· Letters ·

Pregnane Glycosides from Stems of *Marsdenia tenacissima*

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Abstract: Objective To study the chemical constituents from the stems of *Marsdenia tenacissima.* **Methods** The chemical constituents were isolated by various column chromatography and their structures were identified by spectral and chemical analysis. **Results** Two pregnane glycosides were isolated from the stems of *M. tenacissima* and identified as 3-*O*-β-*D*-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-β-*D*-allopyranosyl-(1→4)-β-*D*-oleandropyranosyl-11α-*O*-tigloyltenacigenin B, named as tenacigenoside I (**1**) and 3-*O*-β-*D*-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-β-*D*-allopyranosyl-(1→4)-β-*D*-oleandropyranosyl-11α,12β-di-*O*-acetyltenacigenin B, named as tenacigenoside K (**2**). **Conclusion** Compound **1** is a new compound, ^{1}H-NMR and ^{13}C-NMR data of compound **2** are reported for the first time.

Key words: *Marsdenia tenacissima*; pregnane glycosides; stems; tenacigenoside I; tenacigenoside K

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Introduction

The stems of *Marsdenia tenacissima* (Roxb.) Wight et Arn. (Asclepiadaceae), known as *Tongguangsan*, have been used as a remedy to treat asthma, cancer, trachitis, tonsillitis, pharyngitis, cystitis, and pneumonia in China (Jiangsu New Medicinal College, 1977). Previous studies on this plant led to isolation of 42 pregnane glycosides and 18 pregnane genins (Liu *et al*, 2008; Deng, Liao, and Chen, 2005a; 2005b; 2005c; Wang *et al*, 2006a; 2006b; Wang, Peng, and Ding, 2010; Li *et al*, 2006; 2007; 2009). A method based on HPLC-ESI-MS^{n} analysis has been established for the analysis of polyoxypregnane glycosides in the stems of the plant (Chen *et al*, 2008). The presence of compound **2** was predicted by the ESI-MS^{n} fragmentation of polyoxypregnane glycosides in the plant. Our further investigation on the title plant led to the isolation of compounds **1** and **2** (Fig. 1). Compound **1** is a new compound, and compound **2** is obtained from nature for the first time. The present paper deals with the isolation and structural elucidation of compounds **1** and **2**.

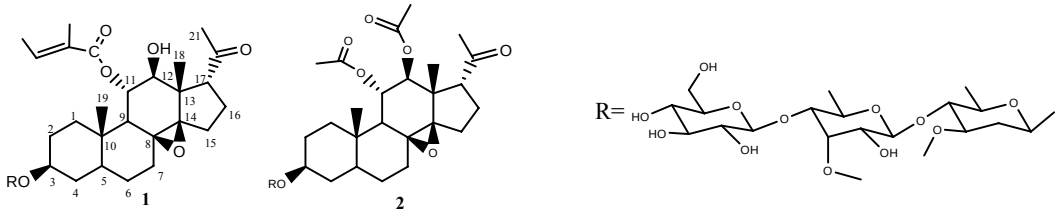

Fig. 1 Structures of compounds 1 and 2

Materials and methods

Plant material

The stems of *Marsdenia tenacissima* (Roxb.) Wight et Arn. were collected in September 2005 in Yunnan Province, China and identified by Prof. ZHAO Zuo-cheng (Chengdu Institute of Biology, Chinese Academy of Sciences). A voucher specimen (No. W2289) was deposited in the Herbarium of Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, China.

Equipments

Melting points were determined on X—4 Micro Melting Point Apparatus. The IR spectra were recorded on a Perkin—Elmer Spectrum one FT-IR Spectrometer.

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MS spectra were recorded on Finnigen LCQ Advantage Max Spectrometer. NMR spectra were measured on a Bruker Avance—600 spectrometer. $[\alpha]_D^{20}$ was measured on a PE—341 Polarimeter. Silica gel (160－200, 200－300 mesh) for column chromatography and GF_{254} silica gel plates for TLC were purchased from Qingdao Marine Chemistry Co., Ltd. ODS (230－400 mesh) was produced by Nacalai Tesque. Sephadex LH-20 was produced by Pharmacia and MCI was produced by Mitsubishi Chemical Corporation.

Extraction and isolation

The stems of *M. tenacissima* were air dried and powdered, and then boiled with water for 2 h. After concentration, the crude aqueous phase was extracted with EtOAc. The EtOAc extract (260 g) was subjected to repeated column chromatography on silica gel and ODS reversed phase silica gel chromatography to obtain compounds **1** (15 mg) and **2** (20 mg).

Results and discussion

Compound **1**: colorless powder. The molecular formula $C_{46}H_{72}O_{18}$ was established by HR-ESI-MS *m/z*: 935.4624 (calculated 935.4616 for $[M + Na]^+$). The IR spectrum showed absorption bands at 3441 cm^{-1} for hydroxyl group, and at 1709 and 1737 cm^{-1} for carbonyl groups. In its ^{1}H-NMR spectrum (Table 1), characteristic signals δ_H 4.65 (1H, t, J = 8.0 Hz), 4.71 (1H, d, J = 8.1 Hz), and 4.35 (1H, d, J = 7.8 Hz), with corresponding carbon signals at δ_C 96.9, 100.1, and 104.3 (Table 1), suggested that the sugar moiety of compound **1** consisted of three units. All of the glycosidic linkages had a β orientation from the coupling constants of anomeric proton signals. One of three anomeric protons, displaying triplet, was assigned to 2-deoxy pyranose. The two methyl signals at δ_H 1.34 (3H, d, J = 6.1 Hz) and 1.29 (3H, d, J = 6.2 Hz), and the two methoxyl groups at δ_H 3.40 and 3.59 (each 3H, s) suggested that two of the three sugar units were 6-deoxy-3-*O*-methyl-pyranose. Further more, the monosaccharides were identified as oleandrose, allose, and glucopyranose by comparing the acidic hydrolysate products of compound **1** with corresponding authentic sample on TLC. The ^{1}H-NMR and ^{13}C-NMR data (Table 1) of compound **1** supported the basic structure of marsdenoside G (Deng *et al*, 2005a; 2005b), except for an additional β-*D*-glucopyranosyl (Glc) unit information in compound **1**. Comparing with the literature, the carbon signal of C-4 of allose (δ_C 81.0) was about 8 downfield shifted and the signal of C-5 (δ_C 71.8) of allose was about 3.2 upfield shifted, respectively. This indicated that the Glc unit was linked to the C-4 of allose. Combining with the HMBC correlations, the sequence of the sugar units was deduced as β-*D*-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-β-*D*-allopyranosyl-(1→4)-β-*D*-oleandropyranosyl. This was supported by the ^{1}H-NMR and ^{13}C-NMR data of the sugar moiety of marsdenoside H. The ^{1}H-NMR and ^{13}C-NMR spectra of aglycone of compound **1** displayed tigloyl signals at δ_H 6.90 (1H, m), 1.80 (3H, d, J = 7.0 Hz), 1.83 (3H, s), with corresponding carbon signals at δ_C 128.8, 137.9, 12.2, and 14.5, respectively. Carbonyl signal in the tigloyl group is at δ_C 168.3 and linkage was assigned at C-11 by analyzing its HMBC spectrum (Fig. 2) in which δ_H 5.13 (1H, t, J = 9.5 Hz, H-11β) was correlated with the carbonyl carbon at δ_C 168.3 belonging to the tigloyl group. Thus, compound **1** was identified as 3-*O*-β-*D*-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-β-*D*-allopyranosyl-(1→4)-β-*D*-oleandropyranosyl-11α-*O*-tigloyltenacigenin B, and named as tenacigenoside I.

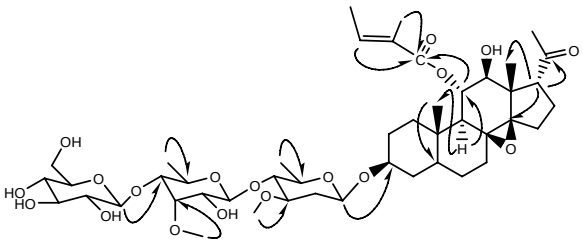

Fig. 2 Key HMBC (H→C) correlations of compound 1

Compound **2**: colorless powder. The molecular formula $C_{45}H_{70}O_{19}$ was established by HR-ESI-MS *m/z*: 937.4425 (calculated 937.4409 for $[M + Na]^+$). IR spectrum showed the presence of hydroxyl (3435 cm^{-1}) and carbonyl (1702, 1727, and 1737 cm^{-1}) groups. In the ^{1}H-NMR and ^{13}C-NMR data (Table 1) of compound **2**, characteristic anomeric proton signals δ_H 4.64 (1H, t, J = 8.1 Hz), 4.72 (1H, d, J = 8.1 Hz), and 4.35 (1H, d, J = 7.7 Hz) in combination with corresponding carbon signals at δ_C 97.3, 100.7, and 104.8 suggested that the sugar moiety of compound **2** consisted of three units. The NMR data due to the sugar moiety of compound **2** are in agreement with those of compound **1** (Table 1), so

Table 1 ^{1}H-NMR (600 MHz, in MeOD) and ^{13}C-NMR (150 MHz, 1 in $CDCl_3$ and 2 in MeOD) data

No.	1		2		No.	1		2	
	δ_H	δ_C	δ_H	δ_C		δ_H	δ_C	δ_H	δ_C
1	1.18 m	37.4	1.06 m	37.2	Ole-1	4.65 t, 8.0	96.9	4.64 t, 8.0	97.3
	1.80 m		1.72 m		Ole-2	2.26 m	36.2	2.26 m	36.5
2	1.45 m	29.3	1.83 m	28.9		1.43 m		1.44 m	
	1.75 m		1.80 m		Ole-3	3.35 m	79.0	3.34 m	79.2
3	3.65 m	76.1	3.65 m	76.4	Ole-4	3.17 m	81.0	3.17 m	82.5
4	1.54 m	34.1	1.54 m	34.5	Ole-5	3.39 m	71.8	3.40 m	71.5
	1.80 m		1.44 m		Ole-6	1.34 d, 6.1	18.5	1.35 d, 6.1	17.5
5	1.15 m	44.1	1.25 m	43.7	3-OMe	3.40 s	56.0	3.40 s	56.1
6	1.36 m	28.0	1.32 m	26.4	Allo-1	4.71 d, 8.1	100.1	4.72 d, 8.1	100.7
	2.17 m		2.22 m		Allo-2	3.82 m	71.1	3.82 m	71.1
7	1.32 m	32.5	1.35 m	31.4	Allo-3	3.95 m	80.5	3.95 m	81.8
	1.65 m		1.66 m		Allo-4	3.19 m	81.0	3.19 m	82.6
8		65.9		66.8	Allo-5	3.89 m	68.3	3.89 m	68.8
9	1.82 d, 10.4	52.1	1.82 d, 10.4	51.2	Allo-6	1.29 d, 6.2	18.0	1.29 d, 6.2	17.5
10		39.3		38.7	3-OMe	3.59 s	61.3	3.59 s	61.7
11	5.13 t	76.8	5.29 t	68.7	Glc-1	4.35 d, 7.7	104.3	4.35 d, 7.7	104.8
12	5.16 d, 10.3	79.4	4.96 d, 10.3	76.5	Glc-2	3.19 m	75.5	3.28 m	76.5
13		47.5		45.6	Glc-3	3.35 m	77.2	3.35 m	76.7
14		71.3		71. 4	Glc-4	3.28 t	70.0	3.35 m	70.5
15	1.24 m	27.0	1.25 m	26.2	Glc-5	3.20 m	73.7	3.24 m	74.1
	1.54 m		1.56 m		Glc-6	3.90 dd	61.9	3.90 dd	61.7
16	2.22 m	25.5	2.24 m	24.3		3.65 dd, 2.0		3.65 dd, 2.0	
	2.27 m		2.17 m						
17	3.37 d, 7.5	63.4	3.02 d, 7.5	60.6					
18	1.05 s	10.4	1.08 s	16.7					
19	1.02 s	12.8	1.02 s	11.8					
20		211.6		211.7					
21	2.23 s	31.6	2.16 s	31.4					
1'		168.3		170.7					
2'		128.8	1.95 s	20.1					
3'	6.90 m	137.9							
4'	1.80 d, 7.0	12.2							
5'	1.83 s	14.5							
1″				170.8					
2″			1.97 s	19.1					

it should contain the same sugar moiety as compound **1**. The conclusion was confirmed by mild acid hydrolysis of compound **2**. The sequence of the sugar units was deduced as β-*D*-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-β-*D*-allopyranosyl-(1→4)-β-*D*-oleandropyranosyl in combination with HMBC correlations (Fig. 3). The ^{1}H-NMR and ^{13}C-NMR data of compound **2** aglycone resembled 11α,12β-di-*O*-acetyltenacigenin B (Deng *et al*, 2005a; 2005b). Two methyl signals at δ_H 1.95 and 1.97 (each 3H, s, CH_3-2′, 2″) in combination with δ_C 20.1 and 19.1 (C-2′, 2″), and two carbonyl signals assigned to acetyl groups at δ_C 170.7 and 170.8 were observed in its ^{1}H-NMR and ^{13}C-NMR spectra. Meanwhile, HMBC spectrum (Fig. 3) in which δ_H 5.29 (1H, t, J = 9.5 Hz) and 4.96 (1H, d, J =10.3 Hz) were correlated with the carbonyl carbon at δ_C 170.7 and 170.8, revealed two acetyl groups linked at C-11 and C-12.

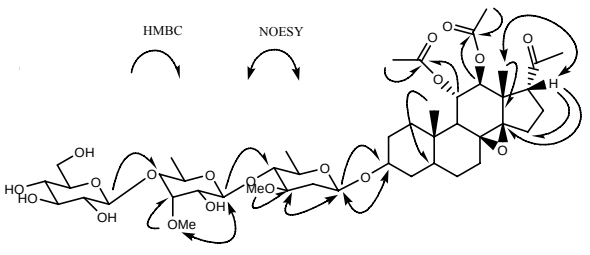

Fig. 3 Key HMBC (H→C) and NOESY correlations of compound 2

Then, the aglycone was deduced as 11α,12β-di-*O*-acetyltenacigenin B. The glycosidation shifts observed for C-2 (–1.4), C-3 (+4.9), and C-4 (–4.2) by comparing the ^{13}C-NMR data of compound **2** with those of 11α,12β-di-*O*-acetyltenacigenin B indicated that the sugar moiety in compound **2** was linked to C_3-OH of the aglycone. Finally, compound **2** was identified as 3-*O*-β-*D*-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-β-*D*-allopyranosyl-(1→4)-β-*D*-oleandro-pyranosyl-11α,12β-di-*O*-acetyltenacigenin B, named as tenacigenoside K.

Compound **1**: colorless needles, mp 152－156 ℃; $[\alpha]_D^{20}$+30° (*c* 0.12, MeOH); IRν_{max}^{KBr} (cm^{-1}): 3441, 2961, 2933, 2860, 1709, 1737, 1461, 1442, 1382. ^{1}H-NMR (600 MHz, MeOD) and ^{13}C-NMR ($CDCl_3$) data are listed in Table 1.

Compound **2**: colorless amorphous powder, mp 158－161 ℃; $[\alpha]_D^{20}$−25° (*c* 0.12, MeOH); IRν_{max}^{KBr} (cm^{-1}): 3435, 2933, 2975, 2934, 2880, 1737, 1727, 1702, 1262, 1158; ^{1}H-NMR (600 MHz, MeOD) and ^{13}C-NMR data are listed in Table 1.

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Captions of Cover Photo

Convallaria majalis L., also named lily of the valley, is the unique species in *Convallaria* L. It is a common used herb medicine mainly distributed in Europe, North America, Korea, Japan, and China. The main constituents include convallatoxin, convallarin, convallamarin, *etc*. *C. majalis* can strengthen heart and promote diuresis, and is clinically used to treat congestive heart failure, atrial fibrillation, and left heart failure caused by high blood pressure and nephritis.

Provided by ZHOU You
Tonghua Normal University