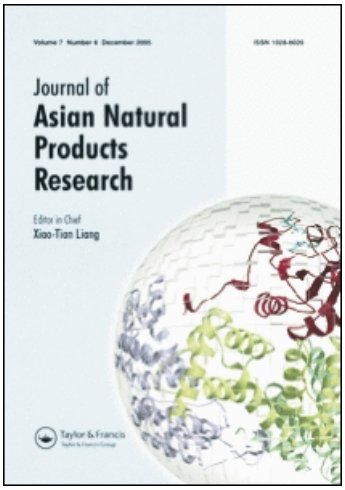

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Pregnane glycosides from the stems of *Marsdenia tenacissima*

Zeng-Hua Xia[a]; Wang-Xing Xing[b]; Shi-Long Mao[a]; Ai-Na Lao[a]; Jun Uzawa[c]; Shigeo Yoshida[c]; Yasuo Fujimoto[d]
[a] Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Shanghai, China [b] Department of Pharmacy, Hangzhou, China [c] The Institute of Physical and Chemical Research (RIKEN), Saitama, Japan [d] College of Pharmacy, Nihon University, Chiba, Japan

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PREGNANE GLYCOSIDES FROM THE STEMS OF *MARSDENIA TENACISSIMA*

ZENG-HUA XIA[a], WANG-XING XING[b], SHI-LONG MAO[a], AI-NA LAO[a,*], JUN UZAWA[c], SHIGEO YOSHIDA[c] and YASUO FUJIMOTO[d]

[a]*Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, 200031, China;* [b]*Department of Pharmacy, 117th Hospital, PLA., Hangzhou, 310013, China;* [c]*The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 351-0198, Japan;* [d]*College of Pharmacy, Nihon University, 7-7-1 Narashinodai, Funabashi, Chiba 274-8555, Japan*

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Four new pregnane glycosides, named marstenacissides A (**1**), B (**2**), C (**3**), and D (**4**), have been isolated from the stems of *Marsdenia tenacissima*. Their structures were established on the basis of chemical and spectral methods.

Keywords: *Marsdenia tenacissima*; Asclepiadaceae; Pregnane glycoside; Marstenacissides A, B, C, D

INTRODUCTION

Marsdenia tenacissima (Roxb.) Wight et Arn. is distributed in the Southwest of China. Its stems are used to treat cancer and asthma in Chinese folk medicine [1]. Previous studies on the stems of this plant have led to the isolation of 9 pregnane glycosides, tenacissiosides A–E [2], F–I [3]. During our search for bioactive compounds from this plant, four new pregnane glycosides, named marstenacissides A (**1**), B (**2**), C (**3**), and D (**4**), were isolated. This paper deals with their isolation and structural elucidation.

RESULTS AND DISCUSSION

Marstenacisside A (**1**), an amorphous solid, had a molecular formula $C_{54}H_{92}O_{24}$, determined from its negative ion FAB-MS spectrum (*m/z* 1123 $[M - H]^-$) as well as from ^{13}C NMR and DEPT data. The IR spectrum of **1** showed a hydroxy absorption (3348 cm^{-1}) and a glycoside linkage (1000–1100 cm^{-1}). Compound **1** showed positive Liebermann–Burchard and Keller–Kiliani reactions. Its spectral features and physicochemical properties suggested that

*Corresponding author. Tel.: +86-021-64437625. Fax: +86-021-64437625. E-mail: ainalao@hotmail.com

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it is a steroid glycoside with 2-deoxy sugar units. Of the 54 carbons, 21 were assigned to the aglycon part, 33 to the oligosaccharide moiety. The ^{1}H and ^{13}C NMR spectra of the aglycon of **1** (Table I) showed the signals of three methyl groups [δ 1.20; 1.94 (each 3H, s), 1.50 (3H, d, $J = 5.7$ Hz), δ 11.9, 13.1, 17.7], three oxygenated methine protons (δ 3.88, 1H, m; 3.92, 1H, m; 4.44, 1H, q, $J = 5.7$ Hz) and five quaternary carbons (δ 36.5, 58.9, 75.0, 88.7, 89.0), but no olefinic protons and carbons. This evidence suggested that the aglycon of **1** was a highly oxidized pregnane at C-3, C-8, C-12, C-14, C-17, and C-20. Comparison of the ^{13}C NMR spectrum of the aglycon moiety of **1** (Table I) with that of dihydrosarcostin [4] showed that the aglycon of **1** was dihydrosarcostin and the glycosylation shift of **1** [C-3 (+5.8 ppm), C-2 (−2.4 ppm), C-4 (−4.4 ppm)] indicated that its sugars were bound to the C-3 position of the aglycon.

In the ^{1}H NMR spectrum of the sugar moiety of **1**, the signals of five anomeric protons (δ 4.89, 1H, d, $J = 8.8$ Hz; 5.12, 1H, d, $J = 7.8$ Hz; 4.79, 1H, dd, $J = 9.8$, 2.0 Hz; 5.18, 1H, dd, $J = 9.8$, 2.0 Hz; 5.54, 1H, dd, $J = 9.8$, 2.1 Hz) and four methyl groups [δ 1.33; 1.45; 1.67; 1.76 (each 3H, d)] suggested the presence of five sugar units including three 2-deoxy sugars and four 6-deoxy sugars. Moreover, three methoxy groups [δ 3.52, 3.60, 3.91 (each 3H, s)] were also observed. By a combination of TOCSY, FOCSY, HMQC, HMQCTOCSY, HMBC and ROESY spectra of **1**, the sugars of **1** were found to be composed of one 2,6-dideoxypyranose (S1), two 2,6-dideoxy-3-*O*-methylpyranoses (S2, S3), one 6-deoxy-3-*O*-methylpyranose (S4), and one glucose (S5). In the ^{1}H NMR spectrum of **1**, the signals for H-4 of S2 and S3 were observed at δ 3.40 (dd, $J = 9.1$, 3.0 Hz) and 3.62 (t, $J = 9.1$ Hz), respectively. In the ROESY spectrum of **1**, the H-1 of S2 correlated with H-5, but the H-1 of S3 correlated with both H-3 and H-5. All these evidences suggested that the H-3 of S2 was equatorial, and the H-4, H-5 were axial, but that the H-3, H-4, and H-5 of S3 were all in axial orientations. Furthermore, S2 and S3 showed anomeric proton signals at δ 5.18 (dd, $J = 9.8$, 2.0 Hz) (cymarose: δ 5.08–5.27 [5]), and δ 4.79 (dd, $J = 9.8$, 2.0 Hz) (oleandrose: δ 4.73–4.89 [5]), respectively. Therefore, S2 was suggested to be cymarose (Cym) and S3 as oleandrose (Ole). In the light of the assigned ^{1}H and ^{13}C NMR spectra of **1** (Table II), S1, S4

TABLE I ^{13}C NMR spectral data for the aglycon part of compounds **1**–**4** and dihydrosarcostin (δ in ppm)

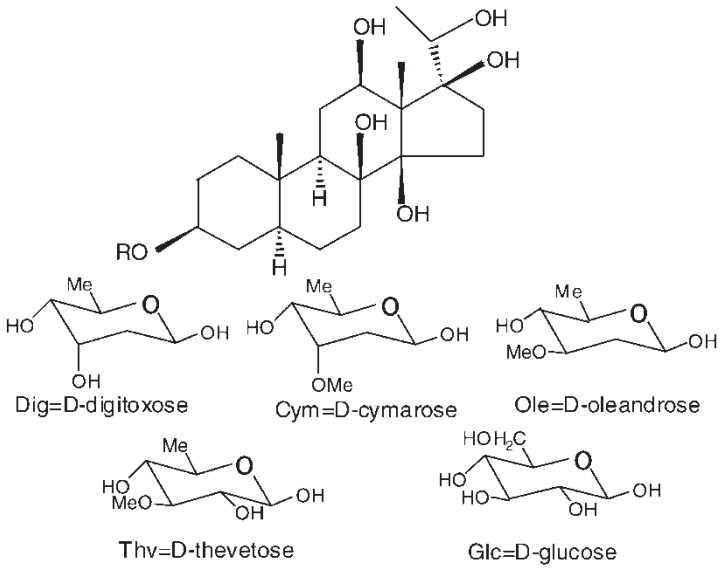
Position	***1***	***2***	***3***	***4***	*Dihydrosarcostin* [*4*]
1	38.3	38.3	38.3	38.3	38.6
2	29.6	29.6	29.6	29.6	32.0
3	76.7	76.8	76.7	76.5	70.9
4	34.8	34.6	34.5	34.5	38.9
5	45.3	45.4	45.4	45.4	46.1
6	25.3	25.3	25.3	25.3	25.5
7	28.3	28.1	28.2	28.2	28.2
8	75.0	75.9	75.8	75.8	76.1
9	47.4	47.4	47.4	47.4	47.6
10	36.5	36.5	36.5	36.5	36.6
11	34.5	34.5	34.8	34.8	34.9
12	71.5	71.8	71.5	71.4	72.9
13	58.9	59.0	59.0	59.0	59.2
14	88.7	88.7	88.7	88.7	89.0
15	34.2	34.1	34.1	34.1	34.1
16	34.0	34.5	34.2	34.2	34.1
17	89.0	89.0	89.0	89.0	88.8
18	11.9	11.9	12.0	11.9	11.7
19	13.1	13.2	13.2	13.2	13.3
20	73.0	73.0	73.1	73.0	71.7
21	17.7	17.7	17.8	17.7	17.7

TABLE II ^{1}H NMR and ^{13}C NMR spectral data of the sugar moieties of compounds **1**–**4** (δ in ppm, J in Hz)

	1		***2***		***3***		***4***	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
Dig							Cym^{I}	
1	5.54, dd, (9.8, 2.1)	95.8					5.34,dd, (9.8, 2.0)	95.9
2	2.45, 2.06	39.1					2.35, 1.82	37.3
3	4.65, q, (2.5)	67.6					4.11, q, (3.1)	78.1
4	3.51, dd, (9.0, 2.5)	83.1					3.53, dd, (9.0, 3.1)	83.5
5	4.35	68.6					4.28	69.0
6	1.33, d, (6.1)	18.5					1.42, d, (6.0)	18.7
OCH_3							3.65	58.9
Cym								
1	5.18, dd, (9.8, 2.0)	99.8	5.34, dd, (9.2, 2.1)	95.9	5.32, dd, (9.8, 2.0)	95.9	5.13, dd (9.8, 2.1)	100.5
2	2.34, 1.80,	36.7	2.33, 1.89	37.3	2.35, 1.90	37.5	2.33, 1.88	37.0
3	3.93, q, (3.0)	77.9	4.07, q, (3.0)	77.9	4.05, q, (3.1)	77.8	4.02, q, (3.0)	77.8
4	3.40, dd, (9.1, 3.0)	83.4	3.51, dd, (9.1, 3.0)	83.6	3.52, dd, (9.0, 3.1)	83.6	3.44, dd, (9.1, 3.0)	83.2
5	4.17	69.0	4.30	69.0	4.30	68.8	4.18	68.9
6	1.45, d, (5.5)	18.7	1.48, d, (6.1)	18.7	1.47, d, (6.1)	18.6	1.38, d, (6.0)	18.5
OCH_3	3.52	58.9	3.51	58.9	3.51	58.9	3.52	59.0
Ole								
1	4.79, dd, (9.8, 2.0)	101.9	4.80, dd, (9.9, 2.0)	101.9	4.79, dd, (9.8, 2.0)	101.9	4.79, dd (9.9, 2.1)	101.9
2	2.46, 1.75	38.3	2.50, 1.76	37.7	2.50, 1.75	37.5	2.50, 1.76	37.6
3	3.57	79.2	3.58	79.3	3.57	79.1	3.57	79.3
4	3.62, t, (9.1)	83.1	3.61, t, (9.1)	83.3	3.60, t, (9.1)	83.4	3.61,t, (9.0)	83.3
5	3.54	72.0	3.56	72.0	3.57	71.9	3.57	72.0
6	1.67, d, (6.0)	18.8	1.67, d, (6.1)	18.8	1.68, d, (6.0)	18.7	1.68, d, (6.1)	18.8
OCH_3	3.60	57.4	3.61	57.4	3.62	57.4	3.57	57.4
Thv								
1	4.89, d, (8.8)	103.9	4.89, d, (8.6)	104.0	4.89, d, (8.8)	104.0	4.88,d, (8.7)	104.0
2	3.88	74.9	3.91	74.9	3.90	74.8	3.96	75.0
3	3.70, t, (9.2)	86.3	3.70, t, (9.2)	86.3	3.68, t, (9.1)	86.4	3.67, t, (9.1)	86.4
4	3.87, t, (9.2)	83.2	3.89, t, (9.2)	83.2	3.85, t, (9.1)	83.5	3.83, t, (9.1)	83.5
5	3.75	72.0	3.75	72.0	3.74	72.0	3.75	72.0
6	1.76, d, (5.8)	18.7	1.78, d, (6.0)	18.7	1.76, d, (6.1)	18.7	1.76, d, (6.1)	18.7
OCH_3	3.91	60.7	3.91	60.7	3.93	60.7	3.89	60.7
Glc								
1	5.12, d, (7.8)	104.8	5.13, d, (8.0)	104.8	5.10, d, (7.8)	104.7	5.10, d, (7.7)	104.7
2	4.03	75.8	4.11	75.8	4.03	75.4	4.02	75.4
3	4.25	78.4	4.24	78.6	4.22	76.9	4.26	76.8
4	4.20	72.1	4.21	72.0	4.33	81.7	4.32	81.5
5	3.97	78.1	4.04	78.2	3.94	76.3	3.93	76.5
6	4.55, 4.35	63.1	4.56,4.29	63.1	4.52, 4.32	62.5	4.53, 4.49	62.5
Glc^{I}								
1					5.21, d, (7.8)	105.0	5.20, d, (7.8)	105.0
2					4.12	75.0	4.11	74.8
3					4.22	78.3	4.23	78.3
4					4.20	71.6	4.20	71.6
5					4.10	78.5	4.04	78.5
6					4.56, 4.31	62.5	4.56, 4.31	62.5

and S5 were suggested to be digitoxose (Dig), thevetose (Thv) and glucose (Glc), respectively. Finally, acid hydrolysis of **1** (see Experimental section) further confirmed that the sugars of **1** were composed of digitoxose (S1), cymarose (S2), oleandrose (S3), thevetose (S4), and glucose (S5). The β configurations for all of the sugars were determined from their large $^{3}J_{H1H2}$ coupling constants (7.8–9.8 Hz).

In the HMBC spectrum of **1**, cross peaks (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, H-4 of Thv/C-1 of Glc) were observed. Furthermore, in the ROESY spectrum of **1**, cross peaks (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, H-4 of Thv/H-1 of Glc)



1 R = Glc(1→4)Thv(1→4)Olc(1→4)Cym(1→4)Dig

2 R = Glc(1→4)Thv(1→4)Olc(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)Cym′

FIGURE 1 Structures of **1**–**4**.

were also observed. Therefore **1** was established as 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-digitoxopyranosyl dihydrosarcostin (Fig. 1).

Marstenacisside B (**2**), an amorphous solid, has a molecular formula $C_{48}H_{82}O_{21}$, determined from its negative ion FAB-MS (*m/z*: 993 $[M-H]^-$) as well as from ^{13}C NMR and DEPT data. Of the 48 carbons, 21 were assigned to the aglycon part, 27 to the sugar moiety. The spectral evidence indicated that compound **2** had the same aglycon, dihydrosarcostin, as that of **1** but differed in the sugar part (Tables I, II). In the ^1H and ^{13}C NMR spectra of **2**, the signals of four anomeric protons (δ 4.80, dd, $J = 9.9$, 2.0 Hz; 4.89, d, $J = 8.6$ Hz; 5.13, d, $J = 8.0$ Hz; 5.34, dd, $J = 9.2$, 2.1 Hz) and carbons (δ 95.9, 101.9, 104.0, 104.8) 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, except for the absence of a digitoxose moiety in **2**, the remaining four sugars were identical to those of **1**. The sequence and the linkage sites of the sugar units were established based on HMBC and ROESY spectra of **2**. In the HMBC spectrum of **2**, H-3 of the aglycon with C-1 of Cym, H-4 of Cym with C-1 of Ole, H-4 of Ole with C-1 of Thv, and H-4 of Thv with C-1 of Glc had cross peaks. Furthermore, in the ROESY spectrum of **2**, H-3 of the aglycon with H-1 of Cym, H-4 of Cym with H-1 of Ole, H-4 of Ole with H-1 of Thv, and H-4 of Thv with H-1 of Glc had cross peaks. Therefore, the structure of **2** was established to be 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl dihydrosarcostin.

Marstenacisside C (**3**), an amorphous solid, had a molecular formula $C_{54}H_{92}O_{26}$ determined from its negative ion FAB-MS (*m/z*: 1155 $[M-H]^-$) as well as from ^{13}C NMR and DEPT data. Of the 54 carbons, 21 were assigned to the aglycon part, 33 to

the sugar moiety. The spectral evidence indicated that compound **3** had the same aglycon as that of **2** but differed in the sugar part (Tables I, II). The pentasaccharide feature of **3** was manifested by its ^{1}H (δ 4.79, dd, $J = 9.8$, 2.0 Hz; 4.89, d, $J = 8.8$ Hz; 5.10, d, $J = 7.8$ Hz; 5.21, d, $J = 7.8$ Hz; 5.32, dd, $J = 9.8$, 2.0 Hz) and ^{13}C NMR (δ 95.9, 101.9, 104.0, 104.7, 105.0) data. Comparison of the ^{13}C NMR, ^{1}H—^{1}H COSY, TOCSY, ROESY, HMQC, and HMBC spectra of **3** with those of **2** indicated that, except for the presence of an additional glucose (Glc′) moiety in **3**, the remaining four sugars were identical to those of **2**. The glycosylation shift (+9.7 ppm) at C_4 of the glucose (Glc) indicated that the additional glucose (Glc′) should be bound to C_4 of Glc. The sequence and the linkage sites of the oligosaccharide of **3** were further confirmed by HMBC and ROESY experiments. In the HMBC spectrum of **3**, H-3 of the aglycon with C-1 of Cym, H-4 of Cym with C-1 of Ole, H-4 of Ole with C-1 of Thv, H-4 of Thv with C-1 of Glc, and H-4 of Glc with C-1 of Glc′, and in the ROESY spectrum of **3**, H-3 of the aglycon with H-1 of Cym, H-4 of Cym with H-1 of Ole, H-4 of Ole with H-1 of Thv, H-4 of Thv with H-1 of Glc, and H-4 of Glc with H-1 of Glc′ had cross peaks. 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 dihydrosarcostin.

Marstenacisside D (**4**), an amorphous solid, has the molecular formula $C_{61}H_{104}O_{29}$, as determined from its negative ion FAB-MS (*m/z*: 1299 $[M - H]^-$) and ^{13}C NMR and DEPT data. Of the 61 carbons, 21 were assigned to the aglycon part, 40 to the sugar moiety. The spectral evidence indicated that **4** had the same aglycon, dihydrosarcostin, as that of **3** (Table I), but differed in the sugar moiety (Tables I, II). The hexasaccharide feature of **4** was manifested by its ^{1}H (δ 4.79, dd, $J = 9.9$, 2.1 Hz; 4.88, d, $J = 8.7$ Hz; 5.10, d, $J = 7.7$ Hz; 5.13, dd, $J = 9.8$, 2.1 Hz; 5.20, d, $J = 7.8$ Hz; 5.34, dd, $J = 9.8$, 2.0 Hz) and ^{13}C NMR (δ 95.9, 100.5, 101.9, 104.0, 104.7, 105.0) data. Comparison of ^{13}C NMR, ^{1}H—^{1}H COSY, TOCSY, ROESY, HMQC, and HMBC spectra of **4** with those of **3** indicated that, except for the presence of an additional cymaropyranose (Cym′) moiety in **4**, five of the sugars were identical to those of **3**. The sequence and the linkage sites of the sugar units of **4** were established based on HMBC and ROESY experiments. In the HMBC spectrum of **4**, H-3 of the aglycon with C-1 of Cym′, H-4 of Cym′ with C-1 of Cym, H-4 of Cym with C-1 of Ole, H-4 of Ole with C-1 of Thv, H-4 of Thv with C-1 of Glc, and H-4 of Glc with C-1 of Glc′ had cross peaks. Furthermore, in the ROESY spectrum of **4**, H-3 of the aglycon with H-1 of Cym′, H-4 of Cym′ with H-1 of Cym, H-4 of Cym with H-1 of Ole, H-4 of Ole with H-1 of Thv, H-4 of Thv with H-1 of Glc, and H-4 of Glc with H-1 of Glc′ also had cross peaks. Therefore, **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-cymaropyranosyl dihydrosarcostin.

EXPERIMENTAL

General Experimental Procedures

Optical rotations were obtained on a JASCO-DIP-181 Polarimeter. IR spectra were recorded on a Perkin-Elmer 599 infrared spectrometer. ^{1}H and ^{13}C NMR and all 2D spectra were recorded on a JEOL α600 with an NM-AFG type field gradient unit, TMS as internal standard, and C_5D_5N as solvent. FAB-MS spectra were measured on a MAT-95 Mass spectrometer. Lichroprep RP-18 (25–40 μm, Merck), Diaion HP-20 (Mitsubishi Kasei), and silica gel 60H (Qingdao Haiyang Chemical Group Co. of China) were used for column

chromatography. TLC was performed on silica gel $HSGF_{254}$ (Zhifu Huangwu Co. Ltd. of Yantai, China). Spots were visualized by spraying with 10% H_2SO_4 in 95% EtOH followed by heating.

Plant Material

The stems of *Marsdenia tenacissima* were purchased in Kunming, Yunnan Province (China) in 2000. Botanical identification was made by Dr Wangxing Xing (117th. Hospital, PLA.). A voucher specimen (No. 7) is deposited at the Herbarium of the Department of Phytochemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and Isolation

The powdered stems of *Marsdenia tenacissima* (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 light petroleum, EtOAc and n-BuOH. The n-BuOH fraction (180 g) was subjected to Diaion HP-20 using an EtOH–H_2O gradient system (0–95%). The fraction (12 g) eluted by 95% EtOH was subjected to silica gel column chromatography with a $CHCl_3$–MeOH–H_2O (7:1:0.05-4:1:0.1) solvent system. Fractions A (1.2 g) eluted with $CHCl_3$–MeOH–H_2O (7:1:0.05) and B (0.8 g) with $CHCl_3$–MeOH–H_2O (4:1:0.1) were subjected to RP-18 silica gel column chromatography with 65% MeOH–H_2O, and then to silica gel column chromatography with EtOAc–MeOH–H_2O (11:1.1:1), respectively, Compounds **1** (45 mg) and **2** (42 mg) from A and compounds **3** (25 mg) and **4** (18 mg) from B were isolated.

Marstenacisside A (**1**), an amorphous solid, IR (KBr) ν_{max} (cm^{-1}): 3425, 1000–1100. FAB-MS *m/z*: 1123 $[M - H]^-$, ^1H NMR of the aglycon part of **1**: δ (ppm): 1.20 (H-19, s), 1.50 (H-21, d, $J = 5.7$ Hz), 1.93 (H-18, s), 3.88 (H-12, m); δ 3.92 (H-3, m), 4.44 (H-20, q, $J = 5.7$ Hz). ^{13}C NMR of the aglycon part of **1**: Table I; ^1H NMR and ^{13}C NMR of the sugar moiety of **1**: Table II.

Marstenacisside B (**2**), an amorphous solid, IR (KBr) ν_{max} (cm^{-1}): 3438, 1000–1100. FAB-MS *m/z*: 993 $[M - H]^-$, ^1H NMR of the aglycon part of **2**: δ (ppm): 1.21 (H-19, s), 1.50 (H-21, d, $J = 5.7$ Hz), 1.95 (H-18, s), 3.89 (H-12, m), 3.90 (H-3, m), 4.45 (H-20, q, $J = 5.7$ Hz). ^{13}C NMR of the aglycon part of **2**: Table I; ^1H NMR and ^{13}C NMR of the sugar moiety of **2**: Table II.

Marstenacisside C (**3**), an amorphous solid, IR (KBr) ν_{max} (cm^{-1}): 3423, 1000–1100. FAB-MS *m/z*: 1155 $[M - H]^-$, ^1H NMR of the aglycon part of **3**: δ (ppm): 1.19 (H-19, s), 1.53 (H-21, d, $J = 5.7$ Hz), 1.91 (H-18, s), 3.88 (H-12, m), 3.90 (H-3, m), 4.46 (H-20, q, $J = 5.7$ Hz). ^{13}C NMR of the aglycon part of **3**: Table I; ^1H NMR and ^{13}C NMR of the sugar moiety of **3**: Table II.

Marstenacisside D (**4**), an amorphous solid, IR (KBr) ν_{max} (cm^{-1}): 3448, 1000–1100. FAB-MS *m/z*: 1299 $[M - H]^-$, ^1H NMR of the aglycon part of **4**: δ (ppm): 1.20 (H-19, s), 1.49 (H-21, d, $J = 5.7$ Hz), 1.92 (H-18, s), 3.88 (H-12, m), 3.90 (H-3, m), 4.45 (H-20, q, $J = 5.7$ Hz). ^{13}C NMR of the aglycon part of **4**: Table I; ^1H NMR and ^{13}C NMR of the sugar moiety of **4**: Table II.

Acid Hydrolysis of Compound 1

Compound **1** (5 mg) was heated at 95°C with 5 ml of 0.1 M HCl–dioxane (1:1) for 2 h. Dioxane was evaporated *in vacuo* and H_2O (3 ml) was added. The reaction mixture was then neutralized with 10% KOH and partitioned with $CHCl_3$–H_2O to obtain the aglycon and

sugar fractions. The sugar fraction was identified by comparison with authentic samples on a TLC silica gel plate developed with $CHCl_3$–MeOH–H_2O (3:1:0.1) and EtOAc–MeOH–H_2O (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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