Steroidal Saponins from the Rhizomes of *Smilacina henryi*

by **Xiao Zhang**[a]), **Yan-Fang Su***[a])[b]), **Lei Chen**[a]), **Xiong Huang**[a]), **Shi-Lun Yan**[a]), **Xin Chai**[b]), and **Xiu-Mei Gao**[b])

[a]) Tianjin Key Laboratory for Modern Drug Delivery and High-Efficiency, School of Pharmaceutical Science and Technology, Tianjin University, No. 92 Weijin Road, Nankai District, Tianjin 300072, P. R. China (phone: +86-22-27402885; fax: +86-22-27892025; e-mail: yfsuphd@yahoo.com)

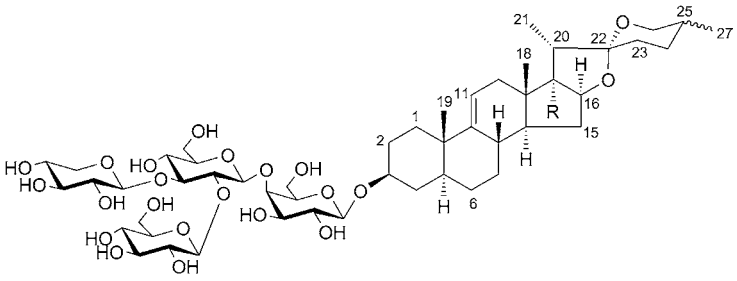
[b]) Tianjin State Key Laboratory of Modern Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin 300193, P. R. China

Five steroidal saponins, namely henryiosides A – E (**1** – **5**), were isolated from the EtOH extract of the rhizomes of *Smilacina henryi*. Their structures were elucidated by the extensive use of 1D- and 2D-NMR experiments, along with HR-MALDI-MS analysis and the results of acid hydrolysis. The aglycones of henryiosides A – E possess a C(7)=C(8) or C(9)=C(11) bond and were not previously found in saponins.

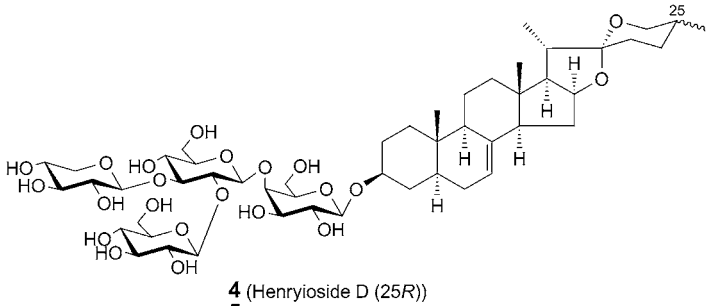
Introduction. – The *Smilacina* genus belongs to the Liliaceae family and comprises *ca.* 25 species distributed in East Asia, North America, and Central America [1]. Previous phytochemical research on the genus mainly focused on the *Smilacina atropurpurea* and *S. japonica* species and led to the isolation of a series of saponins [2][3], nucleosides [4][5], and flavonoids [6][7].

Smilacina henryi (BAKER) H. HARA (syn. *Maianthemum henryi* (BAKER) LA-FRANKIE) is mainly distributed in the northwest of China. The rhizomes of *S. henryi*, locally called '*BingPanQi*', are used for the treatment of rheumatism, traumatic injury, and impotence [8][9]. Previous studies addressed the nutritional components and inorganic elements of the leaf and stalk [9][10]. However, to the best of our knowledge, no phytochemical information on the rhizomes has been reported. For this reason, investigation on the chemical constituents of *BingPanQi* was performed which resulted in the isolation of five steroidal saponins, henryiosides A – E (**1** – **5**; *Fig. 1*). Their structures were elucidated by 1D- and 2D-NMR spectroscopic techniques, acid hydrolysis, as well as HR-MALDI-MS analysis.

Results and Discussion. – Compound **1** was obtained as a white amorphous powder. Its molecular formula was established to be $C_{50}H_{80}O_{23}$ with HR-MALDI-MS (*m*/*z* 1071.4989 ($[M+Na]^+$)). The ^{1}H-NMR spectrum (*Table 1*) showed the presence of two tertiary Me groups at δ(H) 0.87 (*s*) and 0.80 (*s*), two secondary Me groups at δ(H) 1.17 (*d*, $J = 7.0$ Hz) and 1.02 (*d*, $J = 7.0$ Hz), one olefinic H-atom at δ(H) 5.39 (*d*, $J = 5.0$ Hz) as well as four anomeric H-atoms at δ(H) 4.83 (*d*, $J = 7.5$ Hz), 5.14 (*d*, $J = 8.0$ Hz), 5.19 (*d*, $J = 8.0$ Hz), and 5.54 (*d*, $J = 8.0$). The ^{13}C-NMR and DEPT spectra (*Table 2*) exhibited fifty C-atom signals, including those of four Me groups at δ(C) 9.3, 16.5, 17.5, and 18.4, fourteen CH_2 units, twenty-seven CH groups (including four anomeric C-



1 R = OH (Henryioside A (25*S*))
2 R = H (Henryioside B (25*S*))
3 R = H (Henryioside C (25*R*))



4 (Henryioside D (25*R*))
5 (Henryioside E (25*S*))

Fig. 1. *The Structures of Henryiosides A – E* (**1** – **5**), *isolated from* Smilacina henryi

atoms at δ(C) 102.9, 105.1, 105.2, and 105.3 and one olefinic C-atom at δ(C) 117.3), five quaternary C-atoms (including an olefinic C-atom at δ(C) 146.5). These ^{1}H-NMR data and the quaternary C-atom signal at δ(C) 110.8 (C(22)) supported the fact that **1** had a spirostanol skeleton. The correlation δ(H) 1.17/δ(C) 110.8 observed in the HMBC spectrum (*Fig. 2*) allowed to ascribe the secondary Me group at δ(H) 1.17 to Me(21). Thus the other secondary Me group at δ(H) 1.02 was ascribed to Me(27). δ(C) 46.0 could be assigned to C(20) based on the HMBC from Me(21) to δ(C) 46.0. A further long-range correlation was also observed from Me(21) to the quaternary C-atom at δ(C) 90.1 (C(17)), revealing an OH group at C(17) due to its high chemical shift. The α-equatorial orientation of OH–C(17) was deduced from the NOESY cross-peak (*Fig. 2*) between δ(H) 0.87 (Me(18)) and δ(H) 2.19 (H–C(20)) [2]. The resonances at δ(C) 44.0 and δ(H) 0.87 were attributed to C(13) and Me(18), respectively, on the basis of the HMBCs from δ(H) 0.87 to δ(C) 44.0 and C(17). Thus the tertiary Me group at δ(H) 0.80 was ascribable to Me(19). The HMBC between the Me(19) signal and δ(C) 38.5 (C(10)) allowed the determination of the latter. Moreover, the HSQC showed the correlations δ(C) 17.5 (C(18))/δ(H) 0.87 (Me(18)), δ(C) 18.4 (C(19))/δ(H) 0.80 (Me(19)), δ(C) 9.3 (C(21))/δ(H) 1.17 (Me(21)), and δ(C) 16.5 (C(27))/δ(H) 1.02 (Me(27)). The location of a C(9)=C(11) bond was confirmed from the HMBC spectrum displaying correlations from the olefinic H-atom at δ(H) 5.39 to the C-atom resonances of C(10) and C(13). Full assignments of the H-and C-atoms of the aglycone

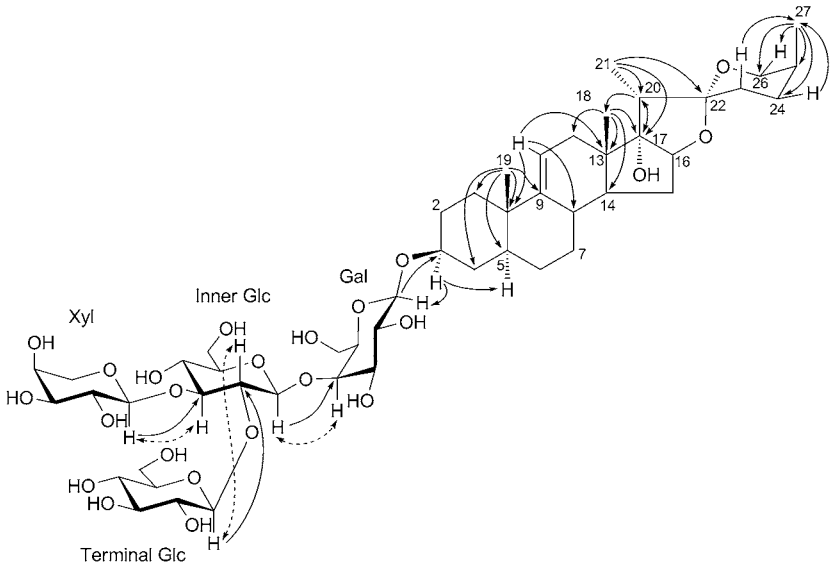


Fig. 2. *Selected HMBCs* (H → C) *and NOESY correlations* (H ↔ H) *of* **1**

(*Tables 1* and *2*) were achieved based on the combined analysis of ^{1}H- and ^{13}C-NMR, COSY, DEPT, HSQC, HMBC, TOCSY, and NOESY data. The C(25) configuration was deduced as (*S*) on the basis of NOESY cross-peaks δ(H) 1.02 (Me(27))/δ(H) 1.90 (H_a–C(23)), δ(H) 1.31 (H_b–C(24))/δ(H) 3.24 (H_b–C(26)) [11] and of the difference of chemical shifts of the CH_2(26) protons, *i.e.*, δ(H) 4.00 (H_a–C(26)) and δ(H) 3.24 (H_b–C(26)) [12]. Besides, the C-atom resonances related to ring *F*, δ(C) 26.8 (C(23)), δ(C) 26.0 (C(24)), δ(C) 27.7 (C(25)), δ(C) 65.4 (C(26)), and δ(C) 16.5 (C(27)), verified the (25*S*) configuration [13]. The α-equatorial orientation of H–C(5) was deduced from the chemical shift of Me (19) at δ(C) 18.4 [14] and the α-equatorial orientation of H–C(3) from the NOESY cross-peak δ(H) 3.84(H–C(3))/δ(H) 0.99 (H–C(5)) [2]. The four anomeric H- and C-atoms in the ^{1}H- and ^{13}C-NMR spectrum indicated the presence of four sugar units. Acid hydrolysis of **1** with HCl in dioxane gave xylose, galactose, and glucose, as showen by TLC analysis. The β-configuration at the anomeric centers of the sugar units were supported by the *J* values of their anomeric H-atoms. The absolute D-configuration of the xylose, glucose, and galactose residues were assumed from biogenetic considerations. The chemical shifts of all the individual sugar H-atoms and C-atoms were ascertained by a combination of ^{1}H- and ^{13}C-NMR, COSY, DEPT, HSQC, HMBC, TOCSY, and NOESY analysis, starting from the anomeric H-atoms, which established the presence of one β-D-galactopyranosyl (Gal), two β-D-glucopyranosyl (inner Glc and terminal Glc), and one β-D-xylopyranosyl (Xyl) unit. The sequence and linkage sites among the four sugar moieties and the aglycone were determined by the HMBCs δ(H) 4.83 (Gal H–C(1))/δ(C) 77.8 (aglycone C(3)), δ(H) 5.14 (inner Glc H–C(1))/δ(C) 80.2 (Gal C(4)), δ(H) 5.54 (terminal Glc H–C(1))/δ(C) 81.6 (inner Glc C(2)), δ(H) 5.19 (Xyl H–C(1))/δ(C) 87.2 (inner Glc C(3)). The deduction was supported by the NOESY plot, which showed the cross-

Table 1. *^{1}H-NMR Data* ((D_5) pyridine, 500 MHz)) *of Compounds* **1**–**5**. δ in ppm, J in Hz.

H-Atom	**1**	**2**	**3**	**4**	**5**
Aglycone:					
CH_2(1)	1.25, 1.56	1.21, 1.50	1.21, 1.49	0.87 (*br t*, $J=12.5$), 1.57	0.87, 1.57
CH_2(2)	1.68, 2.10	1.63, 2.07	1.63, 2.07	1.50, 1.98	1.26, 1.98
H–C(3) or CH_2(3)	3.84	3.81	3.81	3.84	3.83
CH_2(4)	1.34, 1.79	1.29, 1.76 (*d*, $J=12.5$)	1.30, 1.75	1.28, 1.78	1.29, 1.89
H–C(5)	0.99	0.97	0.96	1.19	1.17
CH_2(6)	1.13, 1.24	1.12, 1.18	1.11, 1.17	1.60	1.22, 1.59
CH_2(7) or H–C(7)	0.88, 1.76	0.83, 1.70	0.83, 1.68	5.06 (br. *s*)	5.06 (br. *s*)
H–C(8) or C(8)	2.05	1.97	1.96	–	–
C(9) or H–C(9)	–	–	–	1.51	1.51 (br. *d*, $J=10.5$)
C(10)	–	–	–	–	–
H–C(11) or CH_2(11)	5.39 (*d*, $J=5.0$)	5.22 (br. *s*)	5.22 (br. *s*)	1.34, 1.42 (br. *d*, $J=13.0$)	1.30, 1.41
CH_2(12)	1.70, 3.03 (br. *d*, $J=17.0$)	1.89, 1.89	1.88, 1.88	1.09, 1.62	1.07, 1.62
C(13)	–	–	–	–	–
H–C(14)	2.07	1.24	1.26	1.84	1.83
CH_2(15)	1.48, 2.28	1.39, 2.08	1.38, 2.08	1.63, 1.98	1.58, 1.97
H–C(16)	4.43	4.47	4.49	4.54	4.52
C(17) or H–C(17)	–	1.85	4.29	1.83	4.32
Me(18)	0.87 (*s*)	0.73 (*s*)	0.74 (*s*)	0.65 (*s*)	0.65 (*s*)
Me(19)	0.80 (*s*)	0.73 (*s*)	0.74 (*s*)	0.61 (*s*)	0.61 (*s*)
H–C(20)	2.19 (*q*, $J=7.0$)	1.87	1.92	1.86	1.82 (*d*, $J=7.0$)
Me(21)	1.17 (*d*, $J=7.0$)	1.06 (*d*, $J=6.0$)	1.05 (*d*, $J=6.5$)	1.07 (*d*, $J=6.0$)	1.08 (*d*, $J=6.0$)
C(22)	–	–	–	–	–
CH_2(23)	1.44, 1.90 (*ddd*, $J=13.5, 13.5, 5.0$)	1.38, 1.84	1.60, 1.60	1.60, 1.27	1.36, 1.83
CH_2(24)	1.31, 2.12	1.29, 2.08	1.18,1.49	1.50, 1.22	1.07, 1.29
H–C(25)	1.54	1.53	1.51	1.51	1.53
CH_2(26)	3.24 (*d*, $J=11.0$), 4.00	3.30 (*d*, $J=10.5$), 3.99	3.45, 3.53 (*d*, $J=8.5$)	3.42 (*t*-like, $J=9.5$), 3.51 (br. *d*, $J=9.5$)	3.30 (*d*, $J=11.0$), 3.99
Me(27)	1.02 (*d*, $J=7.0$)	1.01 (*d*, $J=7.5$)	0.64 (*d*, $J=5.5$)	0.63 (*d*, $J=4.5$)	1.01 (*d*, $J=7.0$)

Table 1 (cont.)

H-Atom	**1**	**2**	**3**	**4**	**5**
Galactose:					
H–C(1)	4.83 (*d*, *J* = 7.5)	4.80 (*d*, *J* = 7.5)	4.81 (*d*, *J* = 7.5)	4.79 (*d*, *J* = 7.5)	4.80 (d, *J* = 7.5)
H–C(2)	4.36	4.33	4.33	4.35	4.35
H–C(3)	4.07	4.03	4.03	4.05	4.04
H–C(4)	4.56	4.53	4.52	4.54	4.53
H–C(5)	3.96	3.92	3.93	3.91	3.92
CH_2(6)	4.18, 4.64 (*dd*, *J* = 9.5, 10.0)	4.13, 4.61 (br. *s*)	4.14, 4.61	4.1 3, 4.61 (*t*-like, *J* = 9.0)	4.14, 4.62 (*t*-like, *J* = 9.0)
Inner glucose:					
H–C(1)	5.14 (*d*, *J* = 8.0)	5.11 (*d*, *J* = 8.0)	5.12 (*d*, *J* = 7.5)	5.11 (*d*, *J* = 7.0)	5.13 (*d*, *J* = 8.0)
H–C(2)	4.35	4.34	4.33	4.34	4.36
H–C(3)	4.11	4.09	4.08	4.08	4.09
H–C(4)	3.77 (*dd*, *J* = 9.0, 9.0)	3.74 (*dd*, *J* = 9.0, 9.3)	3.74 (br. *d*, *J* = 8.5)	3.76 (br. *d*, *J* = 8.5)	3.76 (*t*-like, *J* = 8.0)
H–C(5)	4.06	4.04	4.04	4.04	4.04
CH_2(6)	4.00, 4.48 (br. *d*, *J* = 9.5)	3.97, 4.44	3.98, 4.45 (*d*, *J* = 10.0)	3.98, 4.45 (br. *d*, *J* = 11.0)	3.99, 4.46 (*d*, *J* = 10.5)
Xylose:					
H–C(1)	5.19 (*d*, *J* = 8.0)	5.15 (*d*, *J* = 7.5)	5.16 (*d*, *J* = 8.0)	5.16 (*d*, *J* = 7.5)	5.18 (*d*, *J* = 8.0)
H–C(2)	3.93 (*dd*, *J* = 8.0, 8.5)	3.89 (*dd*, *J* = 8.0, 8.5)	3.88	3.90	3.92 (*d*, *J* = 8.0)
H–C(3)	4.05	4.02	4.01	4.01	4.02
H–C(4)	4.08	4.03	4.03	4.04	4.04
CH_2(5)	3.64 (*dd*, *J* = 10.0, 11.0), 4.18	3.60 (*t*, *J* = 10.5), 4.15	3.61 (*t*-like, *J* = 10.5), 4.15	3.62 (*t*-like, *J* = 10.0), 4.16	3.62 (*t*-like, *J* = 10.5), 4.16
Terminal glucose:					
H–C(1)	5.54 (*d*, *J* = 8.0)	5.49 (*d*, *J* = 7.5)	5.51 (*d*, *J* = 7.5)	5.50 (*d*, *J* = 7.5)	5.52 (*d*, *J* = 7.5)
H–C(2)	4.02	3.96	3.99	3.99	4.01
H–C(3)	4.07	4.04	4.04	4.05	4.05
H–C(4)	4.17	4.13	4.12	4.13	4.15
H–C(5)	3.87	3.83	3.83	3.84	3.86
CH_2(6)	4.34, 4.53 (br. *d*, *J* = 10.5)	4.29, 4.48 (br. *d*, *J* = 10.5)	4.29, 4.48 (*d*, *J* = 9.0)	4.31, 4.49	4.32, 4.52

[a]) Overlapped signals are reported without multiplicity.

Table 2. *^{13}C-NMR* ((D_5) pyridine, 125 MHz) *Data of Compounds* **1**–**5**. δ in ppm.

C-Atom	**1**	**2**	**3**	**4**	**5**
Aglycone:					
CH_2(1)	36.2	35.7	35.7	37.2	37.2
CH_2(2)	30.3	29.8	29.9	29.8	29.9
CH(3)	77.8	77.2	77.3	77.3	77.2
CH_2(4)	35.4	34.9	34.9	34.6	34.6
CH(5)	43.7	43.0	43.0	40.2	40.2
CH_2(6)	29.1	28.6	28.6	29.8	29.8
CH_2(7) or CH(7)	33.9	33.3	33.3	118.1	118.1
CH(8) or C(8)	37.1	35.9	35.9	138.9	138.9
C(9) or CH(9)	146.5	147.3	147.3	49.1	49.1
C(10)	38.5	38.1	38.1	34.4	34.4
CH(11) or CH_2(11)	117.3	116.0	116.0	21.5	21.5
CH_2(12)	34.0	41.9	41.9	39.4	39.4
C(13)	44.0	39.0	39.0	41.5	41.5
CH(14)	51.6	54.0	54.0	55.0	55.0
CH_2(15)	33.0	33.2	33.2	31.4	31.4
CH(16)	91.3	81.2	81.2	80.8	80.9
C(17) or CH(17)	90.1	62.0	62.2	62.7	62.5
Me(18)	17.5	15.9	15.9	16.3	16.3
Me(19)	18.4	17.9	17.9	12.9	12.9
CH(20)	46.0	42.9	42.4	42.4	42.9
Me(21)	9.3	14.4	14.6	14.8	14.7
C(22)	110.8	109.7	109.2	109.2	109.6
CH_2(23)	26.8	26.2	31.7	31.7	26.2
CH_2(24)	26.0	26.1	29.1	29.1	26.1
CH(25)	27.7	27.4	30.5	30.5	27.4
CH_2(26)	65.4	65.1	66.9	66.8	65.0
Me(27)	16.5	16.2	17.2	17.2	16.2
Galactose:					
CH(1)	102.9	102.4	102.4	102.5	102.5
CH(2)	73.5	73.1	73.1	73.1	73.1
CH(3)	75.9	75.5	75.5	75.4	75.5
CH(4)	80.2	79.8	79.8	79.8	79.8
CH(5)	75.6	75.2	75.2	75.2	75.2
CH_2(6)	61.0	60.5	60.5	60.5	60.5
Inner glucose:					
CH(1)	105.3	105.0	105.0	105.0	105.0
CH(2)	81.6	81.2	81.2	81.2	81.2
CH(3)	87.2	86.7	86.7	86.7	86.7
CH(4)	70.7	70.4	70.4	70.3	70.4
CH(5)	77.8	77.5	77.5	77.5	77.5
CH_2(6)	63.3	62.9	62.9	62.9	62.9
Xylose:					
CH(1)	105.2	104.8	104.8	104.8	104.8
CH(2)	75.4	75.0	75.0	75.0	75.0
CH(3)	78.9	78.5	78.5	78.5	78.5
CH(4)	71.1	70.6	70.6	70.6	70.6
CH_2(5)	67.6	67.2	67.2	67.2	67.2

Table 2 (cont.)

C-Atom	**1**	**2**	**3**	**4**	**5**
Terminal glucose:					
CH(1)	105.1	104.7	104.7	104.7	104.7
CH(2)	76.5	76.1	76.1	76.1	76.1
CH(3)	78.1	77.6	77.6	77.6	77.6
CH(4)	71.4	70.9	70.9	70.9	70.9
CH(5)	79.0	78.6	78.6	78.6	78.6
CH_2(6)	62.8	62.3	62.3	62.3	62.4

peaks δ(H) 4.83 (Gal H–C(1))/δ(H) 3.84 (aglycone H–C(3)), δ(H) 5.14 (inner Glc H–C(1))/δ(H) 4.56 (Gal H–C(4)), δ(H) 5.54 (terminal Glc H–C(1))/δ(H) 4.35 (inner Glc H–C(2)), and δ(H) 5.19 (Xyl H–C(1))/δ(H) 4.11 (inner Glc H–C(3)) [15]. The sugar chain was further confirmed by comparison with literature data [16]. Consequently, the structure of compound **1** was determined to be (3β,5α,17α,25*S*)-17-hydroxyspirost-9(11)-en-3-yl *O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside and named henryioside A.

Compound **2** was obtained as a white amorphous powder. Its molecular formula was established to be $C_{50}H_{80}O_{22}$ with HR-MALDI-MS (*m*/*z* 1055.5034 ([*M* + Na]$^+$)), which displayed one less O-atom than **1**. The ^{13}C-NMR spectrum of **2** (*Table 2*) showed a close similarity to that of **1**, except for the missing of the signals assigned to C(12), C(13), C(14), C(15), C(16), C(17), C(20), and C(21) of **1** and the appearance instead of δ(C) 41.9, 39.0, 54.0, 33.2, 81.2, 62.0, 42.9, and 14.4. In addition, the ^{13}C-NMR and DEPT spectra indicated the presence of only four quaternary C-atoms in **2** instead of the five in **1**. These observations suggested that the difference between **1** and **2** was the absence of OH–C(17) in **2** according to [2]. Full assignments of the H- and C-atoms of the aglycone (*Tables 1* and *2*) were achieved based on the combined analysis of ^{1}H- and ^{13}C-NMR, COSY, DEPT, HSQC, HMBC, TOCSY, and NOESY data. 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 HCl in dioxane gave xylose, galactose, and glucose. By a combined analysis of ^{1}H- and ^{13}C-NMR, COSY, DEPT, HSQC, HMBC, TOCSY, and NOESY data, the structure of compound **2** was assigned as (3β,5α,25*S*)-spirost-9(11)-en-3-yl *O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside and named henryioside B.

Compound **3** was obtained as a white amorphous powder. Its molecular formula was established to be $C_{50}H_{80}O_{22}$ with HR-MALDI-MS (*m*/*z* 1055.5034 ([*M* + Na]$^+$)), which was the same as that of **2**. Comparison of the ^{1}H- and ^{13}C-NMR data of **2** and **3** (*Tables 1* and *2*) established the presence of identical sugar chains. The δ(C) of the aglycone of **3** were in good agreement with those of **2**, except for the peaks corresponding to ring *F, i.e.*, expect for δ(C) 109.2, 31.7, 29.1, 30.5, 66.9, and 17.2, inconsistent with those of **2** but closely related to those of the *F*-ring atoms C(22), C(23), C(24), C(25), C(26), and C(27) of a (25*R*)-spirostane [14]. The configuration at C(25) was deduced as (*R*) on the basis of the difference of chemical shifts of the CH_2(26) protons, *i.e.*, of δ(H) 3.45 (H_a–C(26)) and δ(H) 3.53 (H_b–C(26)), and of the

NOESY correlations δ(H) 0.64 (Me(27))/δ(H) 1.49 (H_a–C(24)), and 3.45 (H_a–C(26)). By a detailed analysis of ^{1}H- and ^{13}C-NMR, COSY, DEPT, HSQC, HMBC, TOCSY, and NOESY data, compound **3** was shown to be (3β,5α,25*R*)-spirost-9(11)-en-3-yl *O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside and named henryioside C.

Compound **4** was obtained as a white amorphous powder. Its molecular formula was established to be $C_{50}H_{80}O_{22}$ with HR-MALDI-MS (*m*/*z* 1055.5034 ([*M* + Na]$^+$)), which was the same as that of **3**. Comparison of the ^{1}H-and ^{13}C-NMR data of **3** and **4** (*Tables 1* and *2*) established the presence of identical sugar chains and a strong resemblance of ring *F*. The obvious differences in the chemical shifts of the olefinic C-atoms suggested that the location of the C=C bond of **4** was different from that of **3**. Comparison of the ^{13}C-NMR data of **4** with those of agapanthussaponin B [17] allowed to assign the position of the C=C bond between C(7) and C(8); this location was confirmed by the HMBC spectrum displaying the correlations δ(H) 5.06 (H–C(7))/δ(C) 40.2 (C(5)), δ(C) 49.1 (C(9)), and δ(C) 55.0 (C(14)). Based on a combined analysis of ^{1}H- and ^{13}C-NMR, COSY, DEPT, HSQC, HMBC, TOCSY, and NOESY data, the structure of **4** was elucidated as (3β,5α,25*R*)-spirost-7(8)-en-3-yl *O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside and named henryioside D.

Compound **5** was obtained as a white amorphous powder. Its molecular formula was established to be $C_{50}H_{80}O_{22}$ with HR-MALDI-MS (*m*/*z* 1055.5034 ([*M* + Na]$^+$)), identical to that of **4**. The ^{1}H- and ^{13}C-NMR data of **5** (*Tables 1 and 2*) were similar to those of **4**, except for the peaks corresponding to ring *F*, including C(23), C(24), C(25), C(26), and C(27). Similarly to the structural relationship observed between **2** and **3**, **5** was different from **4** only in the configuration at C(25). By an extensive use of ^{1}H- and ^{13}C-NMR, COSY, HSQC, HMBC, TOCSY, and NOESY data, compound **5** was established to be (3β,5α,25*S*)-spirost-7(8)-en-3-yl *O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside and named henryioside E.

According to previous investigations, steroidal saponins with C(5)=C(6) bonds are common in plants belonging to the *Smilacina* genus and related genera. On the other hand, the C(7)=C(8) or C(9)=C(11) bond found in henryiosides A – E (**1** – **5**) is unusual for *Smilacina* species. In particular, henryiosides A(**1**), B(**2**), and D(**4**) are based on steroidal aglycones never reported before. Although the aglycones of henryioside C(**3**) and E(**5**) have been synthesized before [18][19], they are reported in natural products for the first time.

This work was supported by the *Program for New Century Excellent Talents in University* (NCET-09 – 0589)

Experimental Part

General. TLC: silica gel GF_{254} plates (*Qingdao Haiyang Chemical Co. Ltd.*, P. R. China); spots visualized by UV light (254 and 365 nm) and by spraying with 5% PMA (phosphomolybdic acid) reagent followed by heating. Column chromatography (CC): silica gel (SiO_2; 100 – 200 and 200 – 300 mesh; *Qingdao Haiyang Chemical Co. Ltd.*, P. R. China), *LiChroprep RP-18* (40 – 63 μm; *Merck*, Germany), *Sephadex LH-20* (*Amersham Pharmacia Biotech AB*, Sweden), and *D101* macroporous resin (*Tianjin*

Haiguang Chemical Technology Co. Ltd., P. R. China). Prep. HPLC: *ODS* column (*Agilent Zorbax SB-C18*, 21.2 mm × 250 mm, 7 μm); 4 ml/min; t_R in min. Anal. HPLC: *ODS* column (*Baseline C18*, 250 × 4.6 mm, 5 μm); 1 ml/min. M.p.: *XT4A* microscope apparatus. Optical rotation: *Rudolph-Research-Analytical-Autopol-II* automatic polarimeter. IR: *Bruker-Tensor-27* spectrometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker-AV-500* instruments; in (D_5) pyridine; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *Varian-7.0T-FT-ICR* mass spectrometer; in *m*/*z*.

Plant Material. The rhizomes of *Smilacina henryi* were collected from Mei County, Shaanxi Province, P. R. China, during August and September of the year 2006, and were authenticated by Professor *Zhen-Hai Wu* of the North West Agriculture and Forestry University. A voucher specimen (S200608003) representing this collection was deposited with the Laboratory of the School of Pharmaceutical Science and Technology, Tianjin University, P. R. China.

Extraction and Isolation. The air-dried rhizomes (3 kg) were pulverized and refluxed with 95% (*v*/*v*) EtOH twice (each time 7 l) and then with 60% (*v*/*v*) EtOH once (7 l). The combined extracts were concentrated to give a residue (925 g) which was suspended in H_2O to a final volume of 6 l, and then partitioned sequentially with petroleum ether, $CHCl_3$, AcOEt, and BuOH. The BuOH extract (120 g) was submitted to CC (*D101* macroporous resin, EtOH/H_2O 0:100, 30:70, 50:50, 70:30, and 95:5): 5 fractions. The fraction *A* (29.3 g), eluted with 50% EtOH, was separated into 57 fractions by CC (SiO_2, AcOEt/MeOH 100:0, 93:7, 85:15, 80:20). *Frs. A28–A30* (1.7 g) were subjected to repeated CC (*LiChroprep RP-18*, MeOH/H_2O 47:53, 60:40, 65:35, 67:33, 69:31, 71:29, 75:25, 85:15): **1** (200 mg). The fraction *B* (38.0 g) of the CC (*D101* macroporous resin), eluted with 70% EtOH, was partitioned by CC (*LiChroprep RP-18*, MeOH/H_2O 60:40, 70:30, 80:20 and 90:10): *Frs. B1–B4*. Fraction *Fr. B2* (11.0 g), eluted with 70% MeOH, was separated by repeated CC (*LiChroprep RP-18,* MeOH/H_2O 80:20): *Frs. B2.1* and *B2.2. Fr. B2.1* (1.8 g) was subjected to prep. HPLC (MeCN/H_2O 42:58): **2** (t_R 107.2; 80 mg) and **3** (t_R 110.3; 6 mg). *Fr. 2B2.2* (934.7 mg) was purified by prep. HPLC (MeOH/H_2O 80:20): **4** (t_R 105.9; 5 mg) and **5** (t_R 108.0; 8 mg).

Henryioside A (*=(3β,5α,25*S*)-17-Hydroxy-spirost-9(11)-en-3-yl* O-*β*-D-*Glucopyranosyl-(1→2)*-O-*[β*-D-*xylopyranosyl-(1→3)]*-O-*β*-D-*glucopyranosyl-(1→4)*-*β*-D-*galactopyranoside*; **1**): Amorphous powder. M.p. 296–297°. $[\alpha]_D^{20} = -47.1$ (*c* = 0.85, pyridine). IR (KBr): 3427, 2930, 2877, 1069, 1042, 980. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-MALDI-MS: 1071.4989 ($[M+Na]^+$, $C_{50}H_{80}NaO_{23}^+$; calc. 1071.4983).

Henryioside B (*=(3β,5α,25*S*)-Spirost-9(11)-en-3-yl* O-*β*-D-*Glucopyranosyl-(1→2)*-O-*[β*-D-*xylopyranosyl-(1→3)]*-O-*β*-D-*glucopyranosyl-(1→4)*-*β*-D-*galactopyranoside*; **2**): Amorphous powder. M.p. 267–268°. $[\alpha]_D^{20} = -60.5$ (*c* = 0.35, pyridine). IR (KBr): 3394, 2930, 2873, 1038, 1065, 991. ^{1}H- and ^{13}C-NMR: *Table 1* and *2*. HR-MALDI-MS: 1055.5034 ($[M+Na]^+$, $C_{50}H_{80}NaO_{22}^+$; calc. 1055.5039).

Henryioside C (*=(3β,5α,25*R*)-Spirost-9(11)-en-3-yl* O-*β*-D-*Glucopyranosyl-(1→2)*-O-*[β*-D-*xylopyranosyl-(1→3)]*-O-*β*-D-*glucopyranosyl-(1→4)*-*β*-D-*galactopyranoside*; **3**): Amorphous powder. M.p. 261–262°. $[\alpha]_D^{20} = -176.5$ (*c* = 0.34, pyridine). IR (KBr): 3423, 2928, 2874, 1039, 919. ^{1}H- and ^{13}C-NMR: *Table 1* and *2*. HR-MALDI-MS: 1055.5034 ($[M+Na]^+$, $C_{50}H_{80}NaO_{22}^+$; calc. 1055.5039).

Henryioside D (*=(3β,5α,25R)-Spirost-7(8)-en-3-yl* O-*β*-D-*Glucopyranosyl-(1→2)*-O-*[β*-D-*xylopyranosyl-(1→3)]*-O-*β*-D-*glucopyranosyl-(1→4)*-*β*-D-*galactopyranoside*; **4**): Amorphous powder. M.p. 257°. $[\alpha]_D^{20} = -103.7$ (*c* = 0.27, pyridine). IR (KBr): 3395, 2931, 1062, 1042, 984. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*; HR-MALDI-MS: 1055.5034 ($[M+Na]^+$, $C_{50}H_{80}NaO_{22}$ calc. 1055.5039).

Henryioside E (*=(3β,5α,25*S*)-Spirost-7(8)-en-3-yl* O-*β*-D-*Glucopyranosyl-(1→2)*-O-*[β*-D-*xylopyranosyl-(1→3)]*-O-*β*-D-*glucopyranosyl-(1→4)*-*β*-D-*galactopyranoside*; **5**): Amorphous powder. M.p. 263°. $[\alpha]_D^{20} = -10.0$ (*c* = 0.30, pyridine). IR (KBr): 3434, 2932, 1014, 986, 920, 895. ^{1}H- and ^{13}C-NMR: *Table 1* and *2*. HR-MALDI-MS: 1055.5034 ($[M+Na]^+$, $C_{50}H_{80}NaO_{22}^+$; calc. 1055.5039).

Acid Hydrolysis of **1** *and* **2**. A soln. of **1** (42.2 mg) in 2M HCl/dioxane 2:1 (6 ml) was refluxed at 95° for 5 h. Then the mixture was diluted with H_2O (2 ml) and extracted with AcOEt (4 × 6 ml). TLC Analysis of the org. layer revealed decomposition of the aglycone. The aq. layer was neutralized by Ag_2CO_3, filtered through a microporous membrane and further concentrated to give a monosaccharide mixture in which xylose (R_f 0.81), galactose (R_f 0.60), and glucose (R_f 0.64) were detected by TLC (SiO_2, AcOEt/MeOH/H_2O/AcOH 13:3:2:4) comparson with the authentic samples. Compound **2** (40.0 mg) was subjected to acid hydrolysis as described for **1** to give an aq. layer and an AcOEt extract.

The aq. layer was treated and analyzed as described for **1**, in which xylose, galactose, and glucose were detected by TLC.

REFERENCES

[1] F.-T. Wang, J. Tang, in 'Flora of China', Science Press, Beijing, China, 1978, Vol. 15, pp. 35 – 36.
[2] Y. Zhang, H.-Z. Li, Y.-J. Zhang, M. R. Jacobb, S. I. Khanb, X.-C. Li, C.-R. Yang, *Steroids* **2006**, *8*, 712.
[3] S.-L. Yang, X.-K. Liu, H. Wu, H.-B. Wang, C. Qing, *Steroids* **2009**, *1*, 7.
[4] S.-J. Zhao, L.-M. Yang, *Shi Zhen Guo Yi Guo Yao* **2009**, *11*, 2856.
[5] S.-L. Yang, X.-K. Liu, *Chin. J. Nat. Med.* **2003**, *1*, 196.
[6] M. M. Rafi, B. C. Vastano, *Food Chem.* **2007**, *1*, 332.
[7] S.-J. Zhao, M. Han, Z.-M. Han, Y.-Y. Li, L.-M. Yang, *Chin. J. Anal. Chem.* **2009**, *9*, 1354.
[8] Z.-J. Guo, 'Shaanxi Qi Yao', Shaanxi Science and Technology Press, Shaanxi, China, 2003, pp. 160 – 162.
[9] X.-Y. Liu, J.-Y. Zhang, H. Fu, *Stud. Trace Elem. Health* **2003**, 3, 17.
[10] J.-Y. Zhang, J. Zhou, X.-Y. Liu, *Nat. Prod. Res. Dev.* **2002**, *4*, 45.
[11] U. Sharma, R. Saini, N. Kumar, B. Singh, *Chem. Pharm. Bull.* **2009**, *57*, 890.
[12] T. Muzashvili, A. Perrone, A. Napolitano, E. Kemertelidze, C. Pizza, S. Piacente, *Phytochemistry* **2011**, *72*, 2180.
[13] A. Yokosuka, Y. Mimaki, Y. Sashida, *Phytochemistry* **2002**, *61*, 73.
[14] P. K. Agrawal, D. C. Jain, R. K. Gupta, R. S. Thakur, *Phytochemistry* **1985**, *24*, 2479.
[15] Y. Su, K. Koike, T. Nikaido, J. Liu, J. Zheng, D. Guo, *J. Nat. Prod.* **2003**, *66*, 1593.
[16] M. Sugiyama, K. Nakano, T. Tomimatsu, T. Nohara, *Chem. Pharm. Bull.* **1984**, *32*, 1365.
[17] O. Nakamura, Y. Mimaki, Y. Sashida, T. Nikaido, T. Ohmoto, *Chem. Pharm. Bull.* **1993**, *41*, 1784.
[18] J. S. Dixon, I. Midgley, C. Djerassi, *J. Am. Chem. Soc.* **1977**, *99*, 3432.
[19] N. Kongkathip, B. Kongkathip, N. Noimai, *Synth. Commun.* **2006**, *36*, 865.

Received April 26, 2012