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Two New Spirostanol Glycosides from the Roots and Rhizomes of *Helleborus thibetanus* Franch.

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Abstract: Two new spirostanol glycosides, thibetanosides J and K (**1** and **2**), along with three known ones (**3**-**5**) were isolated from the roots and rhizomes of *Helleborus thibetanus*. Their structures were elucidated by extensive use of spectroscopic techniques and chemical evidence. In this study, compounds **1**-**5** were evaluated for their cytotoxic activity against HCT116, A549 and HepG2 tumor cell lines. Among them, compound **1** exhibited moderate cytotoxicity against A549 cells (IC_{50} 7.69 ± 1.13 μM) and HepG 2 cells (IC_{50} 8.32 ± 2.63 μM). Compound **2** exhibited moderate cytotoxicity against HCT116 cells (IC_{50} 20.67 ± 1.06 μM).

Keywords: *Helleborus thibetanus*; spirostanol glycosides; cytotoxic activity.

1. Introduction

Helleborus thibetanus Franch., a plant endemic to China, known as "Tigencao" or "Xiao-tao-er-qi", is mainly distributed in Gansu, Sichuan and Shaanxi Provinces [1]. Its dried rhizomes have been used as Chinese folk medicine for the treatment of cystitis, urethritis and traumatic injury [2-3]. Several bufadienolides, ecdysteroids, furostanol saponins, spirostanol saponins and flavonoids have been isolated from *H. thibetanus* [4-5]. Modern pharmacology studies revealed that the extracts and chemical components of *H. thibetanus* possess immune-regulation, anticancer, antibacterial and cytotoxic properties [6-7]. As part of an ongoing search for bioactive constituents

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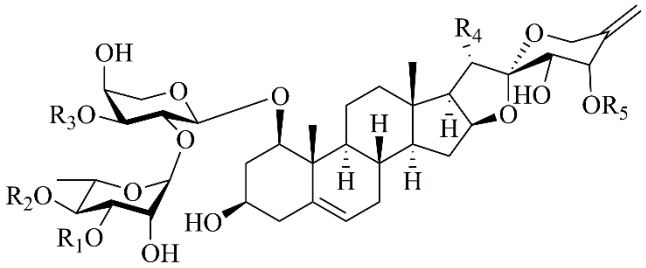
from the medicinal herbs around Qinba Mountains [8-10], two new spirostanol saponins (23*S*,24*S*)-24-{[*O*-*β*-D-glucopyranosyl-(1 → 4)-*β*-D-fucopyranosyl]oxy}-3*β*,23-dihydroxyspirosta-5,25(27)-diene-1*β*-yl*O*-*β*-D-apiofuranosyl-(1 → 3)-*O*-(*α*-L-rhamnopyranosyl)-(1 → 2)-*O*-*α*-L-arabinopyranoside (**1**), and (23*S*,24*S*)-24-{[*O*-*β*-D-glucopyranosyl-(1 → 4)-*β*-D-fucopyranosyl]oxy}-3*β*,23-dihydroxyspirosta 5,25(27)-diene-1*β*-yl*O*-(4-*O*-acetyl-*α*-L-rhamnopyranosyl)-(1→2)-*O*-*α*-L-arabinopyranoside (**2**), and three known saponins (23*S*,24*S*)-24-{[*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-fucopyranosyl]oxy}-3*β*,23-dihydroxypiro-sta-5,25(27)-dien-1*β*-yl*O*-*β*-D-apiofuranosyl-(1→3)-*O*-(4-*O*-acetyl-*α*-L-rhamno-pyranosyl)-(1→2)-*O*-*α*-L-arabinopyranoside (**3**) [5], (23*S*,24*S*)-24-[(*O*-*β*-D-glucopyranosyl)oxy]-3*β*,23-dihydroxyspirosta-5,25(27)-diene-1*β*-yl*O*-*β*-D-apiofuranosyl-1→3)-*O*-(4-*O*-acetyl-*α*-L-rhamnopyranosyl)-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*α*-L-arabinopyranoside (**4**) [11], (23*S*,24*S*)-21-acetoxy-3*β*,23,24-trihydroxyspirosta-5,25(27)-dien-1*β*-yl*O*-*β*-D-apiofuranosyl-(1→3)-*O*-(4-*O*-acetyl-*α*-L-rhamnopyranosyl)-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*α*-L-arabinopyranoside (**5**) [12] (Figure 1), were isolated from the roots and rhizomes of *H. thibetanus*. Herein, the isolation and structure elucidation of the new compounds, and their anti-tumor evaluation against A549, HepG 2 and HCT116 tumor cells were described.



	R_1	R_2	R_3	R_4	R_5
1	Api	H	H	CH_3	Glc(1→4)Fuc
2	H	Ac	H	CH_3	Glc(1→4)Fuc
3	Api	Ac	H	CH_3	Glc(1→4)Fuc
4	Api	Ac	Xyl	CH_3	Glc
5	Api	Ac	Xyl	CH_2OAc	H

Figure 1. Structures of compounds **1–5**

2. Materials and Methods

2.1. General Experimental Procedures

Optical rotations were recorded on a Rudolph Autopol II digital polarimeter. IR spectra were obtained on a Bruker-TENSOR-27 instrument. The HR-ESI-MS analyses were conducted on an Agilent Technologies 6550 Q-TOF (Santa Clara, CA, USA). ESI-MS was performed on a Quattro Premier instrument (Waters, Milford, MA, USA). 1D and 2D NMR spectra were acquired on Bruker-AVANCE 400 instrument (Bruker, Rheinstetten, Germany) with TMS as an internal standard. Semipreparative HPLC separations was performed on a system comprising an LC-6AD pump (Shimadzu, Kyoto, Japan; max pressure: 25 MPa) equipped with a SPD-20A UV detector and a Ultimate XB-C18 (10 mm × 250 mm, 5 μm particles). The GC analysis was performed on an Agilent 6890N apparatus equipped with

an HP-5 capillary column (30 m× 0.32 mm, 0.5 μm) and an FID detector. Standards for D-glucose (D-Glc), D-fucose (D-Fuc), L-arabopyranose (L-Ara), L-rhamnose (L-Rha) and D-apiose (D-Api) was purchased from Herbest Bio-Tech Co. (Baoji, China). Silica gel was purchased from Qingdao Haiyang Chemical Group Corporation (Qingdao, China).

2.2. Plant Material

The roots and rhizomes of *H. thibetanus* Franch were collected in June 2016 from the Taibai region (height: 2276.6 m, longitude: 107°47′28.4581″, latitude: 34°0′54.2781″) of Qinba Mountains in Shaanxi Province, China, and were authenticated by senior experimentalist Jitao Wang. A voucher specimen (herbarium No. 20160915) has been deposited in the Medicinal Plants Herbarium (MPH), Shaanxi University of Chinese Medicine, Xianyang, China.

2.3. Extraction and Isolation

The air-dried underground parts (1.5 kg) of *H. thibetanus* Franch were powdered and extracted three times with 60% EtOH under reflux at 80 °C. After removing the solvent, the concentrated residue was successively partitioned with petroleum ether (PE) and n-BuOH. The n-BuOH extract (200 g) was chromatographed on silica gel column, eluted with gradient solvent system ($CHCl_3$-MeOH-H_2O, 100:0:0-65:35:1) to yield ten fractions (Fr.1 - 10). Fr.5 (40 g) was separated on silica gel column, eluting with gradient solvent system ($CHCl_3$-MeOH, 100:0-50:50) to yield six fractions (Fr.5-1-Fr.5-6). Fr.5-2 (150 mg) was further purified by HPLC (Ultimate XB-C18, 10 mm × 250 mm, 5 μm particles, flow rate: 1.0 mL/min) using CH_3CN-H_2O (32:68) to afford compounds **1** (13 mg; t_R = 27.6 min) and **2** (20 mg; t_R = 35.2 min). Fr.8 (150 g) was subjected to a silica column chromatography, eluting with gradient solvent system ($CHCl_3$-MeOH, 100:0-80:10) to yield five fractions (Fr.8-1-Fr.8-5). Fr. 8-3 (0.7 g) was purified by HPLC (Ultimate XB-C18, 10 mm × 250 mm, 5 μm particles, flow rate: 1.0 mL/min) with CH_3CN– H_2O (20: 80) to get compounds **3** (9 mg; t_R = 24.7 min), **4** (7 mg; t_R = 32.1 min) and **5** (14 mg; t_R = 49.3 min).

2.4. *Spectroscopic Data*

*Thibetanoside J (**1**)*: A white amorphous powder,$[\alpha]_D^{26.6}$-56.8 (*c* 1.4, MeOH); IR (KBr) ν_{max}: 3383, 2932, 1450, 1377, 1250, 1050, 837 and 782 cm^{-1}; ^{1}H-NMR (400 MHz, pyridine-d_5) and ^{13}C-NMR (100 MHz, pyridine-d_5) spectral data, see Table **1**; HR-ESI-MS: *m/z* 1177.5253 $[M - H]^-$ (calcd. for $C_{55}H_{85}O_{27}$ 1177.5278).

*Thibetanoside K (**2**)*: A white amorphous powder,$[\alpha]_D^{27.7}$-69.5 (*c* 1.1, MeOH); IR (KBr) ν_{max}: 3384, 2935, 1732, 1452, 1374, 1243, 1040, 835 and 783 cm^{-1}; ^{1}H-NMR (400 MHz, pyridine-d_5) and ^{13}C-NMR (100 MHz, pyridine-d_5) spectral data, see Table **1**; HR-ESI-MS: *m/z* 1087.4911 $[M - H]^-$ (calcd. for $C_{52}H_{79}O_{24}$ 1087.4961).

3. Results and Discussion

3.1. Structure Elucidation

Thibetanoside J (**1**) was isolated as a white amorphous powder, which showed positive reactions in the Liebermann-Burchard and Molisch tests. Its molecular formula was determined as $C_{55}H_{86}O_{27}$ from the HR-ESI-MS at *m/z* 1177.5253 $[M - H]^-$ (calcd. $C_{55}H_{85}O_{27}$ 1177.5278). In the ^{1}H-

NMR and HSQC spectra, five anomeric protons at δ_H 5.16 (1H, d, J = 7.8 Hz, H-Glc-1), 5.16 (1H, d, J = 7.8 Hz, H-Fuc-1), 4.68 (1H, d, J = 7.9 Hz, H-Ara-1), 6.33 (1H, br s, H-Rha-1), 6.21 (1H, d, J = 2.5 Hz, H-Api-1) as well as two methyl protons at δ_H 1.71 (3H, d, J = 6.1 Hz, H-Rha-6) and 1.54 (3H, d, J = 6.3 Hz, H-Fuc-6) were observed, which were correlated with five anomeric carbon signals at δ_C 107.4 (C-Glc-1), 106.6 (C-Fuc-1), 101.0 (C-Ara-1), 102.0 (C-Rha-1), 112.3 (C-Api-1), 19.5 (C-Rha-6) and 18.0 (C-Fuc-6), respectively. Acid hydrolysis of **1** resulted in the production of apiose (Api), arabinose (Ara), rhamnose (Rha), fucose (Fuc) and glucose (Glc), which were confirmed by GC analysis of the trimethylsilyl-L-cysteine derivatives of the hydrolysate of **1** and the authentic sugars. Coupling constants of the anomeric proton signals suggested *β*-configuration of D-glucose, D-fucose and D-apiose, and *α*-configuration of L-arabinose, respectively. The *α*-configuration of the rhamnose unit was deduced from the absence of intraresidual NOESY correlations between H-1_{rha} and H-3_{rha}/H-5_{rha} [12]. Furthermore, the ^{13}C NMR spectra exhibited 55 carbon signals, of which the distinctive quaternary carbon signal at δ_C 112.3 (C-22) led to the hypothesis that **1** was a spirostanol saponin [13].

For the aglycone of **1**, the ^{1}H NMR spectrum (Table 1) showed three methyl protons at δ_H 0.96 (3H, s, Me-18), 1.43 (3H, s, Me-19) and 1.09 (3H, d, J = 6.9 Hz, Me-21), and two exo-methylene protons at (δ_H 5.22 (1H, br s, H-27a) and 5.11 (1H, br s, H-27b)), as well as one olefinic proton at δ_H 5.57 (1H, d, J = 5.4 Hz, H-6). In addition, three methyl groups at δ_C 17.3 (C-18), 15.6 (C-19), and 15.3 (C-21) were observed in the ^{13}C NMR spectra (Table 1). The presence of a terminal olefinic bond was deduced by a quaternary carbon signal at δ_C 144.4 (C-25), as well as a methylene carbon signal at δ_C 114.3, which exhibited correlations with two olefinic proton signals at δ_H 5.22 (H-27a) and 5.11 (H-27b) in the HSQC spectrum. HSQC spectrum also displayed the correlation from the olefinic proton at δ_H 5.57 (1H, d, J = 5.4 Hz, H-6) to δ_C 125.1 (C-6). ^{1}H-^{1}H COSY correlations from H-1/H_2-2/H-3/H_2-4, from H-6/H_2-7/H-8/H-9/H_2-11/H_2-12, from H-8/H-14/H_2-15/H-16/H-17/H-20/H_3-21, and from H-23/H-24, accompanied with HMBC correlations (Figure **2**) from H-3/C-2, C-4, and C-5, from H_3-19/C-1, C-5, C-9 and C-10, from H-6/ C-4, C-7, C-8 and C-10, from H_3-21/C-17, C-20 and C-22, from H_3-18/C-12, C-13, C-14 and C-17, from H-16/C-13, C-14, C-17, C-20 and C-22, from H-24/C-22, C-23, C-25 and C-26, from H_2-27/C-24, C-25 and C-26, and from H_2-26/C-22, C-24, C-25 and C-27 demonstrated a planar structure of the aglycone moiety as 1,3,23,24-tetraol-spirost-5,25(27)-diene. In addition, in the NOESY spectrum (Figure **2**) of **1**, the NOE correlations of H-1/H-3/H-9 and Me-19/H-2a/H-4a/H-8/Me-18, indicated *α*-axial configurations of H-1 and H-3, and *β*-orientation of Me-19, 1-OH and 3-OH; Furthermore, the configurations of C-23 and C-24 were determined to be *S* by a small coupling constant between H-23 and H-24 (J = 3.5 Hz) and the NOESY correlations of H-23/H-20, H-23/Me-21/H_{27b}, and H-24/H_{27a} [15-18]. Comparison of the ^{1}H and ^{13}C NMR spectroscopic data of the aglycone moiety of **1** with those of **3**, along with the above analysis, the structure of the aglycone of **1** was elucidated as (23S,24S)-1*β*, 3*β*,23,24-tetrahydroxy-spirosta-5,25(27)-diene.

Moreover, HMBC correlations of H-Api-1/C-Rha-3, H-Rha-1/C-Ara-2 and H-Ara-1/C-1 disclosed that the D-apiose unit was linked at C-3 of the L-rhamnose, L-rhamnose unit was linked at C-2 of the inner L-arabinose unit, then the L-arabinose unit was linked at C-1 of the aglycone. In addition, correlations of H-Glc-1/C-Fuc-4 and H-Fuc-1/C-24 disclosed that the terminal D-glucose unit was linked at C-4 of the inner D-fucose unit, then the D-fucose unit was linked at C-24 of the aglycone. Therefore, the structure of **1** was characterized as (23*S*,24*S*)-24-{[*O*-*β*-D-glucopyranosyl-

(1→4)-*β*-D-fucopyranosyl]oxy}-3*β*,23-dihydroxyspirosta-5,25(27)-diene-1*β*-yl*O*-*β*-D-apiofuranosyl-(1→3)-*O*-(*α*-L-rhamnopyranosyl)-(1→2)-*O*-*α*-L-arabinopyranoside.

Thibetanoside K (**2**) was obtained as a white amorphous powder. A [M - H]$^-$ peak at *m/z* 1087.4911 in the HR-ESI-MS indicated that the molecular formula was $C_{52}H_{80}O_{24}$. Comparison of the NMR data of **2** and **1** (Table 1), indicated almost similar NMR spectroscopic features, except an increase of the acetyl linked at C-4 of Rha and an absence of the terminal apiose unit in compound **2**. The proton and carbon NMR signals of [δ_H 4.68 (1H, m, H-Rha-3) and δ_C 80.6 (C-Rha-3)] and [δ_H 4.42 (1H, m, H-Rha-4) and δ_C 73.0 (C-Rha-4)] in **1**, were replaced by [δ_H 4.76 (1H, m, H-3), δ_C 70.5 (C-Rha-3)] and [δ_H 5.83 (1H, t, J =9.6 Hz, H-Rha-4) and δ_C 76.9 (C-Rha-4)] in **2**, which was supported by HSQC, HMBC and NOESY spectrums. The presence of an acetyl group in **2** was shown by the signals at δ_H 2.03 (3H, s) and δ_C 171.3 (C=O) and 21.5 (methyl). Moreover, HMBC correlations of H-Rha-1/C-Ara-2 and H-Ara-1/C-1 disclosed that the L-rhamnose unit was linked at C-2 of the inner L-arabinose unit, then the L-arabinose unit was linked at C-1 of the aglycone. In addition, correlations of H-Glc-1/C-Fuc-4 and H-Fuc-1/C-24 disclosed that the terminal D-glucose unit was linked at C-4 of the inner D-fucose unit, then the D-fucose unit was linked at C-24 of the aglycone. Similarly as compound **1**, the results of the acid hydrolysis procedure and subsequent GC analysis of the hydrolysatesand showed the structure of **2** was defined as (23*S*,24*S*)-24-{[*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-fucopyranosyl]oxy}-3*β*,23-dihydroxyspirosta-5,25(27)-diene-1*β*-yl*O*-(4-*O*-acetyl-*α*-L-rhamnopyranosyl)-(1→2)-*O*-*α*-L-arabinopyranoside.

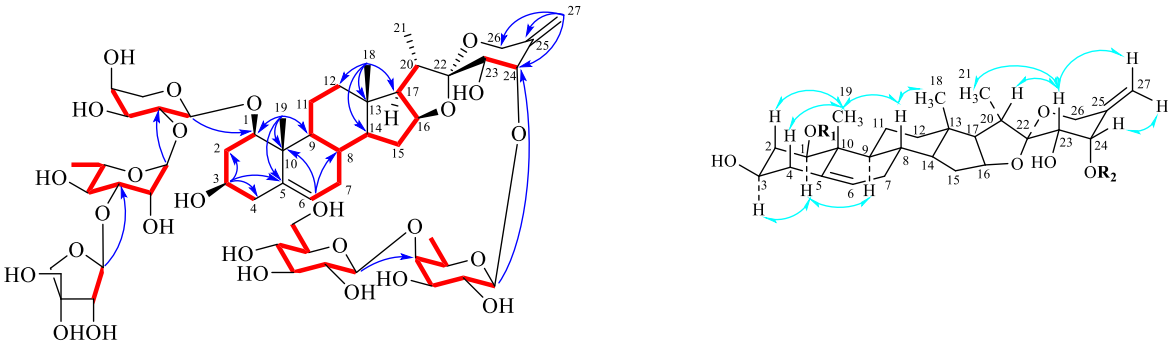


Figure 2. Key ^{1}H-^{1}H COSY, HMBC and NOESY correlations of compound **1**

Table 1. ^{1}H-NMR (400 MHz, in pyr-d_5) and ^{13}C-NMR (100 MHz, in pyr-d_5) spectral data of compounds **1** and **2**

No.	**1**		**2**	
	δC	δ_H (*J* in Hz)	δC	δ_H (*J* in Hz)
1	84.3	3.78 (dd, 11.8, 3.5)	84.1	3.80 (dd, 12.0, 4.6)
2	37.9	2.71 (m, H-2a) 2.37 (dd, 12.0, 12.0, H-2b)	38.0	2.73 (m, H-2a) 2.32 (dd, 13.4, 11.7, H-2b)
3	68.7	3.87, m	68.5	3.90, m
4	44.3	2.72 (m, H-4a) 2.58 (m, H-4b)	44.5	2.75 (m, H-4a) 2.67 (m, H-4b)
5	140.2	—	140.1	—
6	125.1	5.57 (d, 5.4)	125.3	5.64 (d, 4.7)
7	32.4	1.82 (m, H-7a) 1.48 (m, H-7b)	32.5	1.84 (m, H-7a) 1.53 (m, H-7b)
8	33.4	1.46, m	33.5	1.53, m
9	50.9	1.47, m	50.8	1.54, m
10	43.4	—	43.4	—
11	24.4	2.96 (m, H-11a) 1.60 (m, H-11b)	24.4	2.92 (m, H-11a) 1.57 (m, H-11b)
12	40.9	1.51 (m, H-12a) 1.27 (m, H-12b)	40.9	1.54 (m, H-12a) 1.26 (m, H-12b)
13	41.3	—	41.3	—
14	57.2	1.06, m	57.2	1.09, m
15	32.8	1.81 (m, H-15a) 1.37 (m, H-15b)	32.9	1.82 (m, H-15a) 1.37 (m, H-15b)
16	83.5	4.62, m	83.5	4.65, m
17	62.0	1.74, m	62.1	1.72 (dd, 7.2, 7.5)
18	17.3	0.96, s	17.3	1.02, s
19	15.6	1.43, s	15.4	1.41, s
20	37.9	2.89, m	38.0	2.92, m
21	15.3	1.09 (d, 6.9)	15.3	1.1 (d, 6.6)
22	112.3	—	112.3	—
23	70.8	3.98 (d, 3.5)	70.8	3.98 (d, 2.8)
24	82.8	4.79 (d, 3.5)	82.8	4.82 (d, 2.8)
25	144.4	—	144.4	—
26	62.0	4.83 (d, 10.9, H-26a) 4.01 (m, H-26b)	62.0	4.87 (d, 11.7, H-26a) 4.06 (m, H-26b)
27	114.3	5.22 (s, H-27a) 5.11 (s, H-27b)	114.3	5.23 (s, H-27a) 5.12 (s, H-27b)
1-*O*-Ara				
1	101.0	4.68 (d, 7.9)	100.9	4.7 (d, 7.9)
2	75.8	4.58, m	74.7	4.57, m

Table 1 continued..

3	76.3	4.17, m	76.7	4.17, m
4	70.6	4.19, m	70.8	4.19, m
5	67.9	4.20 (m, H-Ara-5a) 3.66 (d, 12.0, H-Ara-5b)	68.2	4.27 (m, H-Ara-5a) 3.68 (d, 12.3, H-Ara-5b)
Rha				
1	102.0	6.33, br s	101.4	6.43, br s
2	72.3	4.93, m	72.8	4.73, m
3	80.6	4.68, m	70.5	4.76, m
4	73.0	4.42, m	76.9	5.83 (t, 9.6)
5	70.0	4.86, m	67.1	4.92, m
6	19.5	1.71 (d, 6.1)	18.8	1.43 (d, 5.8)
CO**C**H$_3$	—	—	21.5	2.03, s
COCH$_3$	—	—	171.3	-
Api				
1	112.3	6.21 (d, 2.5)	—	—
2	78.2	4.84, m	—	—
3	80.6	-	—	—
4	75.6	4.65 (m, H-Api-4a) 4.32 (m, H-Api-4b)	—	—
5	66.1	4.19, m	—	—
24-*O*-Fuc				
1	106.6	5.16 (d, 7.8)	106.5	5.17 (d, 7.7)
2	74.2	4.44, m	74.2	4.44, m
3	76.0	4.08, m	76.0	4.08, m
4	83.8	4.10, m	83.8	4.11, m
5	71.2	3.72, m	71.3	3.74, m
6	18.0	1.54 (d, 6.3)	18.0	1.55 (d, 6.4)
Glc				
1	107.4	5.16 (d, 7.8)	107.4	5.15 (d, 7.7)
2	76.7	4.05, m	76.7	4.07, m
3	79.1	4.22, m	79.1	4.22, m
4	72.0	4.25, m	72.0	4.24, m
5	79.0	3.92, m	79.0	3.88, m
6	63.2	4.48 (m, H-Glc-6a) 4.39 (dd, 11.1, 5.2, H-Glc-6b)	63.3	4.47 (m, H-Glc-6a) 4.37 (dd, 11.6, 4.8, H-Glc-6b)

3.2. Cytotoxicity Assay

The cytotoxic activity assay toward three human tumor cell lines (HCT116, A549 and HepG2) were measured following the procedures that we reported previously [18-20], the details were listed in the Supporting Information.

Table 2. Cytotoxicity of compounds **1-5** (IC_{50} values expressed in μM)

Compounds	Cell lines		
	HCT116	A549	HepG2
1	>100	7.69 ± 1.13	8.32 ± 2.63
2	20.67 ± 1.06	>100	>100
3	>100	>100	>100
4	>100	>100	80.54 ± 1.62
5	>100	>100	>100
5-FU [a]	24.13 ± 2.44	18.92 ± 2.79	41.68 ± 1.58

[a] 5-fluorouracil (5-Fu) as positive control.

*3.3. Sugar Analysis of Compounds **1** and **2***

Sugar moieties of compounds **1** and **2** were confirmed by using the t_R of D-Glc (45.2 min), D-Fuc (35.2 min), D-Api (11.2 min), L-Ara (12.2 min), and L-Rha (14.5 min), following the procedures that we reported previously [21-23], the details were listed in the Supporting information file of the article.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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References

[1] X. M. Song and H. J. Liu (2011). Research and application of "Qi-Medicines" in Taibai Mountains, *People's Medical Publishing House*.

[2] J. Cakar, A. Haveric and S. Haveric (2014). Cytotoxic and genotoxic activity of some *Helleborus* species, *Nat. Prod. Res*. **28**, 883-887.

[3] Q. An, N. W. Lu and Y. M. Dong (2013). Chromatographic fingerprint coupled with hierarchical clustering analysis and principal component analysis for quality evaluation and original discrimination of rhizomes of *Helleborus thibetanus* Franch by HPLC-DAD, *Anal. Meth. UK.* **5**, 5775–5784.

[4] Z. Liu, Y. Liu, B. Xue, W. Chen and R. W. Jiang (2020). The co-occurrence of bufadienolides and podophyllotoxins from *Helleborus thibetanus*, *Biochem. Syst. Ecol*. **90**, 104042-104047.

[5] H. Zhang, Y. F. Su, F. Y. Yang, Z. Q. Zhao and X. M. Gao (2014). Six new steroidal saponins from *Helleborus thibetanus*, *Helv. Chim. Acta*. **97**, 1652-1665.

[6] W. Cheng, Y. F. Tan, H. Y. Tian, X. W. Gong, K. L. Chen and R. W. Jiang (2014). Two new bufadienolides from the rhizomes of *Helleborus thibetanus* with inhibitory activities against prostate cancer cells, *Nat. Prod. Res*. **28**, 901-908.

[7] H. Zhang, Y. F. Su and F. Y. Yang (2016). Four new minor spirostanol glycosides from *Helleborus thibetanus*, *Phytochem. Lett*. **18**, 213-219.

[8] Y. Z. Li, H. W. Zhang, H. Fan, X. F. Liang, B. Song, H. Chen, W. L. Huang, Z. G. Yue, X. M. Song and J. L. Liu (2019). Steroidal constituents from *Helleborus thibetanus* Franch and their cytotoxicities, *Chin. J. Nat. Med.* **17**, 778-784.

[9] D. D. Zhang, Z. L. Zhang, G. Q. Wu, Y. Sun, Y. Jiang, H. W. Zhang, W. Wang, X. M. Song and Y. Z. Li (2022). Iridoids and lignans from *Valeriana officinalis* L. and their cytotoxic activities, *Phytochem. Lett.* 2022, **49**, 125-130.

[10] Y. Z. Li, W. L. Huang, H. W. Zhang, Y. Jiang, C. Deng, W. Wang, J. L. Liu, X. M. Song and D. D. Zhang (2022). Steroidal components from the roots and rhizomes of *Helleborus thibetanus*, *Phytochem. Lett.* **50**, 31-35.

[11] H. Zhang, Y. F. Su, F. Y. Yang and X. M. Gao (2016). New minor spirostanol glycosides from *Helleborus thibetanus*, *Nat. Prod. Res*. **31**, 925-931.

[12] C. Bassarello, T. Muzashvili, A. Skhirtladze, E. Kemertelidze, C. Pizza and S. Piacente (2008). Steroidal glycosides from the underground parts of *Helleborus caucasicus*, *Phytochemistry* **69**, 1227-1233.

[13] Y. Mimaki, T. Inoue, M. Kuroda and Y. Sashida (1996). Steroidal saponins from *sansevieria trifasciata*, *Phytochemistry* **43**, 1325-1331.

[14] K. Watanabe, Y. Mimaki, H. Sakagami and Y. Sashida (2003). Bufadienolide and spirostanol glycosides from the rhizomes of *Helleborus orientalis*, *J. Nat. Prod*. **66**, 236-241.

[15] P. Y. Hayes, R. L. Ehmann, K. Penman, W. Kitching and J. J. Devoss (2009). Steroidal saponins from the roots of *Trillium erectum* (Beth root), *Phytochemistry* **70**, 105-113.

[16] Y. Mimaki and K. Watanabe (2008). Clintoniosides A-C, new polyhydroxylated spirostanol glycosides from the rhizomes of *Clintonia udensis*, *Helv. Chim. Acta*. **91**, 2097-2106.

[17] Y. Mimaki, K. Watanabe, C. Sakuma, H. Sakagami and Y. Sashida (2003). Novel polyoxygenated spirostanol glycosides from the rhizomes of *Helleborus orientalis*, *Helv. Chim. Acta*. **86**, 398-407.

[18] Y. Z. Li, H. W. Zhang, X. F. Liang, B. Song, X. D. Zheng, R. Wang, L. Liu, X. M. Song and J. L. Liu (2020). New cytotoxic bufadienolides from the roots and rhizomes of *Helleborus thibetanus* Franch, *Nat. Prod. Res.* **34**, 950–957.

[19] X. Liang, Y. Li, Y. Cui, Z.Liang, W. Huang, Y. Jiang, H. Zhang 1 and X. Song (2020). A new lignan glycoside from the roots of *Silene tatarinowii* Regel, *Rec. Nat. Prod.* **14**(**6**), 405-409.

[20] G. Wu, Z. Zhang, H. Fan , D. Zhang , W. Huang , H. Zhang , Y. Li and X. Song (2022).A new iridoid glycoside isolated from *Valeriana officinalis* L. *Rec. Nat. Prod.* **16**(**5**), 393-397.

[21] D. D. Zhang, D. Q. Ruan, J. Y. Li, Z. Q. Chen, W. L. Zhu, F. J. Guo, K. X. Chen, Y. M. Li and R. Wang (2020). Four undescribed sulfur-containing indole alkaloids with nitric oxide inhibitory activities from *Isatis tinctoria* L. roots, *Phytochemistry* **174**, 11237.

[22] Z. Chen, X. Xue, S. Zhang, R. Zhang, X. Zhang, Z. Guo and X. Zhang (2020). Steroidal components from the roots and rhizomes of *Smilacina henryi* and their cytotoxic activities, *Rec. Nat. Prod.* **14**(**3**), 225-230.

[23] D. D. Zhang, J. Y. Li, D. Q. Ruan, Z. Q. Chen, W. L. Zhu, Y. H. Shi, K. X. Chen, Y. M. Li and R. Wang (2019). Lignans from Isatis indigotica roots and their inhibitory effects on nitric oxide production, *Fitoterapia* **137**, 104189.