Two New Chemical Constituents from the Rhizome of *Sparganium stoloniferum*

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Received August 30, 2011, Accepted October 15, 2011

Key Words: *Sparganium stoloniferum*, Sparganiaceae, 1H-pyrrole-2-carboxylic acid, 3-hydroxy-3-methylglutaric acid (HMG), Cytotoxicity

*Sparganium* (Bur-reed) is a genus of flowering plants, which contains about 20 species in temperate regions of both the Northern and Southern Hemispheres. Three *Sparganium* species, *S. stoloniferum*, *S. angustifolium*, and *S. japonicum*, grow in Korea. *S. stoloniferum* is widely distributed in the wet valley areas, and has been used as an emmenagogue, a galactagogue, and an antispasmodic agent in Chinese folk medicine,1,2 and also for the treatment of menstrual disorders and chronic hepatitis.3 Previous phytochemical investigations on this plant reported the isolation of pyrrole carboxylic acid ester,4 phenylpropanoid glycosides,5-7 and two sucrose esters.8 Aldose reductase inhibition,9 anti-inflammatory, and anti-thrombotic10 activities of an EtOH extract have also been reported. In our continuing study on the constituents of Korean medicinal plant sources, we have identified molecules from the rhizome of *S. stoloniferum*. Column chromatographic purification of the MeOH extract of the rhizome of this source led to isolation of two new constituents (1-2), together with three known compounds (3-5). The structures of the new compounds (1-2) were determined through spectral analysis, and chemical means. The isolated compounds (1-5) were tested for cytotoxicity against four human tumor cells in vitro using a sulforhodamin B (SRB) bioassay.

Compound 1 was isolated as a colorless gum, [α]$_D^{25}$ +4.0° (c 0.2, MeOH). The molecular formula C$_{11}$H$_{13}$NO$_6$ was determined by the HR-FAB MS m/z 255.0743 [M]$^+$ (calcd. 255.0743). Compound 1 displayed three proton signals at δH 7.02 (1H, m, H-5’), 6.92 (1H, m, H-3’), 6.22 (1H, m, H-4’) in an 1H-NMR spectrum and five carbon signals at δC 160.1, 124.3, 121.0, 116.5, and 109.8 in a 13C-NMR spectrum, which were assignable to 1H-pyrrole-2-carboxylic acid.11 The 1H NMR spectrum also showed signals characteristic of 1,4-dimethyl malate group at δH 5.60 (1H, t, J = 7.0 Hz, H-2), 3.78 (3H, s, OCH$_3$-4), 3.73 (3H, s, OCH$_3$-1), and 3.02 (2H, m, H-3). The corresponding carbon resonances of these protons were observed at δC 170.4, 170.1, 68.2, 51.8, 51.3, and 35.5 in the HMOC spectrum. In addition, 1H-1H COSY correlations between the methine proton signal at δH 5.60 (t, J = 7.0 Hz, H-2), and the methylene proton signals at δH 3.02 (m, H-3) were observed. The HMBC correlations between the methoxy group at δH 3.78 (OCH$_3$-4) and the carbonyl carbon at δC 170.1 (C-4) and the other methoxy group at δH 3.73 (OCH$_3$-1) and carbonyl carbon at δC 170.4 (C-1) implied that two methoxy groups were present at C-1 and C-4. These data indicated the presence of a 1,4-dimethyl malate group.12 The HMBC spectrum showed that the methine proton at δH 5.60 (1H, t, J = 7.0 Hz, H-2) correlated with the carbonyl carbon at δC 160.1 (C-6’) (Fig. 2). Thus, compound 1 was

![Figure 1. Chemical structures of compounds 1-5.](image-url)
deduced as 1,4-dimethyl-2-(1H-pyrole-2-carbonyloxy)malate. Alkaline hydrolysis (0.1 M KOH) afforded 1,4-dimethyl malate (1a), which was identified by the comparison of its optical rotation value, 1H-NMR and MS spectra. The 1,4-dimethyl malate with S configuration at C-2 was reported to show a positive optical rotation (αD 25 +20.4, CHCl3). The optical rotation of 1a exhibited a positive value (αD 25 +17.1, CHCl3), indicating that the absolute configuration of the asymmetric carbon at C-3\(^\text{′}\) of the HMG moiety was determined to be S form. Thus, the structure of 2a was determined to be 3,4-dimethoxyphenyl-1-O-β-D-[6'-O-[(3'S)-3'-hydroxy-3'-methyl-glutaryl]]-glucopyranoside.

Known compounds were identified as dihydrophaseic acid 3-O-β-D-glucopyranoside (3), (+)-lyoniresinol 3α-O-β-D-glucopyranoside (4), and (+)-5,5′-dimethoxy seciosolari- ciresinol 3α-O-β-D-glucopyranoside (5) by comparison of physicochemical and spectroscopic data with previously reported literature values. Compounds 3-5 were isolated for the first time from this plant.

The cytotoxicities of compounds (1-5) were evaluated against the A549, SK-OV-3, SK-MEL-2, and HCT15 human cancer cell lines in vitro using the Sulforhodamine B (SRB) bioassay. All the compounds showed little cytotoxicity against any tested cell line (IC50 > 100 μM).

**Experimental Section**

**Plant Materials.** *Sparganium stoloniferum* Buch.-Hamil. was purchased in Yeongchenon, Korea, in September, 2008, and the plant was identified by one of the authors (K.R.L.). A voucher specimen (SKKU 2008-19) was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

**Extraction and Isolation.** The dried and chopped rhizomes of *S. stoloniferum* (5 kg) were 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, resulting in n-hexane (17 g), CH2Cl2 (3 g), EtOAc (4 g), and n-BuOH (30 g). The EtOAc fraction (4 g) was separated over a silica gel column with a solvent system (CHCl3:MeOH:H2O = 25:3:0.1 – 100% MeOH) to give nine fractions (E1-E9). Fraction E1 (10 mg) was separated on a RP-C18 silica gel column with 100% MeOH and purified with a RP-C18 prep HPLC (95% MeOH) to yield compound 1 (4 mg, Rf = 13 min). The n-BuOH fraction (30 g) was separated over a silica gel column with a solvent system of (CHCl3:MeOH:H2O = 14:3:7:0.1 – 100% MeOH) to give nine fractions (E1-E9). Fraction E1 (10 mg) was separated on a RP-C18 column with a solvent system of 40% MeOH to give two subfractions (B1-B10). Fraction B4 (400 mg) was separated over a RP-C18 prep HPLC (50% MeOH) to yield compound 5 (5 mg, Rf = 15 min). Fraction B7 (590 mg) was subjected to Sephadex LH-20 column chromatography eluted with 100% MeOH as to give seven subfractions (B71-B77). Subfraction B72 (34 mg) was purified with a silica gel
prep HPLC (CH₃Cl:MeOH = 2:1) to yield compound 3 (4 mg, Rᵣ = 16 min). Subfraction B76 (30 mg) was purified with a RP-C₁₈ prep HPLC (50% MeOH) to yield compound 2 (5 mg, Rᵣ = 13 min). Subfraction B77 (19 mg) was purified with a RP-C₁₈ prep HPLC (50% MeOH) to yield compound 4 (5 mg, Rᵣ = 17 min).

(2S)-1,4-Dimethyl-2-[(1H-pyrole-2'-carbonyloxy)-methyl]-3-hydroxy-3'-methyl-glutaryl]-glucopyranoside (2). Pale yellow gum, [α]D₂⁰ = +14.0° (c 0.15 in MeOH); FAB-MS m/z: 460 [M]+; HR-FAB-MS m/z: 460.1584 [M+Na]+ (calculated for C₂₃H₂₂NO₁₂ 460.1584); ¹H-NMR (CDCl₃, 500 MHz): δ 6.95 (1H, d, J = 8.5 Hz, H-5'), 6.46 (1H, d, J = 2.5 Hz, H-2'), 3.20 (1H, br s, OH), 4.51 (1H, d, J = 15.5 Hz, H-4'a), 2.34 (1H, d, J = 15.5 Hz, H-4'b), 1.29 (3H, s, H-6'); ¹³C-NMR (CDCl₃, 125 MHz): δ 178.5 (C-5'), 171.5 (C-1'), 153.9 (C-4), 151.0 (C-3), 139.5 (C-1), 120.0 (C-5), 106.6 (C-2), 103.1 (C-1'), 100.8 (C-2'), 76.5 (C-3'), 74.2 (C-5'), 73.8 (C-2'), 70.4 (C-4'), 69.7 (C-3'), 63.4 (C-6'), 55.6 (OCH₃-4'), 55.4 (OCH₃-1'), 46.8 (C-4'), 46.1 (C-2'), 26.6 (C-4').

Alkaline Hydrolysis of Compound 1: Compound 1 (1.7 mg) was hydrolyzed with 0.1 M KOH (1 mL) at room temperature for 3 hr. Then H₂O (3 mL) was added and the mixture was extracted with CHCl₃, three times, and the CHCl₃ extract was evaporated in vacuo. The CHCl₃ extract was purified over a silica gel Waters Sep-Pak Vac 6cc (CHCl₃:MeOH = 10:1) to give 1a, which was identified by ¹H-NMR, MS and optical rotation.

1a: Colorless gum; FAB-MS m/z: 163 [M+H]+; [α]D₂⁰ = +27.5° (c 0.08 in CHCl₃); ¹H-NMR (500 MHz, CDCl₃): δ 2.85 (2H, m, H-3), 3.72 (3H, s, OCH₃-1), 3.81 (3H, s, OCH₃-4), 3.20 (1H, br s, OH), 4.51 (1H, m, H-2').

Alkaline Methanolysis of Compound 1: Compound 2 (2.0 mg) was treated with 1% NaOMe in MeOH (1 mL) at room temperature for 3 hr. The reaction mixture was neutralized through an Amberlite IR-120B column and chromatographed on Sephadex LH-20 with MeOH to give 2a, which was identified by ¹H-NMR, MS and optical rotation.

2a: Colorless gum; FAB-MS m/z: 176 [M]+; [α]D₂⁰ = +17.1° (c 0.06 in CHCl₃); ¹H-NMR (500 MHz, CDCl₃): δ 3.73 (3H, s, OMe), 2.72 (1H, d, J = 16.0, H-4'a), 2.71 (1H, d, J = 16.0, H-2''a), 2.67 (1H, d, J = 16.0, H-4''b), 2.65 (1H, d, J = 2.0, H-2''b), 1.30 (3H, s, H-6').

A detailed description of the bioassays is available in the Supporting Information. The positive control, doxorubicin (purity ≥ 98%) was purchased from Sigma Corporation.

Acknowledgments. This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (20100029358). We thank Drs. E. J. Bang, S. G. Kim, and J. J. Seo at the Korea Basic Science Institute for their aid in obtaining the NMR and mass spectra.

Supporting Information. Spectral data of compounds 1 and 2, general experimental procedures and bioassay protocols are available upon request from the correspondence author.

References