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Cyclic fatty acyl glycosides in the glandular trichome exudate of *Silene gallica*

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ABSTRACT

Chemical investigation of the glandular trichome exudate from *Silene gallica L.* (Caryophyllaceae) resulted in isolation of 10 cyclic fatty acyl glycosides (gallicasides A–J). The cyclic structures were characterized by a glycosidic linkage of the glucose moiety to either the C-12 or the C-13 position of the octadecanoyl moiety, and by an ester linkage between the C-2 hydroxy group of the glucose moiety and the carboxyl group of the oxygenated octadecanoic acid. The structures of the cyclic fatty acyl glycosides were further distinguished from one another by acetylation and/or malonylation on the glucose moiety. Of these compounds, the 1,2′-cyclic ester of 12(*R*)-(6-*O*-acetyl-3-*O*-malonyl-β-D-glucopyranosyloxy)octadecanoic acid (gallicaside J) was the most abundant (30.7%). These secondary metabolites were found specifically in the glandular trichome exudate rather than in other aerial parts.

1. Introduction

Hairy microstructures developed on the surface of plants are mainly classified into two types: glandular and non-glandular trichomes. Glandular trichomes have uni- or multi-secretory cells at the tops of their stalks and actively exude secondary metabolites. On the other hand, non-glandular trichomes are simple hairy structures without secretory cells (Werker, 2000). From an ecological perspective, glandular trichomes use secondary metabolites to protect the plant from herbivores, fungal infections and UV irradiation among others (Duke et al., 2000; Spring, 2000). By contrast, the non-glandular trichomes may play this type of role using their physiological features (Werker, 2000).

Silene gallica L. (Caryophyllaceae), native to central Europe, grows to about 25 cm in height, and the calyx has both long non-glandular trichomes (*ca.* 2.0 mm) and shorter glandular trichomes (*ca.* 0.1 mm), as shown in Fig. 1. The *Silene* genus is one of the largest genera of the world's flora (Greuter, 1995). Although there have been no reports of chemical studies of *S. gallica*, previous investigations of other *Silene* plants led to isolation of triterpene saponins from *Silene jenisseensis* (Dubois et al., 1997), *Silene vulgaris* (Glensk et al., 1999), *Silene fortunei* (Gaidi et al., 2002) and *Silene rubicunda* (Fu et al., 2005), and of phytoecdysteroids from *Silene brahuica* (Sadikov et al., 2000) and *Silene viridiflora* (Simon et al., 2009). We have been interested in the analysis of secondary metabolites in glandular trichome exudates, and reported the characterization of oxygenated fatty acylglycerols (in the glandular trichome exudate on the leaves) and geranylated flavanones (in the oily secretion on the immature fruit surface) from *Paulownia tomentosa* (Asai et al., 2008, 2009). More recently, we reported the identification of glycosylated fatty acids and dammarane triterpenes from the glandular trichome exudates of *Ibicella lutea* and *Proboscidea louisiana* (Asai et al., 2010). As a continuation of this research, we have now carried out phytochemical studies on the glandular trichome exudate of *S. gallica*.

2. Results and discussion

In a preliminary study, two exudate samples were obtained by either gently wiping the surface of the calyxes with oil-free cotton or by briefly rinsing an upper portion of the aerial part in Et_2O. The two samples showed essentially identical TLC spots. To obtain larger samples, large-scale extraction was carried out using the latter method. This material was subjected to silica gel column chromatography and reversed-phase HPLC to yield the cyclic fatty acyl glycosides (**1**–**10**) as colorless oils. Due to their instability, compounds **9** and **10** were isolated and characterized as the corresponding methyl esters **9a** and **10a**. Negligible amounts of these substances were found in the ether-rinsed aerial part.

Compound **1**, named gallicaside A, showed a pseudo-molecular ion at *m*/*z* 551.3217 $[M+Na]^+$ in the positive HRFABMS that corresponded to the molecular formula $C_{28}H_{48}O_9$. The ^1H NMR spectrum of **1** showed signals of seven protons assignable to a β-glucopyranosyl moiety esterified at the 2′-*O*, 3′-*O* and 4′-*O* positions [δ 4.68 (*d*, *J* = 8.0 Hz, H-1′), 4.98 (*dd*, *J* = 9.5, 8.0 Hz, H-2′), 5.23 (*t*, *J* = 9.5 Hz, H-3′), 5.03 (*t*, *J* = 9.5 Hz, H-4′), 3.51 (*m*, H-5′), 3.71 (*m*, Ha-6′) and 3.61 (*m*, Hb-6′)]. Resonances characteristic of a fatty acyl moiety were apparent (longer-chain methylene protons (δ 1.26) and a methyl triplet (δ 0.91)), in addition to an oxymethine proton at δ 3.85

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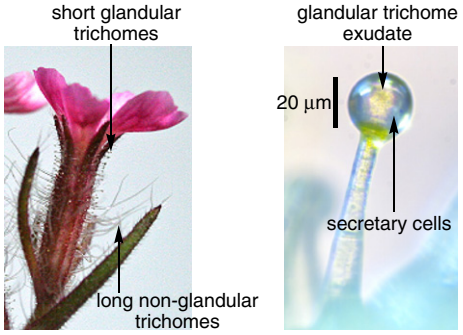


Fig. 1. Flower and calyx of *Silene gallica* (left), and a close-up of glandular trichomes (right).

and two acetyl methyl singlets at δ 2.07 and 2.04 (Table 1). The ^{13}C NMR spectroscopic data of **1** (Table 2) confirmed the presence of the tri-*O*-acylated glucopyranosyl moiety (signals for the ester carbonyls appeared at δ 170.0, 170.4 and 172.2) and further established the fatty acyl group to be linear and mono-oxygenated along the methylene chain (i.e., δ 172.2 and 76.8). The HMBC correlations illustrated in Fig. 3 permitted the assignments of the three ester linkages as the 3′,4′-di-*O*-acetyl and 2′-*O*-fatty acyl glucose. Consideration of the unsaturation number (Δ^5) of the molecule required compound **1** to have an additional ring. An HMBC correlation from the anomeric proton (δ 4.68) to the oxymethine carbon (δ 76.8) provided evidence for an ether linkage that would form a cyclic structure (Fig. 2). The other compounds (**2**–**8**, **9a** and **10a**) were also shown to have an ether linkage between the anomeric carbon of glucose and an oxymethine carbon of the fatty acyl moiety.

Although the spectroscopic data described above demonstrated that compound **1** has a cyclic structure, as shown in Fig. 2, the exact position and the absolute configuration of the oxymethine center in the fatty acyl moiety remained unknown. The oxygenated position along the methylene chain was determined as follows. Alkaline hydrolysis of compound **1** gave a glucosyloxy-fatty acid, which upon treatment with acidic conditions followed by ethereal diazomethane gave the hydroxy-fatty acid methyl ester (**1a**). The EIMS of the trimethylsilyl (TMS) ether of **1a** showed intense fragment ion peaks at *m*/*z* 173 ($[CH(OTMS)(CH_2)_4CH_3]^+$ due to C-12/C-13 cleavage) and at *m*/*z* 315 ($[CH_3OOC(CH_2)_{11}CH(OTMS)]^+$ due to C-13/C-14 cleavage), in addition to a molecular ion peak at *m*/*z* 386. The MS data unequivocally established that compound **1a** is methyl 13-hydroxyoctadecanoate. The homogeneity of the ester **1a** was confirmed by GLC analysis of the trimethylsilylated sample of **1a**, which showed a single peak. Glucose was found in the aqueous layer (identified by TLC and GC–MS as its TMS ether) after the above acidic treatment of the glucosyloxy-fatty acid described above.

The absolute configuration at C-13 of **1a** was determined by application of the advanced Mosher's ester method (Ohtani et al., 1991). The positive $\Delta\delta_{S-R}$ value for H_3-18 (+0.04 ppm) of the (*S*)- and (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) derivatives established the 13*R* configuration of **1a** (Nishioka et al., 1994). Hence, the structure of gallicaside A (**1a**) was determined to be the 1,2′-cyclic ester of 13(*R*)-(3,4-di-*O*-diacetyl-β-D-glucopyranosyloxy)octadecanoic acid, as depicted in Fig. 2.

Compound **2**, named gallicaside B, had the same molecular formula as **1**. The ^{1}H NMR spectrum of **2** resembled that of **1**, and included signals for an oxymethine proton at δ 3.90 and two acetyl methyl singlets. It was evident that the glucose moiety was acylated at the 2-*O*, 3-*O* and 4-*O* positions on the basis of the chemical shifts of H-2′, H-3′ and H-4′ (Table 1). The ^{13}C NMR spectrum of **2** was similar to that of **1**, but minor deviations were observed in the signals of the anomeric carbon (δ 95.6, shifted upfield by 1.0 ppm compared to that of **1**) and the oxygenated fatty acyl moiety (an oxymethine carbon δ 75.4, Table 2). The HMBC correlations indicated the 3′,4′-di-*O*-acetyl and 2′-*O*-fatty acyl structures in the sugar moiety, as well as a glycosylation at the oxymethine carbon in the fattyacyl moiety. The minor deviations of the ^{13}C shifts could be accounted for by assuming that compound **2** is either a positional isomer or a stereoisomer of **1**, with respect to the oxygenated position in the fatty acyl moiety.

Table 1
^{1}H NMR spectroscopic data (500 MHz, $CDCl_3$) for compounds **1**–**8**, **9a** and **10a**.[a]

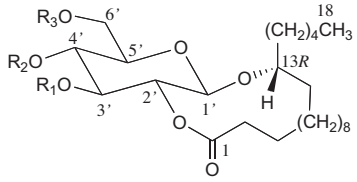
	1	2	3	4	5	6	7	8	9a	10a
2	2.30 (*m*, 2H)	2.27 (*m*, 2H)	2.29 (*m*, 2H)	2.27 (*m*, 2H)	2.29 (*m*, 2H)	2.40 (*m*, 2H)	2.39 (*m*, 2H)	2.39 (*m*, 2H)	2.30 (*m*, 2H)	2.29 (*m*, 2H)
12		3.90 (*m*)		3.88 (*m*)	3.88 (*m*)		3.90 (*m*)	3.90 (*m*)		3.89 (*m*)
13	3.85 (*m*)		3.83 (*m*)			3.83 (*m*)			3.83 (*m*)	
CH_2	1.73–1.20 (*m*)	1.73–1.20 (*m*)	1.73–1.20 (*m*)	1.73–1.20 (*m*)	1.73–1.20 (*m*)	1.73–1.20 (*m*)	1.73–1.20 (*m*)	1.73–1.20 (*m*)	1.73–1.20 (*m*)	1.73–1.20 (*m*)
18	0.91 (*t*, 7.0)	0.90 (*t*, 7.1)	0.91 (*t*, 7.0)	0.90 (*t*, 7.0)	0.90 (*t*, 7.19	0.91 (*t*, 7.0)	0.90 (*t*7.0)	0.90 (*t*, 7.1)	0.91 (*t*, 7.0)	0.90 (*t*, 7.1)
1′	4.68 (*d*, 8.0)	4.73 (*d*, 7.9)	4.61 (*d*, 7.9)	4.67 (*d*, 7.8)	4.71 (*d*, 7.8)	4.58 (*d*, 7.8)	4.63 (*d*, 7.8)	4.67 (*m*)	4.64 (*d*, 7.8)	4.69 (*d*, 7.9)
2′	4.98 (*dd*, 9.5, 8.0)	5.01 (*dd*, 9.5, 7.9)	4.94 (*dd*, 9.6, 7.9)	4.96 (*dd*, 9.6, 7.8)	4.95 (*dd*, 9.6, 7.8)	4.70 (*dd*, 9.6, 7.8)	4.71 (*dd*, 9.6, 7.8)	4.67 (*m*)	4.94 (*dd*, 9.6, 7.8)	4.97 (*dd*, 9.7, 7.8)
3′	5.23 (*t*, 9.5)	5.25 (*t*, 9.5)	5.01 (*t*, 9.6)	5.02 (*t*, 9.6)	5.02 (*t*, 9.6)	3.47 (*td*, 9.6, 2.4)	3.48 (*brt*, 9.6)	3.61 (*brt*, 9.6)	5.08 (*t*, 9.6)	5.10 (*t*, 9.7)
4′	5.03 (*t*, 9.5)	5.03 (*t*, 9.5)	3.59 (*brt*, 9.6)	3.60 (*brt*, 9.6)	3.77 (*brt*, 9.6)	3.59 (*td*, 9.6, 4.0)	3.60 (*brt*, 9.6)	3.64 (*t*, 9.6)	3.66 (*brt*, 9.6)	3.66 (*brt*, 9.7)
5′	3.51 (*m*)	3.51 (*m*)	3.48 (*m*)	3.49 (*m*)	3.42 (*m*)	3.43 (*m*)	3.42 (*m*)	3.37 (*m*)	3.53 (*m*)	3.53 (*m*)
6′	3.71 (*m*)	3.71 (*m*)	4.46 (*dd*, 12.2, 4.5)	4.44 (*dd*, 12.1, 4.6)	3.92 (*dd*, 11.9, 3.3)	4.43 (*dd*, 12.2, 4.5)	4.41 (*dd*, 12.1, 4.8)	3.89 (*m*)	4.37 (*m*)	4.39 (*dd*, 11.9, 2.9)
	3.61 (*m*)	3.61 (*m*)	4.31 (*dd*, 12.2, 2.3)	4.32 (*dd*, 12.1, 1.9)	3.82 (*dd*, 11.9, 4.5)	4.32 (*dd*, 12.2, 2.2)	4.33 (*dd*, 12.1, 2.0)	3.81 (*m*)	4.37 (*m*)	4.37 (*dd*, 11.9, 4.7)
Ac	2.07 (*s*)	2.04 (*s*)	2.11 (*s*)	2.11 (*s*)	2.08 (*s*)	2.11 (*s*)	2.11 (*s*)		2.11 (*s*)	2.10 (*s*)
	2.04 (*s*)	1.99 (*s*)	2.07 (*s*)	2.07 (*s*)						
2″									3.47 (*d*,16.3)	3.47 (*d*,16.2)
									3.38 (*d*, 16.3)	3.38 (*d*, 16.2)
Me									3.75 (*s*)	3.75 (*s*)

[a] Multiplicity and coupling constants (*J* in Hz) are in parentheses.

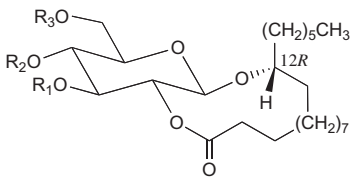
Table 2
^{13}C NMR spectroscopic data (125 MHz, in $CDCl_3$) for compounds **1**–**8**, **9a** and **10a**.[a]

	1	2	3	4	5	6	7	8	9a	10a
1	172.2	172.4	172.3	172.6	172.6	174.7	175.0	175.2	172.4	172.7
2	33.2	33.6	33.3	33.7	33.7	33.7	34.3	34.3	33.2	33.7
CH_2	32.7	32.3	32.8	32.3	32.3	32.7	32.3	32.2	32.7	32.3
CH_2	32.1	31.8	32.1	31.8	31.8	32.1	31.8	31.8	32.1	31.8
CH_2	28.1	29.6	28.1	29.6	29.6	28.0	29.6	29.6	28.1	29.6
CH_2	27.6	27.3	27.6	27.3	27.3	27.8	27.1	27.1	27.6	27.2
CH_2	26.8	26.7	26.8	26.8	26.7	26.9	26.9	27.0	26.7	26.7
CH_2	26.6	26.1	26.6	26.7	26.1	26.7	26.5	26.5	26.6	26.0
CH_2	26.6	26.0	26.6	26.0	26.0	26.6	26.2	26.3	26.6	26.0
CH_2	26.2	25.8	26.2	25.8	25.8	26.4	26.0	26.0	26.2	25.8
CH_2	25.8	24.9	25.8	25.0	24.9	25.9	25.0	24.8	25.7	25.0
CH_2	25.0	24.1	25.1	24.1	24.1	25.0	24.3	24.4	25.1	24.0
CH_2	23.7	23.8	23.7	23.7	23.8	24.1	24.1	24.1	23.7	23.7
CH_2	22.6	22.2	22.8	22.2	22.2	22.7	22.0	22.0	22.7	22.2
12 or 13	76.8	75.4	76.6	76.0	76.5	76.4	74.8	74.9	76.6	75.3
16	31.5	31.5	31.5	31.5	31.5	31.5	31.2	31.2	31.4	31.4
17	22.7	22.6	22.6	22.6	22.6	22.7	22.6	22.6	22.6	22.6
18	14.0	14.1	14.0	14.1	14.1	14.0	14.1	14.1	14.1	14.1
1′	96.6	95.6	96.5	95.5	95.5	96.5	95.3	95.4	96.5	95.5
2′	71.5	71.5	71.3	71.2	71.3	74.7	74.7	74.9	70.8	70.8
3′	73.1	73.1	74.1	74.1	75.4	75.8	75.9	76.2	77.5	77.4
4′	69.0	69.0	69.1	69.3	69.8	71.2	71.3	72.0	69.1	69.2
5′	74.1	74.1	75.9	75.3	75.5	73.5	73.5	75.0	73.5	73.5
6′	61.6	61.5	63.0	63.0	62.4	63.1	63.2	62.5	63.0	63.1
Ac	170.4	170.4	171.6	171.6	171.8	171.7	171.5		171.3	171.3
	20.7	20.7	20.9	20.8	20.9	20.9	20.8		20.9	20.8
Ac	170.0	170.1	1701.5	171.6						
	20.7	20.6	20.8	20.8						
1″									166.3	166.3
2″									41.2	41.2
3″									168.0	167.9
OMe									52.9	52.9

[a] Assignments were based on HSQC and HMBC experiments. Most methylene carbons could not be assigned unambiguously with these 2D NMR studies.



1: R_1=R_2=Ac, R_3=H
1b: R_1=R_2=R_3=Ac
3: R_1=R_3=Ac, R_2=H
6: R_1=R_2=H, R_3=Ac



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2b: R_1=R_2=R_3=Ac
4: R_1=R_3=Ac, R_2=H
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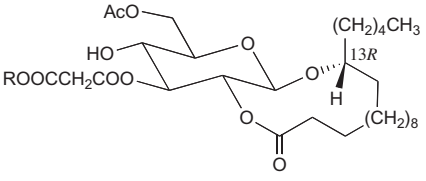
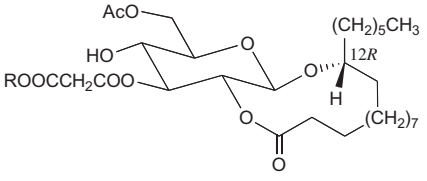
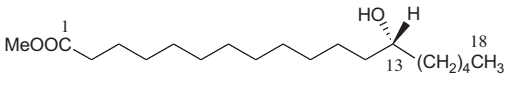
8: R_1=R_2=R_3=H



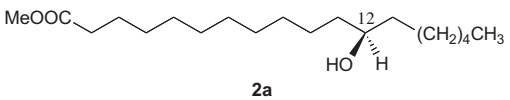
9: R=H
9a: R=Me



10: R=H
10a: R=Me



1a



2a

Fig. 2. Compounds **1**–**10**, **1a**, **2a**, **9a** and **10a**.

The oxygenated position of the fatty acyl moiety was determined in the same manner as described for **1**. The EIMS spectrum of the TMS ether of the hydroxy-fatty acid methyl ester **2a** derived from **2** showed the molecular ion peak at *m*/*z* 386, as well as the intense fragment ion peaks at *m*/*z* 187 ($[CH(OTMS)(CH_2)_5CH_3]^+$ due to C-11/C-12 cleavage) and at *m*/*z* 301 ($[CH_3OOC(CH_2)_{10}CH(OTMS)]^+$ due to C-12/C-13 cleavage). The MS data suggested compound **2a** to be methyl 12-hydroxyoctadecanoate. The TMS ether was found to be free from homologous compounds, since the GLC analysis of the ether exhibited a single peak. The absolute configuration at C-12 of **2a** was determined to be *R* based on the positive $\Delta\delta_{S-R}$ value for H_3-18 (−0.02 ppm) of the (*S*)- and (*R*)-MTPA derivatives of **2a**. Hence, the structure of gallicaside B (**2a**) was determined to be the 1,2′-cyclic ester of 12(*R*)-(3,4-di-*O*-acetyl-β-D-glucopyranosyloxy)octadecanoic acid, as depicted in Fig. 2.

Compound **3**, named gallicaside C, possessed the same molecular formula as **1**. The ^{1}H NMR spectrum of **3** showed signals for the two partial structures, fatty acyl and glucose moieties, as found in compounds **1** and **2**. The ^{1}H signals for the glucose moiety (H-2′ at δ 4.94, H-3′ at δ 5.01, H-4′ at δ 3.59, H_2-6′ at δ 4.46 and 4.31, and two acetyl singlets at δ 2.11 and 2.07) indicated that it was esterified at the 2′-*O*, 3′-*O*, and 6′-*O* positions (Table 1). The ^{13}C NMR spectroscopic data of **3**, particularly the chemical shifts for the anomeric carbon (δ 96.5) and the oxymethine carbon (δ 76.6) were more similar to those of **1** rather than to those of **2** (Table 2), suggesting a C-13 oxygenation in the fatty acyl moiety and a 3′,6′-di-*O*-acetyl-2′-*O*-fatty acyl substitution in the glucose moiety. This structure was confirmed by the fact that acetylation of compounds **1** and **3** furnished the same per-acetyl derivative **1b**. This chemical conversion also unequivocally proved that compound **3** is glycosylated at C-13 of the fatty acyl moiety and that the glucose moiety has a 2′-*O*-fatty acyl substitution. Using the same methods employed with compound **1**, the fatty acyl moiety was determined to possess oxygenation at C-13, and the *R* configuration was assigned to this stereocenter at C-13. Thus, the structure of gallicaside C (**3**) was determined to be the 1,2′-cyclic ester of 13(*R*)-(3,6-di-*O*-acetyl-β-D-glucopyranosyloxy)octadecanoic acid, as depicted in Fig. 2.

Compound **4**, named gallicaside D, had the same molecular formula as **1**. The ^{1}H NMR spectrum of **4** resembled that of **3**, thus suggesting a 3′,6′-di-*O*-acetyl-2′-*O*-fatty acyl substitution in the glucose moiety (Table 1). Comparison of the ^{13}C NMR spectroscopic data (anomeric carbon at δ 95.5) of **4** with those of compounds **2** and **3** suggested glycosylation at C-12 of the fatty acyl moiety rather than at C-13. Per-acetylation of compound **4** gave the per-acetate **2b**. Furthermore, the hydroxy-acid methyl ester derived from **4** (prepared in the same manner as described for **1**) was identified as methyl 12(*R*)-octadecanoate **2a**. Hence, the structure of gallicaside D (**4**) was determined to be the 1,2′-cyclic ester of 12(*R*)-(3,6-di-*O*-acetyl-β-D-glucopyranosyloxy)octadecanoic acid, as depicted in Fig. 2.

Compounds **5**, **7** and **8** furnished the same acetyl derivative, which was identified as **2b**. This observation demonstrated that compounds **5**, **7** and **8** share the 2′-*O*-fatty acyl substitution in the glucose moiety and the glycosylation at C-12 of the fatty acyl moiety to form the cyclic structure.

Compound **5**, named gallicaside E, showed a pseudo-molecular ion at *m*/*z* 487.3307 $[M+H]^+$ in the positive HRFABMS, which corresponded to the molecular formula $C_{26}H_{46}O_8$. The ^{1}H NMR spectrum of **5** was similar to that of **4**, but only one acetyl methyl signal was present (rather than two), and the H_2-6 signals in the glucose moiety (δ 3.92 and 3.82) were shifted upfield relative to those of **4** (Table 1). The NMR spectroscopic data suggested that compound **5** was a C-6′ deacetyl derivative of **4**. The interpretation of the NMR data, together with the fact that compound **2b** was obtained from **5**, permitted the assignment of the structure of gallicaside E as the 1,2′-cyclic ester of 12(*R*)-(3-*O*-acetyl-β-D-glucopyranosyloxy)octadecanoic acid, as depicted in Fig. 2. The chemical shift (δ 95.5) of the anomeric carbon in the ^{13}C NMR spectrum of **5** (Table 2) corroborated the glycosidic linkage at C-12 of the fatty acyl moiety (*vide supra*). The structure was further confirmed by the identification of the methyl hydroxy-fatty acid derived from **5** as methyl 12(*R*)-hydroxyoctadecanoate **2a**.

Compound **6**, named gallicaside F, had the same molecular formula as **5**. The ^{1}H NMR spectroscopic data indicated that the glucose moiety was esterified at the 2-*O* and 6-*O* positions [H-2 at δ 4.70 (*dd*, *J* = 9.6, 7.8 Hz), H_2-6 at δ 4.43 (*dd*, *J* = 12.2, 4.5 Hz) and 4.32 (*dd*, *J* = 12.2, 2.2 Hz)], presumably with fatty acyl and acetyl (δ 2.11) groups, respectively (Table 1). The chemical shift of the anomeric carbon (δ 96.5) in the ^{13}C NMR spectrum (Table 2) predicted the glycosidic linkage to be at C-13 of the fatty acyl moiety (*vide supra*). These NMR data, together with the fact that per-acetylation of compound **6** furnished acetate **1b**, allowed us to assign the structure of gallicaside F as the 1,2′-cyclic ester of 13(*R*)-(6-*O*-acetyl-β-D-glucopyranosyloxy)octadecanoic acid, as shown in Fig. 2.

Compound **7**, named gallicaside G, possessed the same molecular formula as **6**. The ^{1}H NMR data [an acetyl methyl at δ 2.11, H-2′ at δ 4.71 (*dd*, *J* = 9.6, 7.8 Hz), H_2-6′ at δ 4.41 (*dd*, *J* = 12.1, 4.8 Hz) and 4.33 (*dd*, *J* = 12.1, 2.0 Hz)] suggested a 6′-*O*-acetyl-2′-*O*-fatty acyl substitution of the glucose moiety. The chemical shift of the anomeric carbon (δ 95.3) in the ^{13}C NMR spectrum (Table 2) favored a glycosylation at C-12 of the octadecanoyl moiety. These NMR data and the fact that the acetate **2b** was formed from **7** established that the structure of gallicaside G is the 1,2′-cyclic ester of 12(*R*)-(6-*O*-acetyl-β-D-glucopyranosyloxy)octadecanoic acid, as depicted in Fig. 2. The hydroxy-fatty acid methyl ester obtained from **7** was identified as methyl 12(*R*)-hydroxyoctadecanoate **2a**.

Compound **8**, named gallicaside H, showed a pseudo-molecular ion at *m*/*z* 467.3306 $[M+Na]^+$ in the positive HRFABMS, which corresponded to the molecular formula $C_{24}H_{44}O_7$. The ^{1}H NMR spectrum was devoid of acetyl signals and indicated that the glucose moiety was acylated only at the 2-*O* position (2-H′ at δ 4.67) (Table 1). The NMR spectroscopic data, together with the fact that per-acetylation of compound **8** gave the acetate **2b**, enabled the assignment of the structure of gallicaside H as the 1,2′-cyclic ester of 12(*R*)-(β-D-glucopyranosyloxy)octadecanoic acid, as depicted in Fig. 2.

Fraction 6 (a 1:5 mixture of compounds **9** and **10**) showed a pseudo-molecular ion peak at *m*/*z* 571.3118 $[M-H]^-$ in the negative HRFABMS, which corresponded to the molecular formula $C_{29}H_{48}O_{11}$. The ^{1}H NMR spectrum recorded in $CDCl_3$–CD_3OD (9:1) indicated that the fraction was a 1:5 mixture of two components, since several signals were observed as pairs (e.g., anomeric protons at δ 4.66 and 4.71 in a 1:5 intensity ratio; see Section 4). When the ^{1}H NMR spectrum was recorded in $CDCl_3$, additional signals were observed at δ 3.38 (*m*) and 3.28 (*m*) (see Section 4), although the peaks were rather broad. This observation suggested the presence of a malonyl group in the molecule. The minor component was named gallicaside I (**9**), while the major component was named gallicaside J (**10**). Because fraction 6 was found to decompose slowly during storage, it was converted to a mixture of the corresponding methyl esters (**9a** and **10a**) by treatment with diazomethane. Separation of the mixture by reversed-phase HPLC to afforded the methyl esters **9a** and **10a** in pure form as colorless oils.

Compound **9a** showed a pseudo-molecular ion at *m*/*z* 609.3291 $[M+Na]^+$ in the positive HRFABMS, which corresponded to the molecular formula $C_{30}H_{50}O_{11}$. The ^{1}H NMR spectrum of **9a** showed signals of seven protons for a 2,3,6-tri-*O*-acylglucose moiety [δ 4.64 (*d*, *J* = 7.8 Hz, H-1′), 4.94 (*dd*, *J* = 9.6, 7.8 Hz, H-2′), 5.08 (*t*, *J* = 9.6 Hz, H-3′), 3.66 (*brt*, *J* = 9.6 Hz, H-4′), 3.53 (*m*, H-5′) and 4.37 (*m*, H_2-6′)], as well as resonances for an hydroxy-fatty acid moiety (δ 3.83), one acetyl methyl (δ 2.11), a malonate methylene group [δ 3.47 (*d*, *J* = 16.3 Hz) and 3.38 (*d*, *J* = 16.3 Hz)] and a methoxy group (δ

3.75) (Table 1). The ^{13}C NMR spectrum was similar to that of compound **3**; the main difference was the presence of a malonyl methyl ester (δ 168.0, 166.3, 52.9 and 41.2) in place of an acetyl group. The three ester groups in the glucose moiety were now assigned as fatty acyl, acetyl and methylated malonyl. The ester linkages of the three groups were unambiguously determined to be 2′-*O*-fatty acyl, 3′-*O*-malonyl and 6′-*O*-acetyl substitutions by analysis of the HMBC correlations, as illustrated in Fig. 3. The hydroxy-fatty acid methyl ester derived from **9a** was identified as 13(*R*)-hydroxyoctadecanoate. Thus, the structure of compound **9a** was established as depicted in Fig. 2. Accordingly, gallicaside I (**9**) was determined to be the 1,2′-cyclic ester of 13(*R*)-(6-*O*-acetyl-3-*O*-malonyl-β-D-glucopyranosyloxy)octadecanoic acid, as depicted in Fig. 2.

Compound **10a** possessed the same molecular formula as **9a**. The ^{1}H and ^{13}C NMR spectra of **10a** resembled those of **9a**. However, the chemical shift of the anomeric carbon (δ 95.5) in the ^{13}C NMR data indicated a diagnostic difference compared to that of **9a** (δ 96.5), implying that the glycosidic linkage was present at C-12 of the fatty acyl moiety. This structural difference was confirmed by the identification of the hydroxy-fatty acid methyl ester obtained from **10a** as 12(*R*)-hydroxyoctadecanoate **2a**. The 2′-*O*-fatty acyl, 3′-*O*-malonyl and 6′-*O*-acetyl substitutions in the glucose moiety were confirmed using HMBC correlations in the same manner as described for **9a**. Thus, the structure of **10a** was established as described in Fig. 2. Hence, gallicaside J (**10**) was established to be the 1,2′-cyclic ester of 12(*R*)-(6-*O*-acetyl-3-*O*-malonyl-β-D-glucopyranosyloxy)octadecanoic acid, as depicted in Fig. 2.

3. Concluding remarks

The glandular trichome exudate of *S. gallica* specifically contained the new cyclic fatty acyl glycosides (**1**–**10**). The relative abundance of these constituents is summarized in Table 3. Compound **10** was the most abundant constituent (30.7%) of the exudate, followed by compounds **4** (11.7%) and **7** (11.6%). The structures of gallicasides **A**–**J** were characterized by the unique 1,2′-cyclic esters that formed either a 16 or 17-membered ring. Similar cyclic fatty acyl glycosides are uncommon as natural products, but a few examples have been reported, for example, the cyclic 1,2′-ester of 5-(β-D-xylopyranosyloxy)fatty acids from the aerial part of *Stellaria dichotoma* of the Caryophyllaceae family (Ganenko et al., 2001). In compounds **1**–**10**, the octadecanoyl moiety was oxygenated either at the C-12 or the C-13 positions, with the former being predominant, and the two cyclic structures were differentiated by the diagnostic ^{13}C chemical shifts of the anomeric carbon: δ 96.5–96.6 for the 13-*O* linkage (17-membered ring) versus δ 95.3–95.6 for the 12-*O* linkage (16-membered ring). The glucose moiety was either mono- or di-acetylated at the 3′-*O*, 4′-*O* and 6′-*O* positions. The two compounds **9** and **10** were further malonylated at 3-*O*′ of the glucose moiety.

As described in Section 1, we recently reported the isolation of related acyclic compounds, (glucosyloxy-fatty acids and (6-acetylglucosyloxy)-fatty acids) from the glandular trichome exudates of *Ibicella lutea* and *Proboscidea louisiana* (Asai et al., 2010). Isolation of such secondary metabolites suggests that compounds consisting of a covalent bond between sugars and fatty acid derivatives could be common components of glandular trichome exudates. Glucose esters (King and Calhoun, 1988), sucrose esters (King et al., 1990; Matsuzaki et al., 1992) and inositol esters (Spring et al., 2001), all of which were characterized from glandular trichome exudates, can be characterized as this type of secondary metabolite.

Table 3
Relative abundance of constituents in the glandular trichome exudate.

Compound	Relative abundance (%)[a]
Gallicaside A (**1**)	4.5
Gallicaside B (**2**)	9.8
Gallicaside C (**3**)	3.7
Gallicaside D (**4**)	11.7
Gallicaside E (**5**)	8.1
Gallicaside F (**6**)	1.5
Gallicaside G (**7**)	11.6
Gallicaside H (**8**)	4.4
Gallicaside I (**9**)	7.7
Gallicaside J (**10**)	30.7

[a] Compensations were made for the presence of overlapping fractions in the chromatographic separation, and some of the percentage values are thus higher than the quantity of fully purified materials would suggest. The remainder (6.3%) of the exudate could be due to minor related compounds and/or volatile substances.

4. Experimental

4.1. General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer in $CDCl_3$ solution. Tetramethylsilane (δ 0.00) signal was used as an internal standard for ^{1}H shifts, and the $CDCl_3$ (δ 77.00) resonance was used as a reference for ^{13}C shifts. EIMS (70 eV), FABMS and HRFABMS spectra (3-nitrobenzylalcohol as the matrix) were obtained using a JEOL JMS-700 spectrometer, whereas IR spectra were recorded on a JASCO-FT/IR-5300 spectrometer. Optical rotations were measured on a JASCO P-2200 polarimeter. TLC analysis was performed using Merck precoated Si gel 60 F254 glass plates and the spots were detected by treating the plates with a 5% ethanolic solution of phosphomolybdic acid followed by heating at 120 °C. PTLC was performed using the same Si gel plates. Silica gel 60 N (spherical neutral, 40–100 μm, Kanto Chemical, Japan) was used for column chromatography (CC). HPLC analysis was carried out on a Shimadzu LC-6A apparatus equipped with a UV detector (215 nm) using a reversed-phase column (Inertsil ODS-3, 15 cm × 4.6 mm i.d.) under isocratic solvent conditions. GLC was carried out on a Shimadzu GC-14B apparatus equipped with a J&W Scientific DB-5 capillary column (15 m × 0.25 mm, 0.25 mm film thickness) under the following conditions: injection temperature 270 °C, column temperature 200 °C,

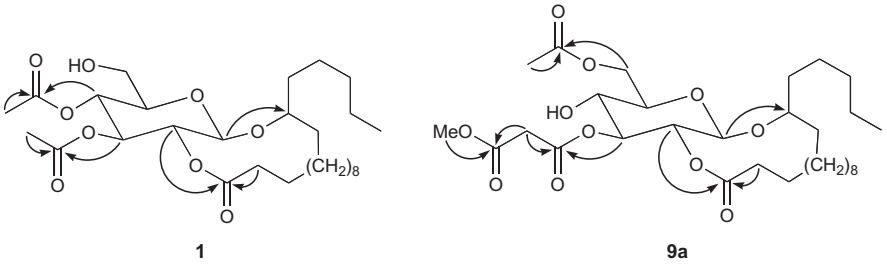


Fig. 3. Pertinent HMBC correlations of **1** and **9a**.

detection temperature 270 °C, He carrier gas flow rate of P1, 50 kPa and P2, 120 kPa, H_2 flow rate, 50 kPa, air flow rate, 50 kPa and split (40:1) injection for the analysis of trimethylsilylated hydroxy-fatty acid methyl esters. GC–MS for the identification of the trimethylsilylated glucose was conducted using a mass spectrometer (JMS-AM SUN200, JEOL) connected to a gas chromatograph (6890A, Agilent Technologies) under the following conditions: EI (70 eV); DB-1 capillary column (30 m × 0.25 mm, 0.25 μm film thickness, J&W Scientific); source temperature 250 °C; injection temperature 250 °C; column temperature 175 °C; interface temperature 280 °C; He carrier gas flow rate of 1.0 mL/min with splitless injection.

4.2. Plant material

Silene gallica L. (Caryophyllaceae) were collected in May, 2008 on the campus of Tokyo Institute of Technology. The plant was identified by Prof. S. Kohshima, Department of Biological Sciences, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology. A voucher specimen (CMS20-01) was deposited in the Department of Chemistry and Materials Science, Tokyo Institute of Technology.

4.3. Extraction and isolation

Fresh upper aerial parts of *S. gallica* (fresh wt. 250 g) were briefly (*ca.* 2 s) rinsed twice in a beaker containing Et_2O (total volume 500 mL) and the Et_2O solution was concentrated to dryness (160 mg) under reduced pressure. The residue was subjected to silica gel (100 g) CC. The column was eluted with a gradient of hexane–EtOAc (8:1 → 1:1, total volume 400 mL) and EtOAc–MeOH (10:1 → 4:1, total volume 200 mL), and the fractions (30 mL each, total 20 fractions) were combined to give six pools (I–V and VI (62.2 mg)) according to the TLC profile. Pool I (23.4 mg, eluted with hexane–EtOAc, 2:1 → 1.5:1) was further separated by HPLC (CH_3CN–H_2O, 6:1; flow rate, 1.0 mL/min) to afford compounds **1** (6.7 mg) and **2** (14.6 mg) at 13.0 and 13.9 min, respectively. Pool II (25.1 mg, eluted with hexane–EtOAc, 1.5:1 → 1:1) was further separated by HPLC (CH_3CN–H_2O, 8:1; flow rate, 1.0 mL/min) to give **3** (5.2 mg) and **4** (16.2 mg) at 13.8 and 14.9 min, respectively. Pool III (13.2 mg, eluted with EtOAc–MeOH, 1:1 → 1:4) was further purified by HPLC (CH_3CN–H_2O, 6:1; flow rate, 1.0 mL/min) to afford **5** (11.2 mg) at 10.3 min. Pool IV (21.5 mg, eluted with EtOAc) was further separated by HPLC (CH_3CN–H_2O, 6:1; flow rate, 1.0 mL/min) to give **6** (2.6 mg) and **7** (18.4 mg) at 9.2 and 10.0 min, respectively. Pool V (7.2 mg, eluted with EtOAc–MeOH, 10:1 → 4:1) was further purified by HPLC (CH_3CN–H_2O, 3:1; flow rate, 1.0 mL/min) to afford **8** (5.3 mg) at 12.6 min.

4.4. Gallicaside A (*1*)

Colorless oil; HRFABMS *m/z*: 551.3217 $[M+Na]^+$ (calcd for $C_{28}H_{48}O_9Na$, 551.3196); $[\alpha]_D^{24}$ −21.0 (*c* = 0.4, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3600, 2910, 2850, 1720, 1440, 1390, 1190, 1090 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2, respectively.

A solution of **1** (1.2 mg) in DME (0.2 mL) was added to a solution of LiOH (2.5 mg) in H_2O (50 μL), and the mixture was stirred at room temperature for 48 h. 2 N HCl was added and the mixture was partitioned between $CHCl_3$ and H_2O. Concentration of the $CHCl_3$ layer gave the glucosyloxy-fatty acid (1.1 mg). A mixture of the material and 1.5 N HCl (0.1 mL) was heated at 80 °C for 6 h and then was cooled to room temperature. The reaction mixture was partitioned between Et_2O and H_2O. The aqueous layer was concentrated and the residual material was identified as glucose by TLC (Rf, 0.55, CH_3CN–H_2O 85:15, developed three times) in comparison with authentic glucose. The residue (0.1 mg) was reacted with TMS-HT (HMDS and TMSCl in anhydrous pyridine) (30 μL) at 70 °C for 1.5 h and cooled to room temperature. Hexane (100 μL) and H_2O (50 μL) were added and a portion of the hexane layer was analyzed by GC–MS. The total ion monitor trace showed two major peaks corresponding to penta-*O*-TMS derivatives (α- and β-anomers) at 12.7 and 18.3 min, respectively. Both peaks exhibited a fragment ion at *m/z* 435 $[M–TMSOH–Me]^+$ and 360 $[M–(TMSOH)_2]^+$. TMS derivative prepared from authentic glucose showed the identical two peaks and mass fragmentation patterns.

The Et_2O layer was concentrated, and the resulting material was treated with excess ethereal diazomethane to give methyl 13(*R*)-hydroxyoctadecanoate **1a** (0.6 mg) after PTLC purification (hexane–EtOAc, 6:1) (the 13(*S*)-antipode has been reported in the literature (Habel et al., 2007)). Amorphous; (+)-FABMS *m/z*: 314 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ: 3.67 (*s*, OMe), 3.59 (*m*, H-13), 2.30 (*t*, *J* = 7.6 Hz, H_2-2), 1.65–1.25 (CH_2), 0.89 (*t*, *J* = 6.7 Hz, H_3-18). A mixture of the methyl ester (100 μg) and TMS-HT (30 μL) was heated at 75 °C for 1.5 h, and then the mixture was cooled to room temperature. Hexane (60 μL) and H_2O (60 μL) were added to the reaction mixture and a part of the hexane layer was analyzed by GLC to confirm the absence of homologous compounds. TMS ether of **1a**: EIMS *m/z*: 386 $[M]^+$ (3), 371 $[M–Me]^+$ (10), 355 $[M–MeO]^+$ (16), 315 (92), 286 (14), 173 (100), 153 (35), 136 (34), 73 (40).

The methyl ester (200 μg) was treated with (*R*)-MTPACl (1.0 μL) in pyridine (30 μL) to give the (*S*)-MTPA ester in a quantitative yield. Colorless oil; (+)-FABMS *m/z*: 531 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ: 7.84–7.48 (*m*), 5.09 (*m*, H-13), 3.67 (*s*, $COOCH_3$), 3.46 (*s*, OCH_3), 2.31, (*t*, *J* = 7.5 Hz, H_2-2), 1.52–1.19 (*m*), 0.88 (*t*, *J* = 6.6 Hz, H_3-18). The (*R*)-MTPA ester was similarly prepared using (*S*)-MTPACl. Colorless oil; (+)-FABMS *m/z*: 531 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ: 7.84–7.48 (*m*), 5.09 (*m*, H-13), 3.67 (*s*, $COOCH_3$), 3.46 (*s*, OCH_3), 2.31, (*t*, *J* = 7.5 Hz, H_2-2), 1.52–1.19 (*m*), 0.84 (*t*, *J* = 6.8 Hz, H_3-18).

Compound **1** (2 mg) was treated with Ac_2O and pyridine at room temperature overnight. Extractive workup gave a crude product which was purified by PTLC (hexane-EtOAc, 3:1) to afford the per-acetyl derivative (**1b**) (2.1 mg, 97% yield): Colorless oil; (+)-FABMS *m/z*: 570 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ: 5.19 (*t*, *J* = 9.2 Hz, H-3′), 5.08 (*t*, *J* = 9.2 Hz, H-4′), 5.00 (*dd*, *J* = 9.2, 8.0 Hz, H-2′), 4.64 (*d*, *J* = 8.0 Hz, H-1′), 4.23 (*dd*, *J* = 12.1, 5.1 Hz, Ha-6′), 4.13 (*dd*, *J* = 12.1, 2.2 Hz, Hb-6′), 3.84 (*m*, H-13), 3.66 (*m*, H-5′), 2.28 (*t*, *J* = 7.0 Hz, H_2-2), 2.08 (*s*, Ac), 2.02 (*s*, Ac), 1.99 (*s*, Ac), 1.70–1.20 (CH_2), 0.91 (*t*, 6.8 Hz, H_3-18); ^{13}C NMR ($CDCl_3$) δ: 172.2, 170.7, 170.5, 169.5, 96.5, 76.8, 73.3, 71.8, 71.4, 68.8, 62.3, 33.2, 32.7, 32.1, 31.5, 28.1, 27.6, 26.8, 26.7, 26.6, 25.1, 23.7, 22.8, 22.6, 20.8, 20.7, 20.6, 14.1.

4.5. Gallicaside B (*2*)

Colorless oil; HRFABMS *m/z*: 529.3378 $[M+H]^+$ (calcd for $C_{28}H_{49}O_9$, 529.3377); $[\alpha]_D^{24}$ −35.7 (*c* = 1.4, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3600, 2910, 2850, 1720, 1440, 1390, 1190, 1090 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2, respectively.

Methyl 12(*R*)-hydroxyoctadecanoate **2a** (Cymerman-Craig and Roy, 1965; Tulloch, 1978) was obtained from **2** in the same manner as described for **1**. (+)-FABMS *m/z*: 314 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ: 3.67 (*s*, OMe), 3.59 (*m*, H-12), 2.30 (*t*, *J* = 7.6 Hz, H_2-2), 1.65–1.25 (CH_2), 0.89 (3H, *t*, *J* = 6.7 Hz, H_3-18). The methyl ester (100 μg) was converted to the corresponding TMS ether in the same manner as described for **1** and was analyzed by GLC and EIMS. TMS ether of **2a**: EIMS *m/z*: 386 $[M]^+$ (2), 371 $[M–Me]^+$ (6), 355 $[M–MeO]^+$ (10), 301 (100), 272 (12), 187 (90), 149 (35), 129 (11), 73 (52).

The methyl ester was converted to the (*S*)- and (*R*)-MTPA esters in the same manner as described for **1a**. The (*S*)-MTPA ester: Colorless oil; (+)-FABMS *m/z*: 531 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ: 7.84–7.48 (*m*), 5.08 (*m*, H-12), 3.67 (*s*, $COOCH_3$), 3.46 (*s*, OCH_3), 2.31, (*t*, *J* = 7.5 Hz, H_2-2), 1.52–1.19 (*m*), 0.88 (*t*, *J* = 6.8 Hz, H_3-18). The (*R*)-MTPA ester: Colorless oil; (+)-FABMS *m/z*: 531 $[M+H]^+$; ^1H NMR

($CDCl_3$) δ: 7.84–7.48 (*m*), 5.08 (*m*, H-12), 3.67 (*s*, $COOCH_3$), 3.46 (*s*, OCH_3), 2.31, (*t*, *J* = 7.5 Hz, H_2-2), 1.52–1.19 (*m*), 0.86 (*t*, *J* = 6.9 Hz, H_3-18).

Compound **2** (3 mg) was treated with Ac_2O and pyridine at room temperature overnight. Extractive workup gave a crude product, which was purified by PTLC (hexane-EtOAc, 3:1) to afford per-acetyl derivative (**2b**) (3.1 mg, 96% yield): Colorless oil; (+)-FABMS *m*/*z*: 570 $[M+H]^+$; ^1H NMR ($CDCl_3$) δ: 5.21 (*t*, *J* = 9.3 Hz, H-3′), 5.08 (*t*, *J* = 9.3 Hz, H-4′), 5.03 (*dd*, *J* = 9.3, 7.9 Hz, H-2′), 4.69 (*d*, *J* = 7.9 Hz, H-1′), 4.22 (*dd*, *J* = 12.2, 5.3 Hz, Ha-6′), 4.13 (*dd*, *J* = 12.2, 2.1 Hz, Hb-6′), 3.89 (*m*, H-12), 3.66 (*m*, H-5′), 2.28 (*t*, *J* = 7.0 Hz, H_2-2), 2.07 (*s*, Ac), 2.02 (*s*, Ac), 1.99 (*s*, Ac), 1.70–1.20 (CH_2), 0.90 (*t*, 6.8 Hz, H_3-18); ^{13}C NMR ($CDCl_3$) δ: 172.4, 170.6, 170.4, 169.4, 95.6, 75.5, 73.4, 71.8, 71.4, 68.9, 62.3, 33.6, 32.3, 31.8, 31.6, 29.6, 27.3, 26.7, 26.0, 26.0, 25.0, 24.2, 23.8, 22.7, 22.3, 20.7, 20.6, 20.6, 14.1.

4.6. Gallicaside C (*3*)

Colorless oil; (+)-HRFABMS *m*/*z*: 529.3390 $[M+H]^+$ (calcd for $C_{28}H_{49}O_9$, 529.3377); $[\alpha]_D^{24}$ −51.5 (*c* = 0.4, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3600, 2910, 2850, 1720, 1440, 1390, 1190, 1090 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2, respectively. The acetate derivative that was prepared from **3** was identified as compound **1b**. The hydroxy-fatty acid methyl ester that was obtained from **3** was identified as **1a**.

4.7. Gallicaside D (*4*)

Colorless oil; (+)-HRFABMS *m*/*z*: 529.3380 $[M+H]^+$ (calcd for $C_{28}H_{49}O_9$, 529.3377); $[\alpha]_D^{24}$ −54.0 (*c* = 1.3, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3600, 2910, 2850, 1720, 1440, 1390, 1190, 1090 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2, respectively. The acetate derivative that was prepared from **4** was identified as compound **2b**. The hydroxy-fatty acid methyl ester that was obtained from **4** was identified as **2a**.

4.8. Gallicaside E (*5*)

Colorless oil; (+)-HRFABMS *m*/*z*: 487.3307 $[M+H]^+$ (calcd for $C_{26}H_{47}O_8$, 487.3271); $[\alpha]_D^{24}$ −50.2 (*c* = 1.5, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3600, 2910, 2850, 1720, 1440, 1390, 1190, 1090 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2, respectively. The acetate derivative that was prepared from **5** was identified as compound **2b**. The hydroxy-fatty acid methyl ester that was obtained from **5** was identified as **2a**.

4.9. Gallicaside F (*6*)

Colorless oil; (+)-HRFABMS *m*/*z*: 487.3260 $[M+H]^+$ (calcd for $C_{26}H_{47}O_8$, 487.3271); $[\alpha]_D^{24}$ −52.5 (*c* = 0.2, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3600, 2910, 2850, 1720, 1440, 1390, 1190, 1090 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 1 and 2, respectively. The acetate derivative that was prepared from **6** was identified as compound **1b**. The hydroxy-fatty acid methyl ester that was obtained from **6** was identified as **1a**.

4.10. Gallicaside G (*7*)

Colorless oil; (+)-HRFABMS *m*/*z*: 487.3298 $[M+H]^+$ (calcd for $C_{26}H_{47}O_8$, 487.3271); $[\alpha]_D^{24}$ −66.8 (*c* = 1.0, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3600, 2910, 2850, 1720, 1440, 1390, 1190, 1090 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2, respectively. The acetate derivative that was prepared from **7** was identified as compound **2b**. The hydroxy-fatty acid methyl ester that was obtained from **7** was identified as **2a**.

4.11. Gallicaside H (*8*)

Colorless oil; (+)-HRFABMS *m*/*z*: 467.3006 $[M+H]^+$ (calcd for $C_{24}H_{44}O_7Na$, 467.2985); $[\alpha]_D^{24}$ −51.5 (*c* = 0.4, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3600, 2910, 2850, 1720, 1440, 1100, 1090 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2, respectively. The acetate derivative that was prepared from **8** was identified as compound **2b**. The hydroxy-fatty acid methyl ester that was obtained from **8** was identified as **2a**.

4.12. Pool VI (a mixture of *9* and *10*)

Colorless oil; (−)-HRFABMS *m*/*z*: 571.3118 $[M-H]^-$ (calcd for $C_{29}H_{47}O_{11}$, 571.3118). The ^1H NMR spectrum recorded in $CDCl_3$ gave only broad signals, but the following resonances were observed: δ 5.11 (*m*, H-3′), 4.91 (*m*, H-2′), 4.75 (*m*, H-1′), 4.41 (*m*, Ha-6′), 4.27 (*m*, Hb-6′), 3.88 (*m*, H-12 or 13), 3.67 (*m*, H-4′), 3.64 (*m*, H-5′), 3.38 (*m*, Ha-2″), 3.28 (*m*, Hb-2″). The ^1H NMR spectrum recorded in $CDCl_3$–CD_3OD (9:1) exhibited sharp signals, several of which resonated as pairs of resonances in a 1:5 ratio. Compound **9**: ^1H NMR (signals of lower intensities) δ: 5.06 (*t*, *J* = 9.5 Hz, H-3′), 4.89 (*dd*, *J* = 9.5, 7.9 Hz, H-2′), 4.66 (*d*, *J* = 7.9 Hz, H-1′), 4.43 (*brd*, *J* = 11.8 Hz, Ha-6′), 4.23 (*dd*, *J* = 11.8, 6.0 Hz, Hb-6′), 3.84 (*m*, H-12), 3.64 (*t*, *J* = 9.5 Hz, H-4′), 3.58 (*m*, H-5′), 3.37 (*m*, H_2-2″), 2.30 (*m*, H_2-2), 2.09 (*s*, Ac), 1.70–1.20 (CH_2), 0.89 (*t*, *J* = 6.8 Hz, H_3-18). **10**: ^1H NMR (signals of higher intensities) δ: 5.09 (*t*, *J* = 9.5 Hz, H-3′), 4.93 (*dd*, *J* = 9.5, 7.9 Hz, H-2′), 4.71 (*d*, *J* = 7.9 Hz, H-1′), 4.43 (*brd*, *J* = 11.8 Hz, Ha-6′), 4.23 (*dd*, *J* = 11.8, 6.0 Hz, Hb-6′), 3.88 (*m*, H-12), 3.64 (*t*, *J* = 9.5 Hz, H-4′), 3.58 (*m*, H-5′), 3.37 (*m*, H_2-2″), 2.30 (*m*, H_2-2), 2.09 (*s*, Ac), 1.70–1.20 (CH_2), 0.89 (*t*, *J* = 6.8 Hz, H_3-18).

A part of Pool VI (30 mg) was treated with ethereal diazomethane, and the resulting methyl ester mixture was separated by HPLC (solvent, CH_3CN–H_2O, 8:1; flow rate, 1.0 mL/min) to give **9a** (4.2 mg) and **10a** (22.3 mg) at 13.9 and 15.1 min, respectively.

4.13. Methyl ester of gallicaside I (*9a*)

Colorless oil; (+)-HRFABMS *m*/*z*: 609.3291 $[M+Na]^+$ (calcd for $C_{30}H_{50}O_{11}Na$, 609.3251); $[\alpha]_D^{24}$ −35.0 (*c* = 0.3, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3600, 2910, 2850, 1720, 1715, 1460, 1440, 1310, 1210, 1170, 1110, 1090 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2, respectively. The hydroxy-fatty acid methyl ester that was obtained from **9a** was identified as **1a**.

4.14. Methyl ester of gallicaside J (*10a*)

Colorless oil; (+)-HRFABMS *m*/*z*: 587.3466 $[M+H]^+$ (calcd for $C_{30}H_{51}O_{11}$, 587.3432); $[\alpha]_D^{24}$ −47.2 (*c* = 2.4, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3600, 2910, 2850, 1720, 1715, 1460, 1440, 1310, 1210, 1170, 1110, 1090 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2, respectively. The hydroxy-fatty acid methyl ester that was obtained from **10a** was identified as **2a**.

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