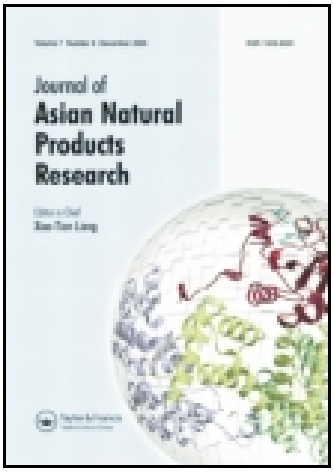

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Two new saponins from Thalictrum fortunei

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Two new saponins from *Thalictrum fortunei*

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Two new cycloartane glycosides were isolated from the aerial parts of *Thalictrum fortunei* (Ranunculaceae). The chemical structures of these compounds were elucidated as 3-*O*-β-D-glucopyranosyl (1 → 4)-β-D-fucopyranosyl-(22*S*,24*Z*)-cycloart-24-en-3β,22,26,30-tetraol 26-*O*-β-D-glucopyranoside and 3-*O*-β-D-glucopyranosyl (1 → 4)-β-D-fucopyranosyl-(22*S*,24*Z*)-cycloart-24-en-3β,22,26,29-tetraol 26-*O*-β-D-glucopyranoside by extensive 1D and 2D NMR methods, HR-ESI-MS, and hydrolysis. Their cytotoxic activities toward human hepatoma Bel-7402 cells, human colon carcinoma LoVo cells, and human non-small-cell lung cancer NCIH-460 cells were evaluated by MTT assay, respectively.

Keywords: *Thalictrum fortunei*; Ranunculaceae; cycloartane glycoside; cytotoxic

1. Introduction

Thalictrum fortunei S. Moore is a perennial plant distributed in the southeastern part of China. The aerial part of this plant has been used in traditional Chinese medicine for the treatment of ophthalmia, dysentery, and jaundice [1]. In our previous study, several new cycloartane glycosides were obtained from *T. fortunei* [2,3]. Further investigation of the plant led to the purification of two minor saponin constituents, named 3-*O*-β-D-glucopyranosyl (1 → 4)-β-D-fucopyranosyl-(22*S*,24*Z*)-cycloart-24-en-3β,22,26,30-tetraol 26-*O*-β-D-glucopyranoside (**1**) and 3-*O*-β-D-glucopyranosyl (1 → 4)-β-D-fucopyranosyl-(22*S*,24*Z*)-cycloart-24-en-3β,22,26,29-tetraol 26-*O*-β-D-glucopyranoside (**2**). In addition, the antitumor activities of the two compounds were investigated against the different cancer cells *in vitro*.

2. Results and discussion

Compound **1** was obtained as a white powder. Positive results from both Liebermann–Burchard and Molish reactions indicated that **1** was a saponin. The HR-ESI-MS showed a quasimolecular ion at *m/z* 943.5280 $[M-H]^-$, consistent with a molecular formula $C_{48}H_{80}O_{18}$. Acid hydrolysis of **1** afforded D-glucose and D-fucose which were identified by gas chromatographic analysis. The ^{1}H NMR spectrum of **1** showed two doublet signals at δ 0.24 (d, $J = 3.8$ Hz) and 0.45 (d, $J = 3.8$ Hz) which indicated the presence of a cyclopropane ring, three tertiary methyls at δ 0.87, 1.06, and 1.63, an olefinic methyl at δ 1.98, two secondary methyls at δ 1.19 (d, $J = 6.4$ Hz) and 1.68 (d, $J = 6.4$ Hz), an olefinic proton at δ 5.83 (1H, t, $J = 6.5$ Hz), and three anomeric protons at δ 4.75 (1H, d, $J = 7.7$ Hz), 4.93 (1H, d, $J = 7.8$ Hz), and 5.22 (1H, d,

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$J = 7.9$ Hz). Similarly, the ^{13}C NMR spectrum showed the corresponding signals due to cyclopropane methylene at δ 29.7, methylene carbons bearing oxygen at δ 63.4 (C-30) and 67.3 (C-26), methine carbons bearing oxygen at δ 73.0 (C-22) and 89.3 (C-3). The ^{13}C NMR spectral data also suggested the presence of three anomeric carbons at δ 102.9, 106.4 and 106.9, which correlated with the protons at δ 4.93, 4.75, and 5.22, respectively. The NMR spectral data of **1** were similar to those of 3-*O*-β-D-glucopyranosyl (1 → 4)-β-D-fucopyranosyl-(22*S*,24*Z*)–cycloart-24-en-3β,22, 26-triol 26-*O*-β-D-glucopyranoside reported previously, except for the appearance of the signals for an additional methylene carbon bearing oxygen group [2]. In the HMBC spectrum of **1**, correlation signals were observed between H-3 (δ 3.44) and C-4 (δ 45.1), C-29 (δ 21.3), and C-30 (δ 63.4), as well as between H-29 (δ 1.63) and C-30 (δ 63.4), C-3 (δ 89.3), respectively. The relative stereochemistry of methylene carbon bearing oxygen group was deduced based on NOESY correlations between H-3 (δ 3.44, α-H) and H-29 (δ 1.63, α-H), as well as between H_b-19 (δ 0.54, β-H) and H_b-30 (δ 4.55, β-H). Therefore, the methylene carbon bearing oxygen group was arranged in β-orientation, and the tertiary methyl group was in α-orientation (Figure 1). These findings were consistent with the stereochemistry previously reported for thalicoside A1 and thalictoside D [4,5]. The interglycosidic linkages of the sugar chains could be deduced from an HMBC experiment. In the HMBC spectrum of **1**, the correlation signals were observed between H-1 (δ 4.75) of fucose and C-3 (δ 89.3) of aglycone, between H-1′ (δ 5.22) of glucose and C-4 (δ 83.5) of fucose, as well as between H-1″ (δ 4.93) of glucose and C-26 (δ 67.3). Thus, the structure of **1** was characterized as 3-*O*-β-D-glucopyranosyl (1 → 4)-β-D-fucopyranosyl-(22*S*,24*Z*)-cycloart-24-en-3β,22,26, 30-tetraol 26-*O*-β-D-glucopyranoside.

Compound **2** was obtained as a white powder. Positive results from both Liebermann–Burchard and Molish reactions indicated that **2** was a saponin. The molecular formula of **2** was determined to be $C_{48}H_{80}O_{18}$ by HR-ESI-MS at *m/z* 943.5280 $[M-H]^-$. Upon acid hydrolysis, **2** afforded D-glucose and D-fucose which were identified by gas chromatographic analysis. The ^1H NMR spectrum of **2** showed two doublet signals at δ 0.30 (d, $J = 3.8$ Hz) and 0.54 (d, $J = 3.8$ Hz), three tertiary methyls at δ 0.82, 1.04, and 1.08, an olefinic methyl at δ 1.97, two secondary methyls at δ 1.20 (d, $J = 6.4$ Hz) and 1.64 (d, $J = 6.4$ Hz), an olefinic proton at δ 5.83 (1H, t, $J = 6.5$ Hz), and three anomeric protons at δ 4.93 (1H, d, $J = 7.8$ Hz), 4.96 (1H, d, $J = 8.0$ Hz), and 5.20 (1H, d, $J = 7.7$ Hz). The interglycosidic linkages of the sugar chains could be deduced from HMBC and HSQC experiments. The ^{13}C NMR spectral

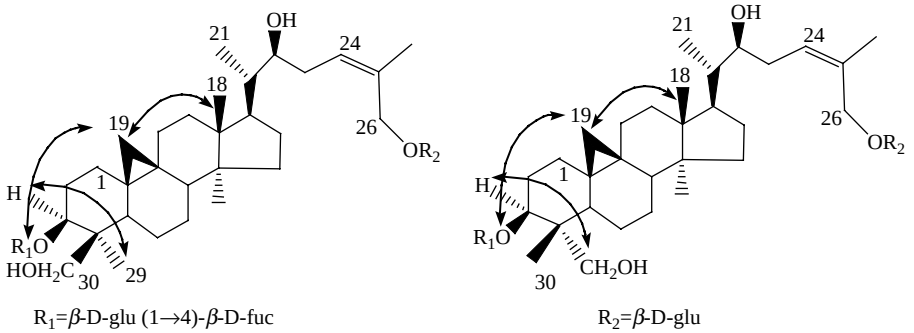

Figure 1. Key NOESY correlations of compounds **1** and **2**.

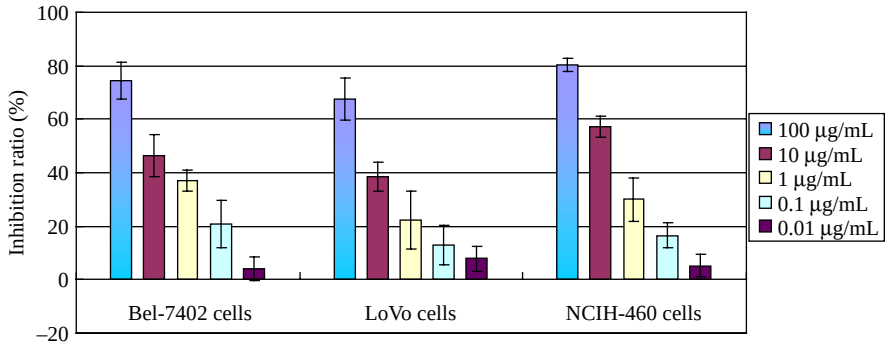

Figure 2. Inhibition ratio of compound **1** at different concentrations toward Bel-7402 cells, LoVo cells, and NCIH-460 cells ($n = 6$).

data of **2** were very similar to those of **1** except signals due to C-3, C-5, C-29 (C-30). In the HMBC spectrum of **2**, correlation signals were observed between H-3 (δ 4.34) and C-29 (δ 64.0), C-30 (δ 12.0), and C-4 (δ 45.4), between H-30 (δ 1.04) and C-29 (δ 64.0), C-5 (δ 40.7), and C-3 (δ 82.4). Moreover, the ROESY spectrum revealed NOESY correlations between H_b-19 (δ 0.54, β-H) and H-30 (δ 1.04, β-H), as well as between H-3 (δ 4.34, α-H) and H-29 (δ 3.74, 4.43, α-H) (Figure 1). Therefore, the structure of **2** was determined as 3-*O*-β-D-glucopyranosyl (1 → 4)-β-D-fucopyranosyl-(22*S*,24*Z*)-cycloart-24-en-3β,22,26,29-tetraol 26-*O*-β-D-glucopyranoside.

As estimated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, the inhibition ratio of the different cancer was markedly increased after a 48-h exposure to the different concentration of compound **1** or compound **2** in the concentration ranges from 100 to 0.1 μg/ml in a dose-dependent manner (Figures 2 and 3). The IC_{50} of compounds **1** and **2** toward human hepatoma Bel-7402 cells, human colon carcinoma LoVo cells, and human non-small-cell lung cancer NCIH-460 cells were 6.83, 24.33, 5.61 μg/ml and 3.32, 7.79, 3.08 μg/ml, respectively.

3. Experimental

3.1 General experimental procedures

Melting points were determined on an X-4 apparatus (Ningbo Hinotek Technology Ltd, Ningbo, China) and are uncorrected. Optical rotations were obtained using a Perkin-Elmer 241 polarimeter (Perkin-Elmer Ltd,

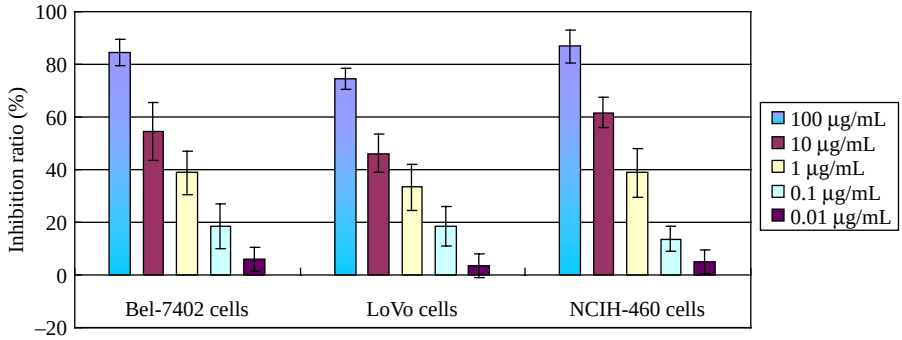

Figure 3. Inhibition ratio of compound **2** at different concentrations toward Bel-7402 cells, LoVo cells, and NCIH-460 cells ($n = 6$).

Norwalk, CT, USA). IR spectra were measured on a Nicolet Impact 410 FT-IR instrument (Nicolet Instrument Ltd, Madison, WI, USA). UV spectra were recorded on a Shimadzu UV-2501 spectrophotometer (Shimadzu Ltd, Kyoto, Japan). The ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AV500 Avance spectrometer (^{1}H, 500 MHz; ^{13}C, 125 MHz; Bruker Ltd, Karlsruhe, Germany) and chemical shifts were given in δ (ppm) with TMS as a reference. HR-ESI-MS were obtained on an Applied Biosystems Mariner 5140 spectrometer (Life Technologies Ltd, New York, NY, USA). Column chromatography (CC) was performed on silica gel (Qingdao Marine Chemical Ltd, Qingdao, China), Sephadex LH-20 (Pharmacia Ltd, New York, NY, USA), and ODS (Merck Ltd, Darmstadt, Germany). Thin-layer chromatography was performed on precoated silica gel GF_{254} plates (Qingdao Marine Chemical Ltd). Preparative HPLC was carried out using a Zobax XDB-18 column (10 mm i.d. × 15 cm, Agilent Technologies Ltd, Wilmington, DE, USA). GC experiments were carried out on an HP-1 TCD instrument (Hewlett-Packard Ltd, Palo Alto, CA, USA) using an HP-Chiral column (30 m × 0.25 mm × 1.0 μm, 20% permethylated β-cyclodextrin; Agilent Technologies Ltd). All chemical reagents were purchased from Nanjing Reagent Co., Ltd (Nanjing, China).

3.2 Plant material

The aerial parts of *T. fortunei* were collected in Wuhu City, Anhui Province, China, in April 2004, and authenticated by Dr Ming-Jian Qin of China Pharmaceutical University. A voucher specimen (No. 040192) has been deposited in the herbarium of China Pharmaceutical University, Nanjing.

3.3 Extraction and isolation

The dried aerial parts (4.8 kg) of *T. fortunei* were extracted with 95% EtOH (3 × 20 liters) under reflux. The EtOH extract was suspended in water and then successively extracted with petroleum ether, EtOAc, and *n*-BuOH. The *n*-BuOH solution was concentrated and given a residue (207 g), which was separated by a silica gel column using $CHCl_3$–MeOH (1:0 → 1:1) as an eluent, affording five fractions (frs A–F). Fr. B (3.5 g) was purified by CC (Sephadex LH-20, MeOH): Fr. B1–B4. Fr. B4 (385 mg) was subjected to reversed-phase CC (ODS, MeOH/H_2O 60:40–80:20 (v/v)), followed by HPLC (MeCN/H_2O 34:66 (v/v)) to give compounds **1** (45 mg, t_R 17 min) and **2** (67 mg, t_R 26 min), respectively.

3.3.1 3-O-β-D-Glucopyranosyl (1 → 4)-β-D-fucopyranosyl-(22S,24Z)-cycloart-24-en-3β, 22,26,30-tetraol 26-O-β-D-glucopyranoside (1)

A white powder, $[\alpha]_D$ 8.80 ($c = 0.68$, MeOH); mp 258–259°C; IR (KBr) ν_{max} 3416, 2937, 1614, 1384, 1363, 1105, 1078, 773, 625, 476 cm^{-1}; ^{1}H and ^{13}C NMR spectral data see Table 1; ESI-MS (negative ion mode) *m/z* 943 $[M-H]^-$, 781 $[M-163]^-$, 619 $[M-325]^-$; HR-ESI-MS: *m/z* 943.5280 $[M-H]^-$ (calcd for $C_{48}H_{79}O_{18}$, 943.5266).

3.3.2 3-O-β-D-Glucopyranosyl (1 → 4)-β-D-fucopyranosyl-(22S,24Z)-cycloart-24-en-3β,22,26,29-tetraol 26-O-β-D-glucopyranoside (2)

A white powder, $[\alpha]_D$ 21.3 ($c = 0.25$, MeOH); mp 235–237°C; IR (KBr) ν_{max} 3434, 2929, 1637, 1383, 1072 cm^{-1}; ^{1}H and ^{13}C NMR spectral data see Table 1; HR-ESI-MS: *m/z* 943.5280 $[M-H]^-$ (calcd for $C_{48}H_{79}O_{18}$, 943.5266).

3.4 Acid hydrolysis

A solution of the compound (10 mg) in 15 ml of 1 M HCl (MeOH–H_2O, 1:1) was heated under reflux for 3 h. After the

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2**.[a]

	1		**2**	
	C	**H**	**C**	**H**
1	31.1	1.52, 1.22	32.2	1.56, 1.25
2	30.0	2.35, 1.97	29.0	2.38, 2.07
3	89.3	3.50, dd (11.7, 4.3)	82.4	4.34
4	45.1		45.4	
5	48.0	1.48	40.7	2.11
6	21.2	1.70, 0.92	20.9	2.18, 1.56
7	26.7	1.04, 0.97	26.7	1.94, 1.51
8	48.0	1.48	47.9	1.48
9	21.4		20.0	
10	25.6		26.0	
11	26.5	1.96, 1.24	26.0	1.96, 1.22
12	33.4	1.66	33.4	1.66
13	45.4		45.4	
14	49.0		49.1	
15	36.0	1.29, 1.27	35.8	1.31, 1.24
16	28.0	2.12, 1.50	28.0	2.12, 1.50
17	49.2	2.36	49.1	2.33
18	18.6	1.06, s	18.7	1.08, s
19	29.7	0.45, d (3.8); 0.24, d (3.8)	29.9	0.54, d (3.8); 0.30, d (3.8)
20	41.7	1.66	41.7	1.66
21	12.1	1.19, d (6.4)	12.1	1.20, d (6.4)
22	73.0	4.09	73.0	4.06
23	35.0	2.78, 2.42	35.0	2.77, 2.42
24	128.6	5.83, t (6.5)	128.6	5.83, t (6.5)
25	133.1		133.2	
26	67.3	4.76, d (11.6); 4.53, d (11.6)	67.4	4.76, d (11.6); 4.53, d (11.6)
27	22.2	1.98, s	22.2	1.97, s
28	19.7	0.87, s	19.5	0.82, s
29	21.3	1.63, s	64.0	4.43, 3.74
30	63.4	4.55, 3.86	12.0	1.04, s
Fuc 1	106.4	4.75, d (7.7)	105.8	4.96, d (8.0)
2	73.2	4.38	73.8	4.40
3	75.8	4.11	75.7	4.05
4	83.5	4.13	83.4	4.07
5	70.6	3.83, dd (13.2, 6.4)	70.5	3.73, dd (13.2, 6.4)
6	17.7	1.68, d (6.4)	17.7	1.64, d (6.4)
Glc′ 1	106.9	5.22, d (7.9)	106.9	5.20, d (7.7)
2	76.0	4.05	76.2	4.05
3	78.6	4.23	78.6	4.25
4	71.6	4.24	71.6	4.26
5	78.5	3.98	78.5	3.98
6	62.8	4.57, d (11.5); 4.43, dd (11.5, 5.75)	62.8	4.59, d (11.5); 4.42, dd (11.5, 5.75)
Glc″ 1	102.9	4.93, d (7.8)	103.0	4.93, d (7.8)
2	75.2	4.09	75.2	4.09
3	78.7	4.29	78.7	4.28
4	71.7	4.33	71.7	4.30
5	78.6	3.96	78.6	3.94
6	62.8	4.51,d (11.0); 4.41, dd (11.0, 4.4)	62.8	4.52, d (11.0); 4.40, dd (11.0, 4.4)

[a] Spectra were measured in pyridine-d_5. Fuc: β-D-fucopyranosyl; Glc: β-D-glucopyranosy.

removal of the solvent, the residue was partitioned between $CHCl_3$ and H_2O. The aqueous layer was neutralized with Dowex (HCO_3-), and then filtered. The filtrate was concentrated to 2 ml, then treated with $NaBH_4$ (20 mg) at room temperature for 3 h. Excessive $NaBH_4$ was removed with 30% AcOH. After evaporation at 60°C and washing with 0.1% hydrochloric acid (in MeOH) repeatedly until the BO_3^{3-} was removed, the reaction mixture was heated to dryness at 105°C for 15 min, followed by the addition of pyridine (0.5 ml) and Ac_2O (0.5 ml). The mixture was incubated in a water bath at 100°C for 1 h and partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was concentrated for GC analysis. The peak of each monosaccharide was observed at t_R (min): **1**. D-fucose 27.832, D-glucose 33.063; **2**. D-fucose 27.826, D-glucose 33.070 (reference D-fucose 27.833, D-glucose 33.077, L-fucose 29.363, L-glucose 34.463).

3.5 *Cytotoxic assay* in vitro

Human hepatoma Bel-7402 cells, human colon carcinoma LoVo cells, and human non-small-cell lung cancer NCIH-460 cells were maintained in RPMI 1640 supplemented with 10% fetal bovine serum, 100 mg/l streptomycin, and 100 mU/l penicillin at 37°C in a humidified atmosphere of 5% CO_2. Cells (100 μl) in their exponential growth phase were seeded in 96-multiwell plates at a density of 1×10^5 cells/ml, after 24 h of incubation to allow cells to adhere. In the experimental group, cells were incubated with different concentrations of compound **1** or compound **2** (100, 10, 1, 0.1, 0.01 μg/ml) for 48 h. At the end of experiments, cells were incubated with 20 μl solution of 5 mg/ml MTT for 4 h at 37°C. Then the medium was carefully removed. The formazan crystals were dissolved in 150 μl of DMSO and the absorbance was measured at 570 nm in a plate reader (Elx 800, Bio-TEK, Winooski, VT, USA). The inhibition ratio was calculated with the following formula. Inhibition ratio (%) = (1 − Absorbance of experimental group/Absorbance of control group) × 100% [6].

3.6 *Statistical analysis*

Data were expressed as mean ± SD of mean and the IC_{50} values were analyzed using the SPSS 17.0 followed by Bliss assay.

Acknowledgements

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