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Marsdenosides A–H, polyoxypregnane glycosides from *Marsdenia tenacissima*

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Abstract

Eight polyoxypregnane glycosides, marsdenosides A–H, were isolated from the $CHCl_3$-soluble fraction of the ethanolic extract of the stem of *Marsdenia tenacissima*, along with six known glycosides and two known polyoxypregnane aglycones. Three polyoxypregnanes, 12β-*O*-2-methylbutyryl-tenacigenin A, 11α,12β-di-*O*-acetyltenacigenin B, and 11α-*O*-tigloyltenacigenin B were also obtained. Their structures were established on the basis of spectroscopic analysis and chemical evidence.

Keywords: *Marsdenia tenacissima*; Asclepiadaceae; Polyoxypregnane glycoside; Marsdenosides A–H; Polyoxypregnane

1. Introduction

The stem of *Marsdenia tenacissima* (Roxb.) Wight et Arn. (Asclepiadaceae), a species widely distributed from tropical to subtropical Asia, and known in Chinese folk medicine as "Tong-guang-san", has been used as a remedy to treat asthma, cancer, trachitis, tonsillitis, pharyngitis, cystitis, and pneumonia in China (Jiangsu New College of Medicine, 1977). Previous chemical investigations on this plant were focused on the polyoxypregnane derivatives. Twelve polyoxypregnane genins, including tenacigenins A, B, and C (Yang et al., 1981), six diester derivatives of tenacigenin B (Luo et al., 1993a,b), and dresgenin, and marstenacigenins A and B (Qiu et al., 1996) were isolated successively from the acidic hydrolysate of the $CHCl_3$-soluble fraction of the methanolic extract of the stem of *M. tenacissima* indigenous to the southwest of China. Nine glycosides of tenacigenin B derivatives, named as tenacissosides A–E (trisaccharides) (Miyakawa et al., 1986) and tenacissosides F–I (disaccharides) (Chen et al., 1999) were also isolated from the $CHCl_3$ extract of the stem of this plant, and cissogenin and its analogues were obtained from the seeds of the plant native to India (Singhal et al., 1980a,b). The reported pharmacological studies showed that the crude glycosides from the stem of *M. tenacissima* exhibited mild anti-asthmatic properties (Zhou et al., 1980) and that three tenacigenin B derivatives displayed weak cytotoxicity against the KB-VI cell line (Luo et al., 1993a,b). Our phytochemical investigation on the title plant led to the isolation of eight polyoxypregnane glycosides, named as marsdenosides A–H (**1**–**8**), together with six known glycosides and two known polyoxypregnane aglycones. Additionally, three polyoxypregnane genins were obtained from the mild acidic hydrolysates of **4**, **6**, and **7**. The present paper reports on the isolation and structural characterization of these new compounds, and the chemotaxonomical significance of these polyoxypregnane derivatives was also discussed herein.

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2. Results and discussion

The $CHCl_3$-soluble fraction of the ethanolic extract of the stem of *M. tenacissima* was subjected to repeated column chromatography on silica gel and reversed phase silica gel chromatography with various solvent systems, followed by HPLC, to afford sixteen polyoxypregnane derivatives. Compounds **4** and **7** are the first-reported monoester derivatives of tenacigenin B.

Marsdenoside A (**1**), obtained as colorless needles, displayed a molecular formula $C_{45}H_{70}O_{14}$ from the quasi-molecular ion peak at *m/z* 857.4675 $[M+Na]^+$ in its HRESI-MS spectrum. The Liebermann–Burchard, Keller–Kiliani, and Xanthydrol tests on **1** gave positive reactions, indicating that **it** was a steroidal glycoside with a 2-deoxy sugar moiety in the molecule (Abisch and Reichstein, 1960; Von Euw and Reichstein, 1948; Lewbart et al., 1963). The IR spectrum showed absorption bands at 3441 and 1083 cm^{-1} for hydroxyl groups, and at 1731, 1705, and 1652 cm^{-1} for three carbonyl groups. The ^1H NMR spectrum of **1** displayed two anomeric proton signals at δ 4.80 (1H, d, $J = 8.1$ Hz) and δ 4.58 (1H, dd, $J = 9.7$, 1.6 Hz), with corresponding carbon signals at δ 99.2 and δ 96.9, respectively, in its HMQC spectrum (Table 1), suggesting that the sugar moiety of **1** consists of two units. Interpretation of the ^1H–^1H COSY, HMQC, and HMBC spectra (Table 1 and Fig. 1) revealed that the two methyl signals at δ 1.26 (3H, d, $J = 6.2$ Hz) and 1.37 (3H, d, $J = 5.2$ Hz) were on the two sugar rings, respectively. In the HMBC spectrum of **1**, two cross-peaks were observed between the methoxyl group at δ 3.38 (3H, s) and the carbon signal at δ 78.8, and between the other methoxyl group at δ 3.66 (3H, s) and the carbon resonance at δ 81.0, indicating that the two methoxyl groups were linked at C-3 in each of the two sugar units, respectively (Fig. 1). So one of the sugar units whose anomeric proton signal was a doublet in the ^1H NMR spectrum was inferred to be 6-deoxy-3-*O*-methyl pyranose and the other, with an anomeric proton signal giving a doublet of doublets, to be 2,6-dideoxy-3-*O*-methyl pyranose. Both of the glycosidic linkages had a β orientation from the coupling constants of the two anomeric proton signals. Starting from the anomeric proton signal at δ 4.80, the proton resonances at δ 3.48 (1H, dd, $J = 7.5$, 2.7 Hz), 3.79 (1H, br s), and 3.18 (1 H, br d, $J = 9.5$ Hz) were assigned to H-2, H-3, and H-4 of the spin system, respectively, from the ^1H–^1H COSY analysis (Table 1). From the

Table 1
^1H–^1H and ^1H–^{13}C correlations exhibited in the 2D NMR spectra of **1**

δ_H	Proton	^1H–^1H COSY	HMQC	HMBC
0.79	4′-CH_3	1.20, 1.51	11.7	25.9, 41.4
0.97	5′-CH_3	2.08	15.2	25.9, 41.4, 175.6
1.05	19-CH_3		12.8	37.5, 51.1, 39.1, 71.4
1.08	18-CH_3		16.8	60.0, 74.7
1.26	Allo-6-CH_3	3.56	17.9	70.6, 72.9
1.37	Ole-6-CH_3	3.36	18.5	71.4, 79.8
1.49	Ole-H_a-2	2.32, 3.41	36.1	78.8, 79.8, 96.9
1.68	H_a-16	1.33	25.0	46.1, 60.0, 71.4, 210.7
1.75	5″-CH_3		15.2	128.1, 138.5, 167.4
1.76	4″-CH_3	6.79	11.9	128.1, 138.5
1.91	H-7	1.25, 1.38, 1.58	31.8	12.8, 39.1, 68.7, 66.9
2.01	H-9	5.40	51.1	
2.08	H-2′	0.97	41.4	11.7, 15.2, 25.9, 175.6
2.16	H_e-16	1.60, 2.02, 2.92	25.0	60.0
2.22	21-CH_3		29.9	60.0, 210.7
2.31	Ole-H_e-2	1.49, 3.41	36.1	78.8, 79.8, 96.9
2.92	H-17	2.16	60.0	16.8, 26.6, 46.1, 71.4, 210.7
3.18	Allo-H-4	3.56, 3.79	72.9	71.9
3.35	Ole-H-4		79.8	99.2
3.36	Ole-H-5	1.37	71.4	18.5
3.38	Ole-3-OMe		56.1	78.8
3.41	Ole-H-3	1.49, 2.31	78.8	
3.48	Allo-H-2	3.79, 4.80	71.9	99.2
3.56	Allo-H-5	1.26, 3.18,	70.6	
3.63	H-3	1.33, 1.48, 1.68, 1.78	76.6	
3.66	Allo-3-OMe		61.9	81.0
3.79	Allo-H-3	3.18, 3.48	81.0	61.9, 72.9, 99.2
4.58	Ole-H-1	1.49	96.9	71.4, 76.6, 79.8
4.80	Allo-H-1	3.48	99.2	79.8
5.04	H-12	5.40	74.7	16.8, 46.1, 51.1, 60.0, 68.7, 167.4
5.40	H-11	2.01, 5.04	68.7	51.1, 74.7, 175.6
6.79	H-3″	1.75	138.5	11.9, 14.4, 167.4

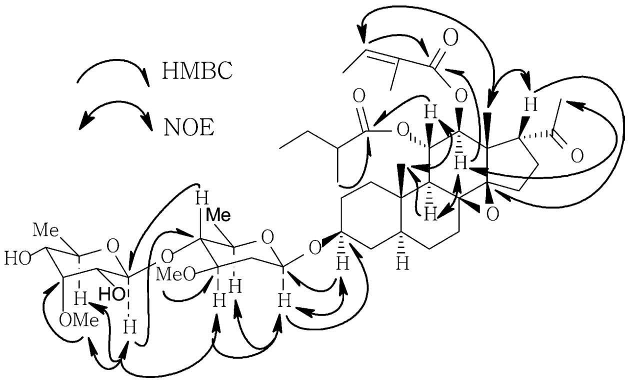


Fig. 1. Key HMBC and NOESY correlations of **1**.

coupling constants, it was concluded that orientation of H-2 and H-4 of the sugar unit should be axial and that of H-3 should be equatorial, so the sugar should be 6-deoxy-3-*O*-methyl allose (Sahu et al., 2002). Beginning with the other anomeric proton signal at δ 4.58, the resonance of H-4 of the spin system should be at δ 3.35 (1H, t, $J = 6.7$ Hz), and the coupling constants suggested that H-3, H-4, and H-5 were each in an axial orientation. In addition, the anomeric proton shows correlations with H-3 and H-5 in the NOESY spectrum (Fig. 1). So the sugar unit was identified as oleandrose (Ma and Fang, 1997). The cross-peaks between H-1_{allo}/C-4_{ole} and H-1_{ole}/C-3_{aglycone} in the HMBC spectrum indicated that oleandrose was the inner sugar and 6-deoxy-3-*O*-methyl allose was the outer. Thus, the sugar moiety of **1** was determined as pachybiose. The conclusion was confirmed when mild acid hydrolysis of **1** gave only one sugar fragment identified as pachybiose by paper chromatography and TLC comparison with an authentic sample. The ^{1}H and ^{13}C NMR spectra of the aglycone moiety of **1** exhibited the patterns for a C_{21}-steroid skeleton. The ^{13}C NMR spectroscopic data of the aglycone of **1** resembled those of a tenacigenin B-11,12-diester (Miyakawa et al., 1986). The proton signals at δ 0.79 (3H, t, $J = 7.3$ Hz), 0.97 (3H, d, $J = 6.9$ Hz) and at δ 1.75 (3H, br s), 1.76 (3 H, d, $J = 6.5$ Hz), 6.79 (1H, q, $J = 6.0$ Hz) in the ^{1}H NMR spectrum of **1** suggested the presence of 2-methylbutyryl and tigloyl ester units, respectively, which was confirmed by the carbon signals due to the two acyl groups in the ^{13}C NMR spectrum of **1** (Table 3). The HMBC spectrum of **1** displayed correlations between the proton signal at δ 5.40 (1H, t, $J = 10.1$ Hz, H-11) and the carbonyl carbon at δ 175.6 of the 2-methylbutyryl unit, and between the proton signal at δ 5.04 (1H, d, $J = 10.1$ Hz, H-12) and the carbonyl carbon at δ 167.4 of the tigloyl moiety. Consequently, the aglycone of **1** was determined as 11α-*O*-2-methylbutyryl-12β-*O*-tigloyltenacigenin B. Mild acid hydrolysis of **1** yielded a genin, **1a**, whose mp, MS, ^{1}H NMR, and ^{13}C NMR spectroscopic data coincided with those of 11α-*O*-2-methylbutyryl-12β-*O*-tigloyltenacigenin B (Luo et al., 1993a,b). The glycosidation shifts (Kasai et al., 1977) were observed at C-2 (−2.2 ppm), C-3 (+6.1 ppm), and C-4 (−3.6 ppm) by comparing the ^{13}C NMR spectroscopic data of **1** and **1a**, which implied that the oligosaccharide chain in **1** was linked to the C-3 hydroxyl of the aglycone. The structure of **1** was thus established as 3-*O*-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandropyranosyl-11α-*O*-2- methylbutyryl-12β-*O*-tigloyltenacigenin B.

The ^{13}C NMR spectroscopic data due to the sugar moieties of **2**–**7** are in agreement with those of **1** (Table 2), so they should contain the same sugar moiety as **1**. The same glycosidation shifts were observed in compounds **2**–**8**, which indicated that they all bear the sugar moiety at the C-3 hydroxyl group. The ^{1}H NMR spectra of **2**, **3**, **5**, and **6** showed patterns analogous to **1**, except for the signals due to their respective diester groups. The differences between the ^{13}C NMR spectra of **2**, **3**, **5**, **6**, and **1** (Table 3) were also reflected in the signals for the diester groups.

Marsdenoside B (**2**) had a molecular formula $C_{45}H_{68}O_{14}$ based on the quasi-molecular ion at *m/z* 855.4492 $[M+Na]^+$ in its HRESI-MS spectrum. In its ^{1}H NMR spectrum, there were signals for two tigloyl groups at δ 1.67 (6H, br s, 5′,5″-CH_3), δ 1.69, 1.71 (each 3H, d, $J = 7.1$ Hz, 4′,4″-CH_3) and δ 6.66, 6.70 (each 1H, qq, $J = 7.0$, 1.2 Hz, H-3′,3″). The ^{13}C NMR spectrum of **2** also displayed resonances due to two tigloyl groups (Table 3). Consequently, the structure of **2** was established as 3-*O*-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandropyranosyl-11α, 12β-di-*O*-tigloyl-tenacigenin B.

Marsdenoside C (**3**) was deduced to have the molecular formula $C_{47}H_{68}O_{14}$ on the basis of the quasi-molecular ion at *m/z* 879.4511 $[M+Na]^+$ in its HRESI-MS spectrum. Comparing the ^{1}H NMR spectrum of **3** with that of **1**, the signals due to a benzoyl group at δ 7.42 (2H, t, $J = 7.7$ Hz, H-4″,6″), δ 7.55 (1H, t, $J = 7.4$ Hz, H-5″), and δ 7.96 (2H, d, $J = 7.3$ Hz, H-3″,7″) appeared, and those due to the tigloyl unit were absent. Correspondingly, comparing with the ^{13}C NMR spectrum of **1**, that of **3** showed the resonances belonging to a benzoyl and none belonging to a tigloyl group (Table 3). In its HMBC spectrum, the proton at δ 5.54 (1H, *t*, $J = 10.1$ Hz, H-11β) was correlated with the carbonyl carbon at δ 175.7 of the 2-methylbutyryl group and the proton at δ 5.25 (1H, d, $J = 10.1$ Hz, H-12α) was coupled with the carbonyl carbon at δ 166.1 of the benzoyl unit. Thus, the structure of **3** was established as 3-*O*-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandropyranosyl-11α-*O*-2-methylbutyryl-12β-*O*-benzoyltenacigenin B.

Marsdenoside E (**5**) gave a quasi-molecular ion at *m/z* 789.4042 $[M+Na]^+$ in its HRESI-MS, and thus its molecular formula was inferred as $C_{40}H_{62}O_{14}$. Beginning with the signal at δ1.07 (3H, t, $J = 7.6$ Hz, 3′-

Table 2
^{13}C NMR spectroscopic data of the sugar moieties of **1–8**

Sugar unit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**8**[a]
Ole									
C-1	96.9	97.0	96.9	97.1	97.0	97.1	96.8	96.9	97.3
2	36.1	36.1	36.1	36.2	36.1	36.2	36.2	36.3	38.5
3	78.8	78.8	78.8	78.8	78.8	78.9	79.0	79.2	79.8
4	79.8	80.0	79.2	79.2	79.2	79.2	79.2	80.5	83.3
5	71.4	71.4	71.4	71.4	71.4	71.4	71.5	58.5	72.0
6	18.5	18.5	18.6	18.6	18.6	18.7	18.7	18.1	18.9
3-OMe	56.1	56.1	55.7	55.6	55.7	55.7	55.7	55.9	57.5
Allo									
C-1	99.2	99.2	99.1	99.1	99.2	99.2	99.3	100.2	102.0
2	71.9	71.9	71.8	71.6	71.9	71.9	71.9	71.0	72.9
3	81.0	81.0	81.0	81.0	81.0	81.1	81.1	80.3	83.4
4	72.9	72.9	72.9	72.9	72.9	72.9	73.0	82.2	83.5
5	70.6	70.5	71.3	71.3	71.4	71.4	71.5	69.3	69.9
6	17.9	17.9	17.9	17.9	17.9	17.9	18.0	18.5	19.7
3-OMe	61.9	61.9	61.9	61.9	62.0	62.0	62.0	61.1	62.0
Glc									
C-1								104.3	106.6
2								75.5	75.7
3								77.2	78.5
4								69.9	72.2
5								73.7	78.6
6								61.9	63.2

δ values (ppm) in $CDCl_3$ (125 MHz).

[a] In pyridine-d_5.

CH_3) the presence of a propionyl group was deduced by interpreting the ^1H-^1H COSY, HMQC, and HMBC spectra of **5**. Additionally, the ^1H NMR spectrum of **5** displayed the signal at δ 1.96 (3H, s, 2″-CH_3) for an acetyl group. From its HMBC spectrum, in which the proton resonance at δ 5.35 (1 H, t, J = 10.1 Hz, H-11β) was correlated with the carbonyl carbon signal at δ 173.5 of the propionyl group, and in which the proton resonance at δ 4.96 (1H, d, J = 10.1 Hz, H-12α) was correlated with the carbonyl carbon signal at δ 170.6 of the acetyl group, the structure of **5** was elucidated as 3-*O*-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandropyranosyl-11α-*O*-propionyl-12β-*O*-acetyltenacigenin B.

Marsdenoside F (**6**) has a molecular formula $C_{39}H_{60}O_{14}$ inferred from the quasi-molecular ion at m/z 775.3891 in its HRESI-MS. Two methyl signals from acetyl groups at δ 1.93 and 1.97 (each 3H, s, 2′,2″-CH_3) and at δ 21.6 and 20.6 (C-2′,2″) were observed in its ^1H and ^{13}C NMR spectra, respectively. Thus, **6** was characterized as 3-*O*-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandropyranosyl-11α,12β-di-*O*-acetyltenacigenin B. From the ether extract of its mild acidic hydrolysate, the aglycone, **6a**, was obtained. On the basis of the NMR spectroscopic data, **6a** was defined as 11α,12β-di-*O*-acetyltenacigenin B.

Marsdenoside D (**4**) displayed a quasi-molecular ion $[M+Na]^+$ at m/z 775.4246 in its HRESI-MS which was consistent with a molecular formula $C_{40}H_{64}O_{13}$. The ^1H NMR spectrum of **4** exhibited a resemblance to the resonance pattern of **1**, except for the higher-field shift of the signal of H-11 from δ 5.40 (1H, t, J = 10.1 Hz) to δ 3.88 (1H, t, J = 10.0 Hz) and the absence of signals due to a tigloyl group observed in the ^1H NMR spectrum of **1**. The resonances of the 2-methylbutyryl group at δ 0.91 (3H, t, J = 7.4 Hz, 4′-CH_3) and δ 1.16 (3H, d, J = 7.0 Hz, 5′-CH_3) in the ^1H NMR spectrum of **4** were a little different from those in the ^1H NMR spectrum of **1**. Correspondingly, the ^{13}C NMR spectroscopic data of **4** coincided well with that of **1**, except that only one ester carbonyl carbon signal was observed in the ^{13}C NMR of **4** and the carbon resonances for the tigloyl group present in that of **1** were absent. Consequently, it was concluded that the aglycone of **4** was a monoester of tenacigenin B. From the HMQC and HMBC (Fig. 2) spectra of **4**, the linkage of the 2-methylbutyryl group was located at C-12 since the proton signal at δ 4.83 (1H, d, J = 10.0 Hz, H-12α) was correlated with the carbonyl resonance at δ 176.7 of the 2-methylbutyryl group. Consequently, **4** was assigned to be 3-*O*-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandropyranosyl-12β-*O*-2-methyltenacigenin B. Mild acid hydrolysis gave **4a**. The pattern of the ^1H NMR spectrum of **4a** was similar to that of tenacigenin A (Zhou et al., 1980), except that the ^1H and ^{13}C NMR spectra of **4a** exhibited the signals belonging to a 2-methylbutyryl group. Comparing the ^1H NMR spectroscopic data

Table 3
^{13}C NMR spectroscopic data of the aglycone moieties of **1**–**8** and **1a**, **4a**, **6a**, **7a**

C	**1**	**1a**	**2**	**3**	**4**	**4a**	**5**	**6**	**6a**	**7**	**7a**	**8**	**8**[a]
1	37.5	37.6	37.3	37.8	38.4	38.3	37.4	37.3	37.2	37.5	37.3	37.7	38.3
2	29.0	31.2	29.7	29.0	29.1	30.5	29.2	29.3	31.4	29.1	31.3	28.9	30.1
3	76.6	70.5	76.2	76.2	76.8	71.0	76.4	76.5	70.5	76.2	70.3	76.1	75.9
4	34.8	38.4	34.8	34.7	34.8	37.1	34.8	34.8	38.4	34.5	38.2	34.7	35.5
5	44.2	44.1	44.1	44.0	44.2	45.9	43.9	44.0	44.0	44.5	44.6	43.9	44.2
6	26.7	26.6	29.3	26.6	26.6	27.6	26.8	26.8	26.6	28.1	28.1	26.8	27.1
7	31.8	31.8	31.9	31.8	31.9	34.1	31.8	31.8	31.8	32.6	32.5	31.8	32.5
8	66.9	66.9	66.9	66.9	66.9	77.9	66.7	66.8	66.8	66.0	65.9	66.8	66.9
9	51.1	51.1	51.3	51.1	52.6	57.4	51.1	51.1	51.0	52.3	52.2	51.1	52.0
10	39.1	38.9	38.9	39.1	39.1	35.2	39.0	39.1	38.9	39.5	39.2	39.1	39.8
11	68.7	68.7	68.9	68.5	67.6	68.6	68.6	68.8	68.8	70.8	70.7	68.5	69.7
12	74.7	74.7	74.8	75.5	77.2	71.7	75.1	75.2	75.1	79.6	79.5	75.2	75.3
13	46.1	46.1	46.1	46.2	45.8	44.4	45.8	45.9	45.8	47.6	47.5	45.8	46.5
14	71.4	71.4	71.5	71.4	71.9	81.0	71.9	71.4	71.4	70.4	70.7	71.3	71.8
15	26.6	26.7	26.7	26.8	26.9	34.4	26.6	26.6	26.5	27.1	26.9	26.5	27.6
16	25.0	25.0	25.0	25.0	25.0	23.0	25.0	25.0	24.9	25.6	25.5	24.9	25.6
17	60.0	60.0	59.8	60.1	60.0	55.3	60.0	60.1	60.0	63.6	63.5	60.2	60.2
18	16.8	16.8	16.7	16.8	16.7	16.2	16.7	16.7	16.7	10.4	10.4	16.8	17.5
19	12.8	12.8	12.7	12.8	12.6	15.8	12.7	12.8	12.8	12.9	12.9	12.7	13.7
20	210.7	210.7	210.9	210.8	210.7	99.7	210.7	210.7	210.6	211.7	211.7	210.6	209.1
21	29.9	29.9	30.2	30.0	30.1	24.2	30.0	30.0	30.0	30.9	30.8	29.7	30.3
	Bu	Bu	Tig	Bu	Bu	Bu	Pro	Ac	Ac	Tig	Tig	Bu	Bu
1′	175.6	175.6	167.3	175.7	176.7	176.3	173.5	170.2	170.1	168.4	168.2	175.6	174.9
2′	41.4	41.3	127.9	41.3	41.4	41.6	28.3	21.6	21.5	128.9	128.9	41.4	41.8
3′	25.9	25.9	137.7	25.8	26.8	26.3	8.9			138.0	137.8	26.2	27.7
4′	11.7	11.6	11.8	11.4	11.8	11.9				12.2	12.2	11.8	12.5
5′	15.2	15.2	14.4	15.1	17.0	17.7				14.6	14.5	15.3	16.2
	Tig	Tig	Tig	Bz			Ac	Ac	Ac			Ac	Ac
1″	167.4	167.4	167.4	166.1			170.6	170.6	170.6			170.7	170.2
2″	128.1	128.1	128.8	129.5			20.6	20.6	20.6			20.9	21.4
3″	138.5	138.5	138.0	129.9									
4″	11.9	11.9	11.8	128.5									
5″	14.4	14.4	14.4	133.3									
6″				128.5									
7″				129.9									

δ values (ppm) in $CDC1_3$ (125 MHz).
[a] In pyridine-d_5.

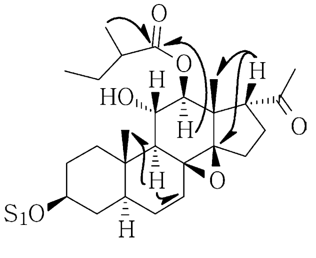


Fig. 2. Key HMBC correlations of **4**.

of **4a** with those of **4**, the resonance for the 21-CH_3 was shifted upfield from δ 2.21 to δ 1.20, the signal for H-11β was shifted downfield from δ 3.88 (1H, t, $J = 10.0$ Hz) to δ 4.30 (1H, dd, $J = 3.8$, 1.6 Hz), and that for the H-12α shifted downfield from δ 4.83 (1H, d, $J = 10.0$ Hz) to δ 5.28 (1H, d, $J = 3.8$ Hz). From the chemical shifts and coupling constants of H-11 and H-12, the conclusion was drawn that the orientation of the two protons had changed from axial to equatorial in the process of mild acid hydrolysis. The ^{13}C NMR spectrum of **4a** showed only one carbonyl carbon signal belonging to the 2-methylbutyryl group. Comparing with the data of **4**, the ^{13}C NMR spectrum of **4a** had an additional signal at δ 99.7, and the resonance at δ 210.7 for C-20 was absent. In the HMBC spectrum of **4a**, the proton signal at δ 4.30 (H-11β) correlating with the carbon signals at δ 68.6 in its HMQC spectrum was correlated with the carbon signal at δ 99.7. Therefore, it was deduced that an intramolecular ketalization product, **4a**, was formed from **4** under the hydrolysis conditions (Fig. 3) (Yang et al., 1981). This conclusion supported the postulate of Yang et al. that tenacigenin A was an artifact formed under the conditions used. The HMBC spectrum of **4a** showed that the proton at δ 5.28 (H-12), coupling with the carbon at δ 71.7 in the HMQC spectrum, was correlated with the carbonyl carbon at δ 176.3 belonging to the 2-methylbutyryl group, suggesting that the 2-methyl-

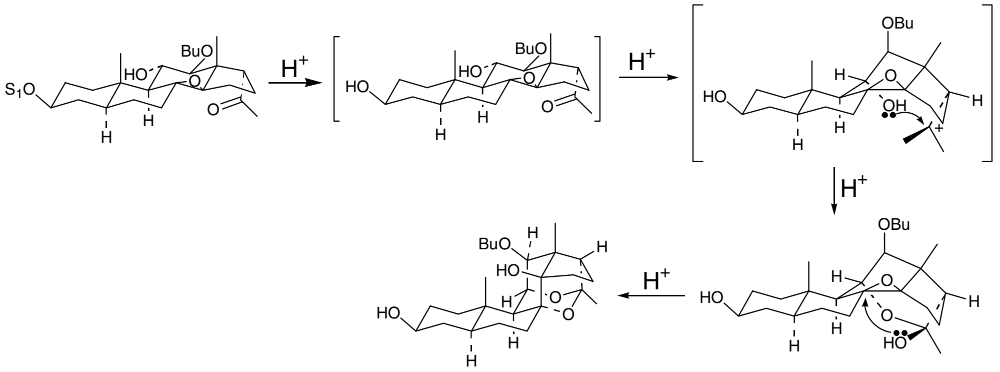


Fig. 3. The transformation of the aglycone of **4** under the mild acidic hydrolysis conditions.

butyryl group was linked at C-12 in **4a**. Thus, the structure of **4a** was established as 12β-*O*-2-methylbutyryltenacigenin A.

Marsdenoside G (**7**) gave a quasi-molecular ion $[M+Na]^+$ at *m/z* 773.4086 in the HRESI-MS, which was in accordance with a molecular formula $C_{40}H_{62}O_{13}$. As in **4**, the ^{1}H NMR spectrum of **7** resembled that of **1**, other than the higher-field shift of the signal of H-12 from δ 5.04 (1H, d, $J = 10.1$ Hz) to δ 3.48 (1H, d, $J = 9.5$ Hz) and the absence of the signals due to the 2-methylbutyryl group in the ^{1}H NMR spectrum of **1**. Likewise, the ^{13}C NMR spectroscopic data of **7** were similar to those of **1**, except that only one ester carbonyl carbon signal was observed and the carbon signals for the 2-methylbutyryl group present in that of **1** were absent. Consequently, the aglycone of **7** was also a monoester of tenacigenin B. The tigloyl group of **7** was fixed at C-11 by analysing its HMBC spectrum (Fig. 4) in which the proton at δ 5.17 (1H, t, $J = 9.5$ Hz, H-11β) was correlated with the carbonyl carbon at δ 168.4 belonging to the tigloyl group. Finally, the structure of **7** was characterized as 3-*O*-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandropyranosyl-11α-*O*-tigloyltenacigenin B. The aglycone **7a**, a monoester derivative of tenaciginin B, was obtained from the ether extract by the mild acidic hydrolysis of **7**. The ^{1}H and ^{13}C NMR spectroscopic data of **7a** coincided well with those of the aglycone moiety of **7** (Table 3), except that glycosidation shifts were observed in the ^{13}C NMR spectrum of **7**. Therefore, the structure of **7a** was elucidated as 11α-*O*-tigloyltenacigenin B.

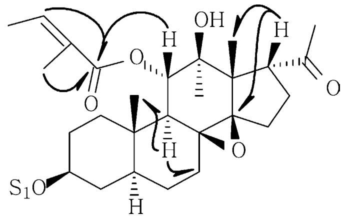
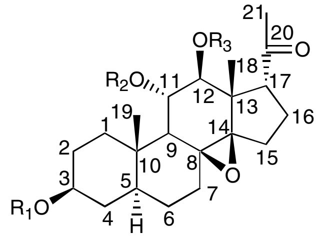
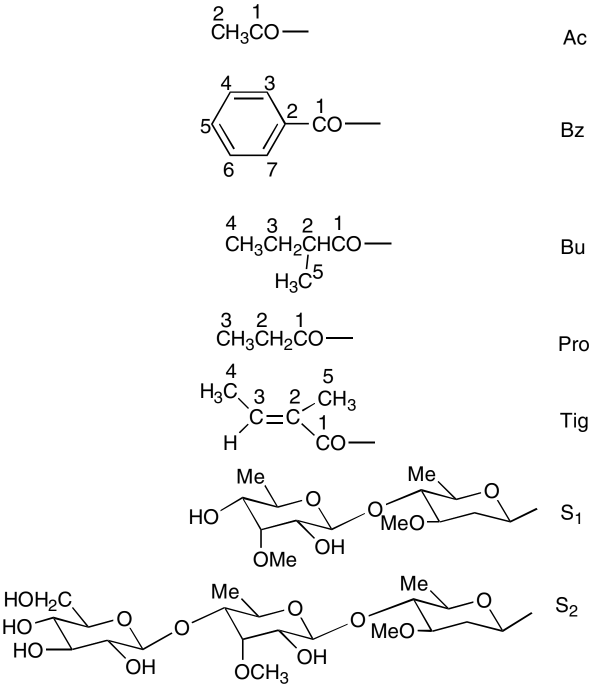


Fig. 4. Key HMBC correlations of **7**.

Marsdenoside H (**8**) was inferred to be $C_{48}H_{76}O_{19}$ on the basis of the quasi-molecular ion at *m/z* 979.4874 $[M+Na]^+$ in its HRESI-MS. Its ^{1}H NMR spectrum exhibited three anomeric proton signals at δ 4.38 (1H, d, $J = 7.8$ Hz), δ 4.58 (1H, dd, $J = 9.5$, 1.6 Hz), and δ 4.80 (1H, d, $J = 7.5$ Hz) which were correlated with the carbon resonances at δ 104.3, 96.9, and 100.2, respectively, in its HMQC spectrum, indicating that **8** contains three sugar units. The unit whose anomeric proton signal was a doublet of doublets was a 2-deoxy pyranose. The two methyl signals at δ 1.28 (3H, d, $J = 6.0$ Hz) and 1.36 (3H, d, $J = 5.4$ Hz) and the two methoxyl group at δ 3.37 and 3.60 (each 3H, s) suggested that two of the three sugar units were 6-deoxy-3-*O*-methyl-pyranose (Ma and Fang, 1997). The signals in the ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY spectra of **8** due to the two 6-deoxy-3-*O*-methyl-pyranose units were identical with those in the spectra of **1**, except for chemical shifts of C-3 (−0.8 ppm), C-4 (+9.2 ppm), and C-5 (−2.2 ppm) of 6-deoxy-3-*O*-methyl allose. The other sugar unit was identified as glucopyranose on the basis of its characteristic coupling pattern of all-axial ring protons and H-6 (δ 3.68 and 3.88, both ABM spin systems) (Sahu et al., 2002). The fact that enzymolysis treatment of **8** by β-glucosidase yielded only one sugar fragment of glucose confirmed this conclusion. The β-linkages of each of the sugars were revealed from the coupling constants of the anomeric proton signals. 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 from the correlations of H-1$_{\text{glc}}$/C-4$_{\text{allo}}$, H-1$_{\text{allo}}$/C-4$_{\text{ole}}$, and H-1$_{\text{ole}}$/C-3$_{\text{aglycone}}$ in its HMBC spectrum. Thus, the sugar moiety of **8** is neo-condurangotriose. This was supported by the fact that the ^{13}C NMR spectroscopic data recorded in pyridine for the sugar moiety of **8** (Table 2) coincided well with those of neo-condurangotriose in literature (Yoshimura et al., 1983). The ^{1}H NMR spectroscopic data of the aglycone moiety of **8** coincided well with those of 1, except for the presence of the signal for an acetyl group at δ 1.97 (3H,



	R_1	R_2	R_3		R_1	R_2	R_3
1	S_1	Bu	Tig	8	S_2	Bu	Ac
1a	H	Bu	Tig	9	H	Bu	Ac
2	S_1	Tig	Tig	10	H	Tig	Ac
3	S_1	Bu	Bz	11	S_1	Tig	Ac
4	S_1	H	Bu	12	S_1	Bu	Ac
5	S_1	Pro	Ac	13	S_1	Bz	Ac
6	S_1	Ac	Ac	14	S_2	Tig	Ac
6a	H	Ac	Ac	15	S_2	Tig	Tig
7	S_1	Tig	H	16	S_2	Bu	Bz
7a	H	Tig	H				



Structures of Compounds **1-16**

s) and the absence of the resonances for the tigloyl group. The ^{13}C NMR spectroscopic data for the aglycone moiety of **8** were also similar to those of **1**, except for signals due to two ester groups (Table 3). In the HMBC spectrum of **8**, the proton resonance at δ 5.36 (1H, t, J = 10.1 Hz, H-11β) was correlated with the carbonyl carbon signal at δ 175.6 of the 2-methylbutyryl group and the proton resonance at δ 4.99 (1H, d, J = 10.1 Hz, H-12α) was coupled with the carbonyl carbon signal at δ 170.7 of the acetyl group. Thus, the structure of **8** was established as 3-*O*-β-D-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandropyranosyl-11α-*O*-2-methylbutyryl-12β-*O*-acetyltenacigenin B. The fact that enzymolysis treatment of **8** yielded tenacissoside H confirmed the structure of **8**.

Compounds **9**–**16** were determined as 11α-*O*-2-methylbutyryl-12β-*O*-acetyltenacigenin B (**9**), 11α-*O*-tigloyl-12β-*O*-acetyltenacigenin B (**10**), tenacissoside H (**11**), tenacissoside G (**12**), tenacissoside I (**13**), tenacissoside A (**14**), tenacissoside B (**15**), and tenacissoside E (**16**), respectively, by comparing their mp, ORD, UV, IR, ^1H NMR, ^{13}C NMR, and MS data with those reported (Luo et al., 1993a,b; Chen et al., 1999; Miyakawa et al., 1986).

Compounds **1**, **3**, **6**, **7**, **9**–**16** were applied to cytotoxic tests against A_{549} and MCF-7 cell lines and anti-HIV test, but no significant activity was observed. Tests on other pharmacological models are in progress.

3. Concluding remarks

Polyoxypregnanes are characteristic constituents of plants in the family Asclepiadaceae (Mu, 2000; Deepak et al., 1997). Their structures are diverse, and the different structural types appear in specific taxa (Luo et al., 1993a,b; Warashina and Noro, 1995, 1998, 2000; El Sayed et al., 1995). Therefore, polyoxypregnanes could be used as taxonomic characters within the Asclepiadaceae. For example, nearly all reported polyoxypregnanes from plants of subfamily Asclepiadoideae have an oxygen atom at C-14 (Luo et al., 1993a,b; Warashina and Noro, 1995, 1998, 2000; El Sayed et al., 1995), whereas those from plants of other subfamilies do not (Zhang et al., 2002). The structural types of polyoxypregnanes are also valuable to the classification of lower taxonomic hierarchies including species. For instance, polyoxypregnanes from *M. condurango* are featured by a 17β side chain (Umehara et al., 1994; Berger et al., 1988; Mitsuhashi et al., 1981; Hayashi et al., 1980), whereas those from *M. tomentosa* bear a hydroxyl group at C-17 and no hydroxyl substitution at C-11 (Abe et al., 1999, 2000; Seto et al., 1977a,b).

According to the reported biosynthetic pathway, pregnane derivatives were formed from C_{27} or C_{29} sterols, by pregnenolone, 5α/β-pregnane-3,20-dione, and pregnane-3α/β-ol-20-one, successively, with every step catalysed by enzymes common in plants. Afterwards, these pregnanes are hydroxylated at different positions, and further esterified and glycosidated catalysed by enzymes specific to species (Lindemann and Luckner, 1997). Therefore, the more oxygen atoms a polyoxypregnane parent bears, the more evolutionary it apparently represents. The fact that plants of family Asclepiadaceae produce plenty of polyoxypregnanes confirms that this family is highly evolutionarily developed (Cronquist, 1981). Whereas polyoxypregnanes from plants of subfamily Asclepiadoideae are hydroxylated to a higher degree than those from plants in subfamily Periplocoideae, suggesting that the former is more evolutionarily developed than the latter, which is consistent with the conclusion of traditional taxonomy (Jiang and Li, 1977). Most of the reported polyoxypregnane glycosides (including the eight described in this paper) and their aglycones from *M. tenacissima* are tenacigenin B derivatives. They all contain a 8β-*O*-14β epoxy ring, which is formed by cyclization of the hydroxyl groups at C-8 and C-14. Many polyoxypregnanes from other plants of subfamily Asclepiadoideae bear hydroxyl groups at C-8 and C-14, but only in *M. tenacissima*, is the 8β-*O*-14β three-membered ring formed. This indicates that*M. tenacissima* differs from the other plants of the same subfamily in the enzyme system related to the biosynthesis of polyoxypregnanes. Consequently, *M. tenacissima* is a highly specialized species in subfamily Asclepiadoideae.

4. Experimental

4.1. General

Melting points were determined on a XT-4 micromelting point apparatus and are uncorr. Optical rotations were measured with a JASCO P-1020 polarimeter at room temp, whereas UV spectra were obtained in MeOH with a 756MC UV–VIS spectrometer and absorption maxima are given in nm. IR spectra were recorded on a Avatar TM 360. E. S. P TM Fourier transform infrared spectroscopy in CH_2Cl_2. EI-MS were obtained using a HP5989A mass spectrometer, and ESI-MS and HR-ESI-MS using a Q-TOF Micro ESI-MS mass spectrometer. The ^1H NMR and ^{13}C NMR spectra were taken on a Bruker DRX-400 spectrometer in $CDCl_3$ with TMS as internal standard. CC was carried out on silica gel (Qingdao, 200–300 mesh and 300–400 mesh) and Cosmosil 140 C_{18}-OPN (Nacalai Tesque). Prep. TLC utilized precoated silica gel F_{254} plate (Yantai, 0.5 mm), whereas analytical TLC was performed on precoated silica gel F_{254} plate (Yantai, 0.15 mm) and Rp-18 F_{254} S plate (Merck). Spots were visualized by spraying 0.5% vanillin-conc. H_2SO_4 and heating at 100 °C. Xinhua filter paper (middle rate) and vanillin-perchloric acid were used for PC. Prep. HPLC was conducted with XDB-C-18 column (0.9 cm × 25 cm) on Agilent HP-1100 apparatus.

4.2. Plant material

The stems of *Marsdenia tenacissima* were collected in August of 2001 from Yunnan Province, People's Republic of China, and identified by Dr. Daofeng Chen. A voucher specimen (DFC-TGS0108) is deposited in the Herbarium of Materia Medica, Department of Pharmacognosy, School of Pharmacy, Fudan University, Shanghai, People's Republic of China.

4.3. Extraction and isolation

The dried and ground stems of *M. tenacissima* (5 kg) were repeatedly percolated with EtOH–H_2O (95:5) at room temp. The solvent was evaporated in vacuo to give a dark brown extract (436 g), which was partitioned between petrol and H_2O, followed by $CHCl_3$ and H_2O. A sample (100 g) of the $CHCl_3$ solubles (314 g) was subjected to CC on silica gel with solvents of increasing polarity from $CHCl_3$ to $CHCl_3$–MeOH (85:15, v/v) to give fractions I (13 g), II (23 g), III (21 g), and IV (27 g). Fraction I was subjected to repeated silica gel CC, eluting with $CHCl_3$–MeOH (95:5) and petrol–Me_2CO (70:30), respectively, followed by prep. TLC with petrol–Me_2CO (60:40) to give **9** (21 mg) and **10** (13 mg). Fraction II was applied to repeated silica gel CC, eluting successively with $CHCl_3$–MeOH (95:5), $CHCl_3$–Me_2CO (85:15), and petrol–Me_2CO (70:30) to afford **1** (76 mg), **11** (2.3 g), fraction II-1 (1.4 g) (a mixture of **2** and **3**), and fraction II-2 (3.7 g) (a mixture of **4** and **12**). Fraction II-1 and fraction II-2 were subjected to Cosmosil 140 C_{18}-OPN using MeOH–H_2O (70:30) as eluting solvent to give **2** (5 mg) and **3** (9 mg), and **4** (14 mg) and **12** (238 mg), respectively. Fraction III was applied to a silica gel column eluted with $CHCl_3$–MeOH (90:10) to afford **6** (31 mg), fraction III-1 (0.38 g) (a mixture of **5** and **13**), and fraction III-2 (0.93 g) containing **7**. Fraction III-1 was subjected to prep. TLC developed with $CHCl_3$–Me_2CO (80:20) to give **5** (3 mg) and **13** (43 mg). Fraction III-2 was applied to a prep. HPLC column eluted with MeOH–H_2O (60:40) to yield **7** (12 mg). Fraction IV was subjected to silica gel CC using $CHCl_3$–MeOH (85:15) as mobile phase, then reapplied to a Cosmosil 140 C_{18}-OPN column eluted with MeOH–H_2O (60:40) yielding **14** (82 mg), **15** (128 mg), **16** (16 mg), and IV-1. Fraction IV-1 was further prepared by prep. HPLC with MeOH–H_2O (50:50) to afford **8** (4 mg).

*4.4. Marsdenoside A (**1**)*

Colorless needles (EtOAc–petrol), mp 206–208 °C, $[\alpha]_D^{25}$: +29.5° (MeOH; *c* 1.7); UV λ_{max}^{MeOH} nm (logε): 220 (4.12); IR ν_{max} cm^{-1} (CH_2Cl_2): 3441, 3050, 2933, 1731, 1705, 1652, 1456, 1383, 1268, 1152, 1130, 1083, 1030, 1005, 989, 840 and 736; ^1H NMR ($CDCl_3$): δ 0.79 (3H, t, J = 7.3 Hz, 4′-CH_3), 0.97 (3H, d, J = 6.9 Hz, 5′-CH_3), 1.05 (3H, s, 19-CH_3), 1.08 (3H, s, 18-CH_3), 1.26 (3H, d, J = 6.2 Hz, Allo-6-CH_3), 1.37 (3H, d, J = 5.2 Hz, Ole-6-CH_3), 1.49 (1H, m, Ole-H_a-2), 1.75 (3H, br s, 5″-CH_3), 1.76 (3H, d, J = 6.5 Hz, 4″-CH_3), 2.01 (1H, d, J = 10.1 Hz, H-9), 2.08 (1H, q, J = 6.9 Hz, H-2′), 2.22 (3H, s, 21-CH_3), 2.31 (1H, dd, J = 11.7, 4.0 Hz, Ole-H_e-2), 2.92 (1H, br d, J = 7.2 Hz, H-17β), 3.18 (1H, br d, J = 9.5 Hz, Allo-H-4), 3.35 (1H, t, J = 6.7 Hz, Ole-H-4), 3.36 (1H, m, Ole-H-5), 3.38 (3H, s, Ole-3-OCH_3), 3.41 (1H, m, Ole-H-3), 3.48 (1H, dd, J = 7.5, 2.7 Hz, Allo-H-2), 3.56 (1H, m, Allo-H-5), 3.63 (1H, m, H-3), 3.66 (3H, s, Allo-3-OCH_3), 3.79 (1H, br s, Allo-H-3), 4.58 (1H, dd, J = 9.7, 1.6 Hz, Ole-H-1), 4.80 (1H, d, J = 8.1 Hz, Allo-H-1), 5.04 (1H, d, J = 10.1 Hz, H-12α), 5.40 (1H, t, J = 10.1 Hz, H-11β), 6.79 (1H, q, J = 6.0 Hz, H-3″); For ^{13}C NMR spectroscopic data, see Tables 2 and 3; HRESI-MS: *m/z* 857.4675, calcd. 857.4663 for $[C_{45}H_{70}O_{14}+Na]^+$.

*4.5. Marsdenoside B (**2**)*

Colorless needles (EtOAc–petrol), mp 198–200.5 °C; $[\alpha]_D^{25}$: −19.3° (MeOH; *c* 0.35); UV λ_{max}^{MeOH} nm (logε): 218 (4.23); IR ν_{max} cm^{-1} (CH_2Cl_2): 3442, 3054, 2931, 1707, 1656, 1450, 1382, 1273, 1160, 1132, 1081, 1032, 1000, 930 and 735; ^1H NMR ($CDCl_3$): δ 1.06 (3H, s, 19-CH_3), 1.11 (3H, s, 18-CH_3), 1.25 (3H, d, J = 6.0 Hz, Allo-6-CH_3), 1.36 (3H, d, J = 5.5 Hz, Ole-6-CH_3), 1.48 (1H, m, Ole-H_a-2), 1.67 (6H, br s, 5′,5″-CH_3), 1.69, 1.71 (each 3H, d, J = 7.1 Hz, 4′,4″,-CH_3), 2.03 (1H, d, J = 10.1 Hz, H-9), 2.20 (3H, s, 21-CH_3), 2.30 (1H, dd, J = 12.5, 2.7 Hz, Ole-H_e-2), 2.92 (1H, br d, J = 7.1 Hz, H-17β), 3.17 (1H, dd, J = 9.6, 3.2 Hz, Allo-H-4), 3.34 (1H, m, Ole-H-5), 3.35 (1H, t, J = 7.2 Hz, Ole-H-4), 3.37 (3H, s, Ole-3-OCH_3), 3.39 (1H, m, Ole-H-3), 3.47 (1H, dd, J = 8.3, 2.7 Hz, Allo-H-2), 3.57 (1H, m, Allo-H-5), 3.66 (3H, s, Allo-3-OCH_3), 3.71 (1H, m, H-3), 3.79 (1H, t, J = 2.9 Hz, Allo-H-3), 4.56 (1H, dd, J = 9.6, 1.6 Hz, Ole-H-1), 4.79 (1H, d, J = 8.3 Hz, Allo-H-1), 5.05 (1H, d, J = 10.1 Hz, H-12α), 5.46 (1H, t, J = 10.1 Hz, H-11β), 6.66, 6.70 (each 1H, qq, J = 7.0, 1.2 Hz, H-3′,3″); For ^{13}C NMR spectroscopic data, see Tables 2 and 3; HRESI-MS: *m/z* 855.4492, calcd. 855.4507 for $[C_{45}H_{68}O_{14}+Na]^+$.

*4.6. Marsdenoside C (**3**)*

Colorless needles (EtOAc–petrol), mp 188–191 °C; $[\alpha]_D^{25}$: +6.9° (MeOH; *c* 0.74); UV λ_{max}^{MeOH} nm (logε): 280 (2.89), 275 (3.04), 230 (4.08); IR ν_{max} cm^{-1} (CH_2Cl_2): 3450, 3062, 2934, 1717, 1700, 1600, 1451, 1366, 1277, 1162, 1127, 1070, 1028, 988, 914 and 736; ^1H NMR ($CDCl_3$): δ 0.56 (3H, t, J = 7.4 Hz, 4′-CH_3), 0.85 (3H, d, J = 7.0 Hz, 5′-CH_3), 1.09 (3H, s, 19-CH_3), 1.16 (3H, s, 18-CH_3), 1.26 (3H, d, J = 6.2 Hz, Allo-6-CH_3), 1.37 (3H, d, J = 4.9 Hz, Ole-6-CH_3), 1.47 (1H, m, Ole-H_a-2), 2.08 (1H, d, J = 10.1 Hz, H-9), 2.27 (3H, s, 21-CH_3), 2.32 (1H, ddd, J = 12.6, 4.8, 1.7 Hz, Ole-H_e-2), 2.99 (1H, br d, J = 7.3 Hz, H-175), 3.18 (1H, dd, J = 9.6, 3.1 Hz, Allo-H-4), 3.34 (2H, m, Ole-H-4, 5), 3.38 (3H, s, Ole-3-OCH_3), 3.40 (1H, m, Ole-H-3), 3.48 (1H, dd, J = 8.2, 2.8 Hz, Allo-H-2), 3.56 (1H, m, Allo-H-5), 3.64 (1H, m, H-3), 3.67 (3H, s, Allo-3-OCH_3), 3.80 (1H, t, J = 2.9 Hz, Allo-H-3), 4.59 (1H, dd, J = 9.7, 1.6 Hz, Ole-H-1), 4.80 (1H, d, J = 8.3 Hz, Allo-H-1), 5.25 (1H, d, J = 10.1 Hz, H-12α), 5.54 (1H, t, J = 10.1 Hz, H-11β), 7.42 (2H, t, J = 7.7 Hz, H-4″,6″), 7.55 (1H, t, J = 7.4 Hz, H-5P), 7.96 (2H, d, J = 7.3 Hz, H-3″,7″); For ^{13}C NMR spectroscopic data, see Tables 2 and 3; HRESI-MS: *m/z* 879.4511, calcd. 879.4507 for $[C_{47}H_{68}O_{14}+Na]^+$.

*4.7. Marsdenoside D (**4**)*

Colorless needles (EtOAc–petrol), mp 179–182.5 °C; $[\alpha]_D^{25}$: +28.5° (MeOH; *c* 0.86); IR ν_{max} cm^{-1} (CH_2Cl_2): 3443, 3054, 2934, 1724, 1704, 1456, 1367, 1268, 1189, 1160, 1128, 1068, 989, 910 and 736; ^1H NMR ($CDCl_3$): δ 0.91 (3H, t, J = 7.4 Hz, 4′-CH_3), 1.05 (3H, s, 18-CH_3), 1.06 (3H, s, 19-CH_3), 1.16 (3H, d, J = 7.0 Hz, 5′-CH_3), 1.26 (3H, d, J = 6.2 Hz, Allo-6-CH_3), 1.37 (3H, d, J = 4.9 Hz, Ole-6-CH_3), 1.50 (1H, m, Ole-H_a-2), 1.71 (1H, d, J = 10.0 Hz, H-9), 2.21 (3H, s, 21-CH_3), 2.33 (1H, ddd, J = 11.8, 4.5, 2.0 Hz, Ole-H_e-2), 2.92 (1H, br d, J = 7.3 Hz, H-17β), 3.18 (1H, dd, J = 9.6, 2.5 Hz, Allo-H-4), 3.37 (2H, m, Ole-H-4, 5), 3.38 (3H, *s*, Ole-3-OCH_3), 3.40 (1H, m, Ole-H-3), 3.48 (1H, br d, J = 9.0 Hz, Allo-H-2), 3.57(1H, m, Allo-H-5), 3.64 (1H, m, H-3), 3.67 (3H, s, Allo-3-OCH_3), 3.80 (1H, t, J = 2.5 Hz, Allo-H-3), 3.88 (1H, t, J = 10.0 Hz, H-11β), 4.60 (1H, dd, J = 9.6, 1.7 Hz, Ole-H-1), 4.80 (1H, d, J = 8.3 Hz, Allo-H-1), 4.83 (1H, d, J = 10.0 Hz, H-12α); For ^{13}C NMR spectroscopic data, see Tables 2 and 3; HRESI-MS: *m/z* 775.4246, calcd. 775.4245 for $[C_{40}H_{64}O_{13}+Na]^+$.

4.8. Marsdenoside E (**5**)

Colorless needles (EtOAc–petrol), mp 188.5–191 °C; $[\alpha]_D^{25}$: −134° (MeOH; *c* 0.12); IR ν_{max} cm^{-1} (CH_2Cl_2): 3406, 3054, 2927, 1736, 1668, 1395, 1265, 1158, 1070, 901 and 739; ^1H NMR ($CDCl_3$): δ 1.04 (3H, s, 19-CH_3), 1.07(3H, t, $J = 7.6$ Hz, 3′-CH_3), 1.09 (3H, s, 18-CH_3), 1.26 (3H, d, $J = 5.8$ Hz, Allo-6-CH_3), 1.38 (3H, d, $J = 8.2$ Hz, Ole-6-CH_3), 1.49 (1H, m, Ole-H_a-2), 1.96 (3H, s, 2″-CH_3), 1.98 (1H, d, $J = 10.1$ Hz, H-9), 2.19 (3H, s, 21-CH_3), 2.31 (1H, ddd, $J = 12.3$, 4.1, 1.6 Hz, Ole-H_e-2), 2.93 (1H, br d, $J = 7.4$ Hz, H-17β), 3.19 (1H, dd, $J = 8.3$, 2.9 Hz, Allo-H-4), 3.35 (2H, m, Ole-H-4, 5), 3.37 (3H, *s*, Ole-3-OCH_3), 3.41 (1H, m, Ole-H-3), 3.49 (1H, br d, $J = 7.5$ Hz, Allo-H-2), 3.56 (1H, m, Allo-H-5), 3.61 (1H, m, H-3), 3.66 (3H, s, Allo-3-OCH_3), 3.79 (1H, br s, Allo-H-3), 4.57 (1H, dd, $J = 9.6$, 1.5 Hz, Ole-H-1), 4.80 (1H, d, $J = 8.5$ Hz, Allo-H-1), 4.96 (1H, d, $J = 10.1$ Hz, H-12α), 5.35 (1H, t, $J = 10.1$ Hz, H-11β); For ^{13}C NMR spectroscopic data, see Tables 2 and 3; HRESI-MS: *m/z* 789.4042, calcd. 789.4037 for $[C_{40}H_{62}O_{14}+Na]^+$.

4.9. Marsdenoside F (**6**)

Colorless needles (MeOH), mp 224–226 °C; $[\alpha]_D^{25}$: −84.5° (MeOH; *c* 0.23); IR ν_{max} cm^{-1} (CH_2Cl_2): 3443, 3062, 2933, 1741, 1703, 1450, 1368, 1252, 1162, 1068, 1031, 989, 910 and 736; ^1H NMR ($CDCl_3$): δ 1.04 (3H, *s*, 19-CH_3), 1.08 (3H, s, 18-CH_3), 1.24 (3H, d, $J = 6.8$ Hz, Allo-6-CH_3), 1.37 (3H, d, $J = 5.3$ Hz, Ole-6-CH_3), 1.51 (1H, m, Ole-H_a-2), 1.93, 1.97 (each 3H, s, 2′,2″-CH_3), 1.98 (1H, d, $J = 10.1$ Hz, H-9), 2.19 (3H, s, 21-CH_3), 2.31 (1H, ddd, $J = 12.2$, 4.6, 1.6 Hz, Ole-H_e-2), 2.93 (1H, br d, $J = 7.3$ Hz, H-17β), 3.19 (1H, dd, $J = 9.5$, 3.2 Hz, Allo-H-4), 3.34 (1H, m, Ole-H-5), 3.35 (1H, t, $J = 7.1$ Hz, Ole-H-4), 3.37 (3H, s, Ole-3-OCH_3), 3.41 (1H, m, Ole-H-3), 3.48 (1H, dd, $J = 8.3$, 2.8 Hz, Allo-H-2), 3.56 (1H, m, Allo-H-5), 3.64 (1H, m, H-3), 3.66 (3H, s, Allo-3-OCH_3), 3.79 (1H, t, $J = 2.6$ Hz, Allo-H-3), 4.57 (1H, dd, $J = 9.6$, 1.5 Hz, Ole-H-1), 4.79 (1H, d, $J = 7.7$ Hz, Allo-H-1), 4.94 (1H, d, $J = 10.1$ Hz, H-12α), 5.31 (1H, t, $J = 10.1$ Hz, H-11β); For ^{13}C NMR spectroscopic data, see Tables 2 and 3; HRESI-MS: *m/z* 775.3891, calcd. 775.3881 for $[C_{39}H_{60}O_{14}+Na]^+$.

4.10. Marsdenoside G (**7**)

Colorless needles (MeOH), mp 221–224.5 °C; $[\alpha]_D^{25}$: −30.6° (MeOH; *c* 0.53); UV λ_{max}^{MeOH} nm (logε): 218 (3.97); IR ν_{max} cm^{-1} (CH_2Cl_2): 3442, 3054, 2932, 1704, 1643, 1456, 1374, 1267, 1162, 1130, 1069, 988, 922 and 734; ^1H NMR ($CDCl_3$): δ 1.01 (3H, s, 18-CH_3), 1.05 (3H, s, 19-CH_3), 1.26 (3H, d, $J = 5.7$ Hz, Allo-6-CH_3), 1.36 (3H, d, $J = 4.6$ Hz, Ole-6-CH_3), 1.49 (1H, m, Ole-H_a-2), 1.79 (3H, d, $J = 7.0$ Hz, 4′-CH_3), 1.82 (1H, d, $J = 9.5$ Hz, H-9), 1.85 (3H, br s, 5′-CH_3), 2.24 (3H, s, 21-CH_3), 2.29 (1H, dd, $J = 12.2$, 3.8 Hz, Ole-H_e-2), 2.68 (1H, br d, $J = 12.6$, 5.4 Hz, H-17β), 3.18 (1H, dd, $J = 9.3$, 2.4 Hz, Allo-H-4), 3.34 (2H, m, Ole-H-4, 5), 3.37 (3H, s, Ole-3-OCH_3), 3.40 (1H, m, Ole-H-3), 3.47 (1H, br d, $J = 9.5$ Hz, Allo-H-2), 3.48 (1H, d, $J = 9.5$ Hz, H-12α), 3.55 (1H, m, Allo-H-5), 3.64 (1H, m, H-3), 3.66 (3H, s, Allo-3-OCH_3), 3.80 (1H, br *s*, Allo-H-3), 4.58 (1H, dd, $J = 8.3$, 1.8 Hz, Ole-H-1), 4.80 (1H, d, $J = 8.1$ Hz, Allo-H-1), 5.17 (1H, t, $J = 9.5$ Hz, H-11β), 6.87 (1H, *q*, $J = 7.0$ Hz, H-3′); For ^{13}C NMR spectroscopic data, see Tables 2 and 3; HRESI-MS: *m/z* 773.4086, calcd. 773.4088 for $[C_{40}H_{62}O_{13}+Na]^+$.

4.11. Marsdenoside H (**8**)

Colorless needles (MeOH–H_2O), mp 177–180 °C; $[\alpha]_D^{25}$: −27.6° (MeOH; *c* 0.7); IR ν_{max} cm^{-1} (CH_2Cl_2): 3400, 3054, 2932, 1720, 1705, 1450, 1366, 1250, 1160, 1070, 989, 910 and 738; ^1H NMR ($CDCl_3$): δ 0.89 (3H, t, $J = 7.4$ Hz, 4′-CH_3), 1.05 (3H, s, 19-CH_3), 1.06 (3H, d, $J = 6.8$ Hz, 5′-CH_3), 1.08 (3H, s, 18-CH_3), 1.28 (3H, d, $J = 6.0$ Hz, Allo-6-CH_3), 1.36 (3H, d, $J = 5.4$ Hz, Ole-6-CH_3), 1.51 (1H, m, Ole-H_a-2), 1.97 (3H, s, 2″-CH_3), 2.01 (1H, d, $J = 10.1$ Hz, H-9), 2.20 (3H, s, 21-CH_3), 2.28 (1H, br d, $J = 7.2$ Hz, Ole-H_e-2), 2.92 (1H, br d, $J = 7.2$ Hz, H-17β), 3.32 (1H, t, $J = 8.3$ Hz, Ole-H-4), 3.33 (1H, br *d*, J = 6.7 Hz, Allo-H-4), 3.37 (3H, s, Ole-3-OCH_3), 3.39 (1H, m, Ole-H-3), 3.49 (1H, br d, $J = 7.3$ Hz, Allo-H-2), 3.60 (3H, s, Allo-3-OCH_3), 3.65 (1H, m, H-3), 3.73 (1H, q, $J = 7.0$ Hz, Ole-H-5), 3.86 (1H, br s, Allo-H-3), 3.90 (1H, m, Ole-H-5), 4.38 (1H, d, $J = 7.8$ Hz, Glc-H-1), 4.58 (1H, dd, $J = 9.5$, 1.6 Hz, Ole-H-1), 4.80 (1H, d, $J = 7.5$ Hz, Allo-H-1), 4.99 (1H, d, $J = 10.1$ Hz, H-12α), 5.36 (1H, t, $J = 10.1$ Hz, H-11β); For ^{13}C NMR spectroscopic data, see Tables 2 and 3; HRESI-MS: *m/z* 979.4874, calcd. 979.4879 for $[C_{48}H_{76}O_{19}+Na]^+$.

4.12. Mild acid hydrolysis of **1**, **2**, **3**, **4**, **6**, and **7**

MeOH (15 ml) and 0.1 M H_2SO_4 (5 ml) were added to **1** (15 mg) and the mixture was kept at 60 °C for 30 min. After evaporation of MeOH under reduced pressure, H_2O (15 ml) was added and kept at around 60 °C for another 30 min, then cooled to room temp. Then the soln. was extracted with Et_2O (10 ml × 3), and the Et_2O solubles were washed with water (5 ml × 4), dried (Na_2SO_4), and evaporated to dryness. The residue was dissolved in EtOAc and **1a** was crystallized from petrol-EtOAc. The aq. acidic layer of the hydrolysate soln. was neutralized with 5% $Ba(OH)_2$ aq. soln. The ppt. was filtered and the soln. was evaporated and verified to contain one sugar fragment which was identified as pachybiose by PC and TLC comparison with an authentic sample developed with the upper layer of *n*–BuOH–HOAc–H_2O (4:1:5, v/v) and $CHCl_3$–MeOH (9:1, v/v), respectively; R_f values were 0.78 and 0.35. The same mild acid hydrolysis procedure was applied to **2** (2 mg), **3** (2 mg), **4** (5 mg), **6** (15 mg), and **7** (5 mg), and the aq. acidic layer of their hydrolysates all contained pachybiose as determined by PC and TLC comparison with an authentic sample. From the Et_2O extracts of the acidic hydrolysates of **4, 6**, and **7**, the genins **4a**, **6a**, and **7a** were obtained, respectively.

4.13. 11α-O-2-Methylbutyryl-12β-O-tigloyltenacigenin B (*1a*)

Colorless needles (EtOAc–petrol), mp 218–220 °C; $[\alpha]_D^{25}$: −18.2° (MeOH; *c* 0.17); UV λ_{max}^{MeOH} nm (log ε): 220 (4.03); IR ν_{max} cm^{-1} (CH_2Cl_2): 3448, 3054, 2932, 1732, 1706, 1651, 1454, 1366, 1266, 1156, 1030, 1000, 938 and 738; ^1H NMR: δ 0.77 (3H, t, J = 7.4 Hz, 4′-CH_3), 0.96 (3H, d, J = 7.0 Hz, 5′-CH_3), 1.04 (3H, s, 19-CH_3), 1.06 (3H, s, 18-CH_3), 1.73 (3H, br s, 5″-CH_3), 1.74 (3H, d, J = 6.5 Hz, 4″-CH_3), 2.20 (3H, s, 21-CH_3), 2.90 (1H, br d, J = 7.4 Hz, H-17β), 3.57 (1H, m, H-3), 5.01 (1H, d, J = 10.1 Hz, H-12α), 5.38 (1H, t, J = 10.1 Hz, H-11β), 6.76 (1H, br. q, J = 6.6 Hz, H-3′); For ^{13}C NMR ($CDCl_3$) spectroscopic data, see Table 3; EI-MS 70eV, *m/z* (rel. int.): 530 $[M]^+$ (0.2), 428 (3), 385 (2), 329 (5), 232 (16), 105 (100), 85 (17).

4.14. 12β-O-2-Methylbutyryltenacigenin A (*4a*)

Amorphous white powder; ^1H NMR: δ 0.91 (3H, t, J = 7.4 Hz, 4′-CH_3), 1.07 (3H, s, 18-CH_3), 1.11 (3H, s, 19-CH_3), 1.20 (3H, s, 21-CH_3), 1.21 (3H, d, J = 7.1 Hz, 5′-CH_3), 1.98 (1H, d, J = 6.1 Hz, H-17β), 2.39 (1H, br s, H-9), 3.63 (1H, m, H-3), 4.30 (1H, dd, J = 3.8, 1.6 Hz, H-11β), 5.28 (1H, d, J = 3.8 Hz, H-12α); For ^{13}CNMR ($CDCl_3$) spectroscopic data, see Table 3; HRESI-MS: *m/z* 471.2727, calcd. 471.2723 for $[C_{26}H_{40}O_6+Na]^+$.

4.15. 11α,12β-Di-O-acetyltenacigenin B (*6a*)

Colorless needles (EtOAc–petrol), mp 217–220.5 °C; $[\alpha]_D^{25}$: +12.7° (MeOH; *c* 0.14); IR ν_{max} cm^{-1} (CH_2Cl_2): 3448, 3054, 2932, 1732, 1706, 1651, 1454, 1366, 1266, 1156, 1030, 1000, 938 and 738; ^1H NMR: δ 1.06 (3H, s, 19-CH_3), 1.09 (3H, s, 18-CH_3), 1.95, 1.98 (each 3H, s, 2′, 2″-CH_3), 2.20 (3H, s, 21-CH_3), 2.94 (1H, d, J = 7.4 Hz, H-17β), 3.60 (1H, m, H-3), 4.96 (1H, d, J = 10.1 Hz, H-12α), 5.33 (1H, t, J = 10.1 Hz, H-11β); For ^{13}C NMR ($CDCl_3$) spectroscopic data, see Table 3; EI-MS 70 eV, *m/z* (rel. int.): 448 $[M]^+$ (0.2), 430 (6), 370 (3), 43 (100); HREI-MS: *m/z* 448.2457, calcd. 448.2461 for $[C_{25}H_{36}O_7]^+$.

4.16. 11α-O-Tigloyltenacigenin B (*7a*)

Amorphous white powder; ^1H NMR: δ 1.02 (3H, s, 18-CH_3), 1.06 (3H, s, 19-CH_3), 1.81 (3H, d, J = 8.1 Hz, 4′-CH_3), 1.85 (3H, br s, 5′-CH_3), 2.25 (3H, s, 21-CH_3), 2.67 (1H, dd, J = 12.7, 5.7 Hz, H-17β), 3.47 (1H, d, J = 9.8 Hz, H-12α), 3.58 (1H, m, H-3), 5.18 (1H, t, J = 9.8 Hz, H-11β), 6.88 (1H, qq, J = 7.1, 1.2 Hz, H-3′); For ^{13}C NMR ($CDCl_3$) spectroscopic data, see Table 3; HRESI-MS: *m/z* 469.2563, calcd. 469.2566 for $[C_{26}H_{38}O_6+Na]^+$.

4.17. β-Glucosidase treatment of *8*

0.1 mol L^{-1} AcOH–AcONa (pH 4.6) buffer (1 ml) and β-glucosidase (30 μl) (Sigma Co.) (250 U/ml) was added to **8** (2 mg). The soln. was incubated at 60 °C for 30 min, then extracted with $CHCl_3$ (5 ml × 3). The $CHCl_3$ layer was evaporated under reduced pressure and the residue was identified as tenacissoside H by TLC comparison with an authentic sample developed with $CHCl_3$–MeOH (9:1, v/v), R_f value was 0.61. The aq. layer contained only one monosaccharide identified as glucose by PC comparison with an authentic sample using the upper layer of *n*-BuOH–HOAc–H_2O (4:1:5, v/v) as developing solvent, R_f value was 0.17.

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