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Two new steroidal saponins isolated from *Anemarrhena asphodeloides*

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[ABSTRACT] Two new steroidal saponins, named timosaponin P (**1**) and timosaponin Q (**2**), were isolated from the rhizome parts of *Anemarrhena asphodeloides* Bunge using various chromatographic methods. Their structures and absolute configurations were elucidated by a combination of spectroscopic and spectrometric data, including 1D, 2D NMR, HR-ESI-MS and ECD calculations, and this is the first time the absolute configuration of C-23 of steroidal saponin was confirmed by ECD calculations.

[KEY WORDS] *Anemarrhena asphodeloides*; Timosaponin P; Timosaponin Q; ECD; Steroidal saponin; Absolute configuration

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Introduction

Anemarrhena asphodeloides Bunge. (*A. asphodeloides*), belonging to the family Liliaceae, is a perennial herb plant mainly distributed in China, Korea, Mongolia and other east Asian region. Previous pharmacological studies indicated that *A. asphodeloides* has beneficial effects on many central nervous system diseases [1-4], blood system diseases [5-6], antitumor [7-8] and anti-oxidation [9]. Steroidal saponins were the main components in *A. asphodeloides* [10], up to the present, 53 steroid saponins have been isolated from it, of which some have displayed markedly anticancer activity [7, 11-14]. In a continued effort to search for new potential anti-cancer bioactive compounds from this plant, an investigation of the chemical constituents from *A. asphodeloides* was undertaken, and this has led to the isolation of two new steroid saponins.

Results and Discussion

Timosaponin P (**1**) was isolated as a white amorphous powder, it gave a positive reaction to Liebermann-Burchard, and it could be deduced to be furostanol saponin on the basis of colour reaction with Ehrlich's spray reagent on TLC. The molecular formula, $C_{45}H_{74}O_{19}$, was deduced from its HR-ESI-MS data (*m/z* 941.471 8 $[M + Na]^+$, Calcd. for $C_{45}H_{74}O_{19}Na$, 941.471 7) and ^{13}C NMR (Table 1), implying nine degrees of unsaturation. The ^1H NMR spectrum of **1** (Table 1) showed three singlet methyl proton signals at δ_H 0.70 (3H, s, H-18), 0.99 (3H, s, H-19), and 1.72 (3H, s, H-21), and one doublet methyl proton signal at δ_H 1.17 (3H, d, J = 6.5 Hz, H-27). Three anomeric proton signals at δ_H 5.29 (1H, d, J = 7.7 Hz), 4.91 (1H, d, J = 7.6 Hz), and 4.86 (1H, d, J = 7.7 Hz). The ^{13}C NMR data of **1** showed four methyl groups at δ_C 24.0, 18.0, 14.5 and 11.7. The downfield-shifted carbonyl carbon at δ_C 154.1 (C-22), δ_C 105.2 (C-20), accounting for the presence of the double bond between C-20 and C-22. There are three anomeric carbons at δ_C 102.6 (Gal C-1'), 106.1 (Glc C-1") and 105.3 (Glc C-1"'). Hence, compound **1** can be regarded as a furostanol saponins with three hexose residues and a double bond between C-20 and C-22. Comparing the spectrometric data of **1** with Timosaponin BIII, it can be found that the same partial structure with rings A, B, C, and D, the main difference is the molecular weight of compound **1** has more 16 than Timosaponin BIII can be regarded

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Table 1 ^{1}H (500 MHz) and ^{13}C (125 MHz) spectral data for compounds 1 and 2 (in pyridine-d_5)

No.	1			2		
	δ_H	δc	HMBC	δ_H	δc	HMBC
1	1.82 (m), 1.48 (m)	31.0	H-5, H-19	1.85 (m), 1.48 (m)	31.0	H-3, H-19
2	1.98 (m), 1.48 (m)	27.0		1.98 (m), 1.48 (m)	27.1	
3	4.35 (m)	75.6	H-1'	4.33 (m)	75.6	H-1’
4	1.86 (m)	30.8		1.82 (m)	30.8	H-3
5	2.16 (m)	37.0	, H-19	2.15 (m)	37.0	H-19
6	1.47 (m)	26.8		1.41 (m)	26.8	
7	1.99 (m), 1.83 (m)	26.8		1.96 (m), 1.83 (m)	26.5	H-9
8	1.41 (m)	35.1	H-9	1.31 (m)	34.9	
9	1.28 (m)	40.2	H-19, H-12	1.26 (m)	40.7	H-11, H-12, H-19
10		35.2	H-9, H-19	–	35.2	H-19
11	1.33 (m), 1.21 (m)	21.3		1.32 (m), 1.29 (m)	20.6	
12	1.27 (m), 1.16 (m)	40.1	H-18	1.81 (m), 1.18 (m)	39.6	H-11, H-17, H-18
13	–	43.9	H-14, H-16, H-17, H-18	–	40.7	H-14, H-17, H-18, H-12
14	0.83 (m)	54.8	H-18	0.88 (m)	56.6	H-18
15	2.08 (m), 1.45 (m)	34.4		1.98 (m), 1.41 (m)	34.8	
16	4.87 (m)	84.7		4.98 (m)	84.0	
17	2.50 (m)	65.0	H-15, H-18, H-21	2.11 (m)	66.9	H-18, H-21, H-15
18	0.70 (s)	14.5	H-14, H-16, H-17	0.85 (s)	13.8	H-14, H-17
19	0.99 (s)	24.0		0.98 (s)	24.0	
20		105.2	H-17, H-21		105.2	
21	1.72 (s)	11.7		1.40 (s)	15.3	H-17
22	–	154.1	H-17, H-21, H-24	–	157.3	H-17, H-21 H-24, H-25
23	4.96 (m)	63.9	H-24	4.89 (m)	81.9	H-21, H-24, H-OCH_3
24	2.14 (m), 2.05 (m)	39.7	H-26, H-27, H-23	2.15 (m), 2.09 (m)	35.2	H-25, H-26, H-27
25	1.87 (m)	31.0	H-24, H-26, H-27	2.2 (m)	29.6	H-24, H-26, H-27
26	4.24 (m), 3.67 (m)	75.2	H-1'", H-27, H-24	4.15 (m), 3.56 (m)	75.2	H-25, H-27, H-1'"
27	1.17 (d, 6.5)	18.0	H-24, H-26	1.11 (d, 6.5)	17.5	H-25, H-26
OCH_3				3.18 (s)	48.8	
C-3						
Gal- 1'	4.91 (d, 7.6)	102.6	H-2', H-3'	4.99 (d, 7.0)	102.6	H-2', H-5'
2'	4.67 (m)	81.9	H-4', H-1"	4.63 (m)	81.9	H-4'
3'	4.03 (m)	76.6		4.21 (m)	76.6	
4'	4.57 (m)	69.9		4.54 (m)	69.9	
5'	4.07 (m)	76.9	H-6'	4.01 (m)	76.9	
6'	4.44 (m)	62.2		4.32 (m)	62.2	H-5’
Glc-1"	5.29 (d, 7.7)	106.1	H-2'	5.29 (d, 7.5)	106.1	H-2', H-2’’
2"	4.35 (m)	75.6	H-3"	4.08 (m)	75.6	
3"	4.19 (m)	78.1		4.89 (m)	82.4	H-1", H-4"
4"	4.18 (m)	71.7	H-3"	4.04 (m)	75.2	
5"	3.86 (m)	78.4		4.34 (m)	78.1	
6"	4.53 (m), 4.46 (m)	62.9		4.51 (m), 4.04 (m)	62.8	H-4"
C-26						
Glc-1'"	4.86 (d, 7.7)	105.3	H-2'"	4.89 (d, 7.5)	105.2	H-26
2'"	4.02 (m)	75.2		4.32 (m)	75.3	
3'"	4.23 (m)	78.5	H-2'"	4.19 (m)	78.1	H-1'", H-4'"
4'"	4.27 (m)	71.8	H-2'", H-3'", H-5'"	4.18 (m)	71.8	
5'"	4.24 (m)	78.6	H-1'"	4.86 (m)	78.4	H-6'"
6'"	4.44 (m), 4.35 (m)	62.9		4.42 (m), 4.30 (m)	62.8	