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Five new pregnane glycosides from the stems of *Marsdenia tenacissima*

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Activity-guided fractionation of the stems of *Marsdenia tenacissima* led to the isolation of five new pregnane glycosides, namely marstenacissides E (1), F (2), G (3), H (4), and I (5). Their structures were determined on the basis of ¹H and ¹³C NMR, COSY, TOCSY, ROESY, and FABMS experiments.

Keywords: *Marsdenia tenacissima*; Asclepiadaceae; pregnane glycoside; Marstenacissides E, F, G, H, I

1. Introduction

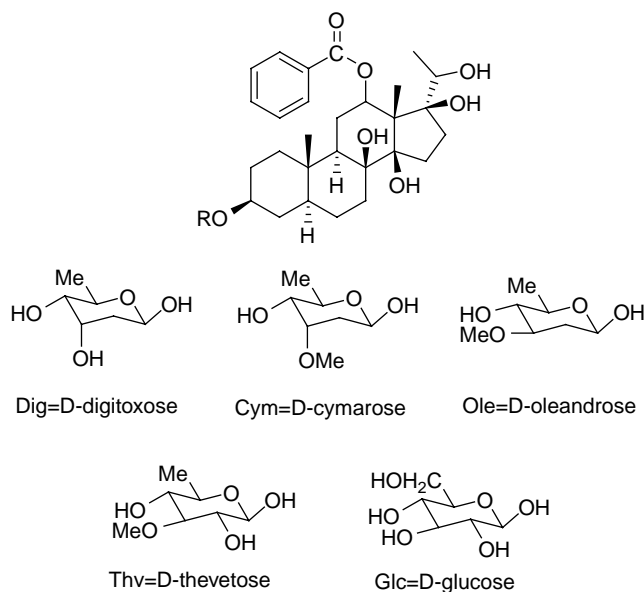
Marsdenia tenacissima (Roxb.) Wight et Arn. is distributed in the southwest of China. Its stems are used for the treatment of cancer and asthma in Chinese folk medicine [1]. In previous studies on the stems of this plant, the isolation of tenacissosides A–E [2], F–I [3], marsdenosides A–H [4], two C₂₁ steroids [5], tenacissosides L, M [6], and five C₂₁ steroidal glycosides [7] has been reported. In the course of our search for bioactive compounds from this plant, we reported the isolation of marstenacissides A–D recently [8]. In this paper, we describe the isolation and structural elucidation of five new pregnane glycosides, namely marstenacissides E (1), F (2), G (3), H (4), and I (5) from *M. tenacissima* (Figure 1).

2. Results and discussion

Marstenacisside E (1), an amorphous solid, has a molecular formula C₆₁H₉₆O₂₅,

determined from its negative ion FAB-MS (m/z 1227 [M – H][–]) as well as ¹³C NMR and DEPT spectral data. The IR spectrum of 1 showed that the absorption bands are due to hydroxyl group (3425 cm^{–1}) and glycoside linkages (1060 cm^{–1}). Compound 1 exhibited positive Liebermann–Burchard and Keller–Kiliani reactions. Its spectral features and physicochemical properties suggested that 1 should be a steroidal glycoside with 2-deoxy sugar units. Of the 61 carbons, 28 were assigned to the aglycon part and 33 to the oligosaccharide moiety. The ¹H and ¹³C NMR spectra of the aglycon moiety of 1 indicated the presence of three methyl groups [δ_{H} 1.19, 2.20 (each 3H, s) and 1.27 (3H, d, $J = 6.1$ Hz), δ_{C} 12.2, 13.0, and 19.5], three oxygenated methine groups [δ_{H} 3.91 (1H, m), 4.09 (1H, q, $J = 6.0$ Hz) and 5.32 (1H, dd); δ_{C} 70.7, 75.9, and 76.7], one benzoyl group, and five quaternary carbons (δ_{C} 36.6, 57.5, 76.0, 88.4, and 88.9)

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- 1 R=Glc(1→4)Thv(1→4)Ole(1→4)Cym(1→4)Dig
- 2 R=Glc(1→4)Thv(1→4)Ole(1→4)Cym
- 3 R=Glc'(1→4)Glc(1→4)Thv(1→4)Ole(1→4)Cym
- 4 R=Glc'(1→4)Glc(1→4)Thv(1→4)Ole(1→4)Cym(1→4)Dig
- 5 R=Glc'(1→4)Glc(1→4)Ole(1→4)Cym(1→4)Cym'

Figure 1. Structure of compounds 1–5.

(Table 1). All of these spectral data suggested that the aglycon of **1** was a pregnane highly oxidized at C-3, C-8, C-12, C-14, C-17, and C-20. The presence of one benzoyl group at C-12 was confirmed by observation of the correlation between H-12 at δ_{H} 5.32 and C=O of the benzoyl group at δ_{C} 166.6 in the HMBC spectrum of **1**. Comparison of the ^{13}C NMR spectrum of the aglycon moiety of **1** with that of 12-*O*-benzoyl-dihydrosarcostin (**A**) [8] (Table 1) showed that the aglycon moiety of **1** was 12-*O*-benzoyl-dihydrosarcostin. The carbon signals at δ_{C} 25.2 and 34.9 of 12-*O*-benzoyl-dihydrosarcostin (**A**) have been assigned to C-7 and C-11, respectively, by Yuan et al. [9]. However, the correlation

between the H-12 at δ_{H} 5.32 and the carbon at δ_{C} 24.6 in the HMBC spectrum of **1** suggested that the signal at δ_{C} 24.6 should be assigned to C-11. Therefore, the assignments for C-7 and C-11 of 12-*O*-benzoyl-dihydrosarcostin (**A**) reported by Yuan et al. should be revised to C-11 and C-7, respectively. The glycosylation shifts of **1** [C-3 (+5.8 ppm), C-2 (−2.4 ppm), and C-4 (−4.4 ppm)] indicated that the sugars of **1** were bound to the C-3 position of the aglycon.

In the ^1H NMR and ^{13}C NMR spectra of the sugar moiety of **1** (Tables 2 and 3), the signals due to five anomeric protons and carbons [δ_{H} 4.67 (1H, dd, J = 9.9, 2.0 Hz), 4.89 (1H, d, J = 8.6 Hz), 5.13

Table 1. ^{13}C NMR spectral data for the aglycon moieties of compounds **1**, **2**, **3**, **4**, **5**, and 12-*O*-benzoyl-dihydrosarcostin (A) (Pyridine- d_5 , δ in ppm, 150 MHz).

	1	2	3	4	5	A ^a
1	38.1	38.0	38.1	38.3	38.0	38.6
2	29.6	29.6	29.6	29.5	29.6	32.0
3	76.7	76.7	76.7	76.9	76.6	70.9
4	34.5	34.7	34.6	34.4	34.5	38.9
5	45.3	45.3	45.3	45.4	45.3	46.1
6	25.2	25.2	25.2	24.5	25.2	25.5
7	34.3	34.7	34.1	34.6	34.5	25.2
8	76.0	76.0	76.0	76.0	75.9	76.1
9	46.9	46.9	46.8	47.0	46.8	47.6
10	36.6	36.6	36.5	36.7	36.5	36.6
11	24.6	24.6	24.6	25.1	24.8	34.9
12	75.9	75.9	75.8	75.9	75.8	75.9
13	57.5	57.5	57.5	58.0	57.5	59.2
14	88.4	88.4	88.9	88.6	88.4	89.0
15	32.8	32.8	32.8	34.0	32.8	34.1
16	33.8	33.8	33.8	34.6	33.8	34.1
17	88.9	88.9	88.8	89.0	88.8	88.8
18	12.2	12.2	12.2	12.1	12.2	11.7
19	13.0	13.0	13.0	13.0	12.9	13.3
20	70.7	70.7	70.7	70.8	70.8	71.7
21	19.5	19.5	19.5	19.5	19.6	17.7
Bez						
C=O	166.6	166.6	166.6	166.7	166.6	167.0
1'	131.6	131.8	131.7	131.6	131.8	131.6
2', 6'	130.4	130.4	130.4	130.4	130.4	130.4
3', 5'	129.0	128.8	128.8	128.7	128.8	129.0
4'	133.0	133.2	133.3	133.3	133.2	133.0

Note: Bez: benzoyl group.

^aReported data for 12-*O*-benzoyl-dihydrosarcostin by Yuan et al.

(1H, d, $J = 8.0$ Hz), 5.19 (1H, dd, $J = 9.8$, 2.1 Hz), and 5.53 (1H, dd, $J = 9.2$, 2.1 Hz); δ_{C} 101.9, 104.0, 104.0, 99.7, and 95.8], four methyl groups [δ_{H} 1.33, 1.49, 1.67, and 1.77 (each 3H, d)], and three methoxyl groups [δ_{H} 3.52, 3.58, and 3.96 (each 3H, s)] suggested that one hexose and four deoxy sugars were present in **1**. Among the four deoxy sugars, three were 2,6-dideoxy sugars and the other one should be 6-deoxy sugar. It was disclosed that the sugars of **1** should be composed of one 2,6-dideoxypyranose (S1), two 2,6-dideoxy-3-*O*-methylpyranoses (S2 and S3), one 6-deoxy-3-*O*-methylpyranose (S4), and one glucose (S5) by detailed analyses of TOCSY, FOCSY, HMQC, SELTOCSY, HMBC, and SELROESY spectra of **1**. The comparison of the

chemical shift values of the anomeric protons [δ 5.19 (1H, dd, $J = 9.8$, 2.1 Hz) and 4.67 (1H, dd, $J = 9.9$, 2.0 Hz)] of S2 and S3 with those of cymarose (Cym: δ 5.08–5.29) and oleandrose (Ole: δ 4.66–4.90, in a higher field than Cym) [10] suggested that S2 and S3 should be Cym and Ole, respectively. In the light of the ^1H and ^{13}C NMR spectra of the sugar moiety of **1** (Tables 2 and 3), S1, S4, and S5 were assigned to be digitoxose (Dig), thevetose (Thv), and glucose (Glc), respectively. Finally, the result of acid hydrolysis of **1** (see Section 3) further confirmed that the sugars of **1** were composed of digitoxose (S1), cymarose (S2), oleandrose (S3), thevetose (S4), and glucose (S5). The connectivity of these sugars was

Table 2. ¹H NMR spectral data for the sugar moieties of compounds **1**, **2**, **3**, **4**, and **5** (Pyridine-*d*₅, δ in ppm, *J* in Hz, 600 MHz).

	1	2	3	4	5
	dig	Cym	cym	Dig	Cym'
1	5.53, dd, (9.2, 2.1)	5.31, dd, (9.6, 2.0)	5.31, dd, (9.2, 2.0)	5.54, dd, (9.8, 2.1)	5.33, dd, (9.8, 2.0)
2	2.43, 2.07	2.32, 1.89	2.34, 1.89	2.45, 2.06	2.35, 1.82
3	4.65, q, (2.5)	4.09, q, (3.1)	4.07, q, (3.1)	4.65, q, (2.5)	4.11, q, (3.1)
4	3.52, dd, (9.0, 2.5)	3.49, dd, (9.1, 3.0)	3.50, dd, (9.0, 3.1)	3.51, dd, (9.0, 2.5)	3.53, dd, (9.0, 3.1)
5	4.35	4.30	4.27	4.35	4.28*
6	1.49, d, (6.1)	1.49, d, (6.1)	1.48, d, (6.1)	1.33, d, (6.1)	1.42, d, (6.0)
OCH ₃		3.61*	3.59*		3.65
	cym	Ole	ole	Cym	Cym
1	5.19, dd, (9.8, 2.1)	4.71, dd, (9.8, 2.1)	4.72, dd, (9.7, 2.0)	5.18, dd, (9.8, 2.0)	5.13, dd, (9.8, 2.1)
2	2.32, 1.78,	2.50, 1.75	2.50, 1.76	2.34, 1.80,	2.33, 1.88
3	4.05, q, (3.0)	3.55	3.58	3.93, q, (3.0)	4.02, q, (3.0)
4	3.40, dd, (9.1, 3.0)	3.60, t, (9.1)	3.60, t, (9.1)	3.40, dd, (9.1, 3.0)	3.44, dd, (9.1, 3.0)
5	4.19	3.57	3.59*	4.17	4.18
6	1.33, d, (5.5)	1.67, d, (6.1)	1.67, d, (6.0)	1.45, d, (5.5)	1.38, d, (6.0)
OCH ₃	3.58	3.61*	3.51	3.52	3.58
	ole	Thv	Thv	Ole	Ole
1	4.67, dd, (9.9, 2.0)	4.89, d, (8.8)	4.87, d, (8.6)	4.79, dd, (9.8, 2.0)	4.70, dd, (9.9, 2.0)
2	2.46, 1.74	3.90	3.91	2.46, 1.75	2.46, 1.79
3	3.57	3.90, t, (9.2)	3.67, t, (9.1)	3.57	3.60
4	3.60, t, (9.1)	3.88, t, (9.2)	3.82, t, (9.1)	3.62, t, (9.1)	3.68, t, (9.1)
5	3.75	3.56	3.74	3.54	3.65
6	1.67, d, (6.0)	1.78, d, (6.0)	1.75, d, (6.1)	1.67, d, (6.0)	1.72, d, (6.0)
OCH ₃	3.52	3.91	3.93	3.60	3.50
	thv	Glc	Glc	Thv	Glc
1	4.89, d, (8.6)	5.13, d, (8.0)	5.08, d, (8.0)	4.89, d, (8.8)	5.08, d, (8.0)
2	3.91	4.05	4.03	3.88	4.03
3	3.70, t, (9.2)	4.24	4.28	3.70, t, (9.2)	4.28*
4	3.88, t, (9.2)	4.21	4.30	3.87, t, (9.2)	4.30
5	3.74	3.98	3.92	3.75	3.92
6	1.77, d, (5.8)	4.55, 4.38	4.49, 4.30	1.76, q, (5.8)	4.55, 4.50
OCH ₃	3.96			3.91	
	glc		glc'	Glc	glc'
1	5.13, d, (8.0)		5.18, d, (7.8)	5.12, d, (7.8)	5.18, d, (7.8)
2	4.03		4.10	4.03	4.10
3	4.24		4.22 [§]	4.25	4.29
4	4.21		4.20	4.20*	4.20
5	3.98		4.22 [§]	3.97	4.04
6	4.53, 4.36		4.54, 4.28	4.55, 4.35	4.54, 4.28
				glc'	
				5.21, d, (7.8)	
				4.12	
				4.22	
				4.20*	
				4.10	
				4.56, 4.31	

Note: *, §, overlapped signals.

Table 3. ^{13}C NMR spectral data for the sugar moieties of compounds **1**, **2**, **3**, **4**, and **5** (Pyridine- d_5 , δ in ppm, 150 MHz).

	1	2	3	4	5
	dig	cym	cym	Dig	Cym'
1	95.8	95.9	95.9	95.8	95.9
2	39.1	37.2	37.5	39.1	37.3
3	67.6	77.9	77.8	67.6	78.1
4	83.5	83.6	83.6	83.1	83.5
5	68.6	68.9	68.8	68.6	69.0
6	18.7	18.8	18.6	18.5	18.7
OCH ₃		58.9	58.9		58.9
	cym	ole	ole	Cym	cym
1	99.7	101.9	101.9	99.8	100.5
2	36.7	37.6	37.5	36.7	37.0
3	77.7	79.2	79.1	77.9	77.8
4	83.2	83.3	83.4	83.4	83.2
5	69.0	71.9	71.9	69.0	68.9
6	18.7	18.6	18.7	18.7	18.5
OCH ₃	58.9	57.4	57.4	58.9	59.0
	ole	thv	thv	ole	ole
1	101.9	104.0	104.0	101.9	101.9
2	37.6	74.8	74.8	38.3	37.6
3	79.3	86.3	86.4	79.2	79.3
4	83.3	83.4	83.5	83.1	83.3
5	72.0	71.9	72.0	72.0	72.0
6	18.7	18.5	18.7	18.8	18.8
OCH ₃	57.4	60.7	60.7	57.4	57.4
	thv	glc	glc	thv	glc
1	104.0	105.0	104.7	103.9	104.3
2	75.0	75.9	75.4	74.9	75.4
3	86.3	78.6	78.2	86.3	76.8
4	83.3	72.0	81.7	83.2	81.5
5	72.1	78.2	76.3	72.0	76.5
6	18.7	62.4	62.5	18.7	62.5
OCH ₃	60.6			60.7	
	glc		glc'	glc	glc'
1	104.8		105.0	104.8	105.0
2	75.8		75.0	75.8	74.8
3	78.4		78.3	78.4	78.3
4	72.1		71.6	82.1	71.6
5	78.1		78.5	78.1	78.5
6	63.1		62.5	63.1	62.5
				glc'	
1				105.0	
2				75.0	
3				78.3	
4				71.6	
5				78.5	
6				62.5	

investigated by analysis of HMBC and ROESY spectra of **1**.

In the HMBC spectrum of **1** (Figure 2), the cross peaks between the following proton and carbon signals were observed:

H-3 of aglycon/C-1 of Dig, H-4 of Dig/C-1 of Cym, H-4 of Cym/C-1 of Ole, H-4 of Ole/C-1 of Thv, and H-4 of Thv/C-1 of Glc. Furthermore, the ROESY spectrum of **1** (Figure 2) exhibited the cross peaks

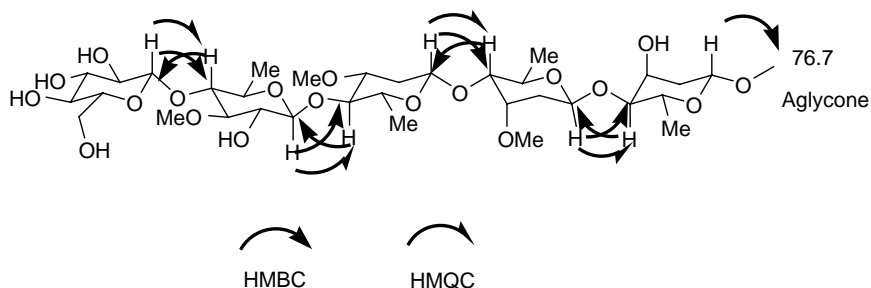


Figure 2. HMBC and NOESY correlations of the sugar moiety of **1**.

between the following proton signals: H-3 of aglycon/H-1 of Dig, H-4 of Dig/H-1 of Cym, H-4 of Cym/H-1 of Ole, H-4 of Ole/H-1 of Thv, and H-4 of Thv/H-1 of Glc. The β configurations for all of the sugars were determined from their large $^3J_{H1,H2}$ coupling constants (7.8–9.8 Hz). Thus, the sequence and the linkage sites of the pentasaccharide of **1** was represented as shown in Figure 2. Therefore, **1** was established as 3-*O*- β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-thvetopyranosyl-(1 \rightarrow 4)- β -D-oleandropyranosyl-(1 \rightarrow 4)- β -D-cymaropyranosyl-(1 \rightarrow 4)- β -D-digitoxopyranosyl-12-*O*-benzoyl-dihydrosarcostin.

Marstenacisside F (**2**), an amorphous solid, had a molecular formula $C_{55}H_{86}O_{22}$ determined from its negative ion FAB-MS (m/z : 1097 [M - H]⁻) as well as ^{13}C NMR and DEPT spectral data. The spectral evidence indicated that compound **2** had the same aglycon as that of **1** (Table 1) and the structural difference should be in the sugar part. In the 1H and ^{13}C NMR spectra of the sugar moiety of **2** (Tables 2 and 3), the signals due to four anomeric protons and carbons [δ_H 4.71 (1H, dd, J = 9.8, 2.1 Hz), 4.89 (1H, d, J = 8.8 Hz), 5.13 (1H, d, J = 8.0 Hz), and 5.31 (1H, dd, J = 9.6, 2.0 Hz); δ_C 101.9, 104.0, 105.0, and 95.9] indicated the presence of four sugars in **2**. Comparison of the ^{13}C NMR, 1H - 1H COSY, TOCSY, ROESY, HMQC, and HMBC spectra of **2** with those of **1** suggested that the four sugars of **2** were identical to those of **1** except for the

absence of a digitoxose moiety. In the HMBC spectrum of **2**, the correlations were observed between the following proton and carbon signals: H-3 of aglycon/C-1 of Cym, H-4 of Cym/C-1 of Ole, H-4 of Ole/C-1 of Thv, and H-4 of Thv/C-1 of Glc. The ROESY spectrum showed the cross peaks between the following proton-proton signals: H-3 of aglycon/H-1 of Cym, H-4 of Cym/H-1 of Ole, H-4 of Ole/H-1 of Thv, and H-4 of Thv/H-1 of Glc. Therefore, the structure of **2** was established to be 3-*O*- β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-thvetopyranosyl-(1 \rightarrow 4)- β -D-oleandropyranosyl-(1 \rightarrow 4)- β -D-cymaropyranosyl-12-*O*-benzoyl-dihydrosarcostin.

Marstenacisside G (**3**), an amorphous solid, had a molecular formula $C_{61}H_{96}O_{27}$ determined from its negative ion FAB-MS (m/z : 1259 [M - H]⁻) as well as ^{13}C NMR and DEPT spectral data. The spectral evidence indicated that **3** had the same aglycon as that of **2** (Table 1), but differed in the sugar part. The 1H and ^{13}C NMR spectra of the sugar moiety of **3** (Tables 2 and 3) [δ_H 4.72 (1H, dd, J = 9.7, 2.0 Hz), 4.87 (1H, d, J = 8.6 Hz), 5.08 (1H, d, J = 8.0 Hz), 5.18 (1H, d, J = 7.8 Hz), and 5.31 (1H, dd, J = 9.2, 2.0 Hz); δ_C 101.9, 104.0, 104.7, 105.0, and 95.9 assignable to the anomeric protons and carbons] manifested the pentasaccharide feature of sugar moiety of **3**. Comparison of the ^{13}C NMR, 1H - 1H COSY, TOCSY, ROESY, HMQC, and HMBC spectra of **3** with those of **2**

indicated that four sugars were identical with those of **2**, except that an additional glucose existed in the sugar chain of **3**. The glycosylation shift (+9.6 ppm) of the C₄ signal of the glucose (Glc) in **3** suggested that the additional glucose (Glc') in **3** should be bound to C₄ of Glc. Finally, the sequence and the linkage sites of the pentasaccharide of **3** were further confirmed by HMBC and ROESY experiments. Thus, **3** was established to be 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-thevetopyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-12-*O*-benzoyl-dihydrosarcostin.

The structure of compound **3** reported by Liu et al. [11] is the same as that of marstenacisside G (**3**). However, compound **3** exists in a fraction of extract from *Dregea sinensis* var. *corrugate* instead of being isolated. Its structure was deduced from HPLC-HR-ESI-MS and HPLC-DADESIMSLC-ESI. Also, the reported data (Table 2 in Liu's article: ESI-MS [M + Na]⁺ 1267 and the molecular formula C₆₁H₉₆O₂₅Na) of compound **3** are in agreement neither with its structure nor with Marstenacisside G (**3**) (FAB-MS 1259 [M - H]⁻ and the molecular formula is C₆₁H₉₆O₂₇). No further data of compound **3** were reported in Liu's article.

Marstenacisside F (**4**), an amorphous solid, had the molecular formula C₆₇H₁₀₆O₃₀ determined from its negative ion FAB-MS (*m/z*: 1389 [M - H]⁻) as well as ¹³C NMR and DEPT spectral data. The spectral evidence indicated that **4** had the same aglycon as that of **3** (Table 1), but differed in the sugar moiety. The ¹H and ¹³C NMR spectra of the sugar moiety of **4** (Tables 2 and 3) [δ_{H} 4.79 (1H, dd, *J* = 9.8, 2.0 Hz), 4.89 (1H, d, *J* = 8.8 Hz), 5.12 (1H, d, *J* = 7.8 Hz), 5.18 (1H, dd, *J* = 9.8, 2.0 Hz), 5.21 (1H, d, *J* = 7.8 Hz), and 5.54 (1H, dd, *J* = 9.8, 2.1 Hz); δ_{C} 101.9, 103.9, 104.8, 99.8, 105.0, and 95.8] indicated that the sugar moiety of **4** was a hexasaccharide. Comparison of ¹³C NMR, ¹H-¹H COSY,

TOCSY, ROESY, HMQC, and HMBC spectra of **4** with those of **3** suggested that the sugar moiety of **4** was similar to that of **3** except for the presence of an additional digitoxopyranose (Dig). By analysis of the HMBC and ROESY spectra, **4** was established as 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-thevetopyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranosyl-12-*O*-benzoyl-dihydrosarcostin.

Marstenacisside F (**5**), an amorphous solid, had the molecular formula C₆₁H₉₆O₂₆ determined from its negative ion FAB-MS (*m/z*: 1243 [M - H]⁻) as well as ¹³C NMR and DEPT spectral data. The spectral evidence indicated that **5** had the same aglycon as that of **4** (Table 1), but differed in the sugar moiety. The pentasaccharide feature of **5** was proved by its ¹H and ¹³C NMR spectra [δ_{H} 4.70 (1H, dd, *J* = 9.9, 2.0 Hz), 5.08 (1H, d, *J* = 8.0 Hz), 5.13 (1H, dd, *J* = 9.8, 2.1 Hz), 5.18 (1H, d, *J* = 7.8 Hz), and 5.33 (1H, dd, *J* = 9.8, 2.0 Hz); δ_{C} 101.9, 104.3, 95.9, 100.5, and 105.0] (Tables 2 and 3). Detailed analysis of the ¹³C NMR, ¹H-¹H COSY, TOCSY, ROESY, HMQC, and HMBC spectra of **5** indicated that the sugar moiety of **5** was composed of one oleandropyranose, two cymaropyranoses, and two glucopyranoses. The connectivity of the sugar units of **5** was deduced from the results of the HMBC and ROESY experiments. Therefore, **5** was established as 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranosyl-12-*O*-benzoyl-dihydrosarcostin.

Finally, these above results showed the presence of the β configuration at 1 → 4 linkage in all of the sugar moieties of five new pregnane glycosides **1**, **2**, **3**, **4**, and **5**. In their HMBC and ROESY spectra, correlations of H-1/C-4 and H-1/H-4 signals were observed without an exception, respectively. On the other hand, all of the C-4 signals at the connected position in

the sugar moieties appeared ca. 10 ppm downfield without regard to 2-hydroxy (Glc, Thev) or 2-deoxy (Cym, Ole) sugars.

3. Experimental

3.1 General experimental procedures

IR was recorded on a Perkin-Elmer 599 infrared spectrometer. The ^1H and ^{13}C NMR and all 2D NMR spectra were recorded on a JEOL α 600 with AFG-type field gradient unit, TMS as internal standard, $\text{C}_5\text{D}_5\text{N}$ as solvent. FAB-MS was measured on a MAT-95 mass spectrometer. Lichroprep RP-18 (25–40 μm , Merck, Darmstadt, Germany), Diaion HP-20 (Mitsubishi Kasei, Tokyo, Japan), and silica gel 60H (Qingdao Haiyang Chemical Group Co. Qingdao, China) were used for column chromatography. TLC was performed on silica gel HSGF₂₅₄ (Zhifu Huangwu Co. Ltd. of Yantai, Yantai, China). Spots were visualized by spraying with 10% H_2SO_4 in 95% EtOH followed by heating.

3.2 Plant materials

The stems of *M. tanecissima* were purchased in Kunming, Yunnan Province (China), in 2000. Botanical identification was made by Dr Wang-Xing Xing and Prof. He-Ming Mi (The Second Military Medical University). A voucher specimen (No. 7) is deposited at the Herbarium of the Department of Phytochemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

3.3 Extraction and isolation

The powdered stems of *M. tanecissima* (15 kg) were extracted with 95% EtOH under reflux. After evaporation of ethanol *in vacuo*, the residue was suspended in water and then extracted successively with petroleum ether, EtOAc, and *n*-BuOH. The *n*-BuOH fraction (180 g) was subjected to a Diaion HP-20 column using an EtOH–

H_2O gradient system (0 to 95%) to afford four fractions (Fr. 1–Fr. 4). Fr. 4 (12 g) eluted with 95% EtOH was subjected to a silica gel column eluted with CHCl_3 –MeOH: H_2O (7:1:0.05; 5:1:0.1; 4:1:0.1) to give three fractions (Fr. 4-1–Fr. 4-3), respectively. Fr. 4-2 (2.0 g) was subjected to an RP-18 column eluted with 65% MeOH– H_2O and further separated by silica gel column chromatography eluted with EtOAc:MeOH: H_2O (8:1:1), to get compounds **1** (60 mg), **2** (52 mg), **3** (105 mg), **4** (40 mg), and **5** (18 mg).

3.3.1 *Marstenacisside E* (1)

An amorphous solid, IR $\nu_{\text{max}}^{\text{KBr}}$: 3425, 1060 cm^{-1} . The ^1H NMR ($\text{C}_5\text{D}_5\text{N}$) spectral data of aglycon moiety of **1**: δ 1.19 (3H, s, H-19), 1.27 (3H, d, $J = 6.1$ Hz, H-21), 2.20 (3H, s, H-18), 3.91 (1H, m, H-3), 4.09 (1H, q, $J = 6.1$ Hz, H-20), 5.32 (1H, dd, $J = 4.3, 11.5$ Hz, H-12), 7.40 (2H, t, $J = 8.2$ Hz, H-3', 5'), 7.49 (1H, t, $J = 8.2$ Hz, H-4'), and 8.50 (2H, d, $J = 8.2$ Hz, H-2', 6'). The ^{13}C NMR spectral data of the aglycon moiety of **1** are given in Table 1. The ^1H and ^{13}C NMR spectral data of the sugar moiety of **1** are given in Tables 2 and 3. FAB-MS m/z : 1227 $[\text{M} - \text{H}]^-$.

3.3.2 *Marstenacisside F* (2)

An amorphous solid, IR $\nu_{\text{max}}^{\text{KBr}}$: 3438, 1060 cm^{-1} . The ^1H NMR spectral data ($\text{C}_5\text{D}_5\text{N}$) of aglycon moiety of **2**: δ 1.19 (3H, s, H-19), 1.27 (3H, d, $J = 6.1$ Hz, H-21), 2.20 (3H, s, H-18), 3.91 (1H, m, H-3), 4.05 (1H, q, $J = 6.1$ Hz, H-20), 5.31 (1H, dd, $J = 4.5, 11.5$ Hz, H-12), 7.40 (2H, t, $J = 8.2$ Hz, H-3', 5'), 7.50 (1H, t, $J = 8.2$ Hz, H-4'), and 8.53 (2H, d, $J = 8.2$ Hz, H-2', 6'). The ^{13}C NMR spectral data of the aglycon moiety of **2** are given in Table 1. The ^1H and ^{13}C NMR spectral data of the sugar moiety of **2** are given in Tables 2 and 3. FAB-MS m/z : 1097 $[\text{M} - \text{H}]^-$.

3.3.3 *Marstenacisside G (3)*

An amorphous solid, IR ν_{\max}^{KBr} : 3423, 1060 cm^{-1} . The ^1H NMR spectral data ($\text{C}_5\text{D}_5\text{N}$) of the aglycon moiety of **3**: δ 1.18 (3H, s, H-19), 1.27 (3H, d, $J = 6.1$ Hz, H-21), 2.19 (3H, s, H-18), 3.91 (1H, m, H-3), 4.08 (1H, q, $J = 6.1$ Hz, H-20), 5.31 (1H, dd, $J = 4.3, 11.5$ Hz, H-12), 7.39 (2H, t, $J = 8.2$ Hz, H-3', 5'), 7.50 (1H, t, $J = 8.2$ Hz, H-4'), and 8.52 (2H, d, $J = 8.2$ Hz, H-2', 6'). The ^{13}C NMR spectral data of the aglycon moiety of **3** are given in Table 1. The ^1H and ^{13}C NMR spectral data of the sugar moiety of **3** are given in Tables 2 and 3. FAB-MS m/z : 1259 $[\text{M} - \text{H}]^-$.

3.3.4 *Marstenacisside H (4)*

An amorphous solid, IR ν_{\max}^{KBr} : 3448, 1060 cm^{-1} . The ^1H NMR spectral data ($\text{C}_5\text{D}_5\text{N}$) of the aglycon moiety of **4**: δ 1.19 (3H, s, H-19), 1.30 (3H, d, $J = 6.1$ Hz, H-21), 2.19 (3H, s, H-18), 3.90 (1H, m, H-3), 4.05 (1H, q, $J = 6.1$ Hz, H-20), 5.31 (1H, dd, $J = 4.3, 11.3$ Hz, H-12), 7.40 (2H, t, $J = 8.2$ Hz, H-3', 5'), 7.50 (1H, t, $J = 8.2$ Hz, H-4'), and 8.51 (2H, d, $J = 8.2$ Hz, H-2', 6'). The ^{13}C NMR spectral data of the aglycon moiety of **4** are given in Table 1. The ^1H and ^{13}C NMR spectral data of the sugar moiety of **4** are given in Tables 2 and 3. FAB-MS m/z : 138 $[\text{M} - \text{H}]^-$.

3.3.5 *Marstenacisside I (5)*

An amorphous solid, IR ν_{\max}^{KBr} : 3448, 1060 cm^{-1} . The ^1H NMR spectral data ($\text{C}_5\text{D}_5\text{N}$) of the aglycon moiety of **5**: δ 1.17 (3H, s, H-19), 1.31 (3H, d, $J = 6.1$ Hz, H-21), 2.19 (3H, s, H-18), 3.88 (1H, m, H-3), 4.07 (1H, q, $J = 6.1$ Hz, H-20), 5.31 (1H, dd, $J = 4.3, 11.5$ Hz, H-12), 7.42 (2H, t, $J = 8.2$ Hz, H-3', 5'), 7.50 (1H, t, $J = 8.2$ Hz, H-4'), and 8.53 (2H, d, $J = 8.2$ Hz, H-2', 6'). The ^{13}C NMR spectral data of the aglycon moiety of **5** are given in Table 1. The ^1H and ^{13}C NMR

spectral data of the sugar moiety of **5** are given in Tables 2 and 3. FABMS m/z : 1243 $[\text{M} - \text{H}]^-$.

3.4 Acid hydrolysis

Compound **1** (5 mg) was heated in 5 ml of 0.1 N HCl:dioxane (1:1) at 95°C for 2 h. The reaction mixture was concentrated *in vacuo* and 3 ml of H_2O was added. The mixture was neutralized with 10% KOH and then partitioned with $\text{CHCl}_3\text{-H}_2\text{O}$ to get the aglycon and the sugar fractions. The sugars were identified by comparison of R_f values with those of authentic samples on TLC [(silica gel, developed with $\text{CHCl}_3\text{:MeOH:H}_2\text{O}$ (3:1:0.1) and EtOAc:MeOH:H₂O (6:1.1:1)], detected by spraying with aniline-phthalic acid reagent [aniline-phthalic acid-*n*-BuOH (4:5:0.5)] and then heating to 110°C.

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