A Phenyl Lipid Alkaloid and Flavone C-Diglucosides from *Spergularia marina*

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Abstract A phenyl lipid alkaloid and seven phenolic compounds were isolated from the aerial part of *Spergularia marina*, a halophyte that grows on salt marshes and tidal flat. These compounds were identified as 2,4-di-*tert*-butylphenol, *N*-hexacosanoylanthranilic acid, tryptophan, 4-hydroxybenzoyl glucopyranoside, luteolin 6-*C*- β -D-glucopyranoside 8-*C*- β -D-(2-*O*-feruloyl)glucopyranoside, luteolin 6-*C*- β -D-(2-*O*-feruloyl)glucopyranoside 8-*C*- β -D-glucopyranoside, apigenin 6-*C*- β -D-glucopyranoside 8-*C*- β -D-(2-*O*-feruloyl)glucopyranoside, and apigenin 6-*C*- β -D-(2-*O*-feruloyl)glucopyranoside 8-*C*- β -D-glucopyranoside. The structures were determined by nuclear magnetic resonance and electrospray ionization-mass spectroscopy.

Keywords: *Spergularia marina*, halophyte, *N*-hexacosanoylanthranilic acid, flavone C-diglycoside, phenolics

Introduction

Epidemiological studies have indicated that vegetables can reduce the risk of diseases, including cardiovascular diseases, cancer, hypertension, and diabetes (1,2). The protective effects of fruits and vegetables have been attributed to dietary fiber, minerals, vitamins, and antioxidative phenolics. Vegetables are a useful source of vitamins and phytochemicals, such as antioxidants. The clinical importance of vegetables has received considerable attention (3). Therefore, vegetable consumption is widely recommended for enhancing health and reducing the incidence of disease.

Halophytes, salt tolerant plants grown in saline environments, have been reported to exert various beneficial effects on health, including antioxidant, anti-inflammation, and anticancer activities (4–6). In addition, halophytes contain high contents of essential minerals, amino acids, phenolic acids, and flavonoids (7,8). These plants also accumulate various secondary metabolites related to various defense responses against salt stress (9). Several halophytes including glasswort (*Salicornia herbacea*), which have been used as vegetable and medicinal herbs in Korea, have various reported biological effects (10). Therefore, it is considered that halophytes may be potentially useful food materials.

Spergularia marina Griseb, which is called ‘sebalnamul’ in Korean, is an annual halophyte (11) and a popular winter seasoned vegetable in Korea. Several studies have reported that *S. marina* has biological

effects, such as reducing insulin resistance, and antioxidant, anti-inflammation, antimicrobial, and anti-adipogenic activities (12–16). *S. marina* contains amino acids, vitamins, and minerals (11). Some chemical constituents, such as β -sitosterol glycosides, tricin, dihydroferulic acid, vanillic acid, 4-hydroxybenzoic acid, uracil, and 8-hydroxycuminoic acid, have been isolated from *S. marina* (16). Therefore, *S. marina* is a potentially useful vegetable. Nevertheless, the investigations on health beneficial effects and chemical constituents of *S. marina* are required to evaluate the value of this plant as a food. In particular, understanding the chemical constituents of *S. marina* is important to gather basic information on its health beneficial effects. However, studies on the chemical constituents of *S. marina* have not been conducted. Therefore, in this study, we investigated the chemical constituents in the aerial parts of *S. marina*, and eight compounds including a phenyl lipid alkaloid and seven phenolic compounds were isolated from *S. marina*. We describe the isolation and identification of a phenyl lipid alkaloid and seven phenolic compounds from *S. marina*.

Materials and Methods

Materials and chemicals The aerial part of *S. marina* Griseb was cultivated in Jeonnam, Korea and harvested in April 2012. This sample was immediately treated after harvesting. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma Chemical Co. (St.

Louis, MO, USA). CDCl_3 and CD_3OD were obtained from Merck Co. (Darmstadt, Germany). All solvents used for analyses were of HPLC grade.

Extraction and solvent fractionation The fresh aerial part (2.8 kg) was homogenized with methanol (MeOH, 18 L). After a 24 h extraction at room temperature, the MeOH extract was filtered under vacuum through No. 2 filter paper (Whatman, Maidstone, UK). The residue was extracted with MeOH (6 L) and then filtered under vacuum through No. 2 filter paper (Whatman). The MeOH-extracted solutions were combined and concentrated in a vacuum at 38°C . The MeOH extracts (79.2 g) were suspended in distilled water (3 L) and successively partitioned with *n*-hexane (3 L, three times), ethyl acetate (EtOAc, 3 L, three times), and water-saturated *n*-butanol (BuOH, 3 L, three times). The fractions were respectively concentrated in a vacuum.

Isolation of 1 and 2 from the EtOAc fraction The EtOAc fraction (4.6 g) was fractionated by a silica gel column (4.0×84 cm; Kieselgel 60, 70-230 mesh, Merck) chromatography and eluted with a step-wise system of *n*-hexane/ CHCl_3 /EtOAc/MeOH=10:0:0:0, 5:5:0:0, 0:10:0:0, 0:5:5:0, 0:0:10:0, 0:0:5:5, and 0:0:0:10 (v/v, each 1.4 L). The eight fractions (E1-E8) were grouped according to their thin-layer chromatography (TLC) profile. Fraction E2 (*n*-hexane/ CHCl_3 =5:5, v/v, 20.1 mg) was subjected to gel permeation chromatography (GPC)-HPLC equipped with a GPC HF-2000 (20×500 mm, Shodex, Tokyo, Japan; flow rate, 3.5 mL/min; wavelength, 220 nm) and with elution of 100% CH_2Cl_2 to yield **1** (t_R 29.6 min, 1.6 mg) and three subfractions (E2a–E2c). E7 (100% EtOAc, v/v, 1,500 mg) was purified by Sephadex LH-20 column (1.5×85 cm; 70-230 mesh, Amersham Biosciences AB, Uppsala, Sweden) chromatography and eluted with CHCl_3 /MeOH (5:5, v/v) to obtain three subfractions (E7a–E7c). Compound **2** (t_R 57.5 min, 40 mg) was isolated from subfraction E7b (120 mg) by ODS-HPLC (ODS-80Ts TSK-gel, 4.6×250 mm, Tosoh, Tokyo, Japan; MeCN/ CH_2Cl_2 /MeOH=75:20:10, v/v; flow rate, 1.0 mL/min; wavelength, 254 nm).

Purification of 3-9 from the BuOH fraction The BuOH fraction (10.87 g) was fractionated by on an Amberlite XAD-2 column (2.5×91.5 cm; 521 μm , Supelco, Bellefonte, PA, USA) chromatography and eluted with a step-wise system of H_2O /MeOH=10:0, 8:2, 6:4, 8:2, 0:10 (v/v, each 1 L). The 21 fractions (B1-B21) were grouped according to TLC profile. Fraction B12 (H_2O /MeOH=6:4, v/v, 328.7 mg) was subjected to semi-preparative ODS-HPLC [Shim-pack Prep-ODS (H) Kit, 5 μm , 20×250 mm, Shimadzu, Kyoto, Japan; flow rate, 9.9 mL/min; wavelength, 254 nm], using a linear gradient system of 10% MeOH (pH 2.65 by TFA) to 100% MeOH for 40 min, to yield **3** (t_R 16.3 min, 62.1 mg). Compounds **4** (t_R 14.2 min, 9 mg), B15b (**5** and **6** mixture, t_R 16.3 min, 29.7 mg), and B15c (**7** and **8** mixture, t_R 17.8 min, 104.8 mg) were purified from fraction B15 (1450 mg) by semi-preparative ODS-HPLC, using a linear gradient system of 10% MeOH

Table 1. ^1H - (600 MHz) and ^{13}C - (150 MHz) NMR data of **2** in CDCl_3

Position	δ_{H} (int., mult., J in Hz)	δ_{C}
1	-	113.2
2	-	142.2
3	8.10 (1H, br. d, 8.4)	120.4
4	7.10 (1H, td, 8.4, 1.8)	135.4
5	7.58 (1H, td, 8.4, 1.8)	122.4
6	8.80 (1H, br. d, 8.4)	131.6
7	-	170.2
1'	-	172.4
2'	2.43 (2H, t, 6.0)	38.7
3'	1.74 (2H, dd, 6.0, 6.0)	25.5
4'-23'	1.25 (40H, m) ¹⁾	29.4
24'	1.25 (2H, m) ¹⁾	31.9
25'	1.25 (2H, m) ¹⁾	22.7
26'	0.87 (3H, d, 8.4)	14.1
-NH-	10.95 (1H, s)	-

¹⁾The H-4'–H-25' signals overlapped.

(pH 2.65 by TFA) 100% MeOH (eluent F) for 40 min.

NMR and MS analysis Nuclear magnetic resonance (NMR) spectra were obtained with a ^1H INOVA 500 and 600 spectrometers (Varian, Walnut Creek, CA, USA). Deuterium chloroform (CDCl_3) and methanol- d_4 (CD_3OD) containing tetramethylsilane (TMS) were used as analytical solvents. All mass spectra were acquired on a hybrid ion-trap time-of-flight mass spectrometer (SYNAPT G2; Waters, Cambridge, UK) equipped with an electrospray ionization source (ESI-MS).

Compound **1**: ^1H -NMR (500 MHz, CDCl_3) δ 7.30 (1H, d, J =2.0 Hz, H-2), 7.07 (1H, dd, J =6.5, 2.0 Hz, H-5), 6.59 (1H, d, J =6.5 Hz, H-6), 1.29 (9H, s, H-8, 9, 10), 1.42 (9H, s, H-12, 13, 14); ^{13}C -NMR (125 MHz, CDCl_3) δ 151.7 (C-1), 142.9 (C-2), 115.9 (C-3), 135.1 (C-4), 123.5 (C-5), 124.5 (C-6), 34.3 (C-7), 34.7 (C-11), 31.6 (C-8, 9,10), 29.6 (C-12, 13, 14); ESI-MS (negative): m/z 205.2 [$\text{M}-\text{H}$][−].

Compound **2**: ^1H - and ^{13}C -NMR data are shown in Table 1. ESI-MS (negative): m/z 514.3, 470.3, 377.3, 136.0, and 92.1. HRESI-MS (negative): m/z 514.4178 [$\text{M}-\text{H}$][−] (calculated for $\text{C}_{33}\text{H}_{56}\text{NO}_3$, m/z 514.4260, +0.18 mDa).

Compound **3**: ^1H -NMR (500 MHz, CD_3OD) δ 7.21 (1H, s, H-2), 7.62 (1H, br. d, J =7.5 Hz, H-4), 7.06 (1H, td, J =7.5, 2.5 Hz, H-5), 7.14 (1H, td, J =7.5, 2.5 Hz, H-6), 7.39 (1H, br. d, J =7.5 Hz, H-7), 3.32 (1H, m, H-10a), 3.51 (1H, dd, J =5.0, 4.5 Hz, H-10b), 4.21 (1H, dd, J =5.0, 4.5 Hz, H-11); ^{13}C -NMR (125 MHz, CD_3OD) δ 125.6 (C-2), 108.2 (C-3), 112.7 (C-4), 120.4 (C-5), 123.1 (C-6), 119.1 (C-7), 138.5 (C-8), 128.4 (C-9), 27.9 (C-10), 54.9 (C-11), 172.1 (C-12); ESI-MS (negative): m/z 203.2 [$\text{M}-\text{H}$][−].

Compound **4**: ^1H -NMR (500 MHz, CD_3OD) δ 7.98 (2H, d, J =7.0 Hz, H-2, 6), 6.94 (2H, d, J =7.0 Hz, H-3, 5), 5.02 (1H, J =9.0 Hz, H-1), 4.11~3.45 (6H, m, H-2–H-6); ESI-MS (negative): m/z 299.3 [$\text{M}-\text{H}$][−].

Compound **5**: ^1H - and ^{13}C -NMR data are shown in Tables 2 and 3. ESI-MS (positive): m/z 787.2 [$\text{M}+\text{H}$]⁺ and 809.2 [$\text{M}+\text{Na}$]⁺.

Compound **6**: ^1H - and ^{13}C -NMR data are shown in Tables 2 and 3. ESI-MS (positive): m/z 787.2 [$\text{M}+\text{H}$]⁺ and 809.2 [$\text{M}+\text{Na}$]⁺.

Table 2. ¹H-NMR spectroscopic data of **5-8** in CD₃OD

Position	δ_H (int., mult., J in Hz)			
	5	6	7	8
3	6.38 (1H, s)	6.64 (1H, s)	6.44 (1H, s)	6.64 (1H, s)
2'	7.43 (1H, br. s) ¹⁾	7.60 (1H, br. s)	8.04 (1H, d, 8.3)	8.04 (1H, d, 8.5)
3'	-	-	6.89 (1H, d, 8.3)	6.97 (1H, d, 8.5)
5'	6.99 (1H, br. d, 8.0)	6.94 (1H, d, 8.0)	6.89 (1H, d, 8.3)	6.97 (1H, d, 8.5)
6'	7.43 (1H, br. d, 8.0) ¹⁾	7.57 (1H, d, 8.0)	8.04 (1H, d, 8.3)	8.04 (1H, d, 8.5)
1''	4.91 (1H, d, 10.0)	5.22 (1H, d, 10.0)	5.41 (1H, d, 10.0)	5.22 (1H, d, 9.0)
2''	4.19 (1H, t, 10.0)	5.70 (1H, t, 10.0)	4.18 (1H, t, 10.0)	5.70 (1H, t, 9.0)
3''	3.49-3.46 (1H, m)	3.82 (1H, m)	3.49-3.46 (1H, m)	3.79 (1H, t, 9.0)
4''	3.49-3.46 (1H, m)	3.78 (1H, m)	3.49-3.46 (1H, m)	3.76 (1H, t, 9.0)
5''	3.49-3.46 (1H, m)	3.51 (1H, m)	3.49-3.46 (1H, m)	3.45 (1H, m)
6''	3.97 (1H, m)	3.86 (1H, m)	3.94 (1H, m)	3.77 (1H, m)
	3.88 (1H, m)	4.00 (1H, m)	3.88 (1H, m)	3.97 (1H, m)
1'''	5.40 (1H, d, 10.0)	4.96 (1H, d, 10.0)	5.42 (1H, d, 10.0)	4.95 (1H, d, 10.0)
2'''	5.35 (1H, t, 10.0)	4.19 (1H, t, 10.0)	5.35 (1H, t, 10.0)	4.18 (1H, t, 10.0)
3'''	3.97 (1H, t, 10.0)	3.45-3.40 (1H, m)	3.92 (1H, t, 10.0)	3.42-3.40 (1H, m)
4'''	3.72 (1H, m)	3.45-3.40 (1H, m)	3.72 (1H, t, 10.0)	3.42-3.40 (1H, m)
5'''	3.51 (1H, m)	3.45-3.40 (1H, m)	3.51 (1H, m)	3.42-3.40 (1H, m)
6'''	3.73 (1H, m)	3.81 (1H, m)	3.76 (1H, m)	3.81 (1H, m)
	3.91 (1H, m)	3.88 (1H, m)	3.97 (1H, m)	3.88 (1H, m)
2''''	6.57 (1H, br. s)	7.10 (1H, d, 2.0)	6.63 (1H, br. s)	7.10 (1H, d, 2.0)
5''''	6.63 (1H, d, 8.0)	6.76 (1H, d, 9.0)	6.62 (1H, d, 8.8)	6.76 (1H, d, 8.5)
6''''	6.50 (1H, br. d, 8.0)	7.01 (1H, dd, 8.5, 2.0)	6.47 (1H, br. d, 8.8)	7.01 (1H, dd, 8.5, 2.0)
7''''	7.19 (1H, d, 16.0)	7.47 (1H, d, 16.0)	7.16 (1H, d, 16.0)	7.46 (1H, d, 16.0)
8''''	5.87 (1H, d, 16.0)	6.16 (1H, d, 16.0)	5.85 (1H, d, 16.0)	6.16 (1H, d, 16.0)
-OCH ₃	3.65 (3H, s)	3.87 (3H, s)	3.66 (3H, s)	3.87 (3H, s)

¹⁾The H-2 and H-6 signals overlapped.

Compound **7**: ¹H- and ¹³C-NMR data are shown in Tables 2 and 3. ESI-MS (positive): *m/z* 771.2 [M+H]⁺ and 793.2 [M+Na]⁺.

Compound **8**: ¹H- and ¹³C-NMR data are shown in Tables 2 and 3. ESI-MS (positive): *m/z* 771.2 [M+H]⁺ and 793.2 [M+Na]⁺.

DPPH radical-scavenging assay DPPH reagent was sprayed on a TLC plate (17). Each fraction purified using various column chromatographic methods and HPLC was spotted on silica gel TLC plates and developed using a mixture of CHCl₃/MeOH=9.5:0.5 (v/v) for the EtOAc fraction and a mixture of *n*-butanol/acetic acid/H₂O=4:1:2 (v/v) for the BuOH fraction. After spraying 100 mM DPPH EtOH solution on the plate, the fractions visualized as decolorizations of the spot were regarded as the reflection of antioxidative activity. The plate was visualized by UV and 1% cerium (IV) sulfate ethanol solution spray.

Results and Discussion

The MeOH extracts of *S. marina* aerial parts were solvent-fractionated to obtain *n*-hexane, EtOAc, and BuOH fractions. These fractions were developed on a TLC plate and sprayed with DPPH solution. The antioxidative spots were contained mainly in the EtOAc and BuOH fractions as detected by the TLC. The free radical-scavenging activity of *S. marina* aerial parts has been reported

previously (14). However, no investigation of the chemical constituents from the EtOAc and BuOH fractions of *S. marina* aerial parts has been carried out. Therefore, the antioxidative compounds in the EtOAc and BuOH fractions were purified. The EtOAc fraction was purified by silica gel column chromatography, GPC-HPLC, and ODS-HPLC to isolate **1** and **2** (Fig. 1). Additionally, the BuOH fraction was purified by an Amberlite XAD-2 column chromatography and ODS-HPLC to obtain **4**, a mixture of **5** and **6**, and a mixture of **7** and **8** (Fig. 1).

The molecular weight of **1** was 206, as established by a pseudomolecular ion peak *m/z* 205.2 [M-H]⁻ in the HRESI-MS (negative). The ¹H-NMR spectrum (CDCl₃) exhibited three *sp*² carbon proton signals of a tri-substituted benzene ring at δ 7.30 (1H, d, *J*=2.0 Hz, H-3), 7.07 (1H, dd, *J*=6.5, 2.0 Hz, H-5), and 6.59 (1H, d, *J*=6.5 Hz, H-6) and six methyl proton signals from two *tert*-butyl groups at δ 1.29 (9H, s, H-8, 9, 10) and 1.42 (9H, s, H-12, 13, 14). This result was supported by the ¹³C-NMR spectrum, which contained 14 carbon signals including one oxygenated quaternary *sp*² carbon signal at δ 151.7 (C-1), two quaternary *sp*² carbon signals at δ 142.9 (C-2) and 135.1 (C-4), and two quaternary *sp*³ carbon signals at δ 34.3 (C-7) and 34.7 (C-11). Compound **1** was suggested to be di-*tert*-butylphenol from the MS and 1D-NMR spectra. This structure was confirmed by heteronuclear single quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC) experiments. The ¹H- and ¹³C-

Table 3. ^{13}C -NMR spectroscopic data of **5-8** in CD_3OD

Position	δ_{C}			
	5	6	7	8
2	165.8	167.1	165.7	167.0
3	103.7	104.1	103.8	104.0
4	184.1	184.4	184.1	184.4
5	162.3	160.8	162.3	160.8
6	111.1	108.0	110.1	108.0
7	164.0	162.3	164.0	162.3
8	103.0	105.0	103.0	105.0
9	155.5	157.9	155.5	157.9
10	105.0	105.8	105.0	105.8
1'	123.5	124.2	123.1	123.7
2'	114.3	115.1	117.4	117.2
3'	147.4	147.1	129.8	130.3
4'	151.6	151.2	163.3	163.0
5'	117.2	116.9	129.8	130.3
6'	120.7	121.2	117.4	117.2
1''	75.3	73.2	75.3	73.1
2''	72.5	74.2	72.4	74.2
3''	80.1	78.3	80.2	78.3
4''	71.9	72.4	71.9	72.5
5''	82.9	83.4	82.9	83.3
6''	62.0	63.2	62.0	63.0
1'''	75.2	76.4	75.2	76.4
2'''	75.0	74.6	75.1	74.6
3'''	77.1	79.4	77.2	79.4
4'''	71.1	70.6	71.1	70.6
5'''	83.1	82.4	83.1	82.4
6'''	63.0	61.6	63.0	61.6
1''''	127.1	127.8	127.1	127.8
2''''	110.0	111.8	111.1	111.8
3''''	149.3	149.4	149.3	149.4
4''''	150.8	150.7	150.8	150.7
5''''	116.4	116.6	116.4	116.6
6''''	124.1	124.4	124.1	124.4
7''''	147.2	147.2	147.0	147.4
8''''	114.4	115.0	114.4	115.1
9''''	168.8	168.7	167.8	168.6
-OCH ₃	56.4	56.5	56.4	56.5

NMR spectra of **3** were consistent with those of di-*tert*-butylphenol, which was isolated from *Pseudomonas monteilii* previously (18). Therefore, **1** was identified as 2,4-di-*tert*-butylphenol (Fig. 2).

The molecular weight of **2** was 515 by detecting pseudomolecular ion peaks at m/z 514.3 $[\text{M-H}]^-$ and fragment ion peaks at m/z 470.3 $[\text{M-COOH}]^-$, 377.3 $[\text{M-C}_8\text{H}_9\text{O}_2]^-$, 136.0 $[\text{M-C}_{27}\text{H}_{50}\text{O}]^-$, and 92.1 $[\text{M-C}_{27}\text{H}_{50}\text{O}_3]^-$ in the ESI-MS (negative) spectrum. The molecular formula of **2** was $\text{C}_{33}\text{H}_{57}\text{NO}_3$, as established by the pseudomolecular ion peak at m/z 514.4178 $[\text{M-H}]^-$ (calculated for $\text{C}_{33}\text{H}_{56}\text{NO}_3$, m/z 514.4260, +0.18 mDa) in HRESI-MS (negative) data. The ^1H -NMR spectrum exhibited four proton signals of an *ortho*-substituted phenyl group at δ 8.10 (1H, br. d, $J=8.4$ Hz, H-3), 7.10 (1H, td, $J=8.4$, 1.8 Hz, H-4), 7.58 (1H, td, $J=8.4$, 1.8 Hz, H-5), and 8.80 (1H, br. d, $J=8.4$ Hz, H-6) and one proton signal of an amide group at δ 10.95 (1H, s) (Table 1). In

addition, a sp^3 methyl proton signal at δ 0.87 (3H, d, $J=6.0$ Hz, H-26') and sp^3 methylene proton signals at δ 1.25–2.43 (48H, m, H-2'-H-25') were observed in the ^1H -NMR spectrum. This result was supported by the ^{13}C -NMR spectrum, which contained 33 carbon signals, including two carbonyl carbons at δ 172.4 (C-1') and 170.2 (C-7), six sp^2 carbons at δ 142.2–113.2, and 26 sp^3 carbons at δ 38.7–14.1. Considering the molecular formula of **2**, the chemical shifts in four sp^2 quaternary carbons indicated that two of them were nitrogen-bearing carbons at δ 172.4 (C-1') and 142.2 (C-2) with evidence of a carboxyl carbon at δ 170.2 (C-7). The partial structure of **2** was suggested to contain aminobenzoic acid and hexacosanoic acid moieties based on the HRESI-MS and 1D-NMR spectra. The complete NMR assignment and connectivity of **2** were further determined by ^1H - ^1H COSY, HSQC, and HMBC experiments. 2-Aminobenzoic acid (anthranilic acid) and hexacosanoic acid moieties were confirmed from the spectra. In particular, HMBC correlations (Fig. 2, arrows) of δ 10.95 to δ 113.2 (C-1), 120.4 (C-3) and 38.7 (C-2') indicated that **2** had an amide bond between C-2 of anthranilic acid and C-1' of hexacosanoic acid. Consequently, the structure of **2** was identified as *N*-hexacosanoylanthranilic acid by comparisons with the ^1H - and ^{13}C -NMR spectral data as reported in relevant literature (19) (Fig. 2).

The molecular weight of **3** was 204, as established by a pseudo-molecular ion peak m/z 203.2 $[\text{M-H}]^-$ in the HRESI-MS (negative). The ^1H -NMR spectrum exhibited the presence of 2,3-benzopyrrole (indole) moiety, including four sp^2 carbon proton signals of an *ortho*-substituted benzene ring at δ 7.62 (1H, br. d, $J=7.5$ Hz, H-4), 7.06 (1H, td, $J=7.5$, 2.5 Hz, H-5), 7.14 (1H, td, $J=7.5$, 2.5 Hz, H-6), and 7.39 (1H, br. d, $J=7.5$ Hz, H-7) and one sp^2 carbon proton signal at δ 7.21 (1H, s, H-2). Additionally, one methylene proton signal at δ 3.51 (1H, dd, $J=5.0$, 4.5 Hz, H-10b) and 3.32 (1H, m, H-10a) and a methine proton signal at δ 4.21 (1H, dd, $J=5.0$, 4.5 Hz, H-11) were observed in the ^1H -NMR spectrum. This result was supported by the ^{13}C -NMR spectrum, which contained 11 carbon signals including one carbonyl carbon at δ 172.1 (C-12), eight sp^2 carbons at δ 138.5–108.2, one methylene carbon at δ 54.9 (C-11), and one methine carbon at δ 27.9 (C-10). Considering the MS and 1D-NMR spectra, **3** was suggested to be tryptophan, which is composed of indole and propanoic acid moieties. Therefore, **3** was identified as tryptophan by comparison of ^1H - and ^{13}C -NMR spectra of this compound isolated previously from *Salvia limbata* (20).

The molecular weight of **4** was 300, as established by a pseudo-molecular ion peak m/z 299.3 $[\text{M-H}]^-$ in the HRESI-MS (negative). The ^1H -NMR spectrum revealed two sp^2 carbon proton signals of *para*-substituted benzene ring at δ 7.98 (2H, d, $J=7.0$ Hz, H-2, 6) and 6.94 (2H, d, $J=7.0$ Hz, H-3, 5). In addition, a β -glucopyranose moiety was indicated by the coupling patterns of the anomeric proton signal at δ 5.02 (1H, $J=9.0$ Hz, H-1') and other proton signals at δ 4.11–3.45 (6H, m, H-2'–H-6') in the ^1H NMR spectrum. Compound **3** was suggested to be benzoic acid connected with a β -glucopyranose moiety based on the MS and ^1H -NMR spectra. The ^1H -NMR spectrum of **3** was consistent with that of 4-hydroxybenzoyl glucopyranoside,

Fig. 1. Procedure used for purification and isolation of 1-8 from the aerial parts of *Spergularia marina*. CC: column chromatography.

which was isolated previously from *Mallotus* cell culture (21). Therefore, **3** was identified as 4-hydroxybenzoyl glucopyranoside.

The molecular weight of fraction B15b was 786, based on the pseudomolecular ion peaks detected at m/z 787.2 $[M+H]^+$ and 809.2 $[M+Na]^+$ in the ESI-MS (positive) spectrum. The 1H - and ^{13}C -NMR spectra of fraction B15b suggested that this compound was composed of a flavonoid diglycoside and ferulic acid. However, the spectra showed evidence of a mixture of two compounds, suggesting that the skeletal structure was similar, but the position of the ferulic acid moiety differed. The 1H -NMR spectrum of fraction B15b showed unequal ferulic acid proton signal intensity at a ratio of 3:2 (main compound, **5**; minor compound, **6**). The 1H -NMR spectrum of **5** exhibited the presence of luteolin due to three proton signals of tri-substituted benzene in the flavonoid B ring at δ 7.43 (1H, br. s, H-2'), 6.99 (1H, d, $J=8.0$ Hz, H-5'), and 7.43 (1H, br. d, $J=8.0$ Hz, H-6') and one singlet proton signal of the flavonoid C ring at δ 6.38 (1H, s, H-3) (Table 2). Two β -D-glucopyranose moieties were assigned by the coupling constant value ($J=10.0$ Hz) of the anomeric proton signal at δ 4.91 (1H, d, $J=10.0$ Hz, H-1'') and 5.40 (1H, d, $J=10.0$ Hz, H-1''') and another proton signal at δ 5.35-3.46 (12H, m) as well as their proton-proton correlations in the 1H - 1H COSY spectrum. In addition, three proton signals of a tri-substituted benzene ring at δ 6.57 (1H, br. s, H-2'''), 6.63 (1H, d, $J=8.0$ Hz, H-5'''), and 6.50 (1H, d, $J=8.0$ Hz, H-6'''), two proton signals of a *trans* olefinic double bond at δ 7.19 (1H, d, $J=16.0$ Hz, H-7''') and 5.87 (1H, d, $J=16.0$ Hz, H-8'''), and one methoxyl proton signal at δ 3.65 (3H, s) in the 1H -NMR spectrum indicated the

presence of ferulic acid as a partial structure. These results were also supported by the ^{13}C -NMR spectrum, which contained 37 carbon signals, including 24 sp^2 carbons of luteolin and ferulic acid moieties at δ 184.1-103.0 and 56.4 and 12 sugar carbons at δ 83.1-62.0 (Table 3). In particular, two anomeric carbons at δ 75.3 (C-1'') and 75.2 (C-1''') detected in the HSQC spectrum were shifted upfield compared to the *oxy* form of glucopyranoside, indicating that **5** is luteolin di-C-glucopyranoside. The skeletal structure of **5** was particularly similar to the ^{13}C -NMR data of luteolin 6,8-di-C-glucopyranoside (lucenin-2) (22). The connectivities of **5** were further determined by 1H - 1H -COSY, HSQC, and HMBC experiments. Luteolin, ferulic acid, and diglucopyranose moieties were confirmed from the spectra. In particular, HMBC correlations (Fig. 2, arrows) of δ 4.91 (H-1) to δ 162.3 (C-5) and 164.0 (C-7) and δ 5.40 (H-1) to δ 155.5 (C-9) and 164.0 (C-7) indicated that two glucose moieties were coupled with C-6 and C-8 of luteolin. A cross peak (Fig. 2, arrows) of δ 5.35 (H-2) to δ 168.8 (C-9) was observed in the HMBC spectrum, indicating that ferulic acid was esterified with the C-2 of glucose coupled with the C-8 of luteolin. Consequently, the structure of **5** was unambiguously determined to be luteolin 6-C- β -D-glucopyranoside 8-C- β -D-(2-O-feruloyl)glucopyranoside (Fig. 2).

The 1H - and ^{13}C -NMR spectrum of **6** were closely related to those of **5** (Table 2 and 3). In particular, the proton signals of the ferulic acid moiety including a tri-substituted benzene ring at δ 7.10 (1H, d, $J=2.0$ Hz, H-2'''), 6.76 (1H, dd, $J=8.8$ Hz, H-5'''), and 7.01 (1H, d, $J=8.8$ Hz, H-6'''), olefinic double bonds at δ 7.47 (1H, d, $J=16.0$ Hz, H-7''') and

Fig. 2. Structures of the compounds isolated from the aerial parts of *Spergularia marina* and the important HMBC correlations (arrows) for **2** and **5-8**.

6.16 (1H, d, $J=16.0$ Hz, H-8'''), and a methoxyl group at δ 3.87 (3H, s) were shifted upfield 0.22-0.53 ppm, as compared to those of **5** (Table 2). Therefore, compound **6** was suggested to be luteolin C-diglucopyranoside connected with a ferulic acid moiety, as an isomer of **5**. Luteolin 6,8-C-diglucopyranoside were assigned by ^1H - ^1H COSY, HSQC, and HMBC experiments. A cross peak (Fig. 2, arrow) of δ 5.70 (H-2'') to δ 168.7 (C-9''') was observed in the HMBC spectrum, indicating that ferulic acid was esterified with the C-2 of glucose. HMBC correlations (Fig. 2, arrows) of δ 5.22 (H-1) to δ 160.8 (C-5) and 162.3 (C-7) indicated that ferulic acid was esterified with C-2 of glucose connected with the C-6 of luteolin. Consequently, the structure of **6** was unambiguously determined to be luteolin 6-C- β -D-(2-O-feruloyl)glucopyranoside 8-C- β -D-glucopyranoside (Fig. 2).

The molecular weight of fraction B15c was 770, based on the pseudomolecular ion peaks detected at m/z 771.2 [$\text{M}+\text{H}$] $^+$ and 793.2 [$\text{M}+\text{Na}$] $^+$ in the ESI-MS (positive) spectrum. The ^1H - and ^{13}C -NMR spectra of fraction B15c were very similar to those of **5** and **6**, except for the chemical shifts and coupling patterns of the flavonoid (Tables 2 and 3). In particular, the flavonoid structure was suggested to be apigenin by the ^1H -NMR spectrum, which contained two proton signals of the AA'XX' system in the B ring at δ 6.89 (2H, d, $J=8.3$ Hz, H-3', 5') and 8.04 (2H, d, $J=8.3$ Hz, H-2', 6') and one singlet proton signal in the A ring at δ 6.44 (1H, s, H-3) (Table 2). However, the ^1H - and ^{13}C -NMR spectra showed evidence of a mixture of two compounds, suggesting that the skeletal structure was apigenin C-diglucopyranoside, but the position of the ferulic acid moiety differed. The ^1H -

NMR spectrum of fraction B15c showed unequal proton signal intensity of ferulic acid at a ratio of 55:45 (main compound, **7**; minor compound, **8**). The ^{13}C -NMR data of **7** and **8** were very similar to those of apigenin 6,8-di-C-glucopyranoside (vicenin-2) (23). Two β -D-glucopyranose and *trans*-ferulic acid moieties were assigned by the coupling constant values in the ^1H -NMR spectrum and the proton-proton correlations in the ^1H - ^1H COSY spectrum. Apigenin, ferulic acid, and diglucopyranose moieties were also confirmed by ^1H - ^1H COSY, HSQC, and HMBC experiments. HMBC correlation (Fig. 2, arrow) of δ 5.35 (H-2''') to δ 167.8 (C-9''') was observed for **7**, indicating that ferulic acid was esterified with the C-2 of glucose connected with the C-8 of apigenin. Therefore, the structure of **7** was determined to be apigenin 6-C- β -D-glucopyranoside 8-C- β -D-(2-O-feruloyl)glucopyranoside (Fig. 2). Additionally, HMBC correlations (Fig. 2, arrow) of δ 5.70 (H-2'') and δ 168.6 (C-9''') were observed for **8**, indicating that ferulic acid was esterified with the C-2 of glucose connected with the C-6 of apigenin. Consequently, the structure of **8** was identified to be apigenin 6-C- β -D-(2-O-feruloyl)glucopyranoside 8-C- β -D-glucopyranoside by comparison with its ^1H - and ^{13}C -NMR spectra isolated from acacia, as reported previously (Fig. 2) (24).

In this study, one *N*-phenyl lipid alkaloid and seven known compounds were isolated from the aerial parts of *S. marina*. Flavone 6,8-C-diglucopyranoside derivatives (**5-8**) have been identified from *Spergularia rubra* by MS analyses (25). However, the NMR studies for **5** and **6** were unavailable. Therefore, their NMR assignments with similar compounds (**7** and **8**) are shown in Tables 2 and 3. All

compounds isolated in this study were identified for the first time from this plant. Compounds **1** and **3** reportedly show DPPH radical-scavenging and inhibitory activities on peroxy radical-mediated oxidation of phosphatidylcholine liposome, respectively (26). Compounds **2** and **4** showed very weak DPPH radical-scavenging activities in the DPPH-TLC assay used in this study (data not shown). Luteolin, apigenin, and ferulic acid, which are contained as partial structure in **5-9**, are as excellent antioxidative compounds (27,28). In particular, apigenin 6,8-C-diglucopyranoside (vicenin 2) exerts various biological effects, including anti-oxidant, anti-hepatotoxic, and anti-inflammatory activities (24,29-31). Compounds **5-9**, possessing flavone 6,8-C-diglucoside and ferulic acid, seem to exert high antioxidative activities, although their activities could not be evaluated as a mixture was isolated. Therefore, these compounds may partially contribute to the antioxidative activity of *S. marina*. These results warrant further studies to understand the chemical constituents and biological effects of *S. marina*.

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