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Studies on cytotoxic triterpene saponins from the leaves of *Aralia elata*

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ABSTRACT

Aralia elata has long been used as a tonic, anticancer and antidiabetic agent in China and Japan, and is widely consumed as food. Phytochemical investigation of the leaves of *A. elata* has led to the isolation of four new compounds, 3-O-[β-D-glucopyranosyl(1 → 3)-β-D-glucopyranosyl] echinocystic acid 28-O-β-D-glucopyranosyl ester (congmuyenoside I, **1**), 3-O-[β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl] hederagenin 28-O-β-D-glucopyranosyl ester (congmuyenoside II, **2**), 3-O-{[β-D-glucopyranosyl(1 → 2)]-[β-D-glucopyranosyl(1 → 3)-β-D-glucopyranosyl(1 → 3)]-β-D-glucopyranosyl} echinocystic acid 28-O-β-D-glucopyranosyl ester (congmuyenoside III, **3**) and 3-O-β-D-glucopyranosyl caulophyllogenin 28-O-β-D-glucopyranosyl ester (congmuyenoside IV, **4**), and eight known triterpene saponins (**5**–**12**). The structural determination was accomplished with spectroscopic analysis, in particularly ^{13}C NMR, 2D NMR and HR-ESI-MS techniques. In addition, compounds **5**–**10** were found for the first time in the genus *Aralia*. Compounds **1**–**12** were tested for their inhibition of the growth of HL60, A549 and DU145 cancer cells. In addition, compound **8** showed significant cytotoxic activities against HL60, A549 and DU145 cancer cells with IC_{50} values of 15.62, 11.25 and 7.59 μM, respectively.

1. Introduction

Aralia elata Seem., locally called "ci lao ya", is widely distributed in northeastern China, Korea, Japan and Russia (Shanghai Scientific and Technological Publishing Co., New Medical College of Jiangsu (Ed.), 1977). *A. elata* has unique flavour and good taste which make it popular ingredients of some Chinese dishes, such as salad, dumplings, pickles, and soup, furthermore its health benefits are of increasing interest (Sun, Zhang, Wang, & Zhang, 2009).

It is a very rich source of proteins necessary for human body, fat, carbohydrates, minerals, vitamin, Plant fibre (Zhou & Geng, 2009) and also bioactive compounds including triterpenoid saponins, oleanolic acid, flavones, polysaccharide (Saito, Sumita, & Tamura, 1990), human body essential amino-acid (Walanabe, 1977), macroelement and microelement (Li & Lu, 2009), hence consumed by people suffering from neurasthenia (Gao & Zhang, 1988), rheumatoid arthritis (Zhou, Shi, & Li, 1984), diabetes mellitus (Chung, Choi, & Lee, 2005), gastrospasm, constipation (Zhou & Geng, 2009) and hepatitis (Du & Chi, 2005).

The aim of this work is the study of triterpene saponins from *A. elata* to make an appropriate nutraceutical labelling available in order to enhance the value of it. It is well known that triterpene saponins are the major biologically active portion in *A. elata* (Saito et al., 1993). Recent years, more and more interest has been expressed in triterpene saponins. Biochemical investigations demonstrated triterpene saponins in *A. elata* were shown to have various biological activities, including anticancer (Tomatsu, Kameyama, & Shibamoto, 2003), anti-inflammatory (Suh, Jin, & Kim, 2007), liver-protecting (Saito et al., 1993), antioxidative (Zhang et al., 2006), antiviral (Li, Tian, & Shi, 1994) and antidiabetic activities (Kim, 1993).

Most of the previous phytochemical investigations of *A. elata* have focused on the buds and root barks (Ma, Song, Li, & Xu, 2005; Ma, Zhang, Song, & Xu, 2008; Song, Nakamura, Ma, Hattori, & Xu, 2001) and not on the leaves. To search for novel and bioactive compounds from natural foodstuffs, the phytochemical investigation of the leaves of *A. elata* was performed, and four new triterpene saponins were obtained. Triterpene saponins are considered the principal components of *A. elata* that are responsible for cytotoxic effects. Oleanane-type triterpene saponins are known to possess cytotoxic activities (Tomatsu & Kameyama, 2003). Compounds **1**–**12** were tested *in vitro* for their cytotoxic activities against the human cancer cell lines HL60, A549 and DU145 using the MTT assay method. In addition, compound **8** showed significant cytotoxic activities against HL60, A549 and DU145 cancer cells with IC_{50} values of 15.62, 11.25 and 7.59 μM, respectively.

2. Materials and methods

2.1. General methods

Column chromatography was performed on a 200–300 mesh silica gel (Qingdao Marine Chemical Factory, PR China). Column

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chromatography was performed using YMC ODS-A gel (12 nm S-75 μm, YMC Co., Ltd., Japan) and D101 Macroporous adsorption resin (Shanghai Hualing Resin Factory, PR China). TLC was performed with precoated silica gel GF_{254} plates (Qingdao Marine Chemical Factory, PR China). NMR spectra were performed on a Bruker ARX-300 or an ARX-600 spectrometer using trimethylchlorosilane as the internal standard. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. HR-ESI-MS were measured with a Bruker Daltonics Inc. micro-TOF-Q spectrometer. HPLC separations were performed on a Hitachi 655-15 series pumping system equipped with a Hitachi L-2490 refractive index detector using a YMC-Park ODS-A column (250 × 10 mm I.D, S-5 μm, 12 nm).

2.2. Plant material

The leaves of *A. elata* were collected from Liaoning Province of China in August 2009 and were taxonomically identified by Prof. Lu Jincai of Shenyang Pharmaceutical University. A voucher specimen was deposited at the School of Traditional Chinese Materia Medica (No. 090811).

2.3. Extraction and isolation

The air-dried leaves (8 kg) were first extracted with 60% ethanol for three times under reflux. The combined solutions were concentrated under vacuum subjected to macroporous resin D101 column chromatography and eluted with an EtOH–H_2O gradient. The solution that was eluted with 60% ethanol was evaporated to dryness under vacuum to get a residue (800 g). The residue was chromatographied on silica gel with a CH_2Cl_2–MeOH gradient to provide eight fractions (fr. I–VIII).

Fr. IV was subjected to column chromatography on silica gel and eluted with an increasing MeOH gradient in CH_2Cl_2 (20–100%), to yield 12 fractions (fr. A–L). Fr. F was subjected to C18 column chromatography and eluted with a MeOH–H_2O gradient to give 14 fractions (F_1–F_{14}). Of these fractions, fr. F_9 was further purified using silica gel column chromatography eluting with a solvent system of CH_2Cl_2–MeOH–H_2O (v/v/v = 8:2:0.25) to yield **5** (81 mg), fr. F_7 was applied to preparative HPLC with a solvent system MeOH–H_2O (v/v = 7:3) to yield **12** (45 mg), fr. F_{11} was purified using C8 column chromatography with a solvent system MeOH–H_2O to yield **8** (35 mg). Fr. G was subjected to column chromatography on C18 and eluted with an increasing MeOH gradient in H_2O (15–100%) to 13 fractions (fr. G_1–G_{13}). Fr. G_6 and fr. G_7 were further purified using silica gel column chromatography with a solvent system of CH_2Cl_2–MeOH–H_2O (v/v/v = 6:1:0.1) to yield **6** (60 mg) and **9** (125 mg). Fr. I was separated using C18 column chromatography with a solvent system MeOH–H_2O to give **7** (65 mg) and fifteen fractions (fr. I_1–I_{15}). Fr. I_8 was further separated using silica gel column chromatography with a solvent system of CH_2Cl_2–MeOH–H_2O (v/v/v = 8:2:0.25) to yield **1** (104 mg). Fr. I_9 was further separated using silica gel column chromatography with a solvent system of CH_2Cl_2–MeOH–H_2O (v/v/v = 8:2:0.25), the fourth fraction was further purified by preparative HPLC using a mobile phase of MeOH–H_2O (v/v = 6:4) to yield **2** (44 mg).

Fr. V was subjected to column chromatography on silica gel and eluted with an increasing MeOH gradient in CH_2Cl_2 (30–100%) to nine fractions (fr. A–I). Fr. C was separated on C8 column chromatography with a solvent system MeOH–H_2O to yield **11** (72 mg). Fr. D was separated using C18 column chromatography with a solvent system MeOH–H_2O to 14 fractions (fr. D_1–D_{14}). Fr.D_7 was chromatographied on silica gel with CH_2Cl_2–MeOH gradient to give eleven fractions, the sixth fraction was applied to preparative HPLC with a solvent system MeOH–H_2O (v/v = 4:1) to yield **4** (30 mg). Fr. D_{11} was further separated on silica gel column chromatography with a solvent system of CH_2Cl_2–MeOH–H_2O (v/v/v = 7:3:0.5) to yield **10** (90 mg). Fr. F was purified using C18 column chromatography with a solvent system MeOH–H_2O to 10 fractions, the sixth fraction was applied to preparative HPLC with a solvent system MeOH–H_2O (v/v = 5:1) to yield **3** (147 mg).

2.4. MTT assays

Cytotoxic assays were performed according to the MTT (3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) method (Hussain, Nouri, & Oliver, 1993). The compounds to be tested were dissolved in DMSO, and the final concentration of DMSO in the culture medium was maintained at less than 0.1% (v/v). Cultivated human cells were seeded in a 96-well plate with 1×10^4 cells/well. The plate was incubated with the compounds for 48 h, MTT solution (2.5 mg/ml in PBS) was added (10 μl/well), and the plate was incubated for an additional 4 h at 37 °C. The formazan crystals that were produced were dissolved in 100 μl of DMSO, and the optical density of the solution was measured at 492 nm using a microplate reader. The percent cytotoxic activity was determined by comparison with the control (DMSO).

The cytotoxicities of the samples on tumour cells were expressed as IC_{50} values (the drug concentration reducing 50% of the absorbance in treated cells when compared to untreated cells), which were calculated by the LOGIT method.

2.5. Acid hydrolysis of *1*–*4*

Compounds **1**–**4** were refluxed with 2 M aqueous HCl for 2 h, respectively. After cooling to room temperature, the reaction mixture was neutralised with NaOH and centrifuged. The supernatant was evaporated to dryness on a water bath and the residue was analysed by TLC on silica gel [using *n*-BuOH–HOAc–H_2O (4:1:5)] using an authentic sample of glucose for comparison. A solution of the residue of compounds **1**–**4** (**1**: 2.1 mg, **2**: 2.0 mg, **3**: 2.1 mg, **4**: 2.2 mg) in H_2O (3 mL) showed a positive optical rotation respectively
($[\alpha]_D^{25} = +42.6°$, $[\alpha]_D^{25} = +38.5°$, $[\alpha]_D^{25} = +57.3°$, $[\alpha]_D^{25} = +46.8°$), as expected for D-glucose ($[\alpha]_D^{25} = +52.5°$).

2.6. Characteristic data of compounds

3-O-[β-D-glucopyranosyl(1 → 3)-β-D-glucopyranosyl] echinocystic acid 28-O-β-D-glucopyranosyl ester (congmuyenoside I, **1**). White amorphous powder (MeOH). (+)-HR-ESI-MS: *m*/*z* 981.5039 $[M+Na]^+$ (Calcd for $C_{48}H_{78}NaO_{19}$: 981.5030). ^1H NMR (C_5D_5N, 600 MHz): δ 5.58 (1H, br s, H-12), 5.30 (1H, br s, H-16), 3.49 (1H, dd, *J* = 3.6 Hz, 13.8 Hz, H-18), 3.37 (1H, dd, *J* = 4.2 Hz, 11.4 Hz, H-3), 1.80 (3H, s, 27-CH_3), 1.25 (3H, s, 23-CH_3), 1.09 (3H, s, 26-CH_3), 1.01 (3H, s, 30-CH_3), 0.96 (3H, s, 24-CH_3), 0.96 (3H, s, 29-CH_3), 0.83 (3H, s, 25-CH_3). ^1H NMR data of the sugar part, see Table 2. ^{13}C NMR (C_5D_5N, 150 MHz): see Tables 1 and 2.

3-O-[β-D-Glucopyranosyl(1 → 2)-β-D-glucopyranosyl] hederagenin 28-O-β-D-glucopyranosyl ester (congmuyenoside II, **2**). White amorphous powder (MeOH). (+)-HR-ESI-MS: *m*/*z* 981.5034 $[M+Na]^+$ (Calcd for $C_{48}H_{78}NaO_{19}$: 981.5030). ^1H NMR (C_5D_5N, 600 MHz): δ 5.38 (1H, br s, H-12), 4.31 (1H, d, *J* = 9.6 Hz, H-23), 4.16 (1H, dd, *J* = 3.6 Hz, 10.4 Hz, H-3), 3.72 (1H, d, *J* = 10.8 Hz, H-23), 3.15 (1H, dd, *J* = 3.6 Hz, 13.8 Hz, H-18), 1.15 (3H, s, 27-CH_3), 1.08 (3H, s, 26-CH_3), 1.02 (3H, s, 24-CH_3), 0.88 (3H, s, 25-CH_3), 0.84 (3H, s, 29-CH_3), 0.83 (3H, s, 30-CH_3). ^1H NMR data of the sugar part, see Table 2. ^{13}C NMR (C_5D_5N, 150 MHz): see Tables 1 and 2.

3-O-{[β-D-Glucopyranosyl(1 → 2)]-[β-D-glucopyranosyl(1 → 3)-β-D-glucopyranosyl(1 → 3)]-β-D-glucopyranosyl} echinocystic acid 28-O-β-D-glucopyranosyl ester (congmuyenoside III, **3**). White amorphous powder (MeOH). (+)-HR-ESI-MS: *m*/*z* 1305.6085 $[M+Na]^+$ (Calcd for $C_{60}H_{98}NaO_{29}$: 1305.6086). ^1H NMR (C_5D_5N,