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PHENOLIC CONSTITUENTS FROM THE STEMS OF *Acanthopanax senticosus*

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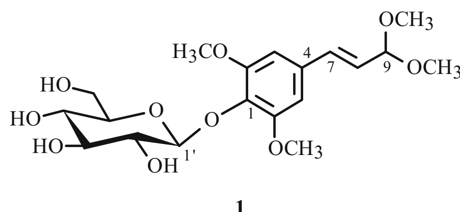
A new phenolic glycoside was isolated from the stems of Acanthopanax senticosus *together with sixteen known compounds. The structure of the new compound was determined to be 2,6-dimethoxy-4-[(1*E*)-3,3-dimethoxy-1-propenyl]phenyl* β*-D-glucopyranoside (**1**) by means of physical, chemical, and spectroscopic methods. Of the known compounds, salvadoraside (7), (7*R*,8*S*)-dihydrodehydrodiconiferyl alcohol 4,9′-di-*O-β*-D-glucopyranoside (**8**), 3-(4-*O-β*-D-glucopyranosylferuloyl)quinic acid (**15**), rel-5-(1*R*,5*S*-dimethyl-3*R*,4*R*,8*S*-trihydroxy-7-oxa-6-oxobicyclo[3,2,1]oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienoic acid (**16**), and lycoperodine-l (**17**) were first reported from the title plant. The inhibitory activities of the isolated compounds against* α*-glucosidase from rat intestine were also reported.*

Keywords: *Acanthopanax senticosus*, Araliaceae, stems, phenolic compound.

Acanthopanax senticosus (Rupr. *et* Maxim.) Harms (Araliaceae) is a shrub that is commonly distributed in the northeast of Asia. Various parts of the plant, including roots, rhizomes, stems, and leaves, have been widely used as Chinese traditional medicines for the treatment of a variety of human diseases, such as ischemic heart diseases, neurasthenia, hypertension, arthritis, and tumors [1]. As for the chemical constituents of these parts, although triterpenoid saponins were reported as the main constituents in the leaves, fruits, seeds, roots, and rhizome [2–6], it was found that the saponins did not occur in the stems. In the stems, lignoids and phenylpropanoid glycosides were reported as the main constituents [7, 8]. The stems were reported to exert several pharmacological effects, such as antioxidant, anti-inflammatory, antiaging, antifatigue, immunoregulatory, anti-stress, etc. [8, 9]. As a part of our continuing studies on *A. senticosus*, we have investigated the chemical constituents of the leaves and fruits of *A. senticosus* [5, 6]. Herein we reported the isolation and structural determination of a new phenolic glycoside (**1**) together with sixteen known compounds from the stems. Of the known compounds, salvadoraside (**7**), (7*R*,8*S*)-dihydrodehydrodiconiferyl alcohol 4,9′-di-*O*-*β*-D-glucopyranoside (**8**), 3-(4-*O*-*β*-D-glucopyranosylferuloyl)-quinic acid (**15**), rel-5-(1*R*,5*S*-dimethyl-3*R*,4*R*,8*S*-trihydroxy-7-oxa-6-oxobicyclo[3,2,1]oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienoic acid (**16**), and lycoperodine-l (**17**) were first reported from the title plant. The inhibitory activities of the isolated compounds against *α*-glucosidase from rat intestine were also reported.



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Compound **1** was isolated as a colorless amorphous solid. The molecular formula of **1** was determined as $C_{19}H_{28}O_{10}$ by HR-ESI-MS. In the ^1H and ^{13}C NMR spectra, it showed the presence of a *β*-glucopyranosyl moiety with the anomeric signals at δ_H 4.89 (1H, d, J = 7.5 Hz) and δ_C 105.3. The *β*-glucopyranosyl moiety was confirmed to be of the D-form by acid hydrolysis of **1**. In the ^1H NMR and ^{13}C NMR spectra, it also showed the presence of a tetrasubstituted aromatic ring with symmetric structure by the characteristic proton signal at δ 6.78 (2H, s) and carbon signals at δ 134.3, 105.9 × 2, 154.5 × 2, and 136.5. The ^1H NMR spectrum further showed two *trans*-relational olefinic protons at δ 6.67 (1H, br.d, J = 16.0 Hz) and 6.12 (1H, dd, J = 16.0, 5.3 Hz), a methine proton at 4.92 (1H, dd, J = 5.3, 1.2 Hz), and the protons due to four methoxyl moieties at 3.85 (6H, s) and 3.35 (6H, s). The correlation between the olefinic proton at δ 6.12 and the methine proton at 4.92 in the ^1H–^1H COSY spectrum suggested the presence of a propenyl moiety. The positions of the *β*-D-glucopyranosyl moiety at C-1, the propenyl moiety at C-4, and two methoxyl moieties at C-2 and C-6 were suggested by the HMBC correlations of δ_H 4.89 (H-1′)/δ_C 136.5 (C-1), δ_H 6.67 (H-7)/δ_C 105.9 (C-3,5), and δ_H 3.85 (2,6-OCH_3)/δ_C 154.5 (C-2,6). The two methoxyl moieties at C-9 were suggested by the HMBC correlation between δ_H 3.35 (9-OCH_3)/δ_C 104.7 (C-9). Thus, the structure of **1** was determined to be 2,6-dimethoxy-4-[(1*E*)-3,3-dimethoxy-1-propenyl]phenyl *β*-D-glucopyranoside.

The known compounds were identified as (+)-syringaresinol (**2**) [10], (+)-syringaresinol 4′-*O*-*β*-D-glucoside (**3**) [11], (+)-syringaresinol di-*O*-*β*-D-glucoside (**4**) [12], (+)-medioresinol di-*O*-*β*-D-glucopyranoside (**5**) [13], (+)-pinoresinol di-*O*-*β*-D-glucopyranoside (**6**) [13], salvadoraside (**7**) [14], (7*R*,8*S*)-dihydrodehydrodiconiferyl alcohol 4,9′-di-*O*-*β*-D-glucopyranoside (**8**) [15], isofraxidin (**9**) [16], isofraxidin-7-*O*-*β*-D-glucoside (**10**) [17], syringin (**11**) [17], sinapaldehyde 4-*O*-*β*-D-glucopyranoside (**12**) [18], glucoferulic acid (**13**) [7], 3-feruloylquinic acid (**14**) [19], 3-(4-*O*-*β*-D-glucopyranosylferuloyl) quinic acid (**15**) [20], rel-5-(1*R*,5*S*-dimethyl-3*R*,4*R*,8*S*-trihydroxy-7-oxa-6-oxobicyclo[3,2,1]oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienoic acid (**16**) [21], and lycoperodine-l (**17**) [22] by interpretation of their physical and spectroscopic data and comparison with values reported in the literature. The known compounds **7**, **8**, **15**, **16**, and **17** were first reported from the title plant.

The isolated compounds **1–17** were subjected to an *α*-glucosidase inhibition assay. Compounds **12** and **17** exhibited IC_{50} values to rat intestinal sucrase at 2.0 and 1.9 mg/mL, respectively. Compounds **12** and **16** exhibited IC_{50} values to rat intestinal maltase at 2.0 and 1.6 mg/mL, respectively.

It was reported that *A. senticosus* could be used to treat diabetic peripheral neuropathy [23, 24] and saponin isolated from the leaves of *A. senticosus* could decrease various cases of experimental hyperglycemias induced by injection of adrenaline, glucose, and alloxan [25]. But this is the first report on α-glucosidase inhibition assay of compounds from the stems of *A. senticosus*. Further research is needed in order to understand the materials foundation and action mechanism of *A. senticosus* in diabetes treatment.

EXPERIMENTAL

Generals. Optical rotations were measured with a JASCO DIP-370 digital polarimeter in a 0.5 dm cell. UV spectra were recorded on a Shimadzu UV-260 spectrometer. IR spectra were measured with a Perkin–Elmer 683 infrared spectrometer (by the KBr disk method). The ^1H and ^{13}C NMR measurements were recorded using a JEOL ECP-500 NMR spectrometer with TMS as the internal reference, and chemical shifts are expressed in δ (ppm). ESI-MS and HR-ESI-MS were conducted using a JEOL JMS-T100LP AccuTOF LC-plus mass spectrometer. Diaion HP-20 resin (Mitsubishi Chemical Corporation, Tokyo, Japan) and ODS (Chromatorex, 100–200 mesh, Fuji Sylisia Chemical, Ltd., Aichi, Japan) were used for column chromatography. For HPLC, a JASCO PU-1580 HPLC system, equipped with a Shodex RI-71 differential refractometer detector, was used. TLC was conducted in Kieselgel 60 F_{254} plates (Merck).

Plant Material. The stems of *A. senticosus* were collected from Hulin city, Heilongjiang Province, P. R. China in September, 2007, and identified by one of the authors (W. L.). A specimen of the plant is kept in the Herbarium of the Faculty of Pharmaceutical Sciences, Toho University.

Extraction and Isolation. The air-dried stems (1 kg) were extracted twice with MeOH at room temperature. Evaporation of the solvent under reduced pressure gave the MeOH extract (36 g). The MeOH extract was applied to a Diaion HP-20 column and eluted with H_2O, 30%, 70%, 100% MeOH and acetone to give five fractions (Fr.1–Fr.5). Fraction 2 (1.75 g) was separated by ODS column chromatography using 50% and 100% MeOH to give two fractions (Fr.21–Fr.22). Fraction 21 was purified by HPLC (15% MeCN containing 0.06% TFA) to give compounds **13** (31 mg), **14** (12 mg), and **15** (3 mg). Fraction 3 (5.25 g) was separated by ODS column chromatography using 30%, 50%, and 100% MeOH to give seven fractions

(Fr. 31–Fr. 37). Fraction 32 was purified by HPLC (15% MeCN containing 0.06% TFA) to give compounds **10** (83 mg), **11** (67 mg), **12** (4 mg), and **16** (3 mg). Fraction 33 was purified by HPLC (30% MeCN containing 0.06% TFA) to give compounds **1** (6 mg), **4** (57 mg), **5** (4 mg), **6** (10 mg), **7** (2.5 mg), **8** (6 mg), and **17** (1 mg). Fraction 34 was purified by HPLC (40% MeCN containing 0.06% TFA) to give compounds **2** (2 mg), **3** (6 mg), and **9** (16 mg).

2,6-Dimethoxy-4-[(1*E*)-3,3-dimethoxy-1-propenyl]phenyl *β*-D-glucopyranoside (1). Colorless amorphous solid; $[\alpha]_D^{19}$ –1.65° (*c* 0.31, MeOH). UV/Vis (MeOH, λ_{max}, nm) (log ε): 230 (1.47), 314 (1.40). IR (KBr, ν, cm^{-1}): 3386, 1671, 1634, 1590, 1506, 1455, 1383, 1312, 1273, 1161, 1074, 1026. ^1H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 6.78 (2H, s, H-3,5), 6.67 (1H, br.d, J = 16.0, H-7), 6.12 (1H, dd, J = 16.0, 5.3, H-8), 4.92 (1H, dd, J = 5.3, 1.2, H-9), 4.89 (1H, d, J = 7.5, H-1′), 3.48–3.40 (3H, m, H-2′, H-3′ and H-4′), 3.21 (1H, m, H-5′), 3.66 (1H, dd, J = 12.0, 5.3, H-6′), 3.78 (1H, dd, J = 12.0, 2.3, H-6′), 3.85 (6H, s, 2 and 6-OCH_3), 3.35 (6H, s, 9-OCH_3). ^{13}C NMR (125 MHz, CD_3OD, δ): 154.5 (C-2 and 6), 136.5 (C-1), 134.6 (C-7), 134.3 (C-4), 126.8 (C-8), 105.9 (C-3 and 5), 105.3 (C-1′), 78.4 (C-3′), 77.9 (C-5′), 104.7 (C-9), 75.8 (C-2′), 71.5 (C-4′), 62.7 (C-6′), 57.1 (2 and 6-OCH_3), 49.5 (2 × 9-OCH_3). ESI-MS: *m/z* 439 $[M + Na]^+$. HR-ESI-MS: *m/z* 439.1571 $[M + Na]^+$, calcd for $C_{19}H_{28}NaO_{10}$, 439.1580.

Acid Hydrolysis of 1. A solution of **1** (0.5 mg) in 1 M HCl (dioxane–H_2O, 1:1, 0.2 mL) was heated at 100°C for 1 h under an Ar atmosphere. After the dioxane was removed, the solution was partitioned between EtOAc and H_2O. The aqueous layer was concentrated under reduced pressure to dryness. The residue was dissolved in pyridine (0.1 mL), to which 0.08 M L-cysteine methyl ester hydrochloride in pyridine (1.5 mL) was added. The mixture was kept at 60°C for 1.5 h. After the mixture was dried in *vacuo*, the residue was trimethylsilylated with 1-trimethylsilylimidazole (0.1 mL) for 2 h. The mixture was partitioned between *n*-hexane and H_2O (0.3 mL each) and the *n*-hexane extract was analyzed by GC-MS under the following conditions: capillary column, Equity TM-1 (30 m × 0.25 mm × 0.25 μm, Supelco), column temperature, 230°C; injection temperature, 250°C; carrier N_2 gas. Based on the acid hydrolysate of **1**, D-glucose was confirmed by comparison of the retention times of their derivatives with those of the D-glucose and L-glucose derivatives prepared in a similar way.

***α*-Glucosidase Inhibition Assay**. *α*-Glucosidase was prepared from rat intestine acetone powder (Sigma-Aldrich Japan Co., Tokyo, Japan). Rat intestine acetone powder (100 mg) was dissolved in 56 μM maleate buffer (pH 6.0, 0.9 mL), sonicated at 4°C for 20 min, and then centrifuged at 15,000 *g* at 4°C for 60 min to obtain the supernatant. The supernatant, diluted by adding a twofold volume of 56 μM maleate buffer, was used as sucrase solution, and by adding a fortyfold volume of 56 μM maleate buffer was used as maltase solution. Sucrose or maltose in 56 μM maleate buffer (20 mg/mL) was used as substrate solution. The above enzyme solution (0.1 mL) was added to a mixture of substrate solution (0.1 mL) and various concentrations of samples in MeOH (0.01 mL) and 56 μM maleate buffer (0.04 mL). The reaction mixture was incubated for 60 min at 37°C in a final volume of 0.25 mL, then heated at 102°C for 10 min to stop the reaction. The glucose released in the solution was determined in a 96-well plate using a glucose assay kit (Glucose CII-test Wako, Wako Pure Chem. Co., Osaka, Japan) based on the glucose oxidase/peroxidase method. The negative control was prepared by adding 56 μM maleate buffer instead of the sample in the same way as the test. Acarbose was used as the positive control. The blank was prepared by adding 56 μM maleate buffer instead of the enzyme solution using the same method.

The inhibition rates (%) = $[(OD_{negative\ control} - OD_{blank}) - (OD_{test} - OD_{test\ blank})]/(OD_{negative\ blank} - OD_{blank}) \times 100\%$. IC_{50} values of the sample were calculated using IC_{50} calculation software.

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