n-Octyl α-L-Rhamnopyranosyl-(1→2)-β-D-glucopyranoside Derivatives from the Glandular Trichome Exudate of *Geranium carolinianum*

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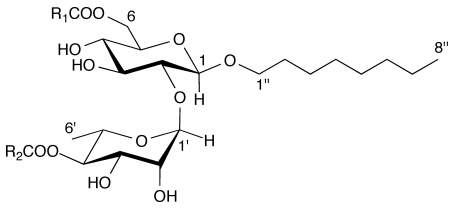
Chemical investigation of the glandular trichome exudate from *Geranium carolinianum* L. (Geraniaceae) led to the characterization of unique disaccharide derivatives, *n*-octyl 4-*O*-isobutyryl-α-L-rhamnopyranosyl-(1→2)-6-*O*-isobutyryl-β-D-glucopyranoside (1), *n*-octyl 4-*O*-isobutyryl-α-L-rhamnopyranosyl-(1→2)-6-*O*-(2-methylbutyryl)-β-D-glucopyranoside (2) and *n*-octyl 4-*O*-(2-methylbutyryl)-α-L-rhamnopyranosyl-(1→2)-6-*O*-isobutyryl-β-D-glucopyranoside (3), named caroliniasides A—C, respectively. These structures were determined by spectral means. *n*-Alkyl glycoside derivatives have been isolated from the glandular trichome exudates for the first time. This rare type of secondary metabolites could be applicable to chemotaxonomic perspective because they are found in glandular trichome exudates of plants belonging to the genus *Geranium*, according to our studies.

Key words *Geranium carolinianum*; caroliniaside; octyl glycoside; Geraniaceae; glandular trichome

Glandular trichomes are micro-organs located on the surface of the leaves, stems, flowers, and fruits of plants that exude oily substances. The chemical and physiological roles of the oily substances have been studied in a limited number of plant species such as those belonging to the Labiatae and Compositae families. It has been suggested that exudate substances are related to a plant's self-protection system, for example, to serve as antifeedants, antifungals, antibiotics, or for UV protection.[1,2] However, our understanding of the chemistry and function of glandular trichome exudates remain insufficient. Additional systematic chemical and biological studies of the exudates would aid in further understanding of glandular trichomes. From this perspective, we have been investigating the glandular trichome exudates of plants belonging to a variety of families. Our previous studies led to the characterization of a series of oxygenated fatty acyl glycerols, in the glandular trichome exudate on the leaves, and geranylated flavanones, in the oily secretion on the immature fruit surface, from *Paulownia tomentosa* (Scrophulariaceae).[3,4] The glandular trichome exudates of *Ibicella lutea* and *Proboscidea louisiana* (Martyniaceae) contained glycosylated fatty acids and dammarane triterpenes.[5] Recently we identified a series of cyclic fatty acyl glycosides from the glandular trichome exudate of *Silene gallica* (Caryophyllaceae)[6] and 2-acetyl-1-(3-glycosyloxy-fatty acyl)octadecanoyl-*sn*-glycerol and dammarane triterpenes from the glandular trichome-like secretory organs in the young stipules and leaves of *Cerasus yedoensis* (Rosaceae).[7] More recently, we found several plants of the Geraniaceae family, such as *Geranium carolinianum*, *G. thunbergii*, *G. robertianum* and *G. maderense*, have capitate glandular trichomes in the upper portion of their stems.

Non-volatile secondary metabolites of the glandular trichome exudates of the Geraniaceae plants have been reported only in a few species. Anacardic acids have been isolated from the glandular trichome exudate of *Pelargonium x hortorum*[8] and their biosynthetic precursors, hexadec-11-enoic and octadec-13-enoic acids, have been characterized in the glandular trichomes of the plant.[9] Flavonoids have been identified in the glandular trichome exudates of *G. macrorrhizum* and *G. lucidum*.[10] As a continuation of our work, we now started to investigate glandular trichome exudates from plants of the Geraniaceae family. This article describes the isolation and structure elucidation of three unique *n*-octyl disaccharides from the glandular trichome exudate of *Geranium carolinianum* (Fig. 1).



1: $R_1 = R_2 =$ -$CHMe_2$
2: $R_1 =$ -$CHMeCH_2Me$, $R_2 =$ -$CHMe_2$
3: $R_1 =$ -$CHMe_2$, $R_2 =$ -$CHMeCH_2Me$

Fig. 1. Structures of Caroliniasides A—C (**1**—**3**)

Geranium carolinianum L. is native to North America and was imported into Japan about 100 years ago. The plant is widely distributed throughout open roadside in Japan and comes into flower between April and May.[11] The upper portion of the aerial part, in particular the calyxes, is rich in capitate glandular trichomes. The aerial part of *G. carolinianum* was reported to exhibit antimicrobial and anti-hepatitis B virus activities.[12,13] Previous phytochemical studies of *G. carolinianum* led to the identification of gallic acid,[14] ethyl gallate,[12] ellagic acid, geraniin and flavones.[13]

Results and Discussion

In a preliminary study, two exudate samples were obtained by either gently wiping the surface of 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, with the latter being contaminated with larger amounts of non-polar materials (exclusively a mixture of hydrocarbons). To obtain larger samples, an extract was prepared using the latter method. This oily material was subjected to silica gel column chromatography to give the exudate-specific fraction, which was a single spot by TLC. HPLC analysis of this fraction showed essentially two major peaks (see

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Experimental), which were separated to yield compounds **1** and **2**/**3**. In contrast, GLC analysis of the fraction as the trimethylsilyl (TMS) ether derivatives exhibited three major peaks A, B and C (Fig. 2). Subsequent studies (*vide infra*) revealed that compounds **1**, **2** and **3** corresponded to the peaks A, B and C, respectively. Negligible amounts of these substances were found in the ether-rinsed residual aerial part, thus indicating that compounds **1**—**3** were distributed in glandular trichome exudate.

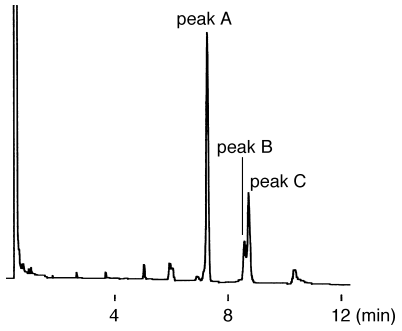

Fig. 2. GLC Chart of the Fraction Containing Compounds **1**—**3**

Compound **1** was obtained as an oil and a major constituent (65%) of the fraction. Its molecular formula $C_{28}H_{50}O_{12}$ was deduced from high resolution (HR)-FAB-MS data, which exhibited a quasi-molecular ion peak at *m*/*z* 601.3203 $[M+Na]^+$ (Calcd for $C_{28}H_{50}O_{12}Na$, 601.3200). GLC analysis of the TMS ether derivative of **1** showed a single peak (peak A in Fig. 2). The ^{1}H-NMR spectrum of **1** exhibited signals ascribable to hexose and 6-deoxyhexose moieties, two isobutyryl groups and a medium-chain *n*-alkyloxy group (Table 1). The hexose moiety was assigned as a β-linked 6-*O*-isobutyrylglucopyranosyl (glc) on the basis of the proton chemical shifts and coupling constants [*e.g.*, δ 4.29 (d, J=7.7 Hz, H-1), 4.33 (dd, J=12.0, 4.0 Hz, H-6a), 4.34 (dd, J=12.0, 4.0 Hz, H-6b)], combined with the data of the ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum. The

Table 1. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) Data of **1**, **2** and **3** in $CDCl_3$–CD_3OD (20 : 1)

Position	**1**		**2**[a)]		**3**[a)]	
	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C
Glucopyranosyl						
1	4.29 (d, 7.5)	101.69	4.29 (d, 7.5)	101.69	4.29 (d, 7.5)	101.69
2	3.50 (dd, 9.0, 7.7)	76.53	3.50 (dd, 9.0, 7.5)	76.44	3.50 (dd, 9.0, 7.5)	76.44
3	3.55 (t, 9.0)	77.62	3.55 (t, 9.0)	77.62	3.55 (t, 9.0)	77.62
4	3.30 (t, 9.0)	70.44	3.30 (t, 9.0)	70.41	3.30 (t, 9.0)	70.41
5	3.42 (m)	73.71	3.42 (m)	73.70	3.42 (m)	73.70
6	4.33 (dd, 12.0, 4.0)	63.48	4.33 (dd, 13.0, 4.0)	63.48	4.33 (dd, 13.0, 4.0)	63.48
	4.34 (dd, 12.0, 4.0)		4.34 (dd, 13.0, 4.0)		4.34 (dd, 13.0, 4.0)	
Rhamnopyranosyl						
1′	5.28 (s)	99.74	5.28 (s)	99.71	5.28 (s)	99.71
2′	3.98 (br s)	70.70	3.97 (br s)	70.71	3.97 (br s)	70.71
3′	3.82 (dd, 9.7, 3.4)	69.71	3.82 (dd, 9.7, 3.4)	69.79	3.82 (dd, 9.7, 3.4)	69.79
4′	4.83 (t, 9.7)	74.65	4.82 (t, 9.7)	74.62	4.83 (t, 9.7)	74.62
5′	4.21 (dq, 9.7, 6.3)	65.98	4.22 (dq, 9.7, 6.3)	65.94	4.22 (dq, 9.7, 6.3)	65.94
6′	1.15 (d, 6.3)	17.13	1.15 (d, 6.3)	17.13	1.15 (d, 6.3)	17.16
n-Octyl						
1″	3.85 (dt, 9.8, 6.3)	70.15	3.86 (dt, 9.3, 7.2)	70.18	3.86 (dt, 9.3, 7.2)	70.18
	3.46 (dt, 9.8, 6.3)		3.46 (dt, 9.3, 7.2)		3.46 (dt, 9.3, 7.2)	
2″	1.61 (m)	29.61	1.62 (m)	29.60	1.62 (m)	29.60
3″	1.22—1.35	25.93	1.22—1.35	25.94	1.22—1.35	25.94
4″	1.22—1.35	29.42	1.22—1.35	29.43	1.22—1.35	29.43
5″	1.22—1.35	29.19	1.22—1.35	29.21	1.22—1.35	29.21
6″	1.22—1.35	31.82	1.22—1.35	31.83	1.22—1.35	31.83
7″	1.22—1.35	22.58	1.22—1.35	22.59	1.22—1.35	22.59
8″	0.88 (t, 7.0)	14.02	0.88 (t, 6.9)	14.03	0.88 (t, 6.9)	14.03
Isobutyryl						
1‴	—	177.87	—	177.91	—	177.91
	—	177.87				
2‴	2.60 (m)	33.93	2.61 (m)	34.18	2.61 (m)	33.93
	2.60 (m)	34.18				
3‴	1.18 (d, 6.3)	18.87	1.18 (d, 6.3)	18.74	1.18 (d, 6.3)	18.87
	1.18 (d, 6.3)	18.74				
4‴	1.19 (d, 6.3)	18.96	1.19 (d, 6.3)	19.00	1.19 (d, 6.3)	18.96
	1.19 (d, 6.3)	18.99				
2-Methylbutyryl						
1⁗			—	177.54	—	177.54
2⁗			2.43 (m)	41.00	2.42 (m)	41.31
3⁗			1.70 (m)	26.75	1.70 (m)	26.63
			1.49 (m)		1.49 (m)	
4⁗			0.92 (t, 7.5)	16.50	0.94 (t, 7.5)	16.61
5⁗			1.18 (d, 6.3)	11.58	1.18 (d, 6.3)	11.48

a) Recorded as a 1 : 2.5 mixture of **2** and **3**.

deoxy hexose moiety was similarly determined to be an α-linked 4′-*O*-isobutyrylrhamnopyranosyl (rha) [*e.g.*, δ 5.28 (s, H-1′), 4.83 (t, J=9.7 Hz, H_3-4′), 1.15 (d, J=6.3 Hz, H_3-6′)]. An α-anomeric configuration of rha was assigned using the nuclear Overhauser effect (NOE) experiments, in which irradiation of H-3′ caused an NOE enhancement toward H-5′, but not toward H-1′. The ^{13}C-NMR spectrum of **1** exhibited 28 signals, among which 12 signals were assigned to the carbon atoms of the two sugars and eight signals were assigned to the carbon atoms of the two isobutyryl groups. The *n*-alkyloxy group was now defined as an *n*-octyloxy group on the basis of the number of the remaining carbon signals, which was in agreement with the calculation from the molecular formula of **1**. Heteronuclear multiple bond correlations (HMBC) illustrated in Fig. 3, allowed us to connect the above-mentioned partial structures. Thus, a cross peak between the anomeric proton of glc and the oxymethylene carbon (δ 70.15) of the *n*-octyloxy group indicated an *n*-octyl glycosidic linkage. A cross peak between the anomeric proton of rha and C-2 of glc (δ 76.53) indicated a rha-(1→2)-glc linkage. The two isobutyryl groups were also connected to the 6-*O* of glc and the 4′-*O* of rha on the basis of HMBC correlations. GC-MS analysis of the TMS ether derivative of **1** exhibited ions at *m*/*z* 851 [M−Me], 737 [M−$C_8H_{17}O$], 488, 361 and 273 (Fig. 4), which supported the presence of the *n*-octyl moiety and the substitution of an isobutyryl group on each sugar moiety. Finally, it was established that the sugar fraction obtained by hydrolysis of **1** was composed of L-rhamnose and D-glucose by the GLC analysis of their TMS ethers of the thiazolidine derivatives according to the method of Hara *et al.*[15] Hence, the structure of compound **1** was determined to be *n*-octyl 4-*O*-isobutyryl-α-L-rhamnopyranosyl-(1→2)-6-*O*-isobutyryl-β-D-glucopyranoside as shown in Fig. 1, and named caroliniaside A.

Compounds **2** and **3** were obtained as an inseparable mixture and showed an $[M+Na]^+$ quasi-molecular ion at *m*/*z* 615.3386 (Calcd for $C_{29}H_{52}O_{12}Na$, 615.3356) in the HR-FAB-MS that corresponded with the molecular formula $C_{29}H_{52}O_{12}$. GLC analysis of the sample as a TMS ether derivative showed two peaks (peaks B and C in Fig. 2) in a 1 : 2.5 ratio. The ^{1}H-NMR spectrum (recorded in $CDCl_3$–CD_3OD 20 : 1) was essentially identical to that of **1** except that signals of only one isobutyryl group were observed instead of the two discerned in **1**, and a doublet methyl and a triplet methyl signals appeared, which are assignable to a branched acyl group. The ^{1}H-NMR data suggested that one of the isobutyryl groups in **1** was replaced by a new, branched acyl group. The ^{13}C-NMR spectrum (Table 1) agreed with this hypothesis and the new acyl group was assigned as a 2-methylbutyryl group on the basis of the newly appeared signals at δ 177.54 (C-1‴), 41.31 (C-2‴), 26.63 (C-3‴), 16.61 (C-4‴) and 11.48 (C-5‴).[16,17] These signals were accompanied by weak (*ca.* 2.5 : 1 ratio) signals at 41.00 (C-2‴), 26.75 (C-3‴), 16.50 (C-4‴) and 11.58 (C-5‴). The ^{13}C signals for the isobutyryl group were also accompanied by weak signals (Table 1). The ^{13}C-NMR data recorded in pyridine-d_5 (Table 2) also supported that the sample is a mixture of two compounds. These NMR data, combined with the GLC behavior of the TMS derivative, suggested that the sample could be a 2.5 : 1 mixture of positional isomers with respect to the isobutyryl and 2-methylbutyryl substitution at the 4′-*O* of rha and 6-*O* of glc. Alternatively, it can be a mixture of due to the co-occurrence of (*R*)- and (*S*)-2-methylbutyryl groups. HMBC correlations observed for the mixture are depicted in

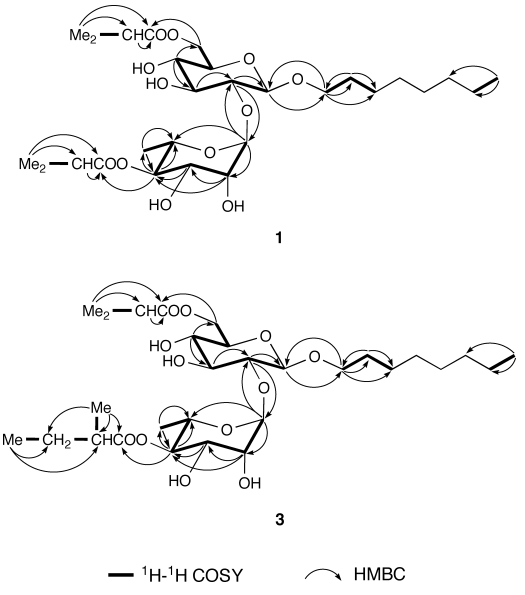

Fig. 3. 2D-NMR Correlations for Compounds **1** and **3**

2D-NMR correlations for **3** were extracted from the spectrum recorded for a 1 : 2.5 mixture of **2** and **3** in pyridine-d_5.

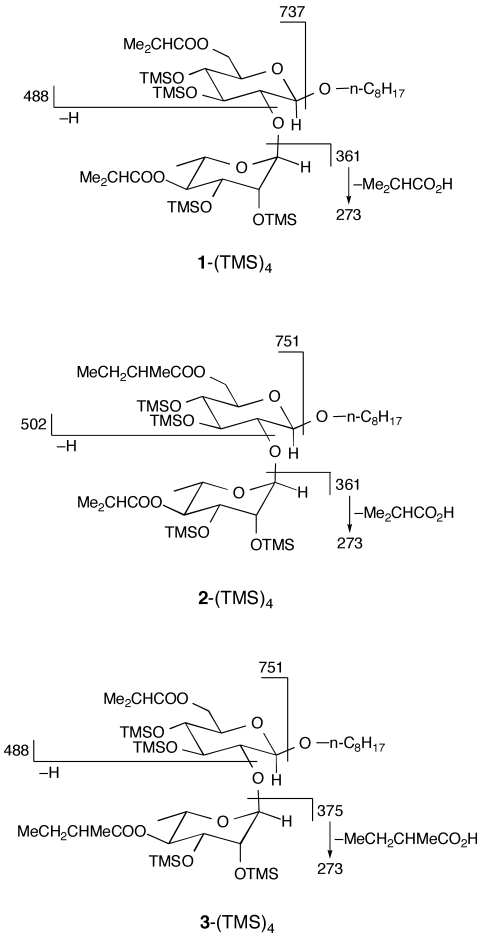

Fig. 4. Mass Fragmentation for the TMS Ether Derivatives of **1**—**3** (Corresponding to Peaks A, B and C, Respectively, in Fig. 2)

Table 2. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) Data of **1**, **2** and **3** in Pyridine-d_6

Position	**1**		**2**[a)]		**3**[a)]	
	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C
Glucopyranosyl						
1	4.77 (d, 7.5)	102.71	4.77 (d, 7.5)	102.69	4.77 (d, 7.5)	102.69
2	4.19 (m)	77.56	4.19 (m)	77.54	4.19 (m)	77.54
3	4.19 (m)	79.22	4.19 (m)	79.18	4.19 (m)	79.18
4	3.94 (m)	71.61	3.94 (m)	71.66	3.94 (m)	71.60
5	3.94 (m)	75.12	3.94 (m)	75.09	3.94 (m)	75.09
6	4.87 (dd, 9.9, 3.6)	64.26	4.87 (dd, 9.9, 3.6)	64.26	4.87 (dd, 9.9, 3.6)	64.26
	4.79 (dd, 9.9, 3.6)		4.79 (dd, 9.9, 3.6)		4.79 (dd, 9.9, 3.6)	
Rhamnopyranosyl						
1′	6.35 (s)	101.89	6.34 (s)	101.88	6.34 (s)	101.88
2′	4.73 (br s)	72.46	4.73 (br s)	72.45	4.73 (br s)	72.45
3′	4.61 (m)	70.32	4.60 (m)	70.30	4.60 (m)	70.30
4′	5.83 (t, 9.9)	75.67	5.83 (t, 9.9)	75.64	5.83 (t, 9.9)	75.52
5′	4.86 (m)	67.02	4.86 (m)	67.00	4.86 (m)	67.00
6′	1.46 (d, 6.0)	18.07	1.46 (d, 6.1)	18.05	1.46 (d, 6.1)	18.09
n-Octyl						
1″	4.11 (dt, 9.3, 7.0)	69.91	4.11 (dt, 9.3, 7.0)	69.90	4.11 (dt, 9.3, 7.0)	69.90
	3.68 (dt, 9.3, 7.0)		3.68 (dt, 9.3, 7.0)		3.68 (dt, 9.3, 7.0)	
2″	1.78 (m)	30.27	1.78 (m)	30.52	1.78 (m)	30.52
3″	1.43 (m)	26.48	1.43 (m)	26.47	1.43 (m)	26.47
4″	1.22—1.35	29.75	1.22—1.35	29.75	1.22—1.35	29.75
5″	1.22—1.35	29.52	1.22—1.35	29.52	1.22—1.35	29.52
6″	1.22—1.35	32.07	1.22—1.35	32.07	1.22—1.35	32.07
7″	1.22—1.35	22.88	1.22—1.35	22.86	1.22—1.35	22.86
8″	0.85 (t, 6.9)	14.24	0.85 (t, 6.9)	14.22	0.85 (t, 6.9)	14.22
Isobutyryl						
1‴	—	176.77	—	176.72	—	176.77
	—	177.73				
2‴	2.56 (m)	34.18	2.69 (m)	34.57	2.56 (m)	34.16
	2.69 (m)	34.58				
3‴	1.084 (d, 7.0)	19.10	1.252 (d, 7.3)	19.09	1.084 (d, 7.3)	19.08
	1.252 (d, 7.0)	19.12				
4‴	1.101 (d, 6.3)	19.03	1.211 (d, 6.3)	19.42	1.101 (d, 6.3)	19.00
	1.211 (d, 6.5)	19.44				
2-Methylbutyryl						
1⁗			—	176.39	—	176.35
2⁗			2.54 (m)	41.22	2.54 (m)	41.67
3⁗			1.83 (m)	27.56	1.83 (m)	27.11
			1.52 (m)		1.52 (m)	
4⁗			0.97 (t, 6.8)	16.68	0.96 (t, 7.4)	17.12
5⁗			1.25 (d, 7.0)	11.72	1.25 (d, 7.0)	11.80

a) Recorded as a 1 : 2.5 mixture of **2** and **3**.

Fig. 3, which indicated that 2-methylbutyryl group was located at the 4′-*O* of rha and isobutyryl group at the 6-*O* of glc in the major constituent **3** (the major and minor constituents were designated as **3** and **2**, respectively).

This was further supported by the GC-MS analysis of the TMS derivative. Fragment ions in the electron impact mass spectra (EI-MS) of peak B (corresponding to **2**-TMS ether) and peak C (corresponding to **3**-TMS ether) are shown in Fig. 4, which exhibited diagnostic fragment ions at *m*/*z* 502 and 361 for peak B and 488 and 375 for peak C, in addition to common ions at *m*/*z* 865 [M−Me] and 751 [M−C_8H_{17}]. These MS data clearly indicated that the isobutyryl and 2-methylbutyryl groups were linked to the 6-*O* of glc and the 4′-*O* of rha, respectively, in compound **3**, and were attached to the 4′-*O* of rha and the 6-*O* of glc, respectively, in compound **2**. The presence of only two sets of ^{13}C-NMR signals in the sample could rule out the possibility of the occurrence of both antipodes of the 2-methylbutyryl group. Thus, compound **2** was determined to be *n*-octyl 4-*O*-isobutyryl-α-L-rhamnopyranosyl-(1→2)-6-*O*-(2-methylbutyryl)-β-D-glucopyranoside, while compound **3** was defined as *n*-octyl 4-*O*-(2-methylbutyryl)-α-L-rhamnopyranosyl-(1→2)-6-*O*-isobutyryl-β-D-glucopyranoside. Compounds **2** and **3** were named caroliniasides B and C, respectively. The chirality of the 2-methylbutyryl group remains unknown and will be investigated in the future.

The present study demonstrated the occurrence of a rare class of secondary metabolites, *n*-alkyl disaccharide derivatives, in the glandular trichome exudate of *G. carolinianum*. This is the first report of the isolation of *n*-alkyl glycoside derivatives from glandular trichome exudates. *n*-Octyl glucosides have been isolated previously from *Rhodiola* species (Crassulaceae),[18,19] *Circaea lutetiana* (Onagraceae)[20] and *Eucalyptus globules* (Myrtaceae).[21] *n*-Hexyl glucosides have been isolated previously from *Rhodiola* species (Crassulaceae).[22,23] *n*-Alkyl di-saccharides, rhodiooctanoside[23,24] and creoside IV,[23] have been isolated from *Rhodiola* species.

Our preliminary study indicated that glandular trichome

exudates of *G. thunbergii*, *G. robertianum* and *G. maderense* also contained *n*-octyl rhamnopyranosyl-(1→2)-glucopyranosides esterified with various shorter-chain acyl groups. These findings suggested that *n*-octyl disaccharide derivatives occur in the *Geranium* genus as components of glandular trichome exudates and this type of compounds can be a taxonomical marker of this genus. To this end, it is interesting to explore glandular trichome exudates of the other major genera of the Geraniaceae family. Sugar esters, in particular esters with shorter-chain acyl groups, glucose esters,[25] sucrose esters[26,27] and inositol esters,[28] have been characterized as glandular trichome exudates from a limited genera of different families. Our present and previous results indicated that secondary metabolites contained in glandular trichome exudates would be much more structurally diverse than ever before expected.

Experimental

General Experimental Procedures ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker DRX-500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer in $CDCl_3$–CD_3OD or pyridine-d_5 solution. Tetramethylsilane (δ 0.00) was used as an internal standard for ^{1}H-NMR shifts, and $CDCl_3$ (δ 77.00) was used as a reference for ^{13}C-NMR shifts. For the spectra taken in pyridine-d_5, the residual nondeuterated solvent signal at δ 7.19 and the solvent signal at δ 123.50 were referenced for ^{1}H and ^{13}C shifts, respectively. Positive mode FAB-MS and HR-FAB-MS spectra, using 3-nitrobenzyl alcohol as the matrix, were obtained on a JEOL JMS-700 spectrometer. 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. Silica gel 60 N (spherical neutral, 40—100 mm, Kanto Chemical, Japan) was used for column chromatography. HPLC was carried out on a Shimadzu LC-6A apparatus equipped with a UV detector (monitored at 215 nm) using a reversed-phase column (Shimadzu Shim-Pack CLC-ODS, 15 cm×4.6 mm i.d.) under isocratic solvent conditions. GLC for the trimethylsilyl derivatives of compounds **1**, **2** and **3** were carried out on a Shimadzu GC-14B apparatus (FID detector) equipped with a DB-5 capillary column (15 m×0.25 mm, 0.25 μm film thickness, J & W Scientific, U.S.A.) under the following conditions: injection temperature of 275 °C, column temperature of 275 °C, detection temperature of 280 °C, He carrier gas flow rate of 50 kPa (P1) and 120 kPa (P2), H_2 flow rate of 50 kPa, air flow rate of 50 kPa and split (40 : 1) injection. GLC of the TMS derivatives of the sugars was carried out under similar conditions except for injection temperature of 270 °C, column temperature of 195 °C, detection temperature of 270 °C. GC-MS of the trimethylsilylated **1**—**3** was conducted using a mass spectrometer (JMS-AM SUN200, JEOL) connected to a gas chromatograph (6890A, Agilent Technologies, U.S.A.) under the following conditions: EI (70 eV), DB-1 capillary column (30 m×0.25 mm, 0.25 μm film thickness, J & W Scientific, U.S.A.), source temperature of 250 °C, injection temperature of 250 °C; column temperature programmed from 80 to 280 °C, which increases at a rate of 20 °C/min and is held at the final temperature for 20 min, interface temperature 280 °C and He carrier gas flow rate of 1.0 ml/min with splitless injection.

Plant Material *Geranium carolinianum* was collected in May 2010 in Kawasaki city of Kanagawa prefecture. The plant was identified by Prof. Shiro Kohshima, Department of Biological Sciences, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology. A voucher specimen (CMS22-04) was deposited in the Department of Chemistry and Materials Science, Tokyo Institute of Technology.

Extraction and Isolation Fresh upper aerial parts (stems and calyxes) of *G. carolinianum* (fresh wt. 399 g) were briefly (*ca.* 10 s) rinsed in a beaker containing Et_2O (1.5 l), and the Et_2O solution was filtered and concentrated to dryness (670 mg) under reduced pressure. The residue was subjected to silica gel column chromatography. Elution of the column with $CHCl_3$–MeOH to give Fr. 1 (439 mg, $CHCl_3$–MeOH 1 : 0—30 : 1, found to be a mixture of hydrocarbons), Fr. 2 (15 mg, $CHCl_3$–MeOH 20 : 1), Fr. 3 (35 mg, $CHCl_3$–MeOH 10 : 1), Fr. 4 (120 mg, $CHCl_3$–MeOH 8 : 1) and Fr. 5 (5 mg, $CHCl_3$–MeOH 6 : 1). Fr. 4 corresponded to the intense spot in a preliminary TLC analysis of the sample, which was obtained by gently wiping the surface of the calyxes with cotton. A part (60 mg) of Fr. 4 was separated by HPLC (solvent, MeOH–H_2O 6 : 1, flow rate 1 ml/min) to give compound **1** (30 mg, eluted at 8.82 min) and compound **2**/**3** (10 mg, eluted at 10.76 min).

Caroliniaside A (**1**): Colorless oil; HR-FAB-MS *m/z*: 601.3203 $[M+Na]^+$ (Calcd for $C_{28}H_{50}O_{12}Na$, 601.3200); $[\alpha]_D^{25}$ −82.0 (c=0.41, $CHCl_3$); IR ($CHCl_3$) cm^{-1}: 3450, 2920, 2880, 2860, 1720, 1480, 1390, 1190, 1160, 1140, 1090; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2.

Caroliniaside B (**2**) and Caroliniaside C (**3**): Colorless oil; HR-FAB-MS *m/z*: 615.3386 $[M+Na]^+$ (Calcd for $C_{29}H_{52}O_{12}Na$, 615.3356); $[\alpha]_D^{25}$ −72.9 (c=0.31, $CHCl_3$); IR ($CHCl_3$) cm^{-1}: 3450, 2980, 2920, 2880, 2860, 1720, 1480, 1390, 1190, 1160, 1140, 1090; ^{1}H- and ^{13}C-NMR data (recoded in $CDCl_3$–CD_3OD 20 : 1 and pyridine-d_5), see Tables 1 and 2.

GLC and GC-MS Analysis A mixture of hexamethyldisilazane and TMSCl in pyridine (TMS-HT) (30 μl) and compounds **1** (0.4 mg) was heated at 75 °C for 1.5 h, and then the mixture was cooled to room temperature. Hexane (50 μl) and H_2O (50 μl) were added to the reaction mixture and a part of the hexane layer was analyzed by GLC and GC-MS. The fraction containing compounds **1**—**3** and a mixture of compounds **2** and **3** were similarly converted to the TMS ether and analyzed. EI-MS of **1** (peak A in Fig. 2) *m/z*: 851 [M−Me] (tr), 737 (tr), 720 (tr), 695 (tr), 607 (1), 578 (1), 488 (1), 361 (3), 273 (17), 204 (43), 143 (17), 73 (100). EI-MS of **2** (peak B in Fig. 2) *m/z*: 865 [M−Me] (tr), 751 (tr), 735 (tr), 709 (tr), 622 (tr), 578 (tr), 502 (tr), 475 (tr), 449 (tr), 361 (3), 273 (17), 231 (3), 204 (40), 182 (6), 143 (14), 103 (3), 73 (100). EI-MS of **3** (peak C in Fig. 2) *m/z*: 865 [M−Me] (tr), 751 (tr), 720 (tr), 695 (tr), 621 (tr), 607 (tr), 592 (tr), 519 (tr), 488 (tr), 375 (3), 361 (3), 273 (26), 231 (6), 204 (66), 182 (11), 143 (25), 103 (9), 73 (100).

Sugar Analysis A solution of LiOH (0.65 mg) in H_2O (100 μl) was added to a solution of **1** (2 mg) in dimethoxyethane (400 μl) and the mixture was stirred at room temperature for 20 h. The mixture was neutralized by the addition of sat. aq. NH_4Cl and partitioned between *n*-BuOH and H_2O. Concentration of the *n*-BuOH layer under reduced pressure gave an oily residue. This was mixed with 2 M HCl (200 μl), and heated at 80 °C for 48 h. The mixture was partitioned between Et_2O and H_2O. The aqueous layer was concentrated and the residual material was identified as glucose and rhamnose by TLC [*Rf* values, 0.24 (glucose) and 0.55 (rhamnose), developed with CH_3CN–H_2O 85 : 15] by comparison with authentic sugars. The sugar fraction was converted to the TMS ether of the thiazolidine derivative, as reported by Hara *et al.*,[15] and analyzed by GLC, which showed two peaks at 11.0 and 18.4 min, identical to those of the TMS ethers derived from L-rhamnose and D-glucose.

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