SHORT REPORT

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Steroidal Components from the Roots and Rhizomes of *Smilacina henryi* and Their Cytotoxic Activities

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Abstract: Nine steroidal components, including a new pregnane glycoside (**1**), were obtained from the roots and rhizomes of *Smilacina henryi*. Their structures were determined via extensive spectroscopic data including, IR, HRESIMS and 1D, 2D NMR data analysis. Furthermore, their cytotoxic activities against human HepG2 and SW620 tumor cells were evaluated by the MTT method and compounds **2**, **3**, **5**, **8** and **9** showed moderate activity with IC_{50} values raging from 18.4 to 86.3 μM.

Keywords: *Smilacina henryi*; steroidal components; structure elucidation; cytotoxicity.

1. Plant Source

In the course of phytochemical studies of a Traditional Chinese Medicine named Pian Tou Qi, the roots and rhizomes of *Smilacina henryi* (bekev) Wang et Tang, which were collected on August in 2017 from Qinba Mountains in Shaanxi Province of China, and authenticated by one of our co-authors Prof. Jing Sun (Shaanxi University of Chinese Medicine). A voucher specimen (herbarium No. SH-201708) has been deposited in School of Pharmacy, Xi'an Jiaotong University, Xi'an 710061, China.

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2. Previous Studies

Steroidal saponins, spirostanes and flavonoids, such as Henryiosides A~G, (25*S*)-5*α*-spirost-9(11)-ene-3*β*, 17*α*, 21-triol, (24*S*, 25*S*)-5*α*-spirost-9(11)-ene-3*β*, 17*α*, 24-triol, and luteolin, have been isolated from genus *Smilacina* which possess anti-tumor, anti-oxidant and anti-inflammatory activities [1-10].

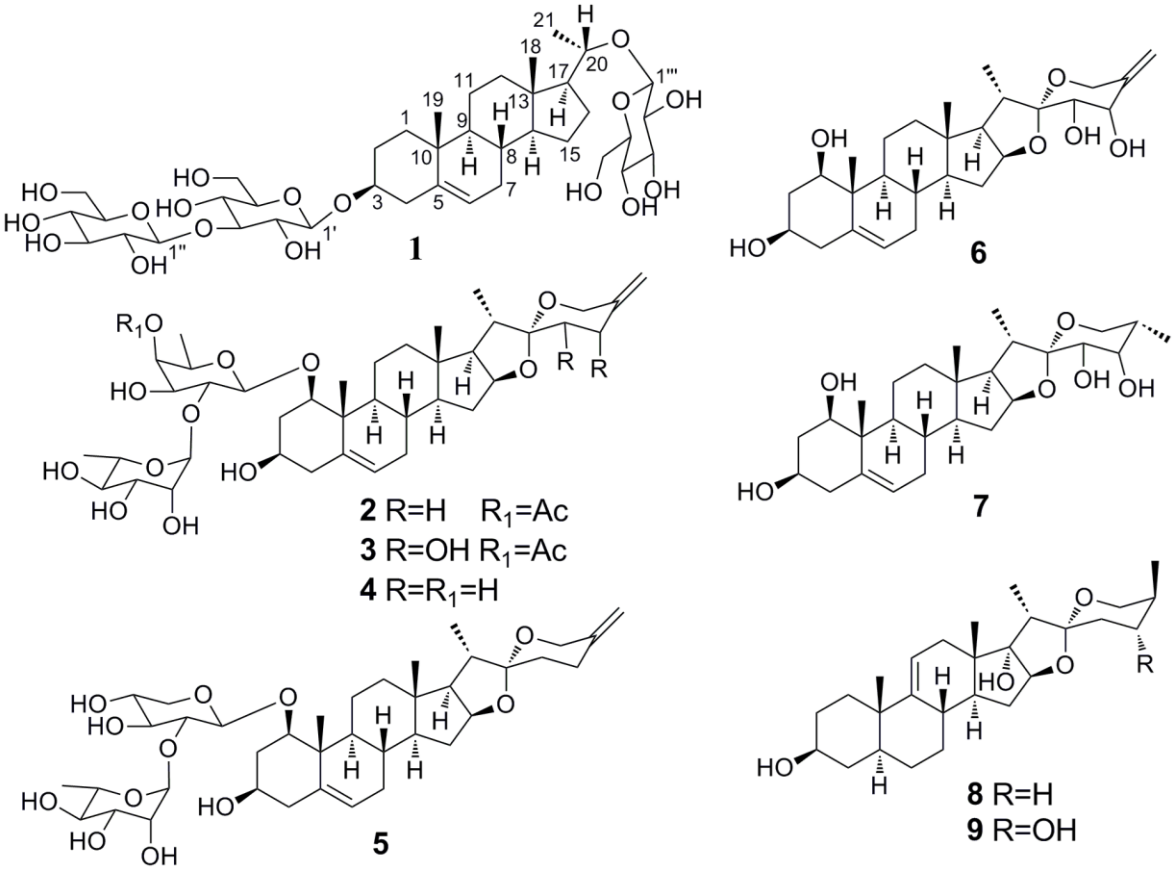

Figure 1. Structures of compounds **1** – **9**

3. Present Study

In our ongoing research project on the plant *S. henryi* [2, 3], a new pregnane glycoside, pregn-5-ene-3*β*,20(*S*)-diol-3-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranosyl-20-*O*-*β*-D-glucopy ranoside (**1**) along with eight known steroids, spirost-5, 25(27)-diene-l*β*,3*β*-diol 1-*O*-*α*-L-rhamnopyranosyl-(1→2)-4-*O*-acetyl-*β*-D-fucopyranoside (**2**) [5], (23*S*, 24*S*)- spirost-5, 25(27)-diene-l*β*,3*β*, 23, 24-tetrol 1-*O*-*α*-L-rhamnopyranosyl-(1→2)-4-*O*-acetyl- *β*-D-fucopyranoside (**3**) [5], spirost-5,25(27)-diene-l*β*,3*β*-diol 1-*O*-*α*-L-rhamnopyranosyl- (1→2)-*β*-D-fucopyranoside (**4**) [5], spirost-5, 25(27)-diene-l*β*, 3*β*-diol 1-*O*-*α*-L- rhamnopyranosyl-(1→2)-*β*-D-xylopyranoside (**5**) [5], (23*S*,24*S*,25*S*)-spirost-5-ene-1*β*, 3*β*, 23, 24-tetraol (**6**) [7], (23*S*, 24*S*)-spirost-5, 25(27)-diene-1*β*, 3*β*,23,24-tetraol (**7**) [10], (25*S*)-5*α*-spirost-9(11)-ene-3*β*, 17*α*-diol (**8**) [8] and (24*S*, 25*S*)-5*α*-spirost-9(11)-ene-3*β*, 17*α*, 24-triol (**9**) [4] were obtained, the extraction and isolation section are presented in the Supporting Information. Their cytotoxic activities against human HepG2 and SW620 tumor cells were also evaluated.

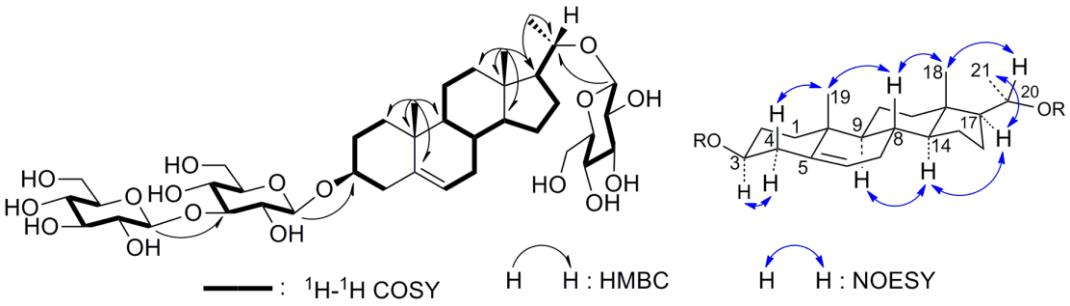

Figure 2. Key ^{1}H-^{1}H COSY, HMBC and NOESY correlations of compound **1**

Table 1. ^{1}H-NMR and ^{13}C-NMR data[a] of **1**

No.	**1**		No.	**1**	
	^{1}H (δ in ppm, *J* in Hz)	^{13}C (δ in ppm)		^{1}H (δ in ppm, *J* in Hz)	^{13}C (δ in ppm)
1	0.92 m 1.72 m	37.4	18	0.67 s	12.4
			19	1.00 s	19.5
2	1.82 m 2.13 m	30.2	20	3.91 m	81.4
			21	1.55 (6.0)	23.3
3	3.88 m	79.3	1'	5.09 (7.9)	101.5
4	2.73 m 2.85 m	39.2	2'	4.39 m	77.8
			3'	4.19 m	84.7
5	-	141.0	4'	4.46 ov	71.6
6	5.35 (4.4)	121.7	5'	4.24 ov	78.2
7	1.42 ov 1.83 m	32.1	6'	4.36 ov 4.62 ov	63.0
8	1.38 m	31.7	1"	5.30 (7.7)	106.6
9	0.82 m	50.3	2"	4.16 ov	77.1
10	-	36.9	3"	4.03 m	78.2
11	1.38 ov 1.42 ov	21.0	4"	4.35 ov	71.7
			5"	4.06 m	78.0
12	0.95 m 1.72 (7.2)	39.1	6"	4.38 ov 4.58 ov	62.6
13	-	41.5	1'''	4.97 (7.7)	106.1
14	0.88 ov	56.7	2'''	4.04 m	75.8
15	1.08 m 1.52 ov	24.4	3'''	4.23 m	78.8
			4'''	4.37 ov	71.7
16	1.94 m 2.37 m	27.2	5'''	4.01 m	78.6
			6'''	4.35 ov 4.59 ov	62.6
17	1.61 ov	58.3			

[a] ^{1}H-NMR and ^{13}C-NMR were measured at 400 MHz and 100 MHz in pyridine-d_5, ov: overlap signals.

Pregn-5-ene-3β20(S)-diol-3-O-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-20-O-β-D-glucopyranoside (**1**): A white amorphous powder; $[\alpha]_D^{20}$ −71.8 (*c* 0.16, MeOH); IR (KBr) ν_{max}: 3400, 2932, 1636, 1455, 1375, 1073, 1038, 889 cm^{-1}; *m/z* 805.4253 $[M + H]^+$ (calculated 805.4222 $C_{39}H_{65}O_{17}^+$). ^{1}H-NMR and ^{13}C-NMR data (400 MHz and 100 MHz in pyridine-d_5) see Table 1.

Compound **1**, a white amorphous powder, with $[\alpha]_D^{20}$ −71.8 (*c* 0.16, MeOH), has the molecular formula of $C_{39}H_{64}O_{17}$ which was supported by the HRESIMS quasimolecular ion peak at *m/z* 805.4253 $[M + H]^+$ (calculated 805.4222 $[M + H]^+$). The ^{1}H NMR spectrum of **1** showed the presence of three steroid methyl groups [4-8] at δ_H 0.67 (s, H-18), 1.00 (s, H-19), and 1.55 (d, *J* = 6.0 Hz, H-21) along with an olefinic proton 5.35 (d, *J* = 4.4 Hz, H-6) and four anomeric protons at 5.09 (1H, d, *J* = 7.7 Hz, Glc-H1'), 5.30 (1H, d, *J* = 7.6 Hz, Glc-H1") and 4.97 (1H, d, *J* = 7.6 Hz, Glc-H1"). The ^{13}C-NMR spectrum displayed 39 carbon signals, in which a set of double bond carbons at δ_C 141.0 (C-5) and 121.7 (C-6), three anomeric carbons 101.5 (Glc-C1'), 106.6 (Glc-C1") and 106.1 (Glc'-C1'") along with three methyl carbons 12.4 (C-18), 19.5 (C-19) and 23.3 (C-21) were observed. These NMR features indicated compound **1** was a pregnane glycoside and this inference was deduced from 2D-NMR data analysis including HSQC, HMBC, NEOSY and ^{1}H-^{1}H COSY experiments. The ^{1}H-^{1}H COSY correlations (Figure 2) from H-1/H-2/H-3/H-4, from H-6/H-7/H-8/H-9/H-11/H-12 and from H-15/H-16/H-17/H-20 along with the HMBC correlations (Figure 2) from H-19/C-1, C-5, C-9 and C-10, from H-18/C-12, C-13, C-14 and C-17 and from H-21/C-17 and C-20 indicated the planar structure of **1** was pregn-5-ene-3, 20-diol. In the NOESY spectrum of **1** [10-12], NOE correlations from H-19/H-4a, H-3/H-4b supported the *β*-orientation of 3-OH, NOE correlations (Figure 2) from H-21/H-16, H-18/H-20 disclosed the C-20*S* absolute configuration of **1** [14, 15]. The sugar chain of 3-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranosyl-20-*O*-*β*-D-glucopyranoside in **1** was determined by the HMBC correlations observed from H-1'/C-3, from H-1"/C-3' and from H-1'"/C-20. Acid hydrolysis of **1** (Supporting Information) resulted in the product of D-glucose which was confirmed by its optical rotation data (Glc:$[\alpha]_D^{20}$ +40.5 in MeOH) and R_f value (BuOH-AcOH-H_2O, 4:1:5 upper layer Glc: 0.36). The structure of **1** was thus deduced as pregn-5-ene-3*β*, 20(*S*)-diol-3-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranosyl-20-*O*-*β*-D-glucopyranoside (**1**, Figure 1).

Bioactivity Test-Cytotoxic activity test: The cytotoxic activity against human HepG2 and SW620 tumor cells of compounds **1**–**9** were evaluated by the MTT method [13], according to the previously reported literature [2,3]. The results (see Table 2) showed that compounds **2**, **3**, **5**, **8** and **9** exhibited moderate effect with IC_{50} values raging from 18.4 to 86.3 μM.

Table 2. Cytotoxicities of compounds **1**–**9** against HepG2 and SW620 cells[a] (IC_{50} μM)

Compounds	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
HepG2	>100	34.5 ± 3.9	47.8 ± 2.0	>100	86.3 ± 4.1	>100	>100	18.4 ± 1.3	52.4 ± 3.9
SW620	>100	52.21 ± 1.6	26.5 ± 0.7	>100	>100	>100	>100	24.1 ± 0.9	15.8±1.2

[a] 5-FU = 5-fluorouracil was used as the positive control (IC_{50} 12.1 ± 0.8 μM).

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Supporting Information

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

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