Six New Steroidal Saponins from *Helleborus thibetanus*

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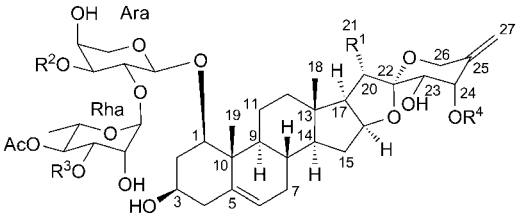
Six steroidal saponins, including five spirostanol glycosides, **1**–**5**, and one furostanol glycoside 1-sulfonate, **6**, previously unknown in nature, together with three known compounds, **7**–**9**, were isolated from dried roots and rhizomes of *Helleborus thibetanus*. Their structures were elucidated by extensive 1D- and 2D-NMR experiments, along with IR and HR-ESI-MS data, as well as the results of acid hydrolysis. Compounds **1**–**5** possessed a C(25)=C(27) bond and were glycosylated at HO–C(1), which was unusual in steroidal saponins.

Introduction. – The genus of *Helleborus* (Ranunculaceae) contains *ca.* 20 species, widely spread in Southeast Europe and West Asia. Previous phytochemical studies on *Helleborus* disclosed that steroids, including bufadienolides, phytoecdystones, and steroidal saponins [1–9], were the most characteristic components. *Helleborus thibetanus* FRANCH., an endemic plant of China, is mainly distributed in Sichuan, Gansu, and Shaanxi. The roots and rhizomes of *H. thibetanus*, locally called '*Xiao-Tao-Er-Qi*', are used for the treatment of traumatic injury, cystitis, and urethritis [2][3]. One spirostanol sulfonate, several bufadienolides, and phytoecdystones have been already isolated from *H. thibetanus* [2][3]. Herein, we report the isolation and structure elucidation of six new steroidal saponins, including five spirostanol glycosides, **1**–**5**, and one furostanol glycoside 1-sulfate, **6**, (*Fig. 1*) from *H. thibetanus*.



	R^1	R^2	R^3	R^4
1	$HOCH_2$	Xyl	Api	Glc(1→4)Fuc
2	Me	Xyl	Api	Glc(1→4)Fuc
3	Me	Xyl	H	Glc(1→4)Fuc
4	Me	H	Api	Glc(1→4)Fuc
5	Me	Xyl	H	Glc

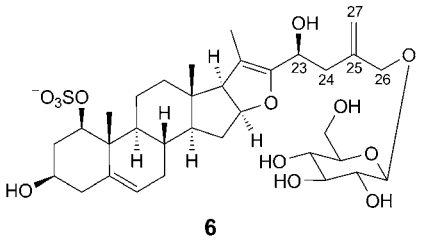


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Fig. 1. *Structures of compounds* **1**–**6**

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Results and Discussion. – Compound **1** was obtained as amorphous solid, and its molecular formula, $C_{62}H_{96}O_{33}$, was deduced from HR-ESI-MS (*m/z* 1391.5727 ($[M+Na]^+$)), as well as its ^{1}H- and ^{13}C-NMR data. The IR spectrum of **1** showed absorptions at 3397 and 1732 cm^{-1}, ascribed to the OH groups and a C=O group, respectively. Its ^{1}H- and ^{13}C-NMR spectra revealed the presence of two angular Me groups (δ(H) 1.02 (*s*), 1.35 (*s*); δ(C) 16.8, 15.0) and one acetal C-atom (δ(C) 111.7), evidencing that **1** had a spirostane skeleton. Full assignments of all individual H- and C-atoms (*Tables 1* and *2*) were ascertained by a combined analysis of ^{1}H- and ^{13}C-NMR, COSY, DEPT, HSQC, HMBC, TOCSY, and NOESY data. The location of a C(5)=C(6) bond was confirmed by the HSQC spectrum displaying correlations of the olefinic H-atom (δ(H) 5.61 (br. *d*, $J=5.5$)) with C(6) (δ(C) 124.9) and the HMBC spectrum displaying correlations of the olefinic H-atom signal at 5.61 with those at δ(C) 43.8 (C(4)), 33.1 (C(8)), and 42.9 (C(10)), along with HMBCs (*Fig. 2*) between the signals at δ(H) 1.35 (Me(19)) and δ(C) 139.5 (C(5)). The presence of a C(25)=C(27) bond was deduced from the HSQC spectrum displaying correlations of the olefinic H-atom signals at δ(H) 5.15 (br. *s*) and 5.03 (br. *s*) with that at δ(C) 113.8 (C(27)), and the HMBCs of olefinic H-atom signals at 5.15 (br. *s*) and 5.03 (br. *s*) with the C-atom resonances at 82.3 (C(24)), 143.7 (C(25)), and 61.5 (C(26)). The location of one OH group at C(21) was established on the basis of HMBCs between the H-atom signals at δ(H) 1.92 (H–C(17)) and 3.28 (H–C(20)) and the C-atom resonance at δ(C) 62.3 (C(21)). The ^{1}H-NMR signal at 3.76 was assigned to the H-atom attached to the O-bearing C(1)-atom by the HMBC between Me(19) signal (δ(H) 1.35) and that of C(1) (δ(C) 84.0), and its HSQC with C(1) (δ(C) 84.0). The H-atom signal at δ(H) 3.85 showed COSY (*Fig. 2*) correlations with those of H_{ax}–C(4)/H_{ax}–C(2), and was assigned as H–C(3). Additionally, the β-configurations of the O-bearing substituents at C(1) and C(3) were verified by the NOESY (*Fig. 3*) cross-peaks H–C(1) (δ(H) 3.76)/H–C(3) (3.85), Me(19) (1.35)/Me(18) (1.02), H_{ax}–C(2) (2.30), and H_{ax}–C(4) (2.56 – 2.63). Besides, the configurations at C(23) and C(24) were deduced as (*S*), based on the small coupling constant ($J=3.5$) between H–C(24) and H–C(23), which were furthermore confirmed by the NOESY cross-peaks H–C(20) (δ(H) 3.28)/H–C(23) (4.44), H–C(23) (4.44)/CH_2(21) (4.14, 3.95), CH_2(27) (5.03, 5.15), and CH_2(27) (5.03, 5.15)/H–C(24) (4.72) [6][8][10][11]. By comparison of the ^{1}H- and ^{13}C-NMR signals of the aglycone moiety of **1** with those of bethoside A [10] and clintonioside B [11], together with the above analysis, the structure of the aglycone of **1** was determined to be (23*S*,24*S*)-1β,3β,21,23,24-pentahydroxyspirosta-5,25(27)-diene.

The six anomeric H-atom signals at δ(H) 6.42 (br. *s*), 5.89 (*d*, $J=3.0$), 5.11 (*d*, $J=8.0$), 5.10 (*d*, $J=8.0$), 4.88 (*d*, $J=7.5$), and 4.60 (*d*, $J=7.5$), and the six C-atom signals at δ(C) 100.6, 100.7, 106.0, 106.7, 106.9, and 112.1 indicated that the sugar chains consisted of six sugar units, and two Me groups (δ(H) 1.34 (*d*, $J=5.5$); and δ(C) 18.3; and δ(H) 1.49 (*d*, $J=6.0$); δ(C) 17.4) suggested two of them were 6-deoxyhexose units. The Me signals at δ(H) 2.17 (*s*) and δ(C) 21.1, and the C=O signal at δ(C) 170.6 indicated an Ac group. Acid hydrolysis of **1** with 1M HCl in dioxane/H_2O 1:1, followed by TLC analysis, revealed the presence of apiose (Api), arabinose (Ara), rhamnose (Rha), xylose (Xyl), fucose (Fuc), and glucose (Glc) units. Comparison of the ^{1}H- and ^{13}C-NMR signals with those of spirostanol glycosides isolated from the rhizomes of *Helleborus orientalis* led to the conclusion that one glycosyl group at C(1) of **1** was the same as

Table 1. *^{1}H-NMR Data* (500 MHz) *of* **1**–**4** ((D$_5$)pyridine) *and* **5** (CD$_3$OD)[a]). δ in ppm, J in Hz.

Position	**1**	**2**	**3**	**4**	**5**
1	3.76 (*dd*, *J* = 12.0, 3.5)	3.77 (*dd*, *J* = 12.0, 4.0)	3.78	3.77 (*dd*, *J* = 12.0, 4.0)	3.38
2	2.30 (*dd*, *J* = 12.0, 11.5, H$_{ax}$)[b]), 2.68 (H$_{eq}$)[c])	2.26 (*dd*, *J* = 12.0, 11.5, H$_{ax}$)[b]), 2.65 (H$_{eq}$)[c])	2.28 (H$_{ax}$)[b]), 2.68 (*dd*, *J* = 12.0, 11.5, H$_{eq}$)[c])	2.26 (*dd*, *J* = 12.0, 11.5, H$_{ax}$)[b]), 2.65 (H$_{eq}$)[c])	1.67 (H$_{ax}$)[b]), 2.11 (H$_{eq}$)[c])
3	3.85	3.84	3.85	3.84	3.40
4	2.56–2.63 (*m*, H$_{ax}$)[b]), 2.70 (H$_{eq}$)[c])	2.57–2.69 (*m*, H$_{ax}$)[b]), 2.69 (H$_{eq}$)[c])	2.60 (H$_{ax}$)[b]), 2.68 (H$_{eq}$)[c])	2.60 (H$_{ax}$)[b]), 2.69 (H$_{eq}$)[c])	2.14–2.30 (*m*, H$_{ax}$)[b]), 2.20 (H$_{eq}$)[c])
6	5.61 (br. *d*, *J* = 5.5)	5.61 (br. *d*, *J* = 5.5)	5.54 (br. *d*, *J* = 5.0)	5.61 (br. *d*, *J* = 5.5)	5.57 (br. *d*, *J* = 5.5)
7	1.75 (H$_a$), 1.46 (H$_b$)	1.77 (H$_a$), 1.45 (H$_b$)	1.77 (H$_a$), 1.45 (H$_b$)	1.77 (H$_a$), 1.45 (H$_b$)	1.97 (H$_a$), 1.52 (H$_b$)
8	1.46	1.45	1.45	1.45	1.54
9	1.47	1.47	1.47	1.47	1.24
11	1.52 (H$_{ax}$)[b]), 2.76–2.92 (*m*, H$_{eq}$)[c])	1.52 (H$_{ax}$)[b]), 2.85 (H$_{eq}$)[c])	1.52 (H$_{ax}$)[b]), 2.85 (H$_{eq}$)[c])	1.52 (H$_{ax}$)[b]), 2.85 (H$_{eq}$)[c])	1.41 (H$_{ax}$)[b]), 2.52 (H$_{eq}$)[c])
12	1.74 (H$_{ax}$)[b]), 1.30 (H$_{eq}$)[c])	1.50 (H$_{ax}$)[b]), 1.24 (H$_{eq}$)[c])	1.51 (H$_{ax}$)[b]), 1.25 (H$_{eq}$)[c])	1.50 (H$_{ax}$)[b]), 1.14–1.29 (*m*, H$_{eq}$)[c])	1.23 (H$_{ax}$)[b]), 1.66 (H$_{eq}$)[c])
14	1.06–1.18 (*m*)	1.06	1.06	1.06	1.14
15	1.79–1.87 (*m*, H$_a$), 1.41 (H$_b$)	1.79 (H$_a$), 1.35 (H$_b$)	1.78 (H$_a$), 1.38 (H$_b$)	1.79 (H$_a$), 1.35 (H$_b$)	1.96 (H$_a$), 1.42 (H$_b$)
16	4.69	4.58	4.58	4.58	4.49
17	1.92 (*dd*, *J* = 7.0, 6.5)	1.70	1.68	1.72–1.85 (*m*)	1.68
18	1.02 (*s*)	0.96 (*s*)	0.94 (*s*)	0.96 (*s*)	0.87 (*s*)
19	1.35 (*s*)	1.37 (*s*)	1.35 (*s*)	1.37 (*s*)	1.09 (*s*)
20	3.28	2.85	2.85	2.85	2.56
21	4.14, 3.95	1.06 (*d*, *J* = 7.0)	1.05 (*d*, *J* = 6.5)	1.06 (*d*, *J* = 7.0)	0.91 (*d*, *J* = 7.0)
23	4.44	3.94	3.92	3.94	3.50
24	4.72 (br. *d*, *J* = 3.5)	4.76 (br. *d*, *J* = 4.0)	4.74	4.76 (br. *d*, *J* = 4.0)	4.34
26	3.98 (H$_{ax}$)[b]), 4.84 (br. *d*, *J* = 12.0, H$_{eq}$)[c])	3.98 (H$_{ax}$)[b]), 4.84 (br. *d*, *J* = 12.0, H$_{eq}$)[c])	3.97 (H$_{ax}$)[b]), 4.80 (br. *d*, *J* = 11.5, H$_{eq}$)[c])	3.98 (H$_{ax}$)[b]), 4.84 (br. *d*, *J* = 12.0, H$_{eq}$)[c])	3.72 (H$_{ax}$)[b]), 4.43 (br. *d*, *J* = 11.5, H$_{eq}$)[c])
27	5.15 (br. *s*, H$_a$), 5.03 (br. *s*, H$_b$)	5.19 (br. *s*, H$_a$), 5.07 (br. *s*, H$_b$)	5.11 (br. *s*, H$_a$), 5.00 (br. *s*, H$_b$)	5.19 (br. *s*, H$_a$), 5.07 (br. *s*, H$_b$)	5.11 (br. *s*, H$_a$), 5.00 (br. *s*, H$_b$)
1-*O*-Ara					
1	4.60 (*d*, *J* = 7.5)	4.62	4.62	4.62	4.30 (*d*, *J* = 7.5)
2	4.53 (*dd*, *J* = 9.5, 9.0)	4.49	4.56	4.49	3.84

Table 1 (cont.)

Position	**1**	**2**	**3**	**4**	**5**
3	3.98	4.00	4.03	4.08	3.73
4	4.37	4.11	4.38	4.11	3.97
5	3.64 (H_{ax})[b]), 4.22 (H_{eq})[c])	3.64 (H_{ax})[b]), 4.22 (H_{eq})[c])	3.64 (H_{ax})[b]), 4.22 (H_{eq})[c])	3.64 (br. *d*, *J* = 12.0, H_{ax})[b]), 4.22 (H_{eq})[c])	3.50 (H_{ax})[b]), 4.33 (*dd*, *J* = 12.0, 2.5, H_{eq})[c])
Rha					
1	6.42 (br. *s*)	6.32 (br. *s*)	6.41 (br. *s*)	6.32 (br. *s*)	5.43 (br. *s*)
2	4.86	4.87	4.69	4.85	3.89
3	4.68	4.70 (*dd*, *J* = 10.0, 3.0)	4.63	4.71 (*dd*, *J* = 10.0, 3.0)	3.85
4	5.86 (*t*-like, *J* = 10.0)	5.87	5.73 (*t*-like, *J* = 9.5)	5.89	4.91 (*dd*, *J* = 10.0, 9.5)
5	4.85	4.87	4.84	4.86	4.31
6	1.34 (*d*, *J* = 5.5)	1.39 (*d*, *J* = 6.0)	1.39 (*d*, *J* = 5.5)	1.41 (*d*, *J* = 6.0)	1.11 (*d*, *J* = 6.5)
Ac	2.17 (*s*)	2.19 (*s*)	2.00 (*s*)	2.17 (*s*)	2.08 (*s*)
Api					
1	5.89 (*d*, *J* = 3.0)	5.90		5.88 (*d*, *J* = 8.5)	
2	4.61	4.61		4.61	
4	4.20 (H_a), 4.48 (*d*, *J* = 9.5, H_b)	4.20 (H_a), 4.48 (*d*, *J* = 9.5, H_b)		4.24 (H_a), 4.52 (*d*, *J* = 9.5, H_b)	
5	3.99	3.99		4.00	
Xyl					
1	4.88 (*d*, *J* = 7.5)	4.89	4.91 (*d*, *J* = 7.0)		4.39 (*d*, *J* = 6.0)
2	3.84	3.84	3.85		3.27
3	4.04	4.06	4.03		3.28
4	4.04	4.05	4.04		3.50
5	3.63 (H_{ax})[b]), 4.23 (H_{eq})[c])	3.62 (H_{ax})[b]), 4.23 (H_{eq})[c])	3.63 (H_{ax})[b]), 4.23 (H_{eq})[c])		3.20 (H_{ax})[b]), 3.86 (H_{eq})[c])
24-*O*-Fuc					
1	5.11 (*d*, *J* = 8.0)	5.11 (*d*, *J* = 8.0)	5.10 (*d*, *J* = 7.0)	5.12 (*d*, *J* = 7.0)	
2	4.38	4.38	4.37	4.40 (*dd*, *J* = 9.0, 8.5)	
3	3.98	3.98	3.98	3.99	
4	4.01	4.02	4.02	4.02	
5	3.67	3.68	3.68	3.70 (*dq*, *J* = 6.5, 6.0)	

Table 1 (cont.)

Position	**1**	**2**	**3**	**4**	**5**
6	1.49 (*d*, *J* = 6.0)	1.50 (*d*, *J* = 6.5)	1.50 (*d*, *J* = 6.0)	1.52 (*d*, *J* = 6.5)	
Glc					
1	5.10 (*d*, *J* = 8.0)	5.10 (*d*, *J* = 7.5)	5.09 (*d*, *J* = 7.0)	5.11 (*d*, *J* = 7.0)	4.51 (*d*, *J* = 8.0)
2	4.04	4.02	4.04	4.00	3.29
3	4.16	4.16	4.15	4.16	3.36
4	4.19	4.19	4.18	4.20	3.30
5	3.85	3.83	3.85	3.85	3.18
6	4.43, 4.32 (*dd*, *J* = 12.0, 5.0)	4.44 (br. *d*, *J* = 11.5), 4.32 (*dd*, *J* = 11.5, 4.5)	4.43 (br. *d*, *J* = 11.0), 4.31 (*dd*, *J* = 11.0, 4.5)	4.44, 4.34 (*dd*, *J* = 12.0, 5.0)	3.75 (*dd*, *J* = 12.0, 2.5), 3.62 (*dd*, *J* = 12.0, 5.0)

[a]) Full assignments of the H-atoms were accomplished by analysis of DEPT, COSY, HSQC, HMBC, and TOCSY spectra, and multiplicities and coupling constants are given in parentheses. Overlapped signals were given without designating the multiplicity. [b]) ax, Axial. [c]) eq, Equatorial.

Table 2. *^{13}C-NMR Data* (125 MHz) *of* **1**–**4** ((D$_5$)pyridine) *and* **5** (CD$_3$OD)[a])

Position	**1**	**2**	**3**	**4**	**5**
1	84.0	84.2	83.9	83.8	85.5
2	37.8	37.9	37.5	37.8	38.1
3	68.0	68.0	68.0	68.0	69.7
4	43.8	43.8	43.9	43.9	44.09
5	139.5	139.5	139.4	139.5	140.1
6	124.9	124.9	124.8	124.8	126.8
7	31.9	31.9	31.9	32.0	33.4
8	33.1	33.0	32.9	33.0	34.7
9	50.3	50.3	50.3	50.4	52.1
10	42.9	42.9	42.8	42.9	44.14
11	23.9	23.9	23.9	23.9	25.5
12	40.2	40.4	40.4	40.4	42.1
13	40.8	40.8	40.7	40.8	42.3
14	56.8	56.7	56.7	56.7	58.6
15	32.5	32.3	32.3	32.4	33.7
16	83.5	83.3	82.9	83.0	84.6
17	57.9	61.50	61.48	61.6	63.2
18	16.8	16.8	16.8	16.8	17.9
19	15.0	15.0	15.0	15.0	16.1
20	46.2	37.4	37.4	37.4	38.9
21	62.3	14.8	14.7	14.8	15.4
22	111.7	111.8	111.7	111.8	113.1
23	71.9	70.3	70.3	70.2	71.4
24	82.3	82.2	82.2	82.3	84.1
25	143.7	143.9	143.8	143.9	145.1
26	61.5	61.46	61.44	61.5	63.3
27	113.8	113.7	113.7	113.7	115.0
1-*O*-Ara					
1	100.6	100.6	100.7	100.5	101.8
2	72.6	72.6	72.8	74.7	73.7
3	85.1	85.2	85.1	76.0	86.4
4	69.7	69.7	69.9	70.3	71.2
5	67.15	67.2	67.3	67.7	67.86
Rha					
1	100.7	100.8	100.8	100.9	101.5
2	71.49	71.49	72.2	71.5	72.9
3	77.9	77.9	69.8	78.0	70.7
4	74.49	74.5	76.4	74.4	76.9
5	66.7	66.7	66.5	66.8	67.83
6	18.3	18.4	19.0	18.3	19.0
Ac	21.1, 170.6	21.1, 170.6	21.0, 170.8	21.0, 170.6	21.8, 173.3
Api					
1	112.1	112.1		112.2	
2	77.7	77.7		77.9	
3	79.9	79.9		79.9	
4	74.9	74.9		75.0	
5	65.3	65.3		65.3	

Table 2 (cont.)

Position	**1**	**2**	**3**	**4**	**5**
Xyl					
1	106.7	106.7	106.6		107.1
2	74.53	74.6	74.5		75.5
3	78.4	78.4	78.4		78.7
4	70.9	70.9	70.7		71.7
5	67.06	67.1	67.1		67.6
24-*O*-Fuc					
1	106.0	106.0	105.9	106.0	
2	73.7	73.7	73.7	73.7	
3	76.2	76.2	76.2	76.2	
4	83.3	83.0	83.3	83.3	
5	70.7	70.7	70.7	70.8	
6	17.4	17.4	17.4	17.4	
Glc					
1	106.9	106.9	106.8	106.9	106.1
2	75.5	75.5	75.5	75.5	76.0
3	78.4	78.4	78.4	78.5	78.6
4	71.5	71.5	71.5	71.6	72.2
5	78.5	78.5	78.5	78.6	78.6
6	62.7	62.7	62.7	62.8	62.8

[a]) Full assignments of the C-atoms were accomplished by analysis of DEPT, COSY, HSQC, HMBC, and TOCSY spectra.

those of the reported compounds [6][8]. When the ^{13}C-NMR spectrum of lβ,3β-dihydroxyspirosta-5,25(27)-diene (neoruscogenin) [12] was compared with that of **1**, the signal due to C(1) was shifted downfield by 5.9 ppm, and was confirmed by the HMBCs between C(1) (δ(C) 84.0) of the aglycone and H–C(1) (δ(H) 4.60) of Ara. An unambiguous determination of the sequence and linkage sites was obtained from the HMBC spectrum which showed key correlations between C(2) (δ(C) 72.6) of Ara and H–C(1) (δ(H) 6.42) of Rha; C(3) (85.1) of Ara and H–C(1) (4.88) of Xyl; C(3) (77.9) of Rha and H–C(1) (5.89) of Api; and C=O of the Ac group (170.7) and H–C(4) (5.86)

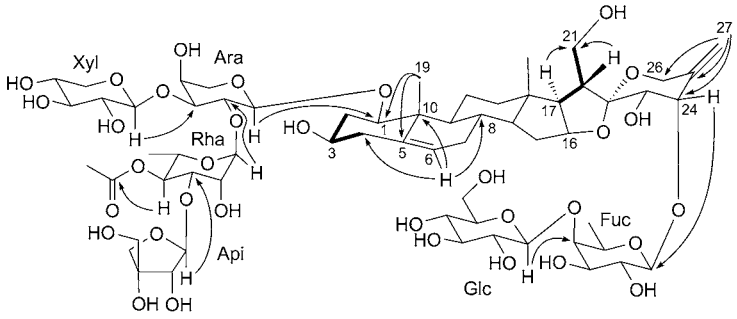

Fig. 2. *Selected ^{1}H,^{1}H-COSY correlations* (—) *and HMBCs* (H → C) *of* **1**

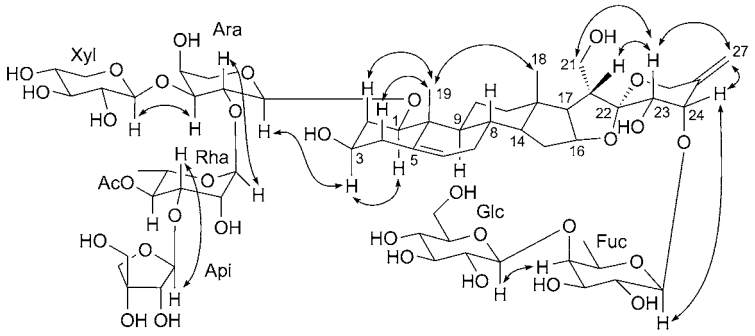

Fig. 3. *Selected NOESY* (H ↔ H) *correlations of* **1**

of Rha; the deduction was supported by the NOESY plot, which showed the cross-peaks between H–C(1) (δ(H) 3.76) of the aglycone and H–C(1) (4.60) of Ara, between H–C(2) (4.53) of Ara and H–C(1) (6.42) of Rha, between H–C(3) (3.98) of Ara and H–C(1) (4.88) of Xyl, between H–C(3) (4.68) of Rha and H–C(1) (5.89) of Api. The linkage of the other sugar chains at C(24) (δ(C) 82.3) of **1** was indicated by a downfield shift of 8.2 ppm of the signal at δ(C) 74.1 (C(24)) compared with clintonioside B [11], and their ^{1}H- and ^{13}C-NMR signals were identical to those of hellebosaponin B from *H. orientalis* [8]; moreover, the linkage was further confirmed by HMBCs between H–C(24) (δ(H) 4.72) of the aglycone and C(1) (δ(C) 106.0) of Fuc, and between H–C(1) (5.10) of Glc and C(4) (83.3) of Fuc, together with the NOESY cross-peaks between H–C(24) of the aglycone and H–C(1) (5.11) of Fuc, between H–C(4) (4.01) of Fuc and H–C(1) (5.10) of Glc. Accordingly, the structure of the new spirostanol glycoside **1** was fully established as (23*S*,24*S*)-21-(hydroxymethyl)-24-{[*O*-β-D-glucopyranosyl-(1 → 4)-β-D-fucopyranosyl]oxy}-3β,23-dihydroxyspirosta-5,25(27)-dien-1β-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.

Compound **2** was obtained as amorphous solid. On the basis of HR-ESI-MS (1375.5771 ([M + Na]$^+$)), and ^{13}C-NMR and DEPT data, its molecular formula was deduced as $C_{62}H_{96}O_{32}$, which displayed one O-atom less than **1**. The assignments of **2** (*Tables 1* and *2*) were achieved by a comprehensive analysis of ^{1}H-, ^{13}C-NMR, DEPT, COSY, HSQC, TOCSY, NOESY, and HMBC spectra. The ^{1}H- and ^{13}C-NMR spectra of **2** and **1** suggested that they possessed similar structures, and these data implied that one more Me group (δ(H) 1.06 (*d*, J = 7.0)) was present in **2**. The HSQC spectrum of **2** displayed correlation to a C-atom signal at δ(C) 14.8, suggesting the presence of a Me group in **2** instead of a CH_2OH group in **1** at C(21) [6][11][13]. The ^{1}H- and ^{13}C-NMR signals of the sugar units of **2** were identical to those of **1**, suggesting the same sugar chains. Acid hydrolysis of **2** with 1M HCl in dioxane/H_2O 1:1 gave apiose, arabinose, rhamnose, xylose, fucose, and glucose. Thus, **2** was identified as (23*S*,24*S*)-24-{[*O*-β-D-glucopyranosyl-(1 → 4)-β-D-fucopyranosyl]oxy}-3β,23-dihydroxyspirosta-5,25(27)-dien-1β-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.

Compound **3** was isolated as amorphous solid. Its molecular formula, $C_{57}H_{88}O_{28}$, was deduced from HR-ESI-MS (1243.5341 ($[M+Na]^+$)), as well as from its ^{13}C-NMR and DEPT spectra, indicating one less pentose (132 amu) than the molecular formula of **2** ($C_{62}H_{96}O_{32}$). The assignments of **3** (*Tables 1* and *2*) were established by a comprehensive analysis of ^{1}H-, ^{13}C-NMR, DEPT, COSY, HSQC, TOCSY, NOESY, and HMBC spectra, and the ^{1}H- and ^{13}C-NMR spectra of **3** were similar to those of **2**, but **3** lacked signals for the terminal β-D-apiose unit, and C(3) signal (δ(C) 69.8) of Rha in **3** also showed an upfield shift of 8.9 ppm compared to that of C(3) (77.9) of Rha in **2**, suggesting there was no β-D-apiose linked at C(3) of Rha in **3**. The resonances of the 4-*O*-acetyl-α-L-rhamnopyranosyl group, including six C-atom signals at δ(C) 100.8, 72.2, 69.8, 76.4, 66.5, and 19.0, together with one anomeric H-atom signal at δ(H) 6.41 (br. *s*) and a Me signal at 1.39 (*d*, $J=5.5$), and the Me signal at δ(H) 2.00 (*s*) and δ(C) 21.0, and C=O signal at 170.8 of the Ac group indicated the presence of a terminal 4-*O*-acetyl-α-L-rhamnopyranosyl moiety [13]. Acid hydrolysis of **3** with 1M HCl in dioxane/H_2O 1:1 gave arabinose, rhamnose, xylose, fucose, and glucose, further verifying the absence of an apiose. By a detailed analysis of ^{1}H-, ^{13}C-NMR, DEPT, COSY, HSQC, TOCSY, NOESY, and HMBC spectra, **3** was deduced as (23*S*,24*S*)-24-{[*O*-β-D-glucopyranosyl-(1 → 4)-β-D-fucopyranosyl]oxy}-3β,23-dihydroxyspirosta-5,25(27)-dien-1β-yl *O*-(4-*O*-acetyl-α-L-rhamnopyranosyl)-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-α-L-arabinopyranoside.

Compound **4** was isolated as amorphous solid. Its molecular formula was determined as $C_{57}H_{88}O_{28}$, based on HR-ESI-MS (1243.5362 ($[M+Na]^+$)), as well as ^{13}C-NMR and DEPT data, suggesting the loss of a pentose (132 amu) compared to the molecular formula of **2** ($C_{62}H_{96}O_{32}$). The assignments of **4** (*Tables 1* and *2*) were achieved by a comprehensive analysis of ^{1}H- and ^{13}C-NMR, DEPT, COSY, HSQC, TOCSY, NOESY, and HMBC spectra, and its ^{13}C-NMR spectra exhibited similar signals to those of **2**, with the difference being the absence of the C-atom signals of a terminal β-D-xylosyl in **4**. When the ^{13}C-NMR spectrum of Ara in **2** was compared with that of Ara in **4**, the signal due to C(3) of Ara was shifted upfield by 9.2 ppm, indicating no terminal β-D-xylosyl was linked at C(3) of Ara in **4**. Additionally, the five C-atom signals of the arabinosyl in **4** at δ(C) 100.5, 74.7, 76.0, 70.3, and 67.7 evidenced that the α-L-arabinopyranosyl unit was only substituted at C(2) [14]. Acid hydrolysis of **4** with 1M HCl in dioxane/H_2O 1:1 gave apiose, arabinose, rhamnose, fucose, and glucose without xylose. By an extensive use of ^{1}H-, ^{13}C-NMR, DEPT, COSY, HSQC, TOCSY, NOESY, and HMBC data, **4** was identified as (23*S*,24*S*)-24-{[*O*-β-D-glucopyranosyl-(1 → 4)-β-D-fucopyranosyl]oxy}-3β,23-dihydroxyspirosta-5,25(27)-dien-1β-yl *O*-β-D-apiofuranosyl-(1 → 3)-*O*-(4-*O*-acetyl-α-L-rhamnopyranosyl)-(1 → 2)-*O*-α-L-arabinopyranoside.

The molecular formula of **5**, an amorphous solid, was established as $C_{51}H_{78}O_{24}$ as deduced from the HR-ESI-MS (1097.4781 ($[M+Na]^+$)), as well as ^{13}C-NMR and DEPT data, suggesting the loss of a deoxyhexose (146 amu) compared to the molecular formula of **3**. The spectroscopic data for **5** showed it was a spirostanol steroid closely related to **3**, except for the absence of one monosaccharide constituent; moreover, its ^{13}C-NMR spectra exhibited six C-atom signals at δ(C) 106.1, 76.0, 78.6, 72.2, 78.6, and 62.8, which were assigned to a terminal β-D-glucopyranosyl unit [4][7][9]. The correlation of the H-atom signal at δ(H) 4.51 (*d*, $J=8.0$) with the C-atom signal at δ(C)

106.1 of **5** in the HSQC spectrum allowed us to ascribe the anomeric H-atom signal at 4.51 ($d, J = 8.0$) to C(1) of Glc. The HMBC of H–C(1) of Glc (δ(H) 4.51) with C(24) of aglycone (δ(C) 84.1), along with the NOESY cross-peak between H–C(24) (δ(H) 4.34) and H–C(1) of Glc (δ(H) 4.51), evidenced that the terminal β-D-glucopyranosyl unit was directly attached to C(24) in **5**. Acid hydrolysis of **5** with 1M HCl in dioxane/H_2O 1:1 gave arabinose, xylose, rhamnose, and glucose, confirming the absence of the β-D-fucose. Eventually, **5** was identified as (23*S*,24*S*)-24-[(*O*-β-D-glucopyranosyl)oxy]-3β,23-dihydroxyspirosta-5,25(27)-dien-1β-yl *O*-(4-*O*-acetyl-α-L-rhamnopyranosyl)-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-α-L-arabinopyranoside.

Compound **6** was isolated as white amorphous solid. Its molecular formula, $C_{33}H_{49}O_{13}S$, was deduced from HR-ESI-MS (685.2897 (M^-)), as well as from its ^{13}C-NMR spectrum. The presence of the OSO_3^- group was further confirmed by a series of characteristic strong absorption bands at 1246, 1213, 1051, and 957 cm^{-1} in its IR (KBr) spectrum. Full assignments of H- and C-atoms (*Table 3*) of **6** were accomplished by analysis of ^{1}H-, ^{13}C-NMR, COSY, HSQC, HMBC, and NOESY spectra. The ^{1}H-NMR spectrum of **6** showed signals for three Me groups (δ(H) 0.68 (*s*), 1.08 (*s*), and 1.65 (*s*)), two CH signals (3.39–3.48 (*m*) and 4.60 (*dd*, $J = 7.5, 7.0$)), indicating the presence of secondary OH substituents, two CH_2 signals (4.31 and 4.14 (br. *d*)), ascribable to a primary OH substituent, three olefinic H-atom signals (5.61 (br. *d*, $J = 5.5$), 5.12 (br. *s*), 4.99 (br. *s*)), along with an anomeric H-atom signal at 4.30 (*d*, $J = 7.5$). The ^{13}C-NMR spectrum for the aglycone moiety displayed signals ascribable to two secondary OH functions at δ(C) 69.2 and 65.3, respectively, and to one primary OH

Table 3. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; (D_5)pyridine) *of* **6**[a]). δ in ppm, J in Hz.

Position	δ(H)	δ(C)	Position	δ(H)	δ(C)
1	4.04 (*dd*, $J = 12.5, 5.0$)	86.6	19	1.08 (*s*)	15.3
2	1.68 (H_{ax})[b]), 2.54 (H_{eq})[c])	39.4	20		108.3
3	3.39–3.48 (*m*)	69.2	21	1.65 (*s*)	12.2
4	4.22	43.5	22		153.4
5		139.1	23	4.60 (*dd*, $J = 7.5, 7.0$)	65.3
6	5.61 (br. *d*, $J = 5.5$)	127.3	24	2.53 (H_a), 2.36 (*dd*, $J = 14.0, 7.0$ H_b)	39.9
7	1.98 (H_a), 1.55 (H_b)	33.3	25		148.3
8	1.53	34.2	26	4.31 (H_a), 4.14 (br. *d*, $J = 12.5$, H_b)	73.2
9	1.34	51.3	27	5.12 (br. *s*, H_a), 4.99 (br. *s*, H_b)	116.3
10		44.2			
11	1.50 (H_{ax})[b]), 2.34 (H_{eq})[c])	24.9	26-*O*-Glc		
12	1.34 (H_{ax})[b]), 1.73 (H_{eq})[c])	41.6	1′	4.30 (*d*, $J = 7.5$)	103.5
13		44.4	2′	3.23	75.5
14	1.05	56.6	3′	3.36	78.5
15	2.16 (H_a), 1.41 (H_b)	35.9	4′	3.30	72.1
16	4.76	86.2	5′	3.27	78.3
17	2.52	66.3	6′	3.86 (*dd*, $J = 12.0, 2.5$, H_a),	63.2
18	0.68 (*s*)	15.5		3.68 (*dd*, $J = 12.0, 5.0$, H_b)	

[a]) Full assignments of the H- and C-atoms were accomplished by analysis of COSY, HSQC, and HMBC spectra, and multiplicities and coupling constants are given in parentheses. Overlapped signals were given without designating the multiplicity. [b]) ax, Axial. [c]) eq, Equatorial.

function at 73.2, suggesting the occurrence of a glycosidic furostanol skeleton. When the spectra of **6** were compared with those of the known compound spirost-5,25(27)-diene-1β,3β-diol 1-sulfonate [3], the signals of ring *A* were similar, indicating presence of the OSO_3^- group at C(1). Comparison with the NMR spectra of the known compound caucasicoside A [4], with differences being only in the chemical shifts corresponding to positions 11, 22, 23, and 24 (**6**: δ(C) 24.9 (C(11))/δ(H) 1.50 (H_{ax}–C(11)), 2.34 (H_{eq}–C(11)); δ(C) 153.4 (C(22)); δ(C) 65.3 (C(23))/δ(H) 4.60 (*dd*, $J = 7.5$, 7.0, H–C(23)); δ(C) 39.9 (C(24))/δ(H) 2.53 (H_a–C(24)), 2.36 (H_b–C(24)); caucasicoside A: δ(C) 67.1 (C(11))/δ(H) 4.0 (H–C(11)); δ(C) 152.6 (C(22)); δ(C) 24.7 (C(23))/δ(H) 2.32 (CH_2(23)); δ(C) 31.1 (C(24))/δ(H) 2.29 (CH_2(24))), indicated that an OH moiety was present at C(23) in **6** rather than at C(11) (δ(C) 67.1) as in caucasicoside A. The sole correlation between the signals at δ(C) 65.3 (C(23)) and δ(H) 4.60 (*dd*, $J = 7.5$, 7.0, H–C(23)) in the HSQC spectrum verified the OH position at C(23). Besides, the configuration at C(23) was assumed to be (*S*) based on biogenetic considerations [4][6][8][15][16]. The sugar unit in **6** was identified as glucose located at C(26) on the basis of the HMBC (*Fig. 4*) between the signal at δ(H) 4.30 (H–C(1) of Glc) and the C-atom resonance at δ(C) 73.2 (C(26)). Acid hydrolysis of **6** with 1M HCl in dioxane/H_2O 1:1, followed by TLC analysis indicated the presence of glucose. Based on all this evidences, **6** was identified as (23*S*)-26-*O*-β-D-glucopyranosyl-3β,23-dihydroxyfurosta-5,20(22),25(27)-trien-1β-yl sulfonate.

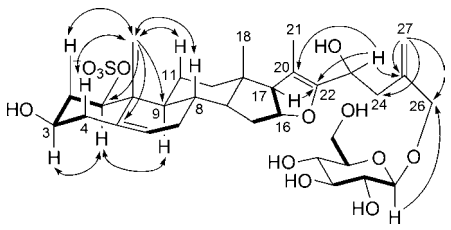

Fig. 4. *Selected $^1H,^1H$-COSY* (—) *and NOESY* (H ↔ H) *correlations, and HMBCs* (H → C) *of* **6**

According to previous investigations, steroidal saponins with a C(5)=C(6) or a C(9)=C(11) bond are common in plants. Also, in most cases the sugar chains are located at C(3). On the other hand, the presence of a C(25)=C(27) bond and a glycosyloxy moiety at C(1) found in spirostanol glycosides **1**–**5** is rather unusual.

In addition to the six new steroidal saponins, **1**–**6**, three previously known compounds, **7**–**9**, were also isolated and identified by comparison with published spectroscopic and physical data as (23*S*,24*S*)-24-[(*O*-β-D-fucopyranosyl)oxy]-3β,23-dihydroxyspirosta-5,25(27)-dien-1β-yl *O*-(4-*O*-acetyl-α-L-rhamnopyranosyl)-(1 → 2)-*O*-[β-D-xylopyranosyl- (1 → 3)]-α-l-arabinopyranoside (**7**) [13], (25*S*)-22α,25-epoxy-26-[(*O*-β-D-glucopyranosyl)oxy]-3β-hydroxyfurosta-5-en-1β-yl *O*-α-L-arabinopyranoside (**8**) [5], and (25*R*)-26-[(*O*-β-D-glucopyranosyl)oxy]-furosta-5,20(22)-dien-3β-yl *O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 4)]-β-D-galactopyranoside (**9**) [17].

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Experimental Part

General. All solvents used were of anal. grade (*Tianjin Jiangtian Chemical Technology Co., Ltd.*). TLC: Precoated silica gel GF_{254} plates (SiO_2; *Qingdao Haiyang Chemical Co., Ltd.*); visualized by UV light or by spraying with 5% aq. H_2SO_4 soln. in 95% EtOH, followed by heating. Column chromatography (CC): SiO_2 (100–200 and 200–300 mesh; *Qingdao Haiyang Chemical Co., Ltd.*), *D101* macroporous resin (*Tianjin Haiguang Chemical Co., Ltd.*), *ODS* SiO_2 (40–63 μm, *LiChroprep RP-18*, *Merck KGaA*), and *Sephadex LH-20* (*Amersham Pharmacia Biotech AB*). Prep. HPLC: *Agilent ZORBAX SB-C18 ODS* columns (21.2 mm × 250 mm, 7 μm). Optical rotations: *Rudolph Research Analytical Autopol II automatic* polarimeter. IR Spectra: *Bruker Tensor 27* spectrometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker AVANCE DRX*-500 spectrometer and *Varian INOVA* 500 MHz spectrometer; δ in ppm rel. to Me_4Si as internal standard, J in Hz. HR-MS: *Varian IonSpec FT 7.0T* mass spectrometer; in m/z.

Plant Material. The roots and rhizomes of *H. thibetanus* were collected from Mei County, Shaanxi Province, P. R. China, in September 2007, and were authenticated by Prof. *Zhen-Hai Wu*. A voucher specimen (No. S200609002) was deposited with the School of Pharmaceutical Science and Technology, Tianjin University, Tianjin 300072, P. R. China.

Extraction and Isolation. Dried roots and rhizomes of *H. thibetanus* (8.0 kg) were pulverized and heated under reflux with 95% EtOH (2 × 6 l) and then with 60% EtOH (1 × 6 l). The combined extracts were concentrated to give a residue (*ca.* 1.5 kg), which was suspended in H_2O to a final volume of 5 l and then partitioned sequentially with petroleum ether (PE; 60–90°), $CHCl_3$, AcOEt, and BuOH.

The BuOH extract (934 g) was submitted to CC (*D101* macroporous resin; EtOH/H_2O 0:100, 30:70, 50:50, 70:30, and 95:5) to give five fractions. *Fr. 1* (378 g), eluted with 30% EtOH, was partitioned into 126 fractions by CC (SiO_2, AcOEt/MeOH 90:10 → 60:40). *Frs. 1.108–1.122* (42 g) were further successively subjected to CC (SiO_2; $CHCl_3$/MeOH/H_2O 74:26:5; SiO_2, AcOEt/MeOH/H_2O 75:25:10 → 60:40:20; *Sephadex LH-20*, MeOH; *RP-C_{18}*, MeOH/H_2O 1:1), and prep. HPLC (MeOH/H_2O 42:58) to furnish **1** (189 mg). *Frs. 1.76–1.93* (50 g) were submitted to CC (SiO_2; $CHCl_3$/MeOH 80:20 → 60:40) to give 54 subfractions. *Subfrs. 42–45* (2 g) were applied to three chromatography steps: *i*) CC (*Sephadex LH-20*; MeOH); *ii*) CC (*RP-C_{18}*; MeOH/H_2O 3:7); *iii*) prep. HPLC (MeOH/H_2O 42:58) to yield **6** (80 mg). *Subfrs. 23–26* (6 g) were separated by CC (SiO_2; AcOEt/MeOH 80:20 → 70:30; *RP-C_{18}*; MeOH/H_2O 47:53), and prep. HPLC (MeOH/H_2O 44:56) to afford **2** (100 mg). *Subfrs. 17–19* (9.1 g) were purified by repeated CC (SiO_2; $CHCl_3$/MeOH 83:17 → 70:30; *RP-C_{18}*; MeOH/H_2O 1:1; *RP-C_{18}*; MeCN/H_2O 19:81), and prep. HPLC (MeOH/H_2O 47:53) to provide **3** (234 mg) and **4** (28 mg). *Subfrs. 14–16* (5 g) were submitted to CC (SiO_2, $CHCl_3$/MeOH/H_2O 74:26:5; SiO_2, AcOEt/MeOH 88:12; *Sephadex LH-20*, MeOH; and *RP-C_{18}*, MeOH/H_2O 1:1) to give **5** (50 mg).

CC (SiO_2; AcOEt/MeOH 90:10 → 60:40) of *Fr. 2* (110 g), eluted with 50% EtOH, gave 78 fractions. *Frs. 2.32–2.51* (22 g) were separated by CC (SiO_2; $CHCl_3$/MeOH) to give 44 subfractions. *Subfrs. 13–18* (3.3 g) were subjected to CC (*RP-C_{18}*; MeOH/H_2O 3:7), followed by CC (*Sephadex LH-20*; MeOH), to give **7** (274 mg). *Subfrs. 29–38* (4.9 g) were separated by CC (*RP-C_{18}*; MeOH/H_2O 3:7; and *Sephadex LH-20*; MeOH), along with washing with MeOH, to obtain **8** (45 mg). *Subfrs. 43* and *44* (2 g) were then subjected to two chromatography steps: *i*) CC (*RP-C_{18}*; MeOH/H_2O 3:7); *ii*) CC (*Sephadex LH-20*; MeOH) to afford **9** (60 mg).

*(1β,3β,23*S*,24*S*)-1-({[β*-D*-Apiofuranosyl-(1 → 3)-4*-O*-acetyl-6-deoxy-α*-L*-mannopyranosyl-(1 → 2)-[β*-D*-xylopyranosyl-(1 → 3)]-α*-L*-arabinopyranosyl]oxy}methyl)-3,21,23-trihydroxyspirosta-5,25(27)-dien-24-yl 6-Deoxy-4*-O*-β*-D*-glucopyranosyl-β*-D*-galactopyranoside* (**1**). White amorphous solid. $[\alpha]_D^{25} = -53.8$ ($c = 1.08$, pyridine). IR (KBr): 3397, 2906, 1732, 1642, 1452, 1379, 1254, 1043, 894, 783, 615. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 1391.5727 ($[M+Na]^+$, $C_{62}H_{96}NaO_{33}^+$; calc. 1391.5726).

*(1β,3β,23*S*,24*S*)-1-({[β*-D*-Apiofuranosyl-(1 → 3)-4*-O*-acetyl-6-deoxy-α*-L*-mannopyranosyl-(1 → 2)-[β*-D*-xylopyranosyl-(1 → 3)]-α*-L*-arabinopyranosyl]oxy}methyl)-3,23-dihydroxyspirosta-5,25(27)-dien-*

24-yl 6-Deoxy-4-O-*β*-D-*glucopyranosyl-β*-D-*galactopyranoside* (**2**). White amorphous solid. $[\alpha]_D^{25} = -47.6$ ($c = 1.08$, pyridine). IR (KBr): 3421, 2976, 2907, 1733, 1642, 1454, 1379, 1252, 1045, 895, 669. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 1375.5771 ($[M+Na]^+$, $C_{62}H_{96}NaO_{32}^+$; calc. 1375.5771).

*(1β,3β,23*S*,24*S*)-1-({[4-*O*-Acetyl-6-deoxy-α-*L*-mannopyranosyl-(1 → 2)-[β-*D*-xylopyranosyl-(1 → 3)]-α-*L*-arabinopyranosyl]oxy}methyl)-3,23-dihydroxyspirosta-5,25(27)-dien-24-yl 6-Deoxy-4*-O-*β*-D-*glucopyranosyl-β*-D-*galactopyranoside* (**3**). White amorphous solid. $[\alpha]_D^{25} = -56.9$ ($c = 1.08$, pyridine). IR (KBr): 3463, 2894, 1730, 1656, 1455, 1380, 1259, 1043, 782, 632. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 1243.5341 ($[M+Na]^+$, $C_{57}H_{88}NaO_{28}^+$; calc. 1243.5354).

*(1β,3β,23*S*,24*S*)-1-({[β-*D*-Apiofuranosyl-(1 → 3)-4*-O-*acetyl-6-deoxy-α-*L*-mannopyranosyl-(1 → 2)-α-*L*-arabinopyranosyl]oxy}methyl)-3,23-dihydroxyspirosta-5,25(27)-dien-24-yl 6-Deoxy-4*-O-*β*-D-*glucopyranosyl-β*-D-*galactopyranoside* (**4**). White amorphous solid. $[\alpha]_D^{25} = -50.2$ ($c = 1.08$, pyridine). IR (KBr): 3420, 2909, 1732, 1646, 1453, 1378, 1253, 1047, 781, 632 cm. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 1243.5362 ($[M+Na]^+$, $C_{57}H_{88}NaO_{28}^+$; calc. 1243.5354).

*(1β,3β,23*S*,24*S*)-1-({[4-*O*-Acetyl-6-deoxy-α-*L*-mannopyranosyl-(1 → 2)-[β-*D*-xylopyranosyl-(1 → 3)]-α-*L*-arabinopyranosyl]oxy}methyl)-3,23-dihydroxyspirosta-5,25(27)-dien-24-yl β-*D*-Glucopyranoside* (**5**). White amorphous solid. $[\alpha]_D^{25} = -62.0$ ($c = 1.08$, pyridine). IR (KBr): 3420, 2906, 1728, 1653, 1454, 1377, 1252, 1040, 896, 836, 782, 603. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 1097.4781 ($[M+Na]^+$, $C_{51}H_{78}NaO_{24}^+$; calc. 1097.4775).

*(1β,3β,23*S*)-26-(β-*D*-Glucopyranosyloxy)-3,23-dihydroxyfurosta-5,20(22),25(27)-trien-1-yl Sulfate* (**6**). White amorphous solid. $[\alpha]_D^{25} = -1.85$ ($c = 1.08$, MeOH). IR(KBr): 3424, 2917, 2853, 1689, 1649, 1452, 1382, 1246, 1213, 1051, 957, 928, 668, 593. ^{1}H- and ^{13}C-NMR: see *Table 3*. HR-ESI-MS: 685.2897 (M^-, $C_{33}H_{49}O_{13}S^-$; calc. 685.2899).

Acid Hydrolyses of **1**–**6**. A soln. of **1** (10 mg) in 1M HCl (dioxane/H_2O 1:1, 6 ml) was heated at 80° for 2 h. After cooling, the mixture was evaporated to remove dioxane and diluted to 8 ml with H_2O, and then extracted with AcOEt (4 × 6 ml). The aq. layer was neutralized by Ag_2CO_3 to pH 6–7, filtered through a microporous membrane, and further concentrated to an appropriate volume. TLC (SiO_2; AcOEt/MeOH/AcOH/H_2O 20:5:3:3 and $CHCl_3$/MeOH/AcOH/H_2O 16:10:3:3) for sugar analysis gave glucose, arabinose, xylose, fucose, apiose, and rhamnose.

In the same way, **2** (5 mg) was subjected to acid hydrolysis to give a sugar fraction. TLC of the sugar fraction under the same conditions as described for **1** revealed the presence of glucose, arabinose, xylose, fucose, apiose, and rhamnose. Compound **3** (5 mg) was subjected to acid hydrolysis as described for **1** to give a sugar fraction. TLC of the sugar fraction under the same conditions as described for **1** evidenced the presence of glucose, arabinose, xylose, fucose, and rhamnose. Compound **4** (4.7 mg) was subjected to acid hydrolysis as described for **1** to give a sugar fraction. TLC of the sugar fraction under the same conditions as described for **1** established the presence of glucose, arabinose, fucose, apiose, and rhamnose. Compound **5** (5.8 mg) was subjected to acid hydrolysis as described for **1** to give a sugar fraction. TLC of the sugar fraction under the same conditions as described for **1** disclosed the presence of glucose, arabinose, xylose, and rhamnose. Compound **6** (6 mg) was subjected to acid hydrolysis as described for **1** to give a sugar fraction. TLC of the sugar fraction under the same conditions as described for **1** showed the presence of glucose.

Sugar analyses of the steroidal saponins isolated from the genus of *Helleborus* [1][3][5][6][10–13] revealed that a particular glycosyl moiety occurred in only one configuration, *e.g*, D-glucose, D-xylose, L-arabinose, D-fucose, L-rhamnose, and D-apiose were reported. Thus, the absolute D-configurations of the glucose, xylose, fucose, and apiose residues and the L-configurations of the rhamnose and arabinose units were deduced from biogenetic considerations.

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