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A New Phenylpropane Glycoside from the Rhizome of *Sparganium stoloniferum*

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The purification of the MeOH extract from the rhizome of *Sparganium stoloniferum* Buch.-Hamil. (Sparganiaceae) using column chromatography furnished one new phenylpropanoid glycoside (**7**) and known phenolic compounds (**1-6**, and **8-13**). The structural elucidation of **7** was based on 1D- and 2D-NMR spectroscopic data analysis to be β-D-(6-*O*-*trans*-feruloyl) fructofuranosyl-α-D-*O*-glucopyranoside. Compounds **1-6**, and **8-13** were elucidated by spectroscopy and confirmed by comparison with reported data; 24-methylenecycloartanol (**1**), *p*-hydroxybenzaldehyde (**2**), ferulic acid (**3**), *p*-coumaric acid (**4**), vanillic acid (**5**), β-D-(1-*O*-acetyl-3-*O*-*trans*-feruloyl)fructofuranosy-α-D-2',4',6'.-*O*-triacetyglucopyranoisde (**6**), β-D-(1-*O*-acetyl-3,6-*O*-*trans*-diferuloyl)fructofuranosyl-α-D-2',4',6'.-*O*-triacetylglucopyranoisde (**8**), hydroxytyrosol acetate (**9**), hydroxytyrosol (**10**), isorhamnetin-3-*O*-rutinoside (**11**), *n*-butyl-α-D-fructofuranoside (**12**), and *n*-butyl-β-D-fructopyranoside (**13**). Compounds **3** and **9-13** were isolated for the first time from this plant. The isolated compounds were tested for cytotoxicity against four human tumor cell lines *in vitro* using a Sulforhodamin B bioassay.

Key words: *Sparganium stoloniferum*, Sparganiaceae, Phenylpropane glycoside

INTRODUCTION

Sparganium stoloniferum Buch.-Hamil. (Sparganiaceae) is widely distributed in the wet valley areas of Korea, Japan, and China. *S. stoloniferum* has been used as an emmenagogue, a galactogogue, and an antispasmodic agent in Chinese folk medicine (Jiangsu, 1977; Hsu, 1986), and also for the treatment of menstrual disorders and chronic hepatitis (Namba, 1980). Previous phytochemical investigation on this plant reported the isolation of pyrrole carboxylic acid ester (Miyaichi et al., 1995), phenylpropanoid glycosides (Osamu et al., 1996, 1997), and two sucrose esters (Xiong et al., 2008). Aldose reductase inhibition (Jung et al., 2004), anti-inflammatory, and antithrombosis (Lee et al., 1995) activities have also been reported. In our continuing study on the constituents of Korean medicinal plant sources, we have investigated the constituents from the rhizome of *S. stoloniferum*. Column chromatography separation of the MeOH extract of *S. stoloniferum* resulted in the isolation of 13 compounds, including one new phenylpropane glycoside. Their structures were determined by spectroscopy. Compound **7** was newly isolated from natural sources, and compounds **3** and **9-13** were isolated for the first time from this plant. The isolated compounds were tested for cytotoxicity against four human tumor cells *in vitro* using a Sulforhodamin B bioassay.

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MATERIALS AND METHODS

General experimental procedure

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1020 Polarimeter. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. UV spectra were recorded with a Schimadzu UV-1601 UV-Visible spectrophotometer. NMR spectra were recorded on a Varian UNITY

INOVA 500 NMR spectrometer. FABMS data were obtained on a JEOL JMS700 mass spectrometer. Preparative HPLC was performed using a Gilson 306 pump with a Shodex refractive index detector and an Apollo Silica 5 μ column (250 × 22 mm) or an Econosil® RP-18 10 μ column (250 × 22 mm). Silica gel 60 (Merck, 70~230 mesh and 230~400 mesh) was used for column chromatography. TLC was performed using Merck precoated Silica gel F_{254} plates and RP-18 F_{254s} plates. The packing material for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co.). Low-pressure liquid chromatography was performed over Merck LiChroprep Lobar®-A Si 60 (240 × 10 mm) or LiChroprep Lobar®-A RP-18 (240 × 10 mm) columns with a FMI QSY-0 pump (ISCO).

Plant materials

Sparganium stoloniferum Buch.-Hamil. was bought at Yeongchenon, Korea, in September, 2008. A voucher specimen (SKKU-2008-09) was deposited at the College of Pharmacy in Sungkyunkwan University.

Extraction and isolation

The dried and chopped *S. stoloniferum* Buch.-Hamil. (5 kg) was extracted at room temperature with 80% MeOH and evaporated under reduced pressure to give a residue (280 g), which was dissolved in water (800 mL) and solvent-partitioned to give *n*-hexane (17 g), CH_2Cl_2 (3 g), EtOAc (4 g), and *n*-BuOH (30 g). The *n*-hexane fraction (17 g) was separated over a silica gel column with *n*-hexane:EtOAc (45:1) as the eluent to yield seven fractions (H1-H7). Fraction H4 (1.8 g) was separated over a silica gel column with a solvent system (*n*-hexane:EtOAc = 9:1 - 1:1) as the eluent to give seven fractions (H41-H47). Subfraction H42 was separated on a RP-C_{18} silica gel column with 100% MeOH and was purified with a silica Lobar A®-column (*n*-hexane:EtOAc = 12:1) to yield compound 1 (35 mg). The CH_2Cl_2 fraction (3 g) was separated over a silica gel column with a solvent system of ($CHCl_3$:MeOH = 25:1 – 100% MeOH) as the eluent to give nine fractions (C1-C9). Fraction C3 was purified with a silica gel prep HPLC (*n*-hexane:EtOAc = 2.5:1) to yield compound 2 (11 mg, R_t = 16 min). Fraction C4 was purified with a RP-C_{18} prep HPLC (60% MeOH) to yield compound **3** (4 mg, R_t = 15 min). Fraction C7 was separated over a RP-C_{18} silica gel column with a solvent system of 60% MeOH as the eluent to give five subfractions (C71-C75). Subfraction C74 (12 mg) was purified with a RP-C_{18} prep HPLC (55% MeOH) to yield compound **4** (5 mg, R_t = 18 min) and a silica gel prep HPLC ($CHCl_3$:MeOH:H_2O = 27:1.8:0.1) to yield compounds **8** (5 mg, R_t = 12 min). Subfraction C72 was subjected to Sephadex LH-20 column chromatography (CH_2Cl_2:MeOH = 1:1) as the eluent to give two fractions (C721-C722) purified with a silica gel prep HPLC ($CHCl_3$:MeOH:H_2O = 27:1.8:0.1) to yield compounds **5** (8 mg, R_t = 11 min) and 6 (10 mg, R_t = 13 min). Subfraction C73 was subjected to Sephadex LH-20 column chromatography (CH_2Cl_2:MeOH = 1:1) purified with a RP-C_{18} prep HPLC (50% MeOH) to yield compound **7** (5 mg, R_t = 14 min). The EtOAc fraction (4 g) was separated over a silica gel column with a solvent system of ($CHCl_3$:MeOH:H_2O = 25:3:0.1 – 100% MeOH) as the eluent to give nine fractions (E1-E9). Fraction E1 was separated over a RP-C_{18} silica gel column with a solvent system of 60% MeOH as the eluent to give two subfractions (E11-E12). Subfraction E12 (12 mg) was purified with a RP-C_{18} prep HPLC (50% MeOH) to yield compound **9** (6 mg, R_t = 16 min). Fraction E2 was separated over a RP-C_{18} Lobar A®-column with a solvent system of 60% MeOH as the eluent to give three subfractions (E21-E23). Subfraction E21 (23 mg) was purified with a RP-C_{18} prep HPLC (50% MeOH) to yield compound **10** (6 mg, R_t = 18 min). Fraction E8 was separated over a RP-C_{18} silica gel column with a solvent system of 30% MeOH as the eluent to give three subfractions (E81-E83). Subfraction E83 (27 mg) was purified with a RP-C_{18} prep. HPLC (50% MeOH) to yield compound **11** (6 mg, R_t = 15 min). The *n*-BuOH fraction (30 g) was separated over a silica gel column with a solvent system of ($CHCl_3$:MeOH:H_2O = 14:3.7:0.1 – 100% MeOH) as the eluent to give nine fractions (B1-B10). Fraction B2 was separated over a RP-C_{18} Lobar A®-column with a solvent system of 40% MeOH as the eluent to give three subfractions (B21-B23). Subfraction B21 and B22 were purified with a silica gel prep HPLC ($CHCl_3$:MeOH = 18:1) to yield compounds **12** (13 mg, R_t = 10 min) and 13 (7 mg, R_t = 12 min).

24-Methylenecycloartanol (1)

White amorphous powder, mp 120-121°C; $[\alpha]_D^{25}$ +41° (*c* 0.1 in $CHCl_3$); FAB-MS *m/z*: 439 $[M-H]^-$; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.73 (1H, br s, C=CH_2), 4.68 (1H, d, *J* = 1.5 Hz, C=CH_2), 3.29 (1H, dd, *J* = 4.6, 11.5 Hz H-3), 1.03 (3H, d, *J* = 6.5 Hz, H-27), 1.02 (3H, d, *J* = 6.5 Hz, H-26), 0.97 (3H, s, H-29), 0.97 (3H, s, H-18), 0.90 (3H, d, *J* = 6.2 Hz, H-21), 0.90 (3H, s, H-28), 0.81 (3H, s, H-30), 0.56 (1H, d, *J* = 4.0 Hz, H-19b), 0.33 (1H, d, *J* = 4.0 Hz, H-19a); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 157.1 (C-24), 106.1 (C-31), 79.1 (C-3), 52.5 (C-17), 49.0 (C-14), 48.2 (C-8), 47.3 (C-5), 45.5 (C-13), 40.7 (C-4), 36.3 (C-20), 35.8 (C-15), 35.2 (C-22), 34.0 (C-25), 33.1 (C-12), 32.2 (C-1), 31.5 (C-23), 30.6 (C-2), 30.1 (C-19), 28.4 (C-16), 26.7 (C-11), 26.3 (C-10), 26.2 (C-7),

25.6 (C-28), 22.2 (C-26), 22.1 (C-27), 21.3 (C-6), 20.2 (C-9), 19.6 (C-30), 18.5 (C-21), 18.2 (C-18), 14.2 (C-29).

***p*-Hydroxybenzaldehyde (2)**
White amorphous powder, mp 113-114°C; FAB-MS *m/z:* 123 $[M+H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.77 (2H, d, *J* = 8.7 Hz, H-2, 6), 6.93 (2H, d, *J* = 8.7 Hz, H-3, 5); ^{13}C-NMR (CD_3OD, 125 MHz): δ 191.6 (C=O), 164.1 (C-4), 132.3 (C-2, 6), 129.1 (C-1), 115.7 (C-3, 5).

Ferulic acid (3)
White amorphous powder, mp 231-232°C; FAB-MS *m/z*: 195 $[M+H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.65 (1H, d, *J* = 15.9 Hz, H-7), 7.19 (1H, d, *J* = 1.3 Hz, H-2), 7.06 (1H, dd, *J* = 8.1, 1.3 Hz, H-6), 6.80 (1H, d, *J* = 8.1 Hz, H-5), 6.38 (1H, d, *J* = 15.9 Hz, H-8), 3.89 (3H, s, -OCH_3); ^{13}C-NMR (CD_3OD, 125 MHz): δ 168.0 (C-9), 149.1 (C-3), 147.9 (C-4), 144.5 (C-7), 123.0 (C-1), 121.3 (C-6), 116.5 (C-8), 115.3 (C-5), 110.6 (C-2), 55.2 (-OCH_3).

***p*-Coumaric acid (4)**
White amorphous powder, mp 210-212°C; FAB-MS *m/z*: 165 $[M+H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.62 (1H, d, *J* = 16.2 Hz, H-7), 7.44 (1H, d, *J* = 8.4 Hz, H-2, 6), 6.89 (2H, d, *J* = 8.4 Hz, H-3, 5), 6.35 (1H, d, *J* = 16.2 Hz, H-8); ^{13}C-NMR (CD_3OD, 125 MHz): δ 167.1 (C-9), 160.2 (C-4), 145.7 (C-7) 135.5 (C-1), 130.1 (C-2, 6), 116.5 (C-3, 5), 115.7 (C-8).

Vanillic acid (5)
White amorphous powder, mp 208-211°C; FAB-MS *m/z*: 169 $[M+H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.56 (1H, dd, *J* = 8.4, 1.5 Hz, H-6), 7.55 (1H, d, *J* = 1.5 Hz, H-2), 6.83 (1H, d, *J* = 8.0 Hz, H-5), 3.90 (3H, s, -OCH_3); ^{13}C-NMR (CD_3OD, 125 MHz): δ 168.8 (-COOH), 151.5 (C-3), 147.5 (C-4), 124.2 (C-1), 122.0 (C-6), 114.7 (C-2), 112.7 (C-5), 55.6 (-OCH_3).

β-D-(1-*O*-Acetyl-3-*O*-*trans*-feruloyl)fructofuranosy-α-D-2',4',6'.-*O*-triacetyglucopyranoisde (6)
Colorless amorphous solid, $[\alpha]_D^{25}$ +50.0° (c 0.1 in $CHCl_3$); FAB-MS *m/z*: 687 $[M+H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.70 (1H, d, *J* = 15.8 Hz, H-7''), 7.28 (1H, d, *J* = 1.7 Hz, H-2''), 7.13 (1H, dd, *J* = 8.2, 1.7 Hz, H-6''), 6.83 (1H, d, *J* = 8.2 Hz, H-5''), 6.42 (1H, d, *J* = 15.8 Hz, H-8''), 5.63 (1H, d, *J* = 3.5 Hz, H-1'), 5.35 (1H, d, *J* = 7.6 Hz, H-3), 4.75 (1H, dd, *J* = 10.0, 3.5 Hz, H-2'), 4.30 (1H, t, *J* = 7.6 Hz, H-4), 4.21, 4.10 (2H, br s, H-1), 4.20 (1H, m, H-6'), 4.10 (1H, m, H-5'), 4.05 (1H, t, *J* = 9.9 Hz, H-3'), 3.95 (3H, s, 3''-OCH_3), 3.94 (1H, m, H-6), 3.78 (1H, m, H-6), 2.11 (3H, s, 2'-OAc), 2.09 (3H, s, 6'-OAc), 2.05 (3H, s, 4'-OAc), 1.98 (3H, s, 1-OAc); ^{13}C-NMR (CD_3OD, 125 MHz): δ 172.2 (2'-OAc; C=O), 171.6 (6'-OAc; C=O), 171.4 (4'-OAc; C=O), 170.9 (1-OAc; C=O), 167.1 (C-9''), 149.2 (C-4''), 148.3 (C-3''), 146.9 (C-7''), 126.4 (C-1''), 123.3 (C-6''), 115.3 (C-5''), 113.3 (C-8''), 110.8 (C-2''), 102.2 (C-2), 89.6 (C-1'), 83.0 (C-5), 78.1 (C-3), 73.2 (C-2'), 73.0 (C-4), 72.4 (C-4'), 70.9 (C-5'), 70.4 (C-3'), 64.8 (C-1), 62.3 (C-6, 6'), 55.4 (3''-OCH_3), 19.8 (2'-OAc; CH_3), 19.6 (6'-OAc; CH_3), 19.4 (4'-OAc; CH_3), 19.3 (1-OAc; CH_3).

β-D-(6-*O*-*trans*-Feruloyl)fructofuranosyl-α-D-*O*-glucopyranoisde (7)
Colorless amorphous solid, $[\alpha]_D^{25}$ +58.8° (*c* 0.1 in MeOH); IR (KBr): ν_{max} cm-^{1} : 3391, 2945, 1684, 1636, 1601, 1519, 1457, 1278, and 1027 cm-^{1}; UV λ_{max} (MeOH) nm (log ε): 237 (4.33), 291 (4.23), and 324 (4.33) nm; HR-FAB-MS *m/z*: 541.1532 $[M+Na]^+$ (calculated for $C_{22}H_{30}O_{14}Na$, 541.1533); ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.63 (1H, d, *J* = 15.8 Hz, H-7''), 7.18 (1H, d, *J* = 1.7 Hz, H-2''), 7.07 (1H, dd, *J* = 8.2, 1.7 Hz, H-6''), 6.81 (1H, d, *J* = 8.2 Hz, H-5''), 6.37 (1H, d, *J* = 15.8 Hz, H-8''), 5.40 (1H, d, *J* = 3.5 Hz, H-1'), 4.44 (2H, m, H-6), 4.44, 3.90 (2H, m, H-6'), 4.08 (1H, d, *J* = 8.2 Hz, H-3), 4.03 (1H, m, H-5), 3.99 (1H, m, H-5'), 3.99 (1H, dd, *J* = 8.2, 7.6 Hz, H-4), 3.90 (3H, s, H-3''-OCH_3), 3.71 (1H, dd, *J* = 9.9, 9.3 Hz, H-3'), 3.64 (2H, br s, H-1), 3.41 (1H, dd, *J* = 9.9, 3.5 Hz, H-2'); ^{13}C-NMR (CD_3OD, 125 MHz): δ 169.1 (C-9''), 150.7 (C-4''), 149.4 (C-3''), 147.0 (C-7''), 128.0 (C-1''), 124.2 (C-6''), 116.5 (C-5''), 115.2 (C-8''), 111.7 (C-2''), 103.4 (C-2), 93.4 (C-1'), 80.8 (C-5), 78.9 (C-3), 76.8 (C-4), 73.6(C-3'), 73.1 (C-2'), 72.1 (C-4'), 71.6 (C-5'), 66.7 (C-6), 65.6 (C-6'), 64.1(C-1), 56.4(3''-OCH_3).

β-D-(1-*O*-Acetyl-3,6-*O*-*trans*-diferuloyl)fructofuranosyl-α-D-2',4',6'.-*O*-triacetylglucopyranoisde (8)
Colorless amorphous solid, $[\alpha]_D^{25}$ +36.3° (*c* 0.16 in $CHCl_3$); FAB-MS *m/z*: 863 $[M+H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.69 (1H, d, *J* = 15.8 Hz, H-7''), 7.67 (1H, d, *J* = 15.8 Hz, H-7'''), 7.10 (2H, br d, *J* = 9.9 Hz, H-6'', H-6'''), 7.10 (2H, br s, H-2'', H-2'''), 6.80 (2H, d, *J* = 8.2 Hz, H-5'', H-5'''), 6.42 (2H, br d, *J* = 15.8 Hz, H-8'', H-8'''), 5.69 (1H, d, *J* = 3.5 Hz, H-1'), 5.38 (1H, d, *J* = 7.6 Hz, H-3), 4.67 (1H, dd, *J* = 9.9, 3.5 Hz, H-2'), 4.51 (1H, br s, *J* = 4.7 Hz, H-6'), 4.45 (1H, t, *J* = 7.6 Hz, H-4), 4.24 (1H, m, H-5), 4.24, 4.12 (2H, br s, H-1), 4.23 (1H, m, H-5'), 4.15 (1H, m, H-4'), 4.13 (1H, m, H-6), 4.11 (1H, d, *J* = 7.6 Hz, H-3'), 3.92 (3H, s, 3'''-OCH_3), 3.90 (3H, s, 3''-OCH_3), 2.12 (3H, s, 6'-OAc), 2.10 (3H, s, 4'-OAc), 2.03 (3H, s, 2'-OAc), 1.88 (3H, s, 1-OAc); ^{13}C-NMR (CD_3OD, 125 MHz): δ 172.2 (6'-OAc; C=O), 172.1 (4'-OAc; C=O), 171.0 (2'-OAc; C=O), 170.9 (1-OAc; C=O), 167.8 (C-9''), 167.6 (C-9'''), 148.3 (C-4'''), 148.2 (C-4'', C-7''), 147.5 (C-3'', C-3'''), 145.6 (C-7'''), 126.5 (C-1'', 1'''),

123.0 (C-6'''), 122.9 (C-6''), 115.3 (C-5'', 5'''), 114.5 (C-8'''), 114.0 (C-8''), 110.6 (C-2''), 110.4 (C-2'''), 103.0 (C-2), 89.0 (C-1'), 81.4 (C-5), 78.2 (C-3), 73.3 (C-4), 72.6 (C-2'), 70.9 (C- 4'), 69.6 (C- 3'), 68.4 (C- 5'), 64.8 (C-1), 63.9 (C- 6'), 62.2 (C- 6), 55.4 (3'''-OCH_3), 55.3 (3''-OCH_3), 19.8 (6'-OAc; CH_3), 19.6 (4'-OAc; CH_3), 19.5 (2'-OAc; CH_3), 19.2 (1-OAc; CH_3).

Hydroxytyrosol acetate (9)
Amorphous gum; FAB-MS *m/z*: 219 $[M+Na]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 6.69 (1H, d, *J* = 8.0 Hz, H-5), 6.66 (1H, d, *J* = 2.3 Hz, H-2), 6.53 (1H, dd, *J* = 2.3, 8.0 Hz, H-6), 4.18 (2H, t, *J* = 7.0 Hz, H-8), 2.76 (2H, t, *J* = 7.0 Hz, H-7), 2.00 (3H, s, CH_3); ^{13}C-NMR (CD_3OD, 125 MHz): δ 171.8 (CO), 145.1 (C-3), 143.8 (C-4), 129.6 (C-1), 120.0 (C-6), 115.8 (C-5), 115.2 (C-2), 65.4 (C-8), 34.2 (C-7), 19.6 (CH_3).

Hydroxytyrosol (10)
Amorphous gum; FAB-MS *m/z*: 155 $[M+H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 6.68 (1H, d, *J* = 8.2 Hz, H-5), 6.66 (1H, d, *J* = 2.0 Hz, H-2), 6.53 (1H, dd, *J* = 2.0, 8.2 Hz, H-6), 3.67 (2H, t, *J* = 7.0 Hz, H-8), 2.66 (2H, t, *J* = 7.0 Hz, H-7); ^{13}C-NMR (CD_3OD, 125 MHz): δ 145.0 (C-3), 144.4 (C-4), 130.6 (C-1), 120.0 (C-6), 115.9 (C-5), 115.1 (C-2), 63.4 (C-8), 38.5 (C-7).

Isorhamnetin-3-*O*-rutinoside (11)
Yellow powder; mp 183-186°C; FAB-MS *m/z*: 647 $[M+Na]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.94 (1H, d, *J* = 1.7 Hz, H-2'), 7.64 (1H, d, *J* = 1.7 Hz, H-6'), 6.92 (1H, d, *J* = 8.2 Hz, H-5'), 6.42 (1H, d, *J* = 1.7 Hz, H-8), 6.22 (1H, d, *J* = 1.7 Hz, H-6), 5.24 (1H, d, *J* = 7.6 Hz, Glc-1), 4.54 (1H, d, *J* = 1.2 Hz, Rha-1), 3.96 (1H, s, OCH_3), 3.84 (1H, br d, *J* = 11.1 Hz, Glc-6b), 3.26-3.47(9H, m, Glc 2-6a, Rha 2-5), 1.11 (3H, d, *J* = 6.4 Hz, Rha-6); ^{13}C-NMR (CD_3OD, 125 MHz): δ 197.4 (C-4), 165.1 (C-7), 163.1 (C-5), 158.9 (C-2), 158.5 (C-9), 150.9 (C-4'), 148.3 (C-3'), 135.5 (C-3), 124.0 (C-6'), 123.1 (C-1'), 116.1 (C-5'), 114.6 (C-2'), 105.6 (C-10), 104.4 (C-1''), 102.5 (C-1'''), 100.1 (C-6), 94.9 (C-8), 78.2 (C-5''), 77.4 (C-3''), 75.9 (C-2''), 73.8 (C-4'''), 72.3 (C-3'''), 72.1 (C-2'''), 71.6 (C-4''), 69.8 (C-5'''), 68.5 (C-6''), 56.8 (OCH_3), 17.9 (C-6''').

***n*-Butyl-α-D-fructofuranoside (12)**
Amorphous gum; $[\alpha]_D^{25}$ +96° (*c* 0.1 in CH_3OH); FAB-MS *m/z*: 259 $[M+Na]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 4.05 (1H, d, *J* = 5.2 Hz, H-3), 3.84-3.85 (1H, m, H-5), 3.75 (1H, dd, *J* = 11.7, 3.0 Hz, H-4), 3.62-3.64 (4H, m, H-1, H-6), 3.51 (2H, td, *J* = 9.4, 3.0 Hz, H-1), 1.55 (2H, m, H-2'), 1.40 (2H, m, H-3'), 0.95 (3H, t, *J* = 7.6 Hz, H-4'); ^{13}C-NMR (CD_3OD, 125 MHz): δ 107.6 (C-2'), 82.7 (C-5'), 82.1 (C-3'), 77.4 (C-4'), 61.5 (C-6'), 60.7 (C-1'), 60.5 (C-1), 32.2 (C-2), 19.2 (C-3), 13.0 (C-4).

***n*-Butyl-β-D-fructopyranoside (13)**
Amorphous gum; $[\alpha]_D^{25}$ -135° (*c* 0.1 in CH_3OH); FAB-MS *m/z*: 259 $[M+Na]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 3.90 (1H, d, *J* = 10.0 Hz, H-3), 3.84-3.85 (1H, m, H-5), 3.77 (1H, dd, *J* = 10.0, 3.5 Hz, H-4), 3.71 (1H, dd, *J* = 12.3, 1.2 Hz, H-6a), 3.70 (1H, d, *J* = 11.1 Hz, H-1a), 3.69 (1H, d, *J* = 11.1 Hz, H-1a), 3.65 (1H, d, *J* = 12.3, 1.8 Hz, H-1b), 3.51 (1H, td, *J* = 9.3, 7.0 Hz, H-1'a), 3.48 (1H, td, *J* = 9.3, 7.0 Hz, H-1'b), 1.55 (2H, m, H-2'), 1.40 (2H, m, H-3'), 0.92 (1H, t, *J* = 7.3 Hz, H-4'); ^{13}C-NMR (CD_3OD, 125 MHz): δ 100.4 (C-2'), 70.4 (C-4'), 69.9 (C-5'), 69.5 (C-3'), 64.0 (C-6'), 62.3 (C-1'), 60.4 (C-1), 32.1 (C-2), 19.3 (C-3), 13.1 (C-4).

Alkaline hydrolysis of compound 7
Compound **7** (0.7 mg) was dissolved in 3% KOH/MeOH and kept at room temperature for 20 min with H_2O and extracted with $CHCl_3$ three times, and the $CHCl_3$ extract was evaporated *in vacuo*, and identified as ferulic acid by co-TLC ($CHCl_3$: MeOH = 10 : 1, R_f value 0.37) with compound **3** (ferulic acid). The H_2O layer gave a mixture of D-glucose and D-fructose, which were identified with authentic samples (Aldrich Co.) using silica gel co-TLC [$CHCl_3$: MeOH : H_2O (6 : 4 : 1, R_f 0.29 D-glucose, R_f 0.37 D-fructose)].

Test for cytotoxicity *in vitro*
Sulforhodamin B bioassays (SRB) were used as cytotoxicity screening methods (Skehan et al., 1990). Cytotoxicity assays for each compound were performed *in vitro* against four cultured human tumor cell lines at the Korea Research Institute of Chemical Technology. The cell lines used were A549 (non-small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma), and HCT15 (colon cancer cells). Doxorubicin was used as a positive control.

RESULTS AND DISCUSSION

Compounds **1-6** and **8-13** were identified by comparing the ^{1}H-, ^{13}C-NMR, and MS spectra with the literature as 24-methylenecycloartanol (**1**) (De Pascual Teresa et al., 1986), *p*-hydroxybenzaldehyde (**2**) (Jang et al., 1990), ferulic acid (**3**) (He et al., 2005), *p*-coumaric acid (**4**) (Iiyama et al., 2008), vanillic acid (**5**) (Sun et al., 2006), β-D-(1-*O*-acetyl-3-*O*-*trans*-feruloyl)fructofuranosy-α-D-2',4',6'.-*O*-triacetyglucopyranoisde (**6**) (Xiong et al., 2008), β-D-(1-*O*-acetyl-3,6-*O*-*trans*-diferuloyl) fructofuranosyl-α-D-2',4',6'.-*O*-triacetylglucopyranoisde

(**8**) (Osamu et al., 1996), hydroxytyrosol acetate (**9**) (Gordon et al., 2001), hydroxytyrosol (**10**) (Capasso et al., 1997), isorhamnetin-3-*O*-rutinoside (**11**) (Nakano et al., 1989), *n*-butyl-α-D-fructofuranoside (**12**) (Li et al., 2004), and *n*-butyl-β-D-fructopyranoside (**13**) (Zhang et al., 1996). Compounds **3** and **9**-**13** were isolated for the first time from this plant.

Compound **7** was isolated as a colorless amorphous solid, $[\alpha]_D^{25}$ +58.8° (c 0.1, CD_3OD). The molecular formula

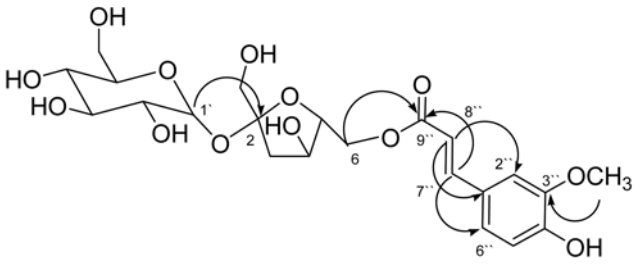

Fig. 1. Key HMBC correlations (H → C) of **7**

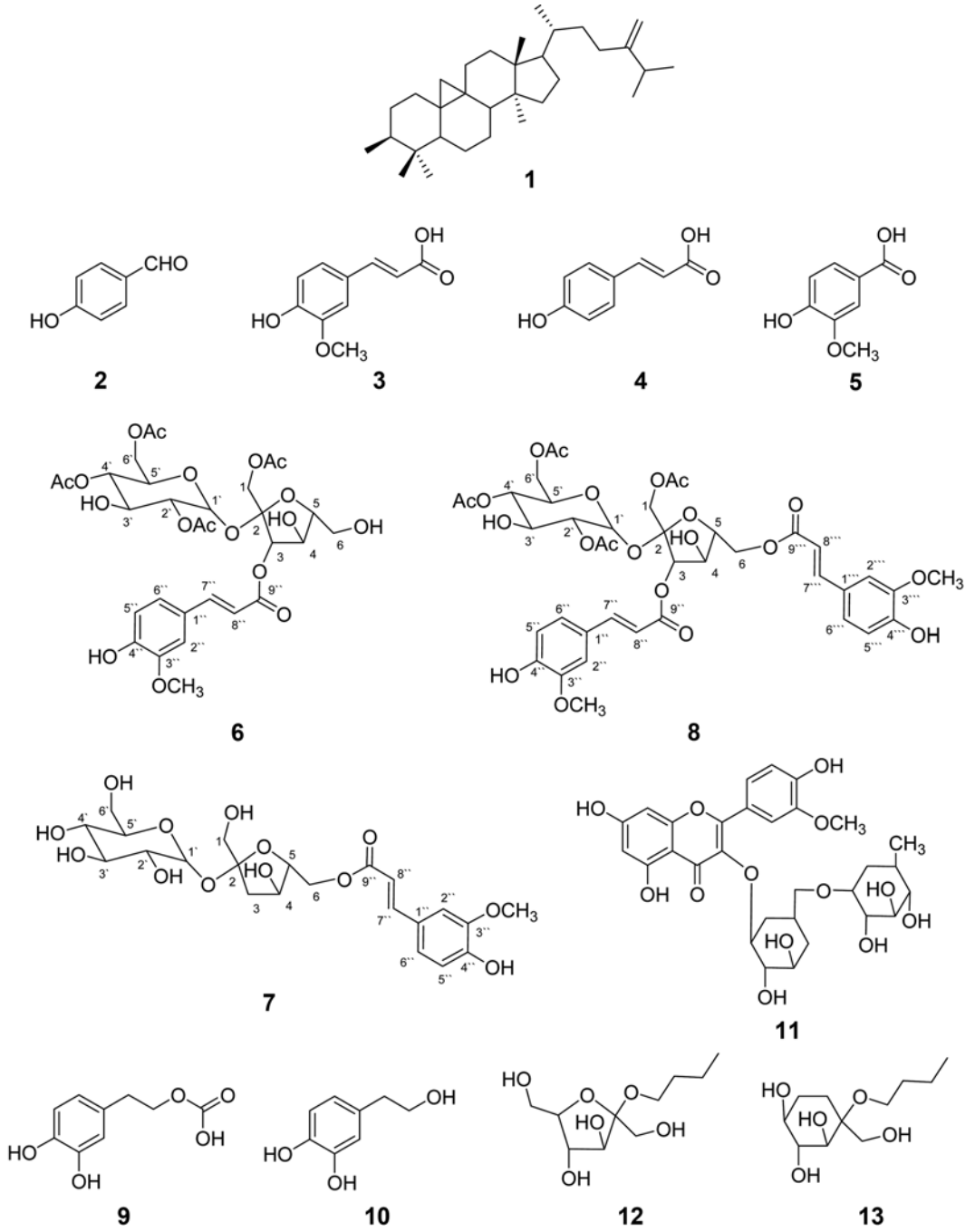

Fig. 2. The structures of compounds **1**-**13**

$C_{22}H_{30}O_{14}$ was determined by the HR-FAB MS *m/z* 541.1532 $[M+Na]^+$ (calcd. 541.1533). The IR spectrum showed the bands of a hydroxyl group at 3391 cm^{-1}, an ester carbonyl at 1684 cm^{-1}, and an aromatic ring at 1666, 1636, and 1519 cm^{-1}. The ^{1}H-NMR spectrum exhibited proton signals characteristic of an *E*-feruloyl moiety: three aromatic proton signals at δ_H 7.07 (1H, dd, *J* = 8.2, 1.7 Hz), 7.18 (1H, d, *J* = 1.7 Hz), and 6.81 (1H, dd, *J* = 8.2 Hz) as an ABX-type system, one methoxy group signal at δ_H 3.90 (3H, s) and *trans*-double bond signals at δ_H 6.37 (1H, d, *J* = 15.8 Hz), and 7.63 (1H, d, *J* = 15.8 Hz). The two olefinic carbon signals at δ_c 115.2, 147.0 and the carbonyl carbon at δ_c 169.1 in the ^{13}C-NMR spectrum indicated the presence of an α,β-unsaturated ketone moiety. The ^{1}H-NMR and ^{13}C-NMR spectrum confirm that 7 contained a feruloyl moieties. In the HMBC spectrum of **7** (Fig. 1), the correlations between the signals of H-6a, and H-6b of the fructosyl group at δ_H 4.44 (2H, m) and the carbon signal at δ_c 169.1 assigned to C-9" of *trans*-feruloyl group indicated that the *trans*-feruloyl group was linked to C-6 of the fructosyl group. The correlations between the signals of H-1' of the glucosyl group at δ_H 5.40 and the carbon signal at δ_c 103.4 assigned to C-2 of the fructosyl group indicated that the fructosyl group was linked to C-1' of the glucosyl group. The methoxyl proton 3"-OCH_3 (δ_H 3.90) and C-3" (δ_c 149.4) were observed, indicated the presence of a feruloyl group. The sugars were further identified by alkaline hydrolysis and compared with authentic samples over co-TLC ($CHCl_3$: MeOH : H_2O = 6: 4: 1, R_f value D-glucose 0.29, D-fructose 0.37). Thus, the structure of **7** was determined to be β-D-(6-*O*-*trans*-feruloyl)fructofuranosyl-α-D-*O*-glucopyranoisde.

The isolated compounds were tested for cytotoxicity against four human tumor cells *in vitro* using the SRB assay. Compound **9** exhibited moderate cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT15 cell lines (IC_{50}: 36.52, 14.11, 23.67, and 64.29 μM, respectively), but the other compounds showed little cytotoxicity (IC_{50} > 100 μM). The cytotoxicities of doxorubicin against the A549, SK-OV-3, SK-MEL-2, and HCT15 cell lines were IC_{50} 0.031, 0.017, 0.001, and 0.020 μM, respectively.

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