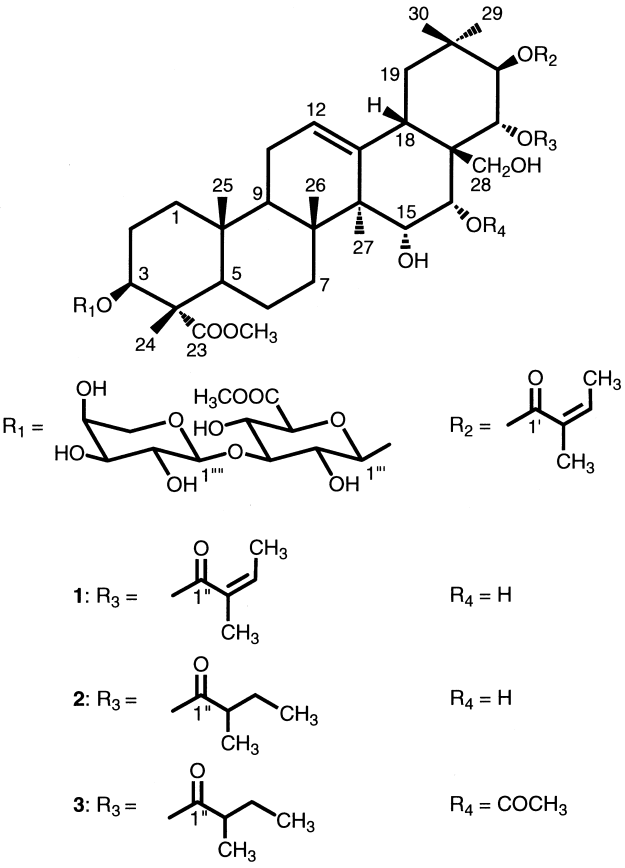
The stereochemistry of the triterpene moiety was established by a NOESY experiment (see Fig. 1) and the information from the vicinal coupling constants of the key protons. The sugar moiety at C-3 was assigned as β-equatorial and the ester carbonyl group at C-4 as α-equatorial because of the cross peaks between H-3 (δ 4.02, *dd*, $J = 4.5$, 11.9 Hz) and H-5 (δ 1.47, *br. d*, $J =$ 12.0 Hz), as well as the carboxymethyl protons at C-23 (δ 3.69, *s*). H-15 (*d*, $J = 4.3$ Hz) was correlated with methyl protons at C-26 (δ 1.00, *s*) in the NOESY spectrum, indicating that the 15-OH group is α-equatorial. The small coupling constant between H-15 and 16 ($J_{15,\,16} = 4.3$ Hz) suggested that H-16 is β-equatorial. The cross peaks between H-21 (δ 5.86, *d*, $J = 10.1$ Hz) and H-19α (δ 2.63, *m*), as well as H-22 (δ 5.55, *d*, $J =$ 10.1 Hz) and H-18 (δ 2.62, *m*) suggested that H-21 and H-22 are α- and β-axial, respectively, which means that the acyl groups at C-21 and C-22 are β- and α-equatorial, respectively.

On the basis of the above evidence, TR-saponin B methyl ester (**2**) was elucidated to be the methyl ester of 3-*O*-α-L-arabinopyranosyl (1 → 3)-β-D-glucuronopyranosyl-21-*O*-angeloyl-22-*O*-2-methylbutanoyl- R_1-barrigenol-23-oic acid.

The FABMS of compound **1** gave an $[M + Na]^+$ peak at *m/z* 1059 and an $[M+H]^+$ peak at *m/z* 1037 (see Table 1). The HR-FABMS showed the elemental composition to be $C_{53}H_{80}O_{20}$, implying that **1** has one more degree of unsaturation than compound **2** (TR-saponin B methyl ester). Comparison of the NMR data (see Tables 2 and 3) demonstrated that the

aglycones and the sugar moieties of **1** and **2** are identical. The only difference is that **1** has an additional carbon–carbon double bond which was assigned to another angeloly moiety (δ 168.7, 137.6, 129.1, 21.1 and 15.8) attached to C-22 via an ester linkage. The downfield shift of C-22 (δ 78.4) can be explained by the unsaturation of the organic acid. Therefore, TR-saponin A methyl ester (**1**) was concluded to be the methyl ester of 3-*O*-α-L-arabinopyranosyl (1 → 3)-β-D-glucuronopyranosyl-21, 22-di-*O*-angeloyl-R_1-barrigenol-23-oic acid.

The FABMS of compound **3** gave an $[M + Na]^+$ peak at *m/z* 1103 and an $[M + H]^+$ peak at *m/z* 1081 (see Table 1). The HR-FABMS showed a molecular formula of $C_{55}H_{84}O_{21}$. Comparison of the NMR data (see Tables 2 and 3) indicated that **3** had the same aglycone and the same sugar chain at C-3 as **2**. The significant differences were the downfield shift of C-16 (δ 74.8), and the addition of an ester carbonyl carbon (δ 170.8) and an acetyl methyl carbon (δ 22.0), which is consistent with the molecular formula. These data suggested the replacement of the C-16 hydroxyl group in **2** with an acetoxy group in **3**. On the basis of the above findings and information from ^1H–^1H-COSY and HMBC, TR-saponin C methyl ester (**3**) was elucidated to be the methyl ester of 3-*O*-α-L-arabinopyranosyl (1 → 3)-β-D-glucuronopyranosyl-16α-*O*-acetyl-21-*O*-angeloyl-22-*O*-2-methylbutanoyl-R_1-barrigenol-23-oic acid.



Saponins in tea leaves and seeds have been investigated for many years. They were determined to be sapogenols, theasapogenol A, B (barringtogenol C), C (camelliagenin C), D (dihydroproverogenin A), E (camelliagenin E), R_1-barrigenol or A_1-barrigenol, with angelic, cinnamic and/or acetic acid as organic acidic components, and with a tetrasaccharide chain (Sagesaka et al., 1994; Yosioka, Nishimura, Matsuda & Kitagawa, 1970a; Yosioka, Nishimura, Matsuda & Kitagawa, 1970b; Yosioka, Nishimura, Matsuda & Kitagawa, 1971; Hashizume, 1973). Saponins of tea roots differ from those mentioned above that no aglycone with a 23-oic acid has been isolated from other parts of tea plants yet, additionally, the major saponins in tea roots contain a disaccharide chain instead of a tetrasaccharide.

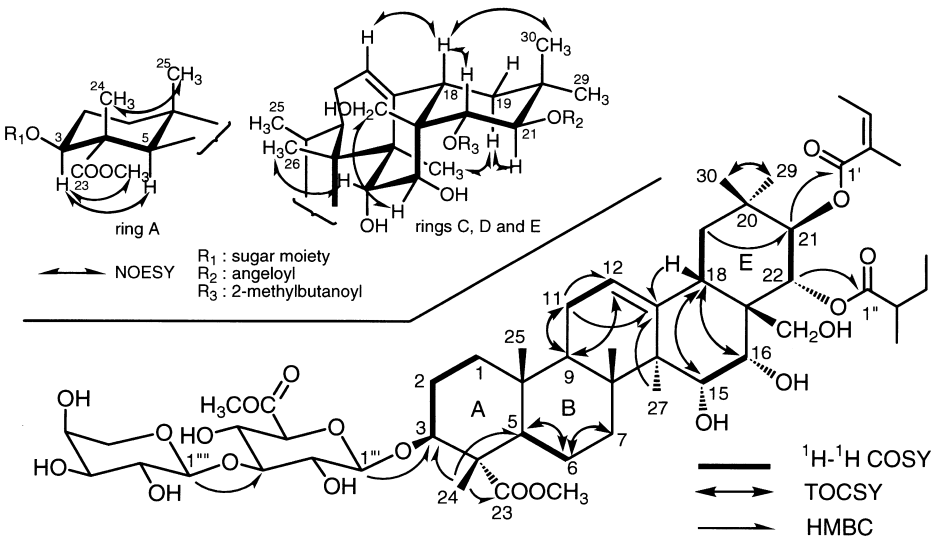


Fig. 1. Key correlations in ^1H–^1H COSY, TOCSY, HMBC and NOESY experiments of TR-saponin B methyl ester (**2**).

Table 3
^{1}H NMR spectral data for compounds **1**–**3**

Proton	**1**[a]	**2**[b]	**3**[a]
1	ca. 1.68 (*m*)[c]	ca. 1.68 (*m*)	1.78 (*m*), ca. 1.69 (*m*)
2	ca. 1.85 (*m*), 1.67 (*m*)	1.85 (*m*), ca. 1.70 (*m*)	1.84 (*m*), ca. 1.68 (*m*)
3	4.02 (*m*)	4.02 (*dd*, 4.5, 11.9)	4.03 (*m*)
5	1.50 (*br. d*, 13.2)	1.47(*d*, 12.0)	1.50 (*m*)
6	1.53 (*m*), ca. 0.95 (*m*)	1.58 (*m*), 0.95 (*m*)	1.53 (*m*), ca. 0.95 (*m*)
7	1.72 (*m*), ca. 1.11 (*m*)	1.69 (*m*)	ca. 1.69 (*m*), 1.11 (*m*)
9	ca. 1.68 (*m*)	ca. 1.66 (*m*)	ca. 1.67 (*m*)
11	1.95 (*m*)	1.95 (*m*)	1.97 (*m*)
12	5.48 (*br. s*)	5.46 (*t*, 3.3)	5.54 (*m*)
15	3.71 (*m*)	3.71 (*d*, 4.3)	4.04 (*m*)
16	3.75 (*m*)	ca. 3.80 (*m*)	5.28 (*d*, 4.0)
			$COCH_3$ 2.29 (*s*)
18	2.70 (*m*)	2.62 (*m*)	2.73 (*dd*, 4.6, 14.3)
19	2.66 (*t*, 14.2), 1.37 (*m*)	2.63 (*m*), 1.19 (*m*)	2.53 (*t*, 14.0), 1.38 (*m*)
21	5.97 (*d*, 10.1)	5.86 (*d*, 10.1)	5.47 (*d*, 10.4)
22	5.59 (*d*, 10.4)	5.55 (*d*, 10.1)	5.53 (*d*, 10.4)
$COOCH_3$	3.67 (*s*)	3.69 (*s*)	3.66 (*s*)
24	1.15 (*s*)	1.17 (*s*)	1.29 (*s*)
25	0.99 (*s*)	0.99 (*s*)	1.00 (*s*)
26	1.00 (*s*)	1.00 (*s*)	1.00 (*s*)
27	1.45 (*s*)	1.41 (*s*)	1.40 (*s*)
28	3.18 (*m*), 3.05 (*m*)	3.26 (*d*, 10.3), 3.00 (*d*, 10.3)	3.39 (*m*), 3.17 (*m*)
29	0.89 (*s*)	0.85 (*s*)	0.91 (*s*)
30	1.10 (*s*)	1.07 (*s*)	1.09 (*s*)
3′	6.04 (*br. q*, 6.0)	6.16 (*qd*, 1.7, 7.3)	6.15 (*br. q*, 5.8)
4′	1.90 (*dd*, 1.5, 4.6)	1.99 (*dd*, 1.7, 7.3)	1.97 (*m*)
5′	1.81 (*m*)	1.85 (*m*)	1.83 (*m*)
2″	–	2.33 (*q*, 7.0)	2.23 (*q*, 7.0)
3″	6.04 (*br. q*, 6.0)	1.63 (*m*), 1.42 (*m*)	1.58 (*q*, 7.4), ca. 1.36 (*m*)
4″	1.91 (*dd*, 1.5, 5.8)	0.87 (*t*, 7.3)	0.84 (*t*, 7.4)
5″	1.81 (*m*)	1.03 (*d*, 7.0)	0.98 (*d*, 7.0)
Glucuronic acid methyl ester			
1‴	4.39 (*d*, 8.0)	4.31 (*d*, 7.9)	4.39 (*d*, 7.6)
2‴	3.29 (*m*)	3.30 (*m*)	3.28 (*m*)
3‴	3.48 (*t*, 9.2)	3.50 (*t*, 9.0)	3.48 (*t*, 9.0)
4‴	ca. 3.63 (*m*)	3.56 (*m*)	ca. 3.60 (*m*)
5‴	3.87 (*d*, 9.7)	3.84 (*d*, 9.8)	3.88 (*d*, 9.0)
$COOCH_3$	3.73 (*s*)	3.77 (*s*)	3.73 (*s*)
Arabinose			
1⁗	4.44 (*d*, 7.1)	4.50 (*d*, 7.0)	4.44 (*m*)
2⁗	ca. 3.63 (*m*)	3.61 (*dd*, 7.0, 9.1)	ca. 3.62 (*m*)
3⁗	ca. 3.62 (*m*)	3.56 (*q*, 4.0, 9.1)	ca. 3.65 (*m*)
4⁗	3.82 (*m*)	3.79 (*m*)	3.83 (*m*)
5⁗	3.94 (*dd*, 2.4, 10.1)	3.90 (*dd*, 3.0, 12.5),	3.94(*br. d*, 12.8)
	ca. 3.60 (*m*)	3.58 (*dd*, 3.0, 12.5)	3.67 (*m*)

[a] Recorded in acetone-d_6.
[b] Recorded in methanol-d_4.
[c] Chemical shifts are expressed as δ values using TMS as an internal standard, with coupling constants in Hz given in parentheses.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were recorded on a JEOL LAMBDA 500 NMR spectrometer. FABMS data were measured with a JEOL JMS-DS-303HF mass spectrometer. HPLC separations were performed with a JASCO PU-980 pump equipped with a JASCO MD-910 detector. GLC analyses were carried out on a Hewlett Packard HP 5890 Series II gas chromatograph.

3.2. Extraction and isolation

The plant material of *C. sinensis* var. *assamica* was collected from 30 year old seedlings of commercially exploited tea bushes uprooted from Jorhat, India. To

the air-dried roots of *Camellia sinensis* var. *assamica* (1 kg), 5 l of MeOH was added and kept at room temperature for seven days. The extraction procedure was repeated three times. The combined extracts were concentrated and partitioned three times between H_2O and EtOAc, and then between H_2O and BuOH. The butanolic fraction was concentrated to yield 33.5 g of dark-brown material, 12.5 g of which was chromatographed over silicic acid twice and then passed through a Sephadex LH-20 column with MeOH as eluent to give a saponin mixture (900 mg). Half of the mixture (450 mg) was further separated on a Sephadex LH-20 column with MeOH before being treated with freshly prepared ethereal diazomethane. The resulting saponin methyl ester mixture (372 mg) was chromatographed three times on a reversed-phase HPLC column (YMC-Pack R and D ODS-5-A ø 20 × 250 mm, 50% MeCN, 0.8 ml/min) to give more than 30 peaks (detected at 210 nm), which included three main saponin methyl esters **1** (R_t = 40.0 min; 2.2 mg), **2** (R_t = 41.7 min; 5.9 mg) and **3** (R_t = 45.2 min; 2.8 mg).

TR-saponin A methyl ester (**1**): Positive FABMS [NOBA (*p*-nitrobenzyl alcohol)]: see Table 1; HR-FABMS (NOBA) calcd. for $C_{53}H_{80}O_{20}Na$ 1059.5140, found 1059.5162; ^{13}C NMR spectral data (125 MHz, acetone-*d*6): see Table 2; ^1H NMR spectral data (acetone-d_6, 500 MHz): see Table 3.

TR-saponin B methyl ester (**2**): Positive FABMS (NOBA): see Table 1; HR-FABMS (NOBA) calcd. for $C_{53}H_{82}O_{20}Na$ 1061.5297, found 1061.5322; ^{13}C NMR (125 MHz, methanol-d_4): see Table 2; ^1H NMR (500 MHz, methanol-d_4): see Table 3.

TR-saponin C methyl ester (**3**): Positive FABMS (NOBA): see Table 1; HR-FABMS (NOBA) calcd. for $C_{55}H_{84}O_{21}Na$ 1103.5981, found 1103.5392; ^{13}C NMR (125 MHz, acetone-d_6): see Table 2; ^1H NMR (500 MHz, acetone-d_6): see Table 3.

3.3. Identification of the component sugars

A saponin methyl ester mixture (2 mg) was stirred with $NaBH_4$ (10 mg) for 6 h in 2 ml of MeOH. After neutralization with dilute HOAc, solvent was evaporated and borate was removed by the addition of dry MeOH and subsequent evaporation. The reaction mixture was heated with 10% methanolic HCl (1 ml) at 100°C for 3 h. After the sapogenins were extracted with EtOAc, the aqueous layer was neutralized with silver carbonate and converted to an alditol mixture with 20 mg of $NaBH_4$ in the conventional method. The alditol mixture was dried over $CaCl_2$; 0.5 ml each of dry acetic anhydride and dry pyridine were added, and the mixture was kept overnight. After excess pyridine and acetic anhydride were removed, the products were extracted with $CHCl_3$ and subjected to GLC (column: GP-3% SP-2340 over 100/200 mesh Supelcoport, ϕ 6.5 mm × 1.8 m, oven temperature: 200°C, injector temperature: 220°C, detector temperature: 250°C, carrier gas: N_2, 26.5 ml/min) to give two main peaks (R_t = 6.11 and 18.34 min). They were identified as arabitol and glucitol, respectively, by comparing with authentic specimens derived from L-arabinose and D-glucose.

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