

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 C₅₀H₈₀O₂₃ with HR-MALDI-MS (*m/z* 1071.4989 ([*M* + Na]⁺)). The ¹H-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 ¹³C-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 CH₂ units, twenty-seven CH groups (including four anomeric C-

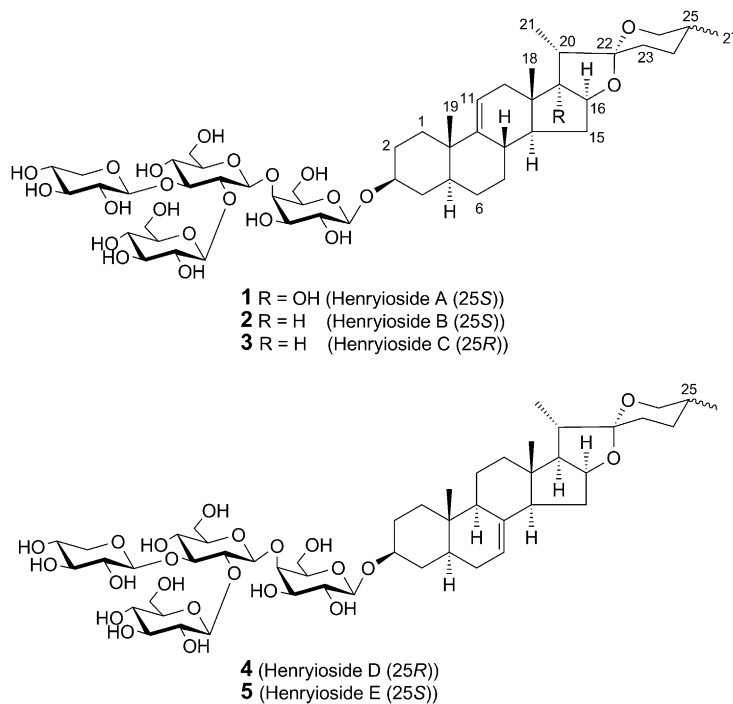


Fig. 1. The Structures of Henryiosides A–E (**1–5**), isolated from *Smilacina henryi*

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

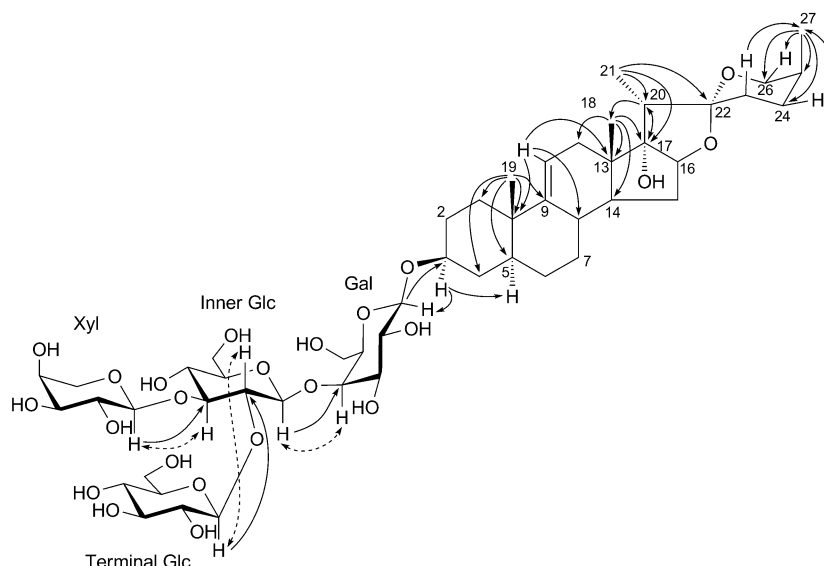


Fig. 2. Selected HMBCs ($H \rightarrow C$) and NOESY correlations ($H \leftrightarrow H$) of **1**

(Tables 1 and 2) were achieved based on the combined analysis of 1H - 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 $\delta(H)$ 1.02 (Me(27))/ $\delta(H)$ 1.90 (H_a -C(23)), $\delta(H)$ 1.31 (H_b -C(24))/ $\delta(H)$ 3.24 (H_b -C(26)) [11] and of the difference of chemical shifts of the CH_2 (26) protons, *i.e.*, $\delta(H)$ 4.00 (H_a -C(26)) and $\delta(H)$ 3.24 (H_b -C(26)) [12]. Besides, the C-atom resonances related to ring F, $\delta(C)$ 26.8 (C(23)), $\delta(C)$ 26.0 (C(24)), $\delta(C)$ 27.7 (C(25)), $\delta(C)$ 65.4 (C(26)), and $\delta(C)$ 16.5 (C(27)), verified the (*25S*) configuration [13]. The α -equatorial orientation of H-C(5) was deduced from the chemical shift of Me (19) at $\delta(C)$ 18.4 [14] and the α -equatorial orientation of H-C(3) from the NOESY cross-peak $\delta(H)$ 3.84(H-C(3))/ $\delta(H)$ 0.99 (H-C(5)) [2]. The four anomeric H- and C-atoms in the 1H - 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 shown 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 1H - 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 $\delta(H)$ 4.83 (Gal H-C(1))/ $\delta(C)$ 77.8 (aglycone C(3)), $\delta(H)$ 5.14 (inner Glc H-C(1))/ $\delta(C)$ 80.2 (Gal C(4)), $\delta(H)$ 5.54 (terminal Glc H-C(1))/ $\delta(C)$ 81.6 (inner Glc C(2)), $\delta(H)$ 5.19 (Xyl H-C(1))/ $\delta(C)$ 87.2 (inner Glc C(3)). The deduction was supported by the NOESY plot, which showed the cross-

Table 1. ¹H-NMR Data ((D₂) 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	–	1.85	4.29	1.83	4.32
H-C(17)	–	–	–	–	–
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 = 6.0)	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 I (cont.)

H-Atom	1	2	3	4	5
Galactose:					
H-C(1)	4.83 (<i>d</i> , <i>J</i> = 7.5)	4.80 (<i>d</i> , <i>J</i> = 7.5)	4.81 (<i>d</i> , <i>J</i> = 7.5)	4.79 (<i>d</i> , <i>J</i> = 7.5)	4.80 (<i>d</i> , <i>J</i> = 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 (<i>dd</i> , <i>J</i> = 9.5, 10.0)	4.13, 4.61 (<i>br. s</i>)	4.14, 4.61	4.13, 4.61 (<i>r</i> -like, <i>J</i> = 9.0)	4.14, 4.62 (<i>r</i> -like, <i>J</i> = 9.0)
Inner glucose:					
H-C(1)	5.14 (<i>d</i> , <i>J</i> = 8.0)	5.11 (<i>d</i> , <i>J</i> = 8.0)	5.12 (<i>d</i> , <i>J</i> = 7.5)	5.11 (<i>d</i> , <i>J</i> = 7.0)	5.13 (<i>d</i> , <i>J</i> = 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 (<i>dd</i> , <i>J</i> = 9.0, 9.0)	3.74 (<i>dd</i> , <i>J</i> = 9.0, 9.3)	3.74 (<i>br. d</i> , <i>J</i> = 8.5)	3.76 (<i>br. d</i> , <i>J</i> = 8.5)	3.76 (<i>r</i> -like, <i>J</i> = 8.0)
H-C(5)	4.06	4.04	4.04	4.04	4.04
CH ₂ (6)	4.00, 4.48 (<i>br. d</i> , <i>J</i> = 9.5)	3.97, 4.44	3.98, 4.45 (<i>d</i> , <i>J</i> = 10.0)	3.98, 4.45 (<i>br. d</i> , <i>J</i> = 11.0)	3.99, 4.46 (<i>d</i> , <i>J</i> = 10.5)
Xylose:					
H-C(1)	5.19 (<i>d</i> , <i>J</i> = 8.0)	5.15 (<i>d</i> , <i>J</i> = 7.5)	5.16 (<i>d</i> , <i>J</i> = 8.0)	5.16 (<i>d</i> , <i>J</i> = 7.5)	5.18 (<i>d</i> , <i>J</i> = 8.0)
H-C(2)	3.93 (<i>dd</i> , <i>J</i> = 8.0, 8.5)	3.89 (<i>dd</i> , <i>J</i> = 8.0, 8.5)	3.88	3.90	3.92 (<i>d</i> , <i>J</i> = 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 (<i>dd</i> , <i>J</i> = 10.0, 11.0), 4.18	3.60 (<i>t</i> , <i>J</i> = 10.5), 4.15	3.61 (<i>r</i> -like, <i>J</i> = 10.5), 4.15	3.62 (<i>r</i> -like, <i>J</i> = 10.0), 4.16	3.62 (<i>r</i> -like, <i>J</i> = 10.5), 4.16
Terminal glucose:					
H-C(1)	5.54 (<i>d</i> , <i>J</i> = 8.0)	5.49 (<i>d</i> , <i>J</i> = 7.5)	5.51 (<i>d</i> , <i>J</i> = 7.5)	5.50 (<i>d</i> , <i>J</i> = 7.5)	5.52 (<i>d</i> , <i>J</i> = 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 (<i>br. d</i> , <i>J</i> = 10.5)	4.29, 4.48 (<i>br. d</i> , <i>J</i> = 10.5)	4.29, 4.48 (<i>d</i> , <i>J</i> = 9.0)	4.31, 4.49	4.32, 4.52

^a) Overlapped signals are reported without multiplicity.

Table 2. $^{13}\text{C-NMR}$ (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 $\delta(\text{H})$ 4.83 (Gal H–C(1))/ $\delta(\text{H})$ 3.84 (aglycone H–C(3)), $\delta(\text{H})$ 5.14 (inner Glc H–C(1))/ $\delta(\text{H})$ 4.56 (Gal H–C(4)), $\delta(\text{H})$ 5.54 (terminal Glc H–C(1))/ $\delta(\text{H})$ 4.35 (inner Glc H–C(2)), and $\delta(\text{H})$ 5.19 (Xyl H–C(1))/ $\delta(\text{H})$ 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 (3 β ,5 α ,17 α ,25 S)-17-hydroxyspirost-9(11)-en-3-yl *O*- β -D-glucopyranosyl-(1 \rightarrow 2)-*O*-[β -D-xylopyranosyl-(1 \rightarrow 3)]-*O*- β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-galactopyranoside and named henryioside A.

Compound **2** was obtained as a white amorphous powder. Its molecular formula was established to be C₅₀H₈₀O₂₂ with HR-MALDI-MS (m/z 1055.5034 ($[M + \text{Na}]^+$)), which displayed one less O-atom than **1**. The ¹³C-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 $\delta(\text{C})$ 41.9, 39.0, 54.0, 33.2, 81.2, 62.0, 42.9, and 14.4. In addition, the ¹³C-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 ¹H- and ¹³C-NMR, COSY, DEPT, HSQC, HMBC, TOCSY, and NOESY data. The ¹H- and ¹³C-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 ¹H- and ¹³C-NMR, COSY, DEPT, HSQC, HMBC, TOCSY, and NOESY data, the structure of compound **2** was assigned as (3 β ,5 α ,25 S)-spirost-9(11)-en-3-yl *O*- β -D-glucopyranosyl-(1 \rightarrow 2)-*O*-[β -D-xylopyranosyl-(1 \rightarrow 3)]-*O*- β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-galactopyranoside and named henryioside B.

Compound **3** was obtained as a white amorphous powder. Its molecular formula was established to be C₅₀H₈₀O₂₂ with HR-MALDI-MS (m/z 1055.5034 ($[M + \text{Na}]^+$)), which was the same as that of **2**. Comparison of the ¹H- and ¹³C-NMR data of **2** and **3** (Tables 1 and 2) established the presence of identical sugar chains. The $\delta(\text{C})$ 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 $\delta(\text{C})$ 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 CH₂(26) protons, *i.e.*, of $\delta(\text{H})$ 3.45 (H_a–C(26)) and $\delta(\text{H})$ 3.53 (H_b–C(26)), and of the

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

Compound **4** was obtained as a white amorphous powder. Its molecular formula was established to be $\text{C}_{50}\text{H}_{80}\text{O}_{22}$ with HR-MALDI-MS (m/z 1055.5034 ($[M + \text{Na}]^+$)), which was the same as that of **3**. Comparison of the ^1H - 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 $\delta(\text{H})$ 5.06 (H-C(7))/ $\delta(\text{C})$ 40.2 (C(5)), $\delta(\text{C})$ 49.1 (C(9)), and $\delta(\text{C})$ 55.0 (C(14)). Based on a combined analysis of ^1H - and ^{13}C -NMR, COSY, DEPT, HSQC, HMBC, TOCSY, and NOESY data, the structure of **4** was elucidated as (3 β ,5 α ,25*R*)-spirost-7(8)-en-3-yl *O*- β -D-glucopyranosyl-(1 \rightarrow 2)-*O*-[β -D-xylopyranosyl-(1 \rightarrow 3)]-*O*- β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-galactopyranoside and named henryioside D.

Compound **5** was obtained as a white amorphous powder. Its molecular formula was established to be $\text{C}_{50}\text{H}_{80}\text{O}_{22}$ with HR-MALDI-MS (m/z 1055.5034 ($[M + \text{Na}]^+$)), identical to that of **4**. The ^1H - 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 ^1H - and ^{13}C -NMR, COSY, HSQC, HMBC, TOCSY, and NOESY data, compound **5** was established to be (3 β ,5 α ,25*S*)-spirost-7(8)-en-3-yl *O*- β -D-glucopyranosyl-(1 \rightarrow 2)-*O*-[β -D-xylopyranosyl-(1 \rightarrow 3)]-*O*- β -D-glucopyranosyl-(1 \rightarrow 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

Hai guang 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; $\bar{\nu}$ 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-70T-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. B2.2* (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 ($= (3\beta,5\alpha,25S)\text{-}17\text{-Hydroxy-spirost-9(11)-en-3-yl O-}\beta\text{-D-Glucopyranosyl-(1}\rightarrow\text{2)-O-}[\beta\text{-D-xylopyranosyl-(1}\rightarrow\text{3)]-O-}\beta\text{-D-glucopyranosyl-(1}\rightarrow\text{4)-}\beta\text{-D-galactopyranoside}$; **1**): Amorphous powder. M.p. 296–297°. $[\alpha]_D^{20} = -47.1$ ($c = 0.85$, pyridine). IR (KBr): 3427, 2930, 2877, 1069, 1042, 980. $^1\text{H-}$ and $^{13}\text{C-NMR}$: *Tables 1* and *2*. HR-MALDI-MS: 1071.4989 ($[M + \text{Na}]^+$, $\text{C}_{50}\text{H}_{80}\text{NaO}_{23}$; calc. 1071.4983).

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

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

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

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