SUPPLEMENTARY MATERIAL

Three new steroidal saponins from *Helleborus thibetanus*

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Three new steroidal saponins including two spirostanol glycosides (**1-2**) and one furostanol glycoside 1-sulfate (**3**) were isolated from the dried roots and rhizomes of *Helleborus thibetanus*. Structures of the compounds were determined on the basis of extensive use of 1D and 2D NMR experiments, together with HR-ESI-MS and IR measurements as well as the results of acid hydrolysis. Compounds **1-2** represented steroidal saponins with an unusual substitution pattern, which possessed a double bond at C-25 and were glycosylated at 1-OH.

Keywords: *Helleborus*; Ranunculaceae; steroidal saponins; spirostanol; furostanol

Index:

Figures S1–S27.

For compound **1**:

^1H NMR spectrum of compound 1 in pyridine- d_5	Figure S1
^{13}C NMR spectrum of compound 1 in pyridine- d_5	Figure S2
DEPT spectrum of compound 1 in pyridine- d_5	Figure S3
HSQC spectrum of compound 1 in pyridine- d_5	Figure S4
^1H – ^1H COSY spectrum of compound 1 in pyridine- d_5	Figure S5
HMBC spectrum of compound 1 in pyridine- d_5	Figure S6
NOESY spectrum of compound 1 in pyridine- d_5	Figure S7
HRESIMS spectrum of compound 1	Figure S8
IR spectrum of compound 1	Figure S9

For compound **2**:

^1H NMR spectrum of compound 2 in pyridine- d_5	Figure S10
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^{13}C NMR spectrum of compound 2 in pyridine- d_5	Figure S11
DEPT spectrum of compound 2 in pyridine- d_5	Figure S12
HSQC spectrum of compound 2 in pyridine- d_5	Figure S13
^1H – ^1H COSY spectrum of compound 2 in pyridine- d_5	Figure S14
HMBC spectrum of compound 2 in pyridine- d_5	Figure S15
NOESY spectrum of compound 2 in pyridine- d_5	Figure S16
HRESIMS spectrum of compound 2	Figure S17
IR spectrum of compound 2	Figure S18

For compound **3**:

^1H NMR spectrum of compound 3 in pyridine- d_5	Figure S19
^{13}C NMR spectrum of compound 3 in pyridine- d_5	Figure S20
HSQC spectrum of compound 3 in pyridine- d_5	Figure S21
^1H – ^1H COSY spectrum of compound 3 in pyridine- d_5	Figure S22
HMBC spectrum of compound 3 in pyridine- d_5	Figure S23
NOESY spectrum of compound 3 in pyridine- d_5	Figure S24
HRESIMS spectrum of compound 3	Figure S25

IR spectrum of compound **3**.....Figure S26

Selected COSY, HMBC and NOESY correlations of compound **1**..... .Figure S27

Tables S1–S3

^1H (600 MHz), ^{13}C (150 MHz) NMR Spectroscopic Data of Compound **1** (pyridine- d_5)Table S1

^1H (600 MHz), ^{13}C (150 MHz) NMR Spectroscopic Data of Compound **2** (pyridine- d_5)Table S2

^1H (600 MHz), ^{13}C (150 MHz) NMR Spectroscopic Data of Compound **3** (pyridine- d_5)Table S3

Figure S1. ^1H NMR spectrum of compound **1** in pyridine- d_5

Figure S2. ^{13}C NMR spectrum of compound **1** in pyridine- d_5

Figure S3. DEPT spectrum of compound **1** in pyridine- d_5

Figure S4. HSQC spectrum of compound **1** in pyridine- d_5

Figure S5. ^1H - ^1H COSY spectrum of compound **1** in pyridine- d_5

Figure S6. HMBC spectrum of compound **1** in pyridine-*d*₅

Figure S7. NOESY spectrum of compound **1** in pyridine- d_5

Figure S8. HRESIMS spectrum of compound **1**

Figure S9. IR spectrum of compound **1**

Figure S10. ^1H NMR spectrum of compound **2** in pyridine- d_5

Figure S11. ^{13}C NMR spectrum of compound **2** in pyridine- d_5

Figure S12. DEPT spectrum of compound **2** in pyridine-*d*₅

Figure S13. HSQC spectrum of compound **2** in pyridine- d_5

Figure S14. ^1H - ^1H COSY spectrum of compound **2** in pyridine- d_5

Figure S15. HMBC spectrum of compound **2** in pyridine- d_5

Figure S16. NOESY spectrum of compound **2** in pyridine- d_5

Figure S17. HRESIMS spectrum of compound **2**

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name D:\Data\Suyanfang\DATAS\20150720\HTB30-8 NEG_P1-D-1_01_3444.d
Method tune_wide-NEG.m
Sample Name HTB30-8 NEG
Comment

Acquisition Date 7/20/2015 9:36:12 AM

Operator bruker

Instrument / Ser# microtof Q II 10387

Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	0.8 Bar
Focus	Active	Set Capillary	2500 V	Set Dry Heater	170 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Waste

Figure S18. IR spectrum of compound **2**

Figure S19. ^1H NMR spectrum of compound **3** in pyridine- d_5

Figure S20. ^{13}C NMR spectrum of compound **3** in pyridine- d_5

Figure S21. HSQC spectrum of compound **3** in pyridine- d_5

Figure S22. ^1H - ^1H COSY spectrum of compound **3** in pyridine- d_5

Figure S23. HMBC spectrum of compound **3** in pyridine-*d*₅

Figure S24. NOESY spectrum of compound **3** in pyridine- d_5

Figure S25. HRESIMS spectrum of compound 3

Figure S26. IR spectrum of compound 3

Figure S27. Selected COSY, HMBC and NOESY correlations of compound **1**

Table S1 ^1H (600 MHz), ^{13}C (150 MHz) NMR Spectroscopic Data of Compound **1** (pyridine- d_5)^a

No.	$\delta_{\text{H}}(\text{ppm})$	$\delta_{\text{C}}(\text{ppm})$	No.	$\delta_{\text{H}}(\text{ppm})$	$\delta_{\text{C}}(\text{ppm})$
1	3.79, m	83.7, CH	1-O-Ara		
2ax^b	2.31	37.4, CH ₂	1	4.67, d (7.5)	100.5, CH
2eq^c	2.71		2	4.59	72.6, CH
3	3.87	67.9, CH	3	4.07	85.0, CH
4ax	2.63	43.8, CH ₂	4	4.42	69.8, CH
4eq	2.72		5ax	3.67	67.2, CH ₂
5		139.4, C	5eq	4.19	
6	5.62, br d (5.0)	124.7, CH	Rha		
7a	1.74	31.8, CH ₂	1	6.46, br s	100.7, CH

7b	1.46		2	4.73,m	72.1, CH
8	1.47	33.0, CH	3	4.67	69.8, CH
9	1.49	50.1, CH	4	5.77, t-like (9.52)	76.3, CH
10		42.8, C	5	4.89	66.4, CH
11ax	1.50	23.8, CH ₂	6	1.36	18.3, CH ₃
11eq	2.85		CO<u>CH</u>₃	2.00,s	20.9, CH ₃
12ax	1.31	40.1, CH ₂	<u>C</u>OCH₃		170.7, C
12eq	1.75		Xyl		
13		40.8, C	1	4.91, d (7.0)	106.6, CH
14	1.1	56.7, CH	2	3.88	74.4, CH
15a	1.83	32.4, CH ₂	3	4.06	78.4, CH
15b	1.40		4	4.08	70.8, CH

16	4.66	83.3, CH	5ax	3.65	67.0, CH ₂
17	1.93	57.8, CH	5eq	4.26	
18	1.02	16.7, CH ₃	24-O-Fuc		
19	1.36, s	14.9, CH ₃	1	5.14, d (7.0)	105.9, CH
20	3.31	46.1, CH	2	4.42	73.7, CH
21a	4.17	62.2, CH ₂	3	4.00	76.1, CH
21b	3.97		4	4.04	83.2, CH
22		111.7, C	5	3.70	70.6, CH
23	4.47	71.8, CH	6	1.50, d (6.0)	17.4, CH ₃
24	4.75, m	82.2, CH	Glc		
25		143.7, C	1	5.13, d (7.0)	106.8, CH
26ax	4.00	61.4, CH ₂	2	4.07	75.4, CH

26eq	4.86, br d (11.5)		3	4.19	78.4, CH
27a	5.16	113.7, CH ₂	4	4.22	71.3, CH
27b	5.04, br s		5	3.86	78.5, CH
			6a	4.47, br d (11.0)	62.6, CH ₂
			6b	4.35, dd (11.0, 4.5)	

a Full assignments of the protons and carbons were accomplished by analysis of COSY, HSQC and HMBC spectra, and coupling pattern and coupling constants (J in Hz) are in parentheses. Overlapped signals were given without designating multiplicity.

b ax=axial.

c eq=equatorial

Table S2 ^1H (600 MHz), ^{13}C (150 MHz) NMR Spectroscopic Data of Compound **2** (pyridine- d_5)^a

No.	$\delta_{\text{H}}(\text{ppm})$	$\delta_{\text{C}}(\text{ppm})$	No.	$\delta_{\text{H}}(\text{ppm})$	$\delta_{\text{C}}(\text{ppm})$
1	3.78,m	83.6, CH	1-O-Ara		
2ax^b	2.35	37.3, CH ₂	1	4.68	100.4, CH
2eq^c	2.71		2	4.61	73.9, CH
3	3.84	68.1, CH	3	4.07	84.4, CH
4ax	2.56	43.7, CH ₂	4	4.39	69.6, CH
4eq	2.67		5ax	3.65	66.9, CH ₂
5		139.6, C	5eq	4.21	
6	5.54, br d (4.62)	124.5, CH	Rha		
7a	1.69	31.7, CH ₂	1	6.31, br s	101.6, CH

7b	1.44		2	4.75~4.78, m	72.4, CH
8	1.44	33.0, CH	3	4.58	72.4, CH
9	1.46	50.1, CH	4	4.26	74.1, CH
10		42.8, C	5	4.75~4.78, m	69.3, CH
11ax	1.53	23.8, CH ₂	6	1.66, d (6.1)	19.0, CH ₃
11eq	2.88		Xyl		
12ax	1.28	40.0, CH ₂	1	4.94, d (7.0)	106.4, CH
12eq	1.72		2	3.88	74.5, CH
13		40.8, C	3	4.07	78.1, CH
14	1.07	56.7, CH	4	4.09	70.8, CH
15a	1.80	32.4, CH ₂	5ax	3.65	66.9, CH ₂
15b	1.41		5eq	4.27	

16	4.65	83.3, CH	24-O-Fuc		
17	1.90	57.8, CH	1	5.13, d (7.0)	106.0, CH
18	1.00	16.7, CH ₃	2	4.43	73.6, CH
19	1.37, s	14.9, CH ₃	3	4.02	76.1, CH
20	3.30	46.1, CH			
21a	4.18	62.2, CH ₂	4	4.03	83.2, CH
21b	3.98		5	3.69	70.6, CH
22		111.5, C	6	1.50, d (6.0)	17.3, CH ₃
23	4.46	71.8, CH	Glc		
24	4.73, m	82.3, CH	1	5.13, d (7.0)	106.8, CH
25		143.7, C	2	4.07	75.4, CH
26ax	4.00	61.4, CH ₂	3	4.19	78.3, CH

26eq	4.86, br d (11.5)		4	4.24	71.3, CH
27a	5.15	113.6, CH ₂	5	3.87	78.5, CH
27b	5.03, br s		6a	4.47, m	62.6, CH ₂
			6b	4.35, dd (11.0, 4.5)	

a Full assignments of the protons and carbons were accomplished by analysis of COSY, HSQC and HMBC spectra, and coupling pattern and coupling constants (*J* in Hz) are in parentheses. Overlapped signals were given without designating multiplicity.

b ax=axial.

c eq=equatorial

Table S3 ^1H (500 MHz), ^{13}C (125 MHz) NMR Spectroscopic Data of Compound **3** (pyridine- d_5)^a

No.	$\delta_{\text{H}}(\text{ppm})$	$\delta_{\text{C}}(\text{ppm})$	No.	$\delta_{\text{H}}(\text{ppm})$	$\delta_{\text{C}}(\text{ppm})$
1	4.69, dd (12.0, 4.0)	85.1, CH	18	0.77, s	16.5, CH ₃
2ax^b	2.19	39.5, CH ₂	19	1.21, s	14.6, CH ₃
2eq^c	3.36, m		20	2.12	40.3, CH
3	3.84, m	67.7, CH	21	1.04, d (7.5)	16.1, CH ₃
4ax	2.49, m	43.4, CH ₂	22		112.3, C
4eq	2.58, m		23a	2.05	31.5, CH ₂
5		138.4, C	23b	1.91	
6	5.53, br d (5.5)	125.5, CH	24a	2.30	28.0, CH ₂
7a	1.79, m	31.7, CH ₂	24b	2.25	

7b	1.41		25		146.4, CH
8	1.44	32.9, CH	26a	4.53, br d (12.5)	71.9, CH ₂
9	1.60	49.7, CH	26b	4.27	
10		43.0, C	27a	5.27, br s	111.0, CH ₂
11ax	1.54	23.5, CH ₂	27b	4.98, br s	
11eq	2.84, m		22-OMe	3.18, s	47.2, CH ₃
12ax	1.24	40.5, CH ₂	26-O-Glc		
12eq	1.52		1'	4.82, d (7.5)	103.7, CH
13		40.4, C	2'	3.98, dd (8.0, 7.5)	74.9, CH
14	0.99	56.4, CH	3'	4.17	78.4, CH
15a	1.88	32.2, CH ₂	4'	4.15	71.6, CH
15b	1.31		5'	3.87	78.3, CH

16	4.35	81.3, CH	6'a	4.45, dd (12.0, 2.5)	62.7, CH ₂
17	1.62	64.2, CH	6'b	4.29	

a Full assignments of the protons and carbons were accomplished by analysis of COSY, HSQC and HMBC spectra, and coupling pattern and coupling constants (J in Hz) are in parentheses. Overlapped signals were given without designating multiplicity.

b ax=axial.

c eq=equatorial