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New steroidal glycosides from the roots of *Asparagus cochinchinensis*

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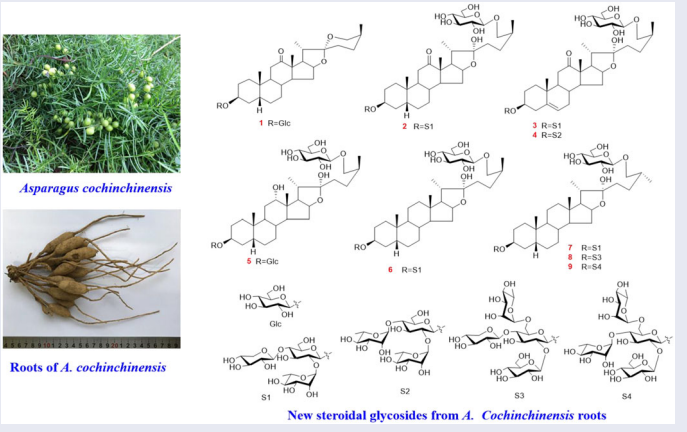
ABSTRACT

Steroidal saponins were the main active constituents of the traditional medicinal herb *Asparagus cochinchinensis*. A phytochemical investigation of *A. cochinchinensis* roots led to the isolation of nine new steroidal glycosides (**1–9**) and seven known analogues (**10**–**16**). Their structures were established by spectroscopic analyses as well as necessary chemical evidence.



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Liliaceae; *Asparagus cochinchinensis*; steroidal saponin

1. Introduction

Asparagus cochinchinensis Merill is a perennial herbaceous plant belonging to Liliaceae family. In China, *A. cochinchinensis* is widely distributed in many regions, and Guizhou, Guangxi and Yunnan provinces are the main produce areas of this species currently. The roots of *A. cochinchinensis* are well known as a yin-nourishing traditional Chinese medicine (Tian-Men-Dong in Chinese) [1]. Pharmacological studies have demonstrated its multiple biofunctional activities including anti-inflammation [2, 3], antioxidant [4, 5],

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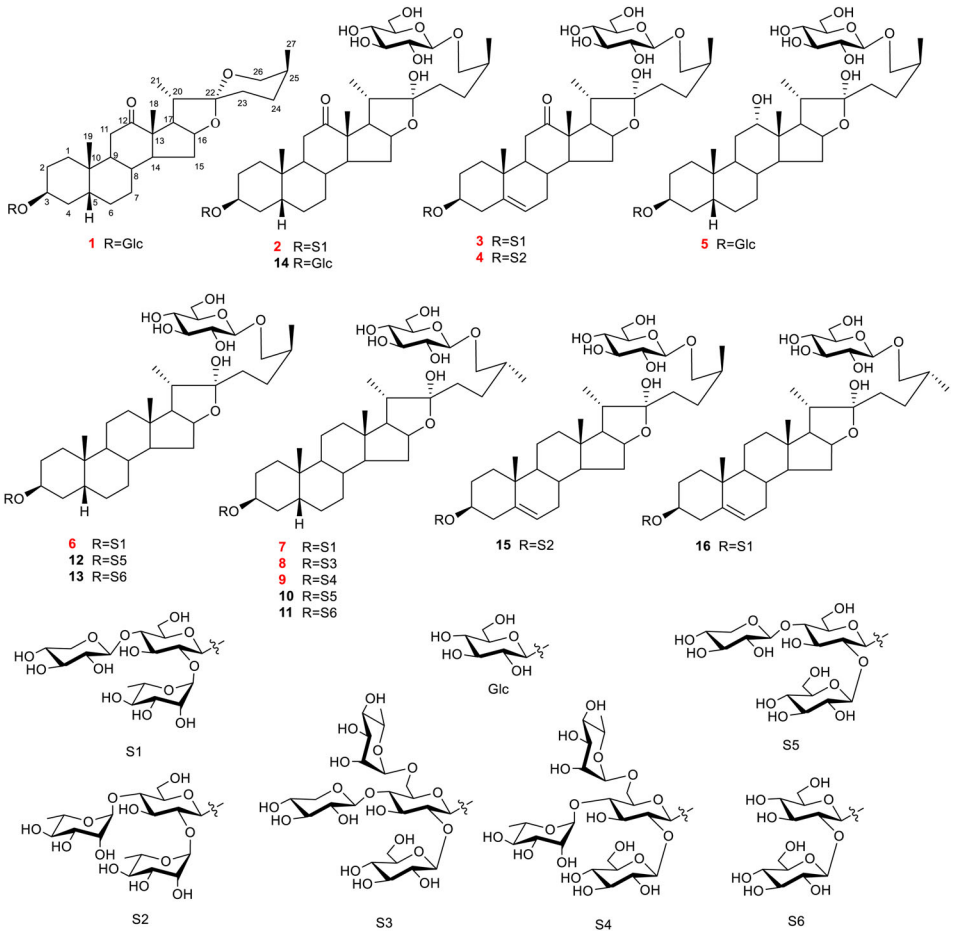

Figure 1. Structures of **1**–**16**.

antitumor [6] and neuroprotection [7, 8]. Steroidal saponin are considered to be the major active components of this herb [9]. Several steroidal glycosides from *A. cochinchinensis* root have been reported by us before [10]. As the ongoing phytochemical investigation, nine new steroidal glycosides (**1**–**9**) and seven known analogues (**10**–**16**) were further obtained (Figure 1). Herein, we mainly presented the isolation and structure elucidation of these new compounds.

2. Results and discussion

By comparing the NMR data with reported values, the known steroidal gycosides were identified as 25-*epi*-officinalisnin II (**10**) [11], disporoside C (**11**) [12], officinalisnin II (**12**) [13], (25*S*)-officinalisnin-I (**13**) [14], (25*S*)-26-*O*-*β*-D-glucopyranosyl-5*β*-furostan-3*β*,22*α*,26-triol-12-one-3-*O*-*β*-D-glucopyranoside (**14**) [15], protoneodioscin (**15**) [16], and pallidifloside I (**16**) [17]. The structures of the new analogous were elucidated based on spectroscopic analysis as well as the chemical evidence.

Compound **1** had a molecular formula of $C_{33}H_{52}O_9$ as determined by HRESIMS. The ^{1}H NMR spectrum showed four typical methyl protons of a steroidal saponin at δ 0.85 (3H, s), 1.08 (3H, s), 1.08 (3H, d, $J = 7.0$ Hz) and 1.38 (3H, d, $J = 7.0$ Hz), along with the characteristic carbon signals of δ 109.8 (C-22), suggested that **1** had a spirostanol skeleton. The anomeric proton at δ 4.94 (1H, d, $J = 7.8$ Hz) and the carbon signal at δ 103.0 indicated that **1** had only one sugar unit, that was also supported by acid hydrolysis of **1** producing a D-glucose. In the ^{13}C NMR spectrum, the carbon signal at δ 213.0 suggested a ketone in the structure, and the HMBC correlations of δ 1.08 (H-18) and δ 213.0 (C-12), 55.6 (C-13), 56.0 (C-14), 54.2 (C-17) further confirmed the position. The carbon signals of C-5 (δ 36.6), C-10 (δ 35.7), and C-19 (δ 23.1) which were same with those of **10**–**14** suggested the A/B *cis*-ring junction of **1**, further indicating the β-configuration of H-5. The 25*S*-configration was determined based on the chemical shifts of C-22 to C-27 [δ 31.7 (C-23), 29.3 (C-24), 30.6 (C-25), 66.9 (C-26), 17.3 (C-27) for 25*R*, and δ 26.4 (C-23), 26.2 (C-24), 27.6 (C-25), 65.1 (C-26), 16.3 (C-27) for 25*S*] [18]. The D-glucose was proved to have a β-configuration due to the large coupling constant ($^3J_{1,2} > 7$ Hz), and the linkage to C-3 was determined by the HMBC correlation between δ 4.94 (H-1′ of Glc) and δ 73.9 (C-3). Confirmed by combined analyses of the ^{1}H–^{1}H COSY, HSQC and HMBC spectra, the structure of **1** was established as (25*S*)-5β-12-one-spirost-3β-ol-3-*O*-β-D-glucopyranoside, and its NMR data were fully assigned and presented in Tables 1–4.

Compound **2** had a molecular formula of $C_{50}H_{82}O_{23}$ as determined by HRESIMS. The ^{1}H NMR spectrum showed four typical methyl protons of a steroidal saponin at δ 1.04 (3H, d, $J = 6.6$ Hz), 1.12 (3H, s), 1.13 (3H, s) and 1.56 (3H, d, $J = 6.7$ Hz), along with the characteristic carbon signals of δ 110.7 (C-22), suggested that **2** had a furostanol skeleton. Moreover, the carbon signal at δ 213.2 further suggested 12-C = O that was the same as in **1**. A detailed comparison of the NMR data suggested that **2** had a similar aglycone (including the 26-*O*-glcucose) as **14**, except the different sugar chain at C-3. The 25*S*-configuration of **2** was assigned according to the chemical shift difference of Ha-26 (δ 4.10, overlap) and Hb-26 (δ 3.51, dd, $J = 9.4$, 6.8 Hz) ($\Delta ab \geq 0.57$ ppm for 25*S*, and $\Delta ab \leq 0.48$ ppm for 25*R*) [19, 20]. Thus, substructure of 26-*O*-β-D-glucopyranosyl-(25*S*)-5β-12-one-furost-3β,26-diol was determined. Acid hydrolysis of **2** produced D-glucose, D-xylcose and L-rhamnose. Except for the anomeric proton of 26-*O*-glucopyranose at δ 4.84 (1H, d, $J = 7.7$ Hz), the other anomeric protons at δ 4.81 (1H, d, $J = 7.1$ Hz), 5.04 (1H, d, $J = 7.8$ Hz) and 6.39 (1H, s) indicated the C-3 sugar chain was made up of one D-glucose, one D-xylcose and one L-rhamnose. Further NMR data comparison indicated that **2** shared the same C-3 sugar chain with **16**. The sequence and the connectivity of C-3 sugar was also supported by the HMBC correlations from δ 6.39 (H-1″ of 2′-*O*-Rha) to 77.6 (C-2′), from 5.04 (H-1‴ of 4′-*O*-Xyl) to 81.7 (C-4′), and from δ 4.81 (H-1′ of 3-*O*-Glc) to 75.6 (C-3). After combined analyses of the ^{1}H–^{1}H COSY, HSQC and HMBC spectra, the structure of **2** was consequently elucidated as 26-*O*-β-D-glucopyranosyl-(25*S*)-5β-12-one-furost-3β,26-diol-3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-xylcopyranosyl-(1→4)]-β-D-glucopyranoside.

Compound **3** had a molecular formula of $C_{50}H_{80}O_{23}$ as determined by HRESIMS. The NMR data indicated that **3** had the very similar structure to **2** except for the

Table 1. ^{1}H NMR spectral data for aglycones of **1-9** in pyridine-d_5 (δ in ppm, 600 MHz).

POS.	1	2	3	4	5	6	7	8	9
1	1.90 m, 1.42 m	1.75 m, 1.54 m	1.91 m, 1.55 m	1.88 m, 1.54 m	1.80 m, 1.52 m	1.83 m, 1.53 m	1.83 m, 1.53 m	1.81 m, 1.53 m	1.81 m, 1.53 m
2	1.81 m, 1.71 m	1.89 m, 1.77 m	2.03 m, 1.81 m	2.03 m, 1.81 m	1.84 m, 1.12 m	1.96 m, 0.91 m	1.96m, 0.98 m	1.78 m, 0.92 m	1.80 m, 0.95 m
3	4.33 m	4.19 m	4.26 m	4.25 m	4.28 m	4.26 m	4.26 m	4.10 m	4.26 m
4	1.84 m, 1.34 m	1.89 m, 1.37 m	2.80m, 2.72 m	2.83 m, 2.73 m	2.08 m, 1.75 m	1.85 m, 1.52 m	1.87 m, 1.53 m	1.87 m, 1.53 m	1.87 m, 1.53 m
5	2.11 m	2.23 m	/	/	2.03 m	2.19 m	2.19 m	2.14 m	2.19 m
6	1.40 m	1.45 m	5.28 br s	5.28 br s	1.38 m	1.84 m	1.35 m	1.48 m	1.45 m
7	1.88 m, 1.32 m	1.93 m, 1.56 m	2.12 m, 1.67 m	2.12 m, 1.67 m	1.91 m, 1.37 m	1.97 m, 1.32 m	1.98 m, 1.32 m	1.91 m, 1.22 m	1.97 m, 1.20 m
8	2.06 m	1.89 m	1.87 m,	1.87 m	1.68 m	1.54 m	1.57 m	1.48 m	1.49 m
9	1.75 m	1.76 m	1.35 m	1.35 m	2.16 m	1.33 m	1.32 m	1.25 m	1.36 m
11	2.37 dd (13.6) 2.21 dd (14.3, 4.8)	2.43 t (13.7) 2.23 overlap	2.58 dd (14.8, 12.8) 2.33 dd (14.6, 5.7)	2.59 m	1.74 m	1.35 m, 1.25 m	1.35 m, 1.27 m	1.33 m, 1.21 m	1.36 m, 1.22 m
12	/	/	/	/	4.03 m	1.65 m, 1.05 m	1.75 m, 1.14 m	1.71 m, 1.10 m	1.70 m,1.20 m
14	1.45 m	1.45 m	1.47 m	1.46 m	2.16 m	1.10 m	1.13 m	1.05 m	1.07 m
15	2.06 m, 1.56 m	2.14 m, 1.59 m	2.10 m, 1.46 m	2.11 m, 1.47 m	2.17 m, 1.57 m	1.99 m, 1.40 m	2.05 m, 1.42 m	2.02 m, 1.43 m	2.05 m, 1.43 m
16	4.52 dd (15.2, 6.0)	4.92 dd (8.5, 6.7)	4.89 dd (8.7, 6.8)	4.90 dd (15.6, 7.0)	5.09 m	5.01 overlap	5.00 overlap	5.00 overlap	5.00 overlap
17	2.80 dd (8.7, 6.6)	2.96 dd (8.5, 6.7)	2.96 dd (8.8, 6.5)	2.96 dd (8.6, 6.7)	3.27 m	1.98 m	1.98 m	1.99 m	1.98 m
18	1.08 s	1.13 s	1.16 s	1.17 s	1.02 s	0.89 s	0.89 s	0.88 s	0.87 s
19	0.85 s	1.12 s	1.09 s	1.10 s	0.91 s	1.11 s	1.11 s	0.97 s	0.95 s
20	1.87 m	2.23 m	2.21 m	2.22 m	2.32 m	2.26 m	2.24 m	2.26 m	2.25 m
21	1.38 d (7.0)	1.56 d (6.7)	1.55 d (6.9)	1.55 d (6.9)	1.38 d (6.8)	1.34 d (7.0)	1.35 d (6.5)	1.35 d (6.9)	1.35 d (6.8)
23	1.70 m, 1.33 m	1.98 m, 0.90 m	1.98 m, 0.90 m	1.98 m, 0.90 m	2.10 m, 1.84 m	2.06 m	2.06 m	2.05 m	2.08 m
24	2.07 m	1.36 m	2.08 m, 1.69 m	2.11 m, 1.71 m	2.12 m	2.06 m, 1.72 m	2.06 m, 1.70 m	2.06 m, 1.71 m	2.06 m, 1.72 m
25	1.59 m	1.94 m	1.94 m	1.94 m	1.95 m	1.93 m	1.93 m	1.95 m	1.94 m
26	3.38 br d (11.1)	4.10 m 3.51 dd (9.4, 6.8)	4.11 m 3.51 dd (9.5, 6.8)	4.11 dd (9.4, 5.8) 3.51 dd (9.5, 6.8)	4.12 dd (9.3, 5.8) 3.49 dd (9.3, 7.0)	4.10 overlap 3.50 dd (9.4, 6.9)	3.95 overlap 3.64 dd (8.8, 6.5)	3.96 overlap 3.63 overlap	3.96 overlap 3.63 dd (9.5, 5.8)
27	1.08 d (7.0)	1.04 d (6.6)	1.04 d (6.6)	1.04 d (6.7)	1.05 d (6.6)	1.05 d (6.7)	1.00 d (6.6)	0.99 d (6.6)	0.99 d (6.6)

Table 2. ^{1}H NMR spectral data for sugar units of **1-9** in pyridine-d_5 (δ in ppm, 600 MHz).

POS.	1	2	3	4	5	6	7	8	9
	3-*O*-Glc	3-*O*-Glc	3-*O*-Glc	3-*O*-Glc	3-*O*-Glc	3-*O*-Glc	3-*O*-Glc	3-*O*-Glc	3-*O*-Glc
1′	4.94 d (7.8)	4.81 d (7.1)	4.97 overlap	4.96 d (7.3)	4.93 d (7.7)	4.85 overlap	4.84 overlap	4.85 overlap	4.82 d (7.4)
2′	4.06 m	4.23 m	4.24 m	4.20 m	4.06 m	4.28 m	4.25 m	4.28 m	4.21 m
3′	4.28 m	4.24 m	4.24 m	4.25 m	4.28 m	4.26 m	4.26 m	4.23 m	4.24 m
4′	4.29 m	4.22 m	4.23 m	4.44 m	4.28 m	5.01 m	4.22 m	4.06 m	4.18 m
5′	3.96 m	3.78 m	3.70 m	3.67 m	3.96 m	3.83 m	3.81 m	3.96 m	4.04 m
6′	4.56 dd (11.7, 2.3)	4.25 m, 4.10 m	4.23 m, 4.10 m	4.24 m, 4.12 m	4.56 m, 4.42 m	4.53 m, 4.45 m	4.53 m, 4.45 m	4.68 m, 4.32 m	4.59 m, 4.25 m
	4.42 dd (11.7, 5.2)								
		2′-*O*-Rha	2′-*O*-Rha	2′-*O*-Rha	26-*O*-Glc	2′-*O*-Rha	2′-*O*-Rha	2′-*O*-Glc	2′-*O*-Glc
1′′		6.39 br s	6.29 overlap	6.46 br s	4.85 d (7.8)	6.41 br s	6.39 br s	5.46 d (7.7)	5.42 d (7.7)
2′′		4.79 m	4.85 m	4.88 m	4.06 m	4.81 m	4.80 m	4.09 m	4.10 m
3′′		4.57 m	4.56 m	4.59 m	4.28 m	4.59 m	4.57 m	4.31 m	4.29 m
4′′		4.35 m	4.38 m	4.41 m	4.28 m	4.37 m	4.35 m	4.33 m	4.35 m
5′′		4.79 m	4.97 m	4.99 m	3.96 m	4.82 m	4.81 m	4.04 m	4.02 m
6′′		1.75 d (6.1)	1.79 d (6.2)	1.79 d (6.2)	4.56 m, 4.42 m	1.77 d (6.1)	1.76 d (5.7)	4.61 m, 4.52 m	4.60 m, 4.51 m
		4′-*O*-Xyl	4′-*O*-Xyl	4′-*O*-Rha		4′-*O*-Xyl	4′-*O*-Xyl	4′-*O*-Xyl	4′-*O*-Rha
1′′′		5.04 d (7.8)	5.05 d (7.8)	5.90 br s		5.05 d (7.7)	5.04 d (7.7)	4.88 d (7.8)	5.63 br s
2′′′		3.99 m	4.05 m	4.73 m		4.01 m	3.99 m	4.04 m	4.62 m
3′′′		4.13 m	4.11 m	4.67 m		4.13 m	4.11 m	4.09 m	4.51 m
4′′′		4.18 m	4.18 m	4.40 m		4.19 m	4.18 m	4.17 m	4.27 m
5′′′		4.30 m, 3.68 m	4.29 m, 3.70 m	4.99 m		4.28 m, 3.67 m	4.26 m, 3.68 m	4.23 m, 3.61 m	4.92 m
6′′′				1.66 d (6.2)					1.66 d (6.2)
		26-*O*-Glc	26-*O*-Glc	26-*O*-Glc		26-*O*-Glc	26-*O*-Glc	6′-*O*-Rha	6′-*O*-Rha
1′′′′		4.84 d (7.7)	4.84 d (7.8)	4.85 d (7.8)		4.84 d (8.0)	4.84 overlap	5.59 br s	53.5 br s
2′′′′		4.04 m	4.10 m	4.07 m		4.06 m	4.04 m	4.65 m	4.63 m
3′′′′		4.26 m	4.26 m	3.98 m		4.27 m	4.25 m	4.55 m	4.51 m
4′′′′		4.25 m	4.26 m	4.27 m		4.28 m	4.25 m	4.29 m	4.33 m
5′′′′		3.97 m	3.95 m	3.98 m		3.96 m	3.96 m	4.40 m	4.92 m
6′′′′		4.59 m, 4.40 m	4.57 m, 4.40 m	4.58 m, 4.42 m		4.58 m, 4.40 m	4.57 m, 4.40 m	1.68 d (6.2)	1.67 d (7.0)
								26-*O*-Glc	26-*O*-Glc
1′′′′′								4.84 d (7.8)	4.84 d (7.1)
2′′′′′								4.26 m	4.25 m
3′′′′′								4.26 m	4.26 m
4′′′′′								4.25 m	4.25 m
5′′′′′								3.96 m	3.96 m
6′′′′′								4.56 m, 4.40 m	4.58 m, 4.40 m

Table 3. ^{13}C NMR spectral data for aglycones of **1-9** in pyridine-d_5 (δ in ppm, 150 MHz).

POS.	1	2	3	4	5	6	7	8	9
1	30.7	30.7	37.0	37.0	31.0	31.0	31.0	31.0	31.1
2	26.8	26.7	29.9	29.9	27.1	26.9	26.9	27.0	27.0
3	73.9	75.6	77.9	77.7	74.4	75.9	76.0	74.7	75.3
4	30.3	30.7	38.8	38.7	30.4	30.9	30.9	30.7	30.7
5	36.6	36.8	140.5	140.5	37.1	37.2	37.2	36.8	36.9
6	26.2	26.6	121.6	121.6	27.0	26.9	26.9	26.8	26.8
7	26.7	26.4	31.8	31.8	26.8	26.8	26.8	26.7	26.6
8	34.7	34.7	30.9	30.9	35.9	35.6	35.6	35.5	35.5
9	41.9	42.0	52.4	52.3	33.7	40.4	40.5	40.3	40.4
10	35.7	35.8	37.6	37.6	35.0	35.3	35.4	35.2	35.2
11	37.8	37.8	37.7	37.7	29.7	21.2	21.2	21.1	21.1
12	213.0	213.2	212.8	213.0	71.7	40.4	40.4	40.4	40.3
13	55.6	56.0	55.3	55.3	45.8	41.2	41.2	41.2	41.2
14	56.0	56.0	55.9	55.8	48.6	56.5	56.5	56.4	56.4
15	31.5	31.7	31.9	31.9	32.5	32.4	32.5	32.4	32.4
16	80.8	79.8	79.7	79.7	81.1	81.2	81.3	81.2	81.2
17	54.2	55.0	54.6	54.8	54.6	64.0	64.1	64.1	64.1
18	16.1	16.2	16.0	16.0	17.6	16.8	16.8	16.7	16.7
19	23.1	23.0	18.8	18.6	23.8	23.9	23.9	24.0	24.0
20	43.1	41.2	41.4	41.3	41.0	40.7	40.7	40.7	40.7
21	13.8	15.3	15.2	15.3	16.3	16.5	16.5	16.5	16.5
22	109.8	110.7	110.8	110.8	110.7	110.6	110.6	110.6	110.6
23	37.1	37.1	37.1	37.1	37.2	37.2	37.3	37.3	37.2
24	26.4	28.3	28.3	28.3	28.4	28.4	28.4	28.4	28.4
25	27.5	34.4	34.4	34.4	34.5	34.4	34.3	34.3	34.3
26	65.2	75.3	75.4	75.4	75.5	75.4	75.3	75.3	75.2
27	16.3	17.5	17.5	17.5	17.5	17.5	17.5	17.5	17.5

difference in the regions surrounding C-5 and C-6. Furthermore, in the ^{13}C NMR spectrum of **3**, the observed characteristic carbon signal of δ 140.5 and 121.6 indicated the double bond between C-5 and C-6. The chemical shift of H-26a at δ 4.11 and H-26b at δ 3.51 ($\Delta ab > 0.57$ ppm) inferred the 25*S*-configuration. Using the ^1H-^1H COSY, HSQC, and HMBC spectra analyses, the structure of **3** was finally determined as 26-*O*-β-D-glucopyranosyl-(25*S*)-$\Delta^{5(6)}$-12-one-furost-3β,26-diol-3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-xylcopyranosyl-(1→4)]-β-D-glucopyranoside.

Compound **4** had a molecular formula of $C_{51}H_{82}O_{23}$ as determined by HRESIMS. The NMR data suggested **4** had the identical furostanol skeleton with **3** and shared same sugar moieties with **15**. The chemical shift difference between H-26a at δ 4.11 and H-26b at δ 3.51 ($\Delta ab > 0.57$ ppm) deduced the 25*S*-configuration for **4**. By combined analyses of ^1H-^1H COSY, HSQC and HMBC data, the structure of **4** was confirmed as 26-*O*-β-D-glucopyranosyl-(25*S*)-$\Delta^{5(6)}$-12-one-furost-3β,26-diol-3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside.

Compound **5** had a molecular formula of $C_{39}H_{66}O_{15}$ as determined by HRESIMS. The NMR data indicated that **5** had the very similar structure to **14** except for the difference in the region surrounding C-12. No carbon signal of 12-C = O was found in the ^{13}C NMR spectrum of **5** and the C-12 was shifted upfield (δ 71.7), indicating a hydroxyl substituent in this position. That was supported by the HMBC correlation of δ 1.02 (H-18) and 71.7 (C-12). And the chemical shift of C-12 indicated the α-configuration for 12-OH [δ 29.4 (C-11), 71.5 (C-12), 45.4 (C-13) for 12α-OH, and δ 31.5 (C-11), 79.1 (C-12) 46.6 (C-11) for 12β-OH] [21, 22]. The chemical shift difference

Table 4. ^{13}C NMR spectral data for sugar units of **1–9** in pyridine-d_5 (δ in ppm, 150 MHz).

POS.	1	2	3	4	5	6	7	8	9
	3-*O*-Glc	3-*O*-Glc	3-*O*-Glc	3-*O*-Glc	3-*O*-Glc	3-*O*-Glc	3-*O*-Glc	3-*O*-Glc	3-*O*-Glc
1′	103.0	101.9	100.0	100.2	103.1	101.6	101.6	101.9	102.1
2′	75.4	77.6	77.5	77.7	75.4	77.7	77.7	81.7	83.1
3′	78.8	76.8	77.3	78.0	78.8	76.9	76.9	76.0	76.3
4′	71.8	81.7	81.5	78.3	71.7	81.7	81.8	80.3	78.1
5′	78.5	76.2	76.3	77.0	78.4	76.3	76.3	76.0	76.1
6′	62.8	61.8	61.7	61.2	62.7	61.8	61.8	66.9	66.6
		2′-*O*-Rha	2′-*O*-Rha	2′-*O*-Rha	26-*O*-Glc	2′-*O*-Rha	2′-*O*-Rha	2′-*O*-Glc	2′-*O*-Glc
1″		101.6	102.0	102.1	105.2	101.9	101.9	105.6	105.9
2″		72.4	72.5	72.6	75.3	72.4	72.4	77.1	77.1
3″		72.7	72.8	72.8	78.6	72.7	72.8	78.0	78.0
4″		74.1	74.2	74.2	71.7	74.1	74.1	71.8	71.7
5″		69.5	69.5	69.6	78.6	69.5	69.5	78.8	78.8
6″		18.8	18.7	18.7	62.8	18.8	18.8	62.9	62.8
		4′-*O*-Xyl	4′-*O*-Xyl	4′-*O*-Rha		4′-*O*-Xyl	4′-*O*-Xyl	4′-*O*-Xyl	4′-*O*-Rha
1‴		105.8	105.8	102.9		105.8	105.8	105.5	102.6
2‴		75.0	75.0	72.6		75.0	75.0	74.9	72.3
3‴		78.4	78.4	72.9		78.4	78.4	78.4	72.7
4‴		70.8	70.8	74.0		70.8	70.8	70.8	73.9
5‴		67.4	67.4	70.4		67.4	67.4	67.4	70.5
6‴				18.6					18.5
		26-*O*-Glc	26-*O*-Glc	26-*O*-Glc		26-*O*-Glc	26-*O*-Glc	6′-*O*-Rha	6′-*O*-Rha
1⁗		105.2	105.2	105.2		105.2	105.0	102.3	101.8
2⁗		75.2	75.3	75.3		75.2	75.2	72.4	72.5
3⁗		78.6	78.7	78.6		78.6	78.7	72.8	72.8
4⁗		71.7	71.7	71.6		71.7	71.7	74.1	74.0
5⁗		78.5	78.6	78.6		78.5	78.5	69.8	69.9
6⁗		62.8	62.8	62.8		62.8	62.9	18.8	18.7
								26-*O*-Glc	26-*O*-Glc
1⁗′								105.0	105.0
2⁗′								75.3	75.2
3⁗′								78.6	78.6
4⁗′								71.7	71.7
5⁗′								78.5	78.5
6⁗′								62.8	62.8

between H-26a at δ 4.12 and H-26b at δ 3.49 ($\Delta ab > 0.57$ ppm) inferred the 25*S*-configuration for **5**. Thus, after combined analyses of ^1H-^1H COSY, HSQC and HMBC spectra, the structure of **5** was determined as 26-*O*-β-D-glucopyranosyl-(25*S*)-5β-furost-3β,12α,26-triol-3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside.

Compound **6** had a molecular formula of $C_{50}H_{84}O_{22}$ as determined by HRESIMS. The NMR data suggested **6** had same sugar moieties to **2** and had the identical skeleton with **10**. The chemical shift difference between H-26a at δ 4.10 and H-26b at δ 3.50 ($\Delta ab > 0.57$ ppm) deduced the 25*S*-configuration for **6**. By combined analyses of ^1H-^1H COSY, HSQC and HMBC data, the structure of **6** was confirmed as 26-*O*-β-D-glucopyranosyl-(25*S*)-5β-furost-3β,26-diol-3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-xylcopyranosyl-(1→4)]-β-D-glucopyranoside.

Compound **7** had a molecular formula of $C_{50}H_{84}O_{22}$ as determined by HRESIMS, which was same as **6**. The identical carbon signals of **7** and **6** suggested they were a pair of 25 *R*/*S*-isomers, sharing same planar structure. The chemical shift difference between H-26a at δ 3.95 and H-26b at δ 3.64 ($\Delta ab < 0.48$ ppm) deduced the 25 *R*-configuration for **7**. Thus, the structure of **7** was confirmed as 26-*O*-β-D-

glucopyranosyl-(25*R*)-5*β*-furost-3*β*,26-diol-3-*O*-α-L-rhamnopyranosyl-(1→2)-[*β*-D-xylcopyranosyl-(1→4)]-*β*-D-glucopyranoside.

Compound **8** had a molecular formula of $C_{56}H_{94}O_{27}$ as determined by HRESIMS. The NMR data suggested that **8** had a very similar structure to **10**, except one extra terminal rhamnose in C-3 sugar chain. In the HMBC spectrum, the correlation between δ 5.59 (H-1′′′′ of 6′-*O*-Rha) and 66.9 (C-6′ of 3-*O*-Glc) confirmed that the connectivity of the terminal rhamnose. The chemical shift difference between H-26a at δ 3.96 and H-26b at δ 3.63 (Δab $<$ 0.48 ppm) confirmed that **8** also had an 25 *R*-configuration. By combined use of ^{1}H-^{1}H COSY, HSQC and HMBC experiments, the structure of **8** was confirmed as 26-*O*-*β*-D-glucopyranosyl-(25*R*)-5*β*-furost-3*β*,26-diol-3-*O*-α-L-rhamnopyranosyl-(1→2)-[*β*-D-xylcopyranosyl-(1→4)]-[α-L-rhamnopyranosyl-(1→6)]-*β*-D-glucopyranoside.

Compound **9** had a molecular formula of $C_{57}H_{96}O_{27}$ as determined by HRESIMS. The NMR data suggested that **9** had a very similar structure to **8**, except the different sugar unit at C-4′. Except for the rhamnose at C-6′, the characteristic anomeric proton of δ 5.63 (1H, s, H-1′′′′ of 4′-*O*-Rha) and methyl proton at δ 1.66 (1H, d, J = 6.2 Hz, H-6′′′′ of 4′-*O*-Rha) deduced that an additional rhamnose unit was linked to C-4′. The chemical shift difference between H-26a at δ 3.96 and H-26b at δ 3.63 (Δab $<$ 0.48 ppm) deduced the 25 *R*-configuration for **9**. By combined analyses of the ^{1}H–^{1}H COSY, HSQC and HMBC spectra, the structure of **9** was confirmed as 26-*O*-*β*-D-glucopyranosyl-(25*R*)-5*β*-furost-3*β*,26-diol-3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-[α-L-rhamnopyranosyl-(1→6)]-*β*-D-glucopyranoside.

The phytochemical study on the roots of *A. cochinchinensis* results in isolation of a series of steroidal glycosides including nine new analogues. The steroidal saponins as the main constituents in *A. cochinchinensis* are diverse in types and similar in structure, which makes it difficult to successfully isolate them, especially the low-content analogues in medicinal materials. The results obtained from this study will be helpful for a further understanding the compositions of steroidal glycosides as main constituents of this plant, and provides a basis for the follow-up chemical study and quality control research of this medicine.

3. Experimental

3.1. General experimental procedures

Optical rotations were performed on a Rudolph Autopol® IV automatic polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA). NMR spectra were measured on a Bruker AVANCE III 600 spectrometer (Bruker Corporation, Karlsruhe, Germany). HRESIMS were recorded on a Waters Synapt MS (Waters Corporation, Milford, MA, USA). HPLC analyses were performed on an Agilent 1100 system (Agilent Technologies, Santa Clara, CA, USA) equipped with an Alltech 2000 ELSD (Alltech Corporation, Cleveland, Ohio, USA) using the columns of Venusil XBP C_{18} (4.6 × 250 mm, 5 μm, Bonna-Agela Technologies, Tianjin, China) and X-Amide (4.6 × 250 mm, 5 μm, Acchrom Technologies, Beijing, China). Preparative HPLC separations were performed using a NP7000 module (Hanbon Co. Ltd., Huaian, China.) equipped with a Shodex RID 102 detector (Showa Denko Group, Tokyo, Japan) and

the columns of Venusil XBP C_{18} (10.0 × 250 mm, 5 μm, Bonna-Agela Technologies, Tianjin, China) and X-Amide (10.0 × 250 mm, 5 μm, Acchrom Technologies, Beijing, China). TLC was performed on silica gel GF254 plates (Qingdao Marine Chemical, Qingdao, China). AB-8 macroporous resin (Solarbio Life Science, Beijing, China), SP825 macroporous resin (Mitsubishi Chemical, Tokyo, Japan), Silica gel H (Qingdao Marine Chemical, Qingdao, China), MCI gel (50 μm, Mitsubishi Chemicals, Tokyo, Japan) and ODS silica gel (120 Å, 50 μm, YMC, Tokyo, Japan) were used for column chromatography.

3.2. Plant material

Asparagus cochinchinensis roots were purchased from the Shibing region of Qiandongnan Miao and Dong Autonomous Prefecture of Guizhou province in November 2017, and identified by Professor Baolin Guo (Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences). A voucher specimen (AC-201711) was deposited in the author's laboratory.

3.3. Extraction and isolation

Dried roots of *A. cochinchinensis* (100 kg) were crushed and extracted with 60% aq. EtOH at reflux three times (each for 1 h). The filtered solution was concentrated *in vacuo* to get the supernatants and sediments. The supernatants were subjected to an AB-8 macroporous resin column eluted with EtOH-H_2O (*v*/*v*, 100:0→10:90→45:55→70:30→90:10) to yield five factions (A ∼ E). Fr.C (1.7 kg) was subjected to MCI gel column chromatography (CC) eluted with a gradient mixture of CH_3CN-H_2O (*v*/*v*, 27:75→80:20) to give five fractions (Fr.C1-Fr.C12). Furthermore, Fr.C4 was separated on ODS CC eluted with CH_3CN-H_2O (*v*/*v*, 23:77) to yield ten fractions of Fr.C4/1∼Fr.C4/10, Fr.C5 was separated on ODS CC eluted with CH_3CN-H_2O (*v*/*v*, 23:77) to afford eleven fractions of Fr.C5/1∼Fr.C5/11, and Fr.C10 was separated on ODS CC eluted with CH_3CN-H_2O (*v*/*v*, 23:77) to afford eight fractions of Fr.C10/1∼Fr.C10/8. Fr.C4/4-5 was subjected to silica gel CC eluted with a gradient mixture of $CHCl_3$-CH_3OH (*v*/*v*, 3:1→1:1) to yield three fractions Fr.C4/4-5/1-Fr.C4/4-5/3. Then, a part of Fr.C4/4-5/1 was separated on preparative HPLC (pHPLC) using the Venusil XBP C_{18} column eluted with CH_3CN-H_2O (*v*/*v*, 20:80) to produce **14** (44.0 mg), and a part of Fr.C4/4-5/1 was separated on pHPLC using the X-Amide column eluted with CH_3CN-H_2O (*v*/*v*, 84:16) to produce **2** (123.0 mg) and **3** (100.0 mg). A part of Fr.C5/7 was first separated by pHPLC using a X-Amide column eluted with $(CH_3)_2CO$-H_2O (*v*/*v*, 82:18) to produce **1** (12.2 mg) and **4** (40.2 mg), and then a part of Fr.C5/7 was subjected to pHPLC using the Venusil XBP C_{18} column eluted with CH_3CN-H_2O (*v*/*v*, 22:78) to yield **8** (8.0 mg) and **9** (7.0 mg). Fr.C5/8 was first separated on pHPLC using the Venusil XBP C_{18} column eluted with $(CH_3)_2CO$-H_2O (*v*/*v*, 28:72) to produce **10** (79.0 mg). Fr.C5/11 was first separated on pHPLC using the Venusil XBP C_{18} column eluted with CH_3CN-H_2O (*v*/*v*, 23:77) to yield **11** (27.4 mg) and **12** (593.6 mg), and then part of Fr.C5/11 was separated on pHPLC using a C_{18} column eluted with $(CH_3)_2CO$-H_2O (*v*/*v*, 27:73) to yield **13** (81.2 mg). Fr.C10/5 was separated on pHPLC using the Venusil XBP C_{18} column eluted with $(CH_3)_2CO$-H_2O (*v*/*v*, 30:70) to

yield **6** (6.9 mg), **7** (42.5 mg), **15** (38.0 mg) and **16** (42.5 mg). Fr.D (490 g) was subjected to an SP825 macroporous resin column eluted with EtOH-H_2O (*v*/*v*, 50:50→70:30) to yield two factions Fr.D1 and Fr.D2. Fr.D2 was first subjected to a Sephadex LH-20 column eluted with CH_3OH and then separated on pHPLC using the Venusil XBP C_{18} column eluted with CH_3OH-H_2O (*v*/*v*, 78:22) to yield **1** (30 mg).

3.3.1. Compound 1

$C_{33}H_{52}O_9$; white amorphous powder; $[\alpha]_D^{25}$ −8.0 (*c* 0.050, MeOH); ^{1}H NMR (600 MHz, pyridine-d_5) spectral data see Tables 1 and 2, and ^{13}C NMR (150 MHz, pyridine-d_5) spectral data see Tables 3 and 4; HRESIMS: *m*/*z* 637.3611 $[M + HCOO^-]^-$ (calcd for $C_{34}H_{53}O_{11}$, 647.3588).

3.3.2. Compound 2

$C_{50}H_{82}O_{23}$; white amorphous powder; $[\alpha]_D^{25}$ −24.0 (*c* 0.050, MeOH); ^{1}H NMR (600 MHz, pyridine-d_5) spectral data see Tables 1 and 2, and ^{13}C NMR (150 MHz, pyridine-d_5) spectral data see Tables 3 and 4; HRESIMS: *m*/*z* 1049.5140 $[M - H]^-$ (calcd for $C_{50}H_{81}O_{23}$, 1049.5169).

3.3.3. Compound 3

$C_{50}H_{80}O_{23}$; white amorphous powder; $[\alpha]_D^{25}$ −73.3 (*c* 0.060, MeOH); ^{1}H NMR (600 MHz, pyridine-d_5) spectral data see Tables 1 and 2, and ^{13}C NMR (150 MHz, pyridine-d_5) spectral data see Tables 3 and 4; HRESIMS: *m*/*z* 1047.4991 $[M - H]^-$ (calcd for $C_{50}H_{79}O_{23}$, 1047.5012).

3.3.4. Compound 4

$C_{51}H_{82}O_{23}$; white amorphous powder; $[\alpha]_D^{25}$ −40.0 (*c* 0.050, MeOH); ^{1}H NMR (600 MHz, pyridine-d_5) spectral data see Tables 1 and 2, and ^{13}C NMR (150 MHz, pyridine-d_5) spectral data see Tables 3 and 4; HRESIMS: *m*/*z* 1061.5140 $[M - H]^-$ (calcd for $C_{50}H_{81}O_{23}$, 1061.5169).

3.3.5. Compound 5

$C_{39}H_{66}O_{15}$; white amorphous powder; $[\alpha]_D^{25}$ −13.3 (*c* 0.030, MeOH); ^{1}H NMR (600 MHz, pyridine-d_5) spectral data see Tables 1 and 2, and ^{13}C NMR (150 MHz, pyridine-d_5) spectral data see Tables 3 and 4; HRESIMS: *m*/*z* 773.4312 $[M - H]^-$ (calcd for $C_{39}H_{65}O_{15}$, 773.4323).

3.3.6. Compound 6

$C_{50}H_{84}O_{22}$; white amorphous powder; $[\alpha]_D^{25}$ −47.3 (*c* 0.055, MeOH); ^{1}H NMR (600 MHz, pyridine-d_5) spectral data see Tables 1 and 2, and ^{13}C NMR (150 MHz, pyridine-d_5) spectral data see Tables 3 and 4; HRESIMS: *m*/*z* 1035.5353 $[M - H]^-$ (calcd for $C_{50}H_{83}O_{22}$, 1035.5376).

3.3.7. Compound 7

$C_{50}H_{84}O_{22}$; white amorphous powder; $[\alpha]_D^{25}$ −60.0 (*c* 0.050, MeOH); ^{1}H NMR (600 MHz, pyridine-d_5) spectral data see Tables 1 and 2, and ^{13}C NMR (150 MHz,

pyridine-d_5) spectral data see Tables 3 and 4; HRESIMS: m/z 1035.5343 $[M-H]^-$ (calcd for $C_{50}H_{83}O_{22}$, 1035.5376).

3.3.8. Compound 8

$C_{50}H_{94}O_{27}$; white amorphous powder; $[\alpha]_D^{25}$ −18.2 (c 0.055, MeOH); ^{1}H NMR (600 MHz, pyridine-d_5) spectral data see Tables 1 and 2, and ^{13}C NMR (150 MHz, pyridine-d_5) spectral data see Tables 3 and 4; HRESIMS: m/z 1197.5842 $[M-H]^-$ (calcd for $C_{54}H_{93}O_{27}$, 1197.5904).

3.3.9. Compound 9

$C_{57}H_{96}O_{27}$; white amorphous powder; $[\alpha]_D^{25}$ −28.0 (c 0.050, MeOH); ^{1}H NMR (600 MHz, pyridine-d_5) spectral data see Tables 1 and 2, and ^{13}C NMR (150 MHz, pyridine-d_5) spectral data see Tables 3 and 4; HRESIMS: m/z 1211.6017 $[M-H]^-$ (calcd for $C_{57}H_{95}O_{27}$, 1211.6061).

3.4. Acid hydrolysis and absolute configuration determination

Standard monosaccharides, L-rhamnose (5 mg) and D-glucose (5 mg), and L-cysteine methyl ester hydrochloride (5 mg) was dissolved in pyridine (5 ml) and heated to 60 °C for 1 h. Then o-tolyl isothiocyanate (10 μl) was added to the mixture and the mixture was kept at 60 °C for 1 h. The reaction mixture was analyzed by UPLC-MS. Compounds **1** − **9** (1.0 mg each) were individually hydrolyzed by heating in 6 M TFA-water (1 ml) at 90 °C for 2 h. After cooling, the reaction mixture was extracted with $CHCl_3$ (3 × 5 ml). Next, each aqueous layer was evaporated to dryness using rotary evaporation. The residue was dissolved in pyridine (1 ml) containing L-cysteine methyl ester hydrochloride (1 mg) (Aldrich, Japan) and heated at 60 °C for 1 h. Then, o-tolyl isothiocyanate (5 μl) was added to each mixture, and heated at 60 °C for another 1 h. The reaction mixtures were directly analyzed by reversed-phase UPLC-MS. Under these conditions, the absolute configurations of the sugars of compounds **1**–**9** were identified as D-glucose (t_R 3.68 min), D-xylose (t_R 4.10 min) and L-rhamnose (t_R 5.38 min).

Supporting information

MS and the NMR spectra of the isolated compounds are available as supporting information.

Disclosure statement

No potential conflict of interest was reported by the authors.

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