

9-HYDROXY-, 9-ACETYLOXY-, AND 9-HEXANOYLOXY FATTY ACIDS FROM THE GLANDULAR TRICHOME EXUDATE OF *Silene latifolia* subsp. *alba*

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Chemical investigation of the glandular trichome exudate from Silene latifolia subsp. alba (Caryophyllaceae) resulted in the identification of 9-hydroxyoctadecanoic acid (1) and five new fatty acids 9-hydroxyicosanoic acid (2), 9-acetyloxyoctadecanoic acid (3), 9-acetyloxyicosanoic acid (4), 9-hexanoyloxyhexadecanoic acid (5) and 9-hexanoyloxyoctadecanoic acid (6). The oxygenated positions in these fatty acid derivatives were determined to be at C-9 by GC-MS analysis of their trimethylsilyl (TMS) derivatives, while the configurations at C-9 of compounds 1 and 2 were established as S by application of the Akasaka-Ohrui method to their methyl esters.

Keywords: *Silene latifolia* subsp. *alba*, Caryophyllaceae, glandular trichome, hydroxy-fatty acid, 9-acetyloxyoctadecanoic acid, 9-hexanoyloxyoctadecanoic acid.

The secondary metabolites secreted by glandular trichomes are suggested to have diverse biological activities, such as protecting the aerial parts of plants against herbivores and pathogens [1, 2]. Glandular trichomes produce secondary metabolites of diverse classes such as terpenoids, phenylpropanoids, polyketides, and fatty acid derivatives [3]. We have recently isolated cyclic fatty acyl glycosides from the glandular trichome exudates of *Silene gallica* [4] and *Cerastium glomeratum* [5] of the Caryophyllaceae family. The occurrence of these unique glycosides prompted us to further investigate secondary metabolites contained in the glandular trichome exudates of other plants that belong to this family.

The genus *Silene* comprises about 700 species found in many areas, mostly in the Northern hemisphere. *Silene latifolia* subsp. *alba* is native to Europe. Nowadays, it is naturalized in worldwide temperate areas, and naturalized in Japan after the Second World War. It can be seen in Hokkaido and a part of Honshu [6]. Flowering stems and calyxes of the plant were found to be rich in glandular trichomes. This article describes the identification and structure elucidation of six fatty acid derivatives from the glandular trichome exudate of *S. latifolia* subsp. *alba*.

The crude exudate sample from flowering stems of the plant showed three major spots on TLC, designated Frs. A, B, and C, in order of decreasing polarity. The three fractions were separated by silica gel column chromatography.

Fraction A was suggested to be a mixture of compounds having the molecular formulas $C_{18}H_{36}O_3$ and $C_{20}H_{40}O_3$ on the basis of HR-FAB-MS data. The 1H NMR spectrum of compounds 1 and 2 showed signals assignable to a mono-hydroxylated linear fatty acid: an oxymethine proton at δ 3.58 (m), methylene protons adjacent to the carboxy group at δ 2.35 (t, $J = 7.0$ Hz), a methyl group at δ 0.88 (t, $J = 7.0$ Hz), and methylene protons centered at δ 1.26 (Table 1). The hydroxylated fatty acid structures were supported by the ^{13}C NMR data, which showed signals of a carboxy carbon (δ 178.8) and an oxymethine carbon (δ 72.1) in addition to a methylene carbon α to the carboxyl group (δ 33.8) and two methylene carbons α to the oxymethine carbon (δ 37.4 and 37.5) (Table 1). These NMR data, taken together with FAB-MS data, suggested that Fr. A could be a mixture of a hydroxy-octadecanoic acid and a hydroxy-icosanoic acid.

The ratio of the two hydroxy-fatty acids in Fr. A was further examined by GC-MS analysis. The total ion chromatogram of the bis-TMS derivative of Fr. A showed two peaks at t_R 12.83 and 13.92 min in a 49:51 ratio.

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Fraction C was thought to be a compound having the molecular formula $C_{24}H_{46}O_4$, which was accompanied by a lesser amount of homologous compound with the molecular formula $C_{22}H_{42}O_4$ on the basis of HR-FAB-MS data. The 1H NMR spectrum of Fr. C was similar to that of Fr. B, except for the appearance of signals due to a linear short-chain acyl group (signals of CH_2 α to a carboxyl group at δ 2.28 (t, $J = 7.5$ Hz) and a terminal methyl group at δ 0.90 (t, $J = 7.0$ Hz) with concomitant disappearance of the acetyl signal. These data, combined with information on the molecular formula, suggested that the major and minor constituents of Fr. C are 9-hexanoyloxyoctadecanoic acid (**6**) and 9-hexanoyloxyhexadecanoic acid (**5**), respectively. The presence of a hexanoyl group was corroborated by the ^{13}C signals assignable to the group δ 173.7 (C-1'), 34.7 (C-2'), 24.7 (C-3'), 31.4 (C-4'), 22.3 (C-5'), 13.9 (C-6') [8]. GC-MS analysis of the TMS derivative of Fr. C displayed a minor peak at t_R 13.85 min and a major peak at t_R 15.30 min in a 17:83 ratio. Both peaks displayed a common fragment ion at m/z 245 assignable to an ion C-1–C-9 fragment arising from C-9/C-10 fission followed by the elimination of the C_4H_9 $CH=C=O$ molecule as found in the Fr. B TMS derivative. The diagnostic fragment ion indicated the C-9 location of the hexanoyloxy moiety in both acids. Thus, Fr. C was characterized as a 17:83 mixture of 9-hexanoyloxyhexadecanoic acid (**5**) and 9-hexanoyloxyoctadecanoic acid (**6**). The C-9 configurations of acids **3–6** remain to be investigated, although it can be assumed that they are *S* by analogy with compounds **1** and **2**.

In addition, careful analysis of the GC-MS data allowed us to identify 9-hydroxyhexadecanoic acid (corresponding to peak at t_R 11.95 min), 9-acetyloxyhexadecanoic acid (corresponding to peak at t_R 12.15 min), and 9-hexanoyloxyicosanoic acid (corresponding to peak at t_R 17.27 min) as very minor constituents in the exudate sample.

In the present study we characterized 9-hydroxyoctadecanoic acid (**1**) and five new fatty acids **2–6** that possess the hydroxy, acetyloxy, or hexanoyloxy group at C-9 from the glandular trichome exudate of *S. latifolia* subsp. *alba*. There was no indication of the occurrence of cyclic glycolipids that were found in *S. gallica* [4] and *C. glomeratum* [5]. Thus, it can be said that Caryophyllaceae plants do not necessarily contain cyclic glycolipids as their glandular trichome exudate constituents. The hydroxy fatty acids can be regarded as a biosynthetic precursor of such cyclic glycolipids. *S. latifolia* subsp. *alba* appears to evolve into an acylation enzyme leading to the *O*-acetate and *O*-hexanoate rather than an *O*-glycosylation enzyme that eventually leads to cyclic glycolipids. It is of note that the configuration at the C-9 stereogenic centers of **1** and **2** was *S*, which is opposite to the *R* configuration at C-9, C-10, C-11, C-12, and C-13 found in the fatty acid moieties of the cyclic glycolipids of *S. gallica* and *C. glomeratum*. These findings further indicate the diversity of plants among the Caryophyllaceae family in the biosynthesis of glandular trichome exudate constituents.

EXPERIMENTAL

General Methods. 1H and ^{13}C NMR spectra were recorded on a Bruker DRX 500 (500 MHz for 1H and 125 MHz for ^{13}C) spectrometer in $CDCl_3$ solution. The tetramethylsilane (δ 0.00) signal was used as an internal standard for 1H shifts, and the $CDCl_3$ (δ 77.00) signal was used as a reference for ^{13}C shifts. FAB-MS spectra (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 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 μm , Kanto Chemical, Japan) was used for column chromatography (CC). GC-MS 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 \times 0.25 mm, 0.25 μm film thickness, J&W Scientific); source temperature 250°C; injection temperature 250°C; column temperature programmed from 80°C to 280°C (held 20 min at the final temperature) at a rate of 20°C; interface temperature 280°C; He carrier gas flow rate 1.0 mL/min with splitless injection.

Plant Material. The seeds of *Silene latifolia* subsp. *alba* (Caryophyllaceae) were collected in Asahikawa City, Hokkaido, Japan in July 2010, and the plants were kept cultivated in a planter at the campus of Tokyo Institute of Technology. The plant was identified by one of the author (Y. F). A voucher specimen (CMS-2202) was deposited in the Department of Chemistry and Materials Science, Tokyo Institute of Technology.

Extraction and Isolation. In a preliminary investigation, the heads of the glandular trichomes were directly pressed to a TLC plate, and the plate was developed with hexane–EtOAc (2:1). The analysis revealed the presence of three major spots designated Frs. A–C with R_f values 0.24, 0.38, and 0.53, respectively. A large-scale sampling was carried out in June 2014 by dipping flower stems (30 stems, ca. 15 cm long) into ether (400 mL) for 1 min. The ether was dried over Na_2SO_4 and filtered.

Concentration of the filtrate under reduced pressure yielded a yellow semi-solid (140 mg). This was subjected to silica gel (5 g) column chromatography eluting with increasing polarities of hexane–EtOAc. Elution with hexane–EtOAc (10:1) removed a surface wax (mainly hydrocarbon, 109 mg). Elution with hexane–EtOAc (4:1) afforded Fr. C (12 mg). Further elution with CHCl₃–MeOH (3:1) furnished Fr. B (5 mg), and further elution with hexane–EtOAc (2:1) furnished Fr. A (4 mg).

Fraction A (49:51 mixture of compounds **1** and **2**). Colorless oil. $[\alpha]_D^{25} -4.7^\circ$ (*c* 0.07, CHCl₃). IR (CHCl₃, ν_{\max} , cm⁻¹): 3690, 3600, 3520, 2930, 2855, 1715. For ¹H and ¹³C NMR data, see Table 1. HR-FAB-MS *m/z*: 299.2567 [M – H]⁻ (calcd for C₁₈H₃₅O₃, 299.2586) and 327.2875 [M – H]⁻ (calcd for C₂₀H₃₉O₃, 327.2899). Two peaks were observed at 12.83 and 13.92 min in a 49:51 ratio. EI-MS of the shorter *t_R* *m/z*: 429 ([M – Me]⁺, 13%), 413 ([M – MeO]⁺, 26%), 339 ([M – Me – TMSOH]⁺, 26%), 317 (C-9/C-10 cleavage, 100%), 288 (19%), 229 (C-8/C-9 cleavage, 82%), 129 (43%), 73 (100%). EI-MS of the longer *t_R* *m/z*: 457 ([M – Me]⁺, 5%), 441 ([M – MeO]⁺, 7%), 367 ([M – Me – TMSOH]⁺, 10%), 317 (C-9/C-10 cleavage, 96%), 288 (7%), 257 (C-8/C-9 cleavage, 41%), 129 (18%), 73 (100%).

Fraction B (74:26 mixture of compounds **3** and **4**). Colorless oil. $[\alpha]_D^{25} -2.6^\circ$ (*c* 0.17, CHCl₃). IR (CHCl₃, ν_{\max} , cm⁻¹): 3675, 3514, 2930, 2860, 1720. For ¹H and ¹³C NMR data, see Table 1. HR-FAB-MS *m/z*: 343.2831 [M + H]⁺ (calcd for C₂₀H₃₉O₄, 343.2848) and 371.3183 [M + H]⁺ (calcd for C₂₂H₄₃O₄, 371.3161). Two peaks were observed at 13.05 and 14.16 min in a 74:26 ratio. EI-MS of the shorter *t_R* *m/z*: 371 ([M – Ac]⁺, 2.0%), 354 ([M – AcOH]⁺, 6.7%), 339 ([354 – Me]⁺, 76.2%), 281 ([354 – TMS]⁺, 5%), 264 ([354 – TMSOH]⁺, 8%), 245 (C-9/C-10 cleavage–CH₂CO, 54%), 155 (53%), 117 (C-1/C-2 fission, 78%), 73 (100%). EI-MS of the longer *t_R* *m/z*: 399 ([M – Ac]⁺, 1%), 382 ([M – AcOH]⁺, 8%), 367 ([382 – Me]⁺, 86%), 309 ([382 – TMS]⁺, 6%), 292 ([382 – TMSOH]⁺, 14%), 245 (C-9/C-10 cleavage–CH₂CO, 57%), 155 (61%), 117 (C-1/C-2 fission, 100%), 73 (100%).

Fraction C (17:83 mixture of compounds **5** and **6**). Colorless oil. $[\alpha]_D^{25} +0.9^\circ$ (*c* 0.27, CHCl₃). IR (CHCl₃, ν_{\max} , cm⁻¹): 3690, 3520, 2930, 2860, 1715. For ¹H and ¹³C NMR data, see Table 1. HR-FAB-MS *m/z*: 399.3463 [M + H]⁺ (calcd for C₂₄H₄₇O₄, 399.3474) and 371.315 [M + H]⁺ (calcd for C₂₂H₄₃O₄, 427.3787). Two peaks were observed at 13.85 and 15.30 min in an 17:83 ratio. EI-MS of the shorter *t_R* *m/z*: 343 ([M – C₅H₁₁CO]⁺, 5%), 326 ([M – C₅H₁₁COOH]⁺, 28%), 311 ([326 – Me]⁺, 74%), 253 ([326 – TMS]⁺, 12%), 236 ([326 – TMSOH]⁺, 14%), 245 (C-9/C-10 cleavage–CH₂CO, 91%), 99 ([C₅H₁₁CO]⁺, 100%), 73 (100%). EI-MS of the longer *t_R* *m/z*: 371 ([M – C₅H₁₁CO]⁺, 5%), 354 ([M – C₅H₁₁COOH]⁺, 28%), 339 ([354 – Me]⁺, 74%), 281 ([354 – TMS]⁺, 12%), 264 ([354 – TMSOH]⁺, 14%), 245 (C-9/C-10 cleavage–CH₂CO, 91%), 99 ([C₅H₁₁CO]⁺, 100%), 73 (100%).

Trimethylsilylation and GC/MS Analysis. A portion (ca. 0.1 mg) of Fr. A, B, or C was heated with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (50 μ L) at 80°C for 0.5 h, and then cooled to room temperature. A portion (1–2 μ L) of the solution was subjected to GC/MS analysis.

Determination of the C-9 Configuration of Fr. A. Fraction A (3 mg) was reacted with trimethylsilyldiazomethane (0.6 M hexane solution, 0.2 mL) in EtOAc (0.3 mL)–MeOH (0.2 mL) at room temperature for 30 min. The crude methyl ester was purified by *p*-TLC to give an oily methyl ester (3 mg). The methyl ester (1 mg each) was further converted to the 9-*O*-(1*R*,2*R*)- and (1*S*,2*S*)-2-(2,3-anthracene dicarboximide)-cyclohexanecarboxylic acid derivatives in the same manner as described in our previous paper [4]. Pertinent ¹H NMR data are described in the text.

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