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Two New C_{21} Steroids from *Marsdenia tenacissima*

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Abstract: Two new C_{21} steroids were isolated from the $CHCl_3$ extract of the stem of *Marsdenia tenacissima*. On the basis of spectroscopic analysis and chemical methods, their structures were elucidated as 17β-tenacigenin B (**2**) and 3-*O*-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandropyranosyl-tenacigenin C (**3**). The structure of the known aglycon tenacigenin C was revised as 5α, 9α, 17β-pregnane-3β, 8β, 11α, 12β, 14β-pentanol-20-one. Compound **3** is the first reported glycoside of tenacigenin C.

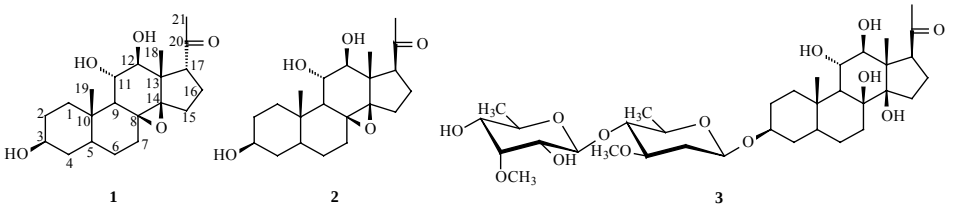
Keywords: *Marsdenia tenacissima*, C_{21} steroids, tenacigenin B, 17β-tenacigenin B, tenacigenin C.

Marsdenia tenacissima, a perennial climber of family Asclepiadaceae, has long been used for the treatment of cancer and asthma in China [1]. Twelve polyoxypregnane genins and nine glycosides have been isolated from the stem of the title plant [2-6]. Two new steroids (**2** and **3**) along with the known tenacigenin B (**1**) were isolated from the $CHCl_3$ extract of the stem of *M. tenacissima*. This paper deals with the structural elucidation of the two new compounds.

Compound **1** was identified as tenacigenin B by comparing its ^1H and ^{13}C NMR data (**Table 1**) with those reported in the literature[2,4]. The 17α side chain of **1** was confirmed by the NOESY experiment (**Figure 2**).

Compound **2**, obtained as colorless needles, gave a quasi-molecular ion at *m/z* 387.2144 ($[M+Na]^+$, calcd. 387.2147) in its HR-ESI-MS spectrum, indicating its molecular formula was $C_{21}H_{32}O_5$. The ^1H and ^{13}C NMR spectral data (**Table 1**) quite resembled to those of **1**. The difference between the ^1H NMR spectra of **2** and **1** was that the resonance for the 17-H was at δ_H 2.59 (*dd*, 1H, *J*= 11.4, 6.7 Hz) in **2** while that

Figure 1 The structures of compounds **1-3**



1 **2** **3**

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was at δ_H 3.02 (*t*, 1H, *J*= 6.1 Hz) in **1**. The split pattern and coupling constants of the signal of 17-H suggested that the C-17 side chain of **2** was in β orientation, while that of **1** was in α orientation [7]. This was supported by the cross peaks between δ_H 0.93 (18-CH_3)/δ_H 2.25 (21-CH_3) and between δ_H 2.59 (17-H)/δ_H 3.26 (12-H) in the NOESY spectrum of **2**, and those between δ_H 1.16 (18-CH_3)/δ_H 3.02 (H-17) and between δ_H 2.28 (21-CH_3)/δ_H 3.30 (12-H) in the NOESY spectrum of **1** (**Figure 2**). Therefore, **2** was determined as 17β-tenacigenin B.

Comparing the ^1H and ^{13}C NMR data of **1** and **2** (recorded in $CDCl_3$), the signal of 18-CH_3 was at δ_H 1.16 in **1** and at δ_H 0.93 in **2**, the signals of C-12 and C-18 were at δ_C 74.3 and δ_C17.6 in **1**, and at δ_C 80.1 and δ_C 10.4 in **2** due to the change of the C-17 configuration. The proton signal of 21-CH_3 of **1** was almost same as that of **2**. In literature, the C-17 configuration of C_{21} steroids was determined by comparing the ^1H NMR data of 21-CH_3 measured in C_5D_5N [4]. It was reported that the chemical shift of 21-CH_3 of marsdenin was δ_H 2.39 and that of 17β-marsdenin was δ_H 2.23 [8]. To understand the relationship between the chemical shift of 21-CH_3 with the C-17 configuration, the ^1H NMR spectra of **1** and **2** were also measured in C_5D_5N. It was found that the signal of 21-CH_3 in **1** was at δ_H 2.37 and that of **2** was at δ_H 2.52, just opposite to that observed with marsdenin and 17β-marsdenin. The above result indicated that the C-17 configuration of polyoxypregnanes can not be determined with the chemical shift of 21-CH_3 protons.

Figure 2 Key NOESY correlations of **1** and **2**

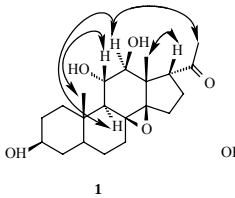
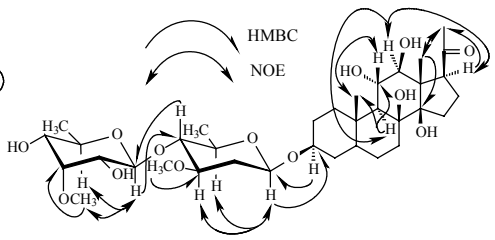


Figure 3 Key HMBC and NOESY correlations of **3**



Compound **3,** was obtained as white amorphous powder. Liebermann-Burchard, Keller-Kiliani, and xanthydrol tests gave positive reactions, indicating that it was a steroidal glycoside with 2-deoxy sugar [9]. It had a molecular formula $C_{35}H_{58}O_{13}$ based on the quasi-molecular ion at *m/z* 725.3513 ($[M+K]^+$, calcd. 725.3515) in the HR-ESI-MS spectrum. The ^1H NMR spectrum displayed two anomeric proton signals at δ_H 4.80 (*d*, 1H, *J*= 8.5 Hz) and δ_H 4.59 (*dd*, 1H, *J*= 9.7, 1.8 Hz), corresponding carbon resonances at δ_C 99.2 and δ_C 97.0 in the ^{13}C NMR spectrum, respectively, suggesting that **3** was a disaccharide glycoside. The ^1H and ^{13}C NMR data of the sugar moiety of **3** (**Table 1**) coincided well with those of pachybiose [3]. Mild acidic hydrolysis of **3** afforded only one sugar fragment which was identified as pachybiose by TLC comparison with an authentic sample. The coupling constants, 8.5 Hz and 9.7 Hz, of the two anomeric proton resonances indicated that the sugar linkages were both in β orientation. The ^1H NMR data of the aglycon moiety of **3** were identical to those of the

known tenacigenin C [4]. The mild acidic hydrolysis of **3** gave a genin **3a**. Its mp, IR, and ^{1}H NMR data were same as those of tenacigenin C. In Yang's work, the C-17 side chain of tenacigenin C was determined as in an α-configuration according to the chemical shift value of 21-CH_3 (δ_H 2.45) which was closer to that of marsdenin [4]. But as mentioned above, the C-17 configuration of C_{21} steroids can not be determined with the chemical shift of 21-CH_3 protons. The NOESY correlations between δ_H 0.93 (18-CH_3) /δ_H 2.26 (21-CH_3) and between δ_H 2.58 (17-H)/δ_H 3.25 (12-H) (**Figure 3**) clearly indicated that the 17-H was in α orientation and that the C-17 side chain was in β orientation in **3**. Therefore, the structure of tenacigenin C was revised as 5α, 9α, 17β-pregnane-3β, 8β, 11α, 12β, 14β-pentanol-20-one. The glycosidation shifts of C-2 (- 2.1 ppm), C-3 (+ 6.1 ppm), and C-4 (- 2.9 ppm) were observed by comparing the ^{13}C NMR data of **3** with those of **3a**, indicating that the oligosaccharide chain was linked at the C-3 hydroxyl group of the aglycon, which was confirmed by the cross peaks between δ_H 4.59 (1-H_{olea})/δ_C 76.8 (C-3) and between δ_H 3.67 (3-H)/δ_C 97.0 (Olea-C-1) in the HMBC spectrum of **3** (**Figure 3**). Consequently, the structure of **3** was elucidated as 3-*O*-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandropyranosyltenacigenin C.

Compound **1**: mp 225.5-228.0℃; $[\alpha]_D^{22}$ -6.2 (*c* 0.6, MeOH); IR (KBr) *v* (cm^{-1}): 3493, 2925, 1699, 1455, 1367, 1178, 1154, 1100, 1037, 940; ESI-MS *m/z* 387.1 $[C_{21}H_{32}O_5+Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 1.06 (*s*, 3H, 19-CH_3), 1.16 (*s*, 3H, 18-CH_3), 1.47 (*d*, 1H, *J*= 9.4 Hz, 9-H), 2.28 (*s*, 3H, 21-CH_3), 3.02 (*t*, 1H, *J*= 6.1 Hz, 17-H), 3.30 (*d*, 1H, *J*= 9.4 Hz, 12α-H), 3.58 (*t*, 1H, *J*= 9.4 Hz, 11β-H), 3.67 (*m*, 1H, 3-H); ^{1}H NMR (400 MHz, C_5D_5N): δ ppm 1.31 (*s*, 3H, 19-CH_3), 1.48 (s, 3H, 18-CH_3), 1.74 (*d*, 1H, *J*= 9.4 Hz, 9-H), 2.37 (*s*, 3H, 21-CH_3), 3.11 (*t*, 1H, *J*= 6.0 Hz, 17-H), 3.75 (*d*, 1H, *J*=9.4 Hz, 12-H), 3.92 (*m*, 1H, 3-H), 3.97 (*t*, 1H, *J*= 9.4 Hz, 11-H); ^{13}C NMR: **Table 1**.

Table 1 The ^{13}C NMR data of **1**-**3** and **3a** (125 MHz; in $CDCl_3$) (δ ppm)

	1	**2**	**3a**		**3**	
C-1	38.1 (t)	38.1 (t)	37.9 (t)	38.1 (t)	Sugar moiety of **3**	
C-2	32.3 (t)	31.3 (t)	31.1 (t)	29.0 (t)	Oleandrose	
C-3	70.8 (d)	71.0 (d)	70.7 (d)	76.8 (d)	C-1	97.0 (d)
C-4	38.5 (t)	38.2 (t)	37.5 (t)	34.6 (t)	C-2	36.1 (t)
C-5	44.6 (d)	44.8 (d)	44.5 (d)	44.6 (d)	C-3	78.9 (d)
C-6	26.9 (t)	27.1 (t)	28.0 (t)	27.1 (t)	C-4	79.2 (d)
C-7	31.2 (t)	31.6(t)	32.4 (t)	32.6 (t)	C-5	71.3 (d)
C-8	66.0 (s)	66.1 (s)	65.9 (s)	66.0 (s)	C-6	18.6 (q)
C-9	54.4 (d)	54.3 (d)	53.2 (d)	53.4 (d)	3-OMe	55.6 (q)
C-10	39.1 (s)	39.1 (s)	39.0 (s)	39.2 (s)	Allose	
C-11	68.6 (d)	67.9 (d)	68.1 (d)	67.8 (d)	C-1	99.2 (d)
C-12	74.3 (d)	80.1 (d)	80.3 (d)	80.0 (d)	C-2	71.9 (d)
C-13	47.3 (s)	46.3 (s)	46.4 (s)	46.1 (s)	C-3	81.0 (d)
C-14	71.5 (s)	71.2 (s)	70.4 (s)	70.3 (s)	C-4	72.9 (d)
C-15	27.7 (t)	27.0 (t)	27.8 (t)	27.9 (t)	C-5	71.4 (d)
C-16	25.5 (t)	25.6 (t)	25.4 (t)	25.4 (t)	C-6	17.9 (q)
C-17	60.4 (d)	64.1(d)	63.8 (d)	64.0 (d)	3-OMe	61.9 (q)
C-18	17.6 (q)	10.4 (q)	10.4 (q)	10.3 (q)		
C-19	13.0 (q)	12.9 (q)	12.8 (q)	12.8 (q)		
C-20	212.4 (s)	213.4 (s)	213.0 (s)	213.2 (s)		
C-21	32.6 (q)	30.3 (q)	30.4 (q)	30.3 (q)		

Compound **2**: mp 229-231.5℃; [α]$_D^{22}$ -85.7 (*c* 0.18, MeOH); IR (KBr) *v* (cm^{-1}): 3491, 2921, 1676, 1450, 1368, 1164, 1122, 1094, 1051, 934; HR-ESI-MS *m/z* 387.2144 (calcd. 387.2147 for [$C_{21}H_{32}O_5$+Na]$^+$); ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 0.93 (*s*, 3H, 18-CH_3), 1.04 (*s*, 3H, 19-CH_3), 1.35 (*d*, 1H, *J*=9.4 Hz, 9-H), 2.25 (*s*, 3H, 21-CH_3), 2.59 (*dd*, 1H, *J*=11.4, 6.7 Hz, 17α-H), 3.26 (*d*, 1H, *J*=9.4 Hz, 12α-H), 3.62 (*t*, 1H, *J*=9.4 Hz, 11β-H), 3.64 (*m*, 1H, 3-H); ^{1}H NMR (400 MHz, C_5D_5N, δ ppm): 1.32 (*s*, 6H, 18, 19-CH_3), 1.82 (*d*, 1H, *J*= 9.4 Hz, 9-H), 2.52 (*s*, 3H, 21-CH_3), 2.94 (*dd*, 1H, *J*=12.1, 6.1 Hz, 17-H), 3.68 (*d*, 1H, *J*= 9.4 Hz, 12-H), 3.94 (*m*, 1H, 3-H), 3.97 (*t*, 1H, *J*= 9.4 Hz, 11-H); ^{13}C NMR: **Table 1**.

Compound **3**: [α]$_D^{22}$ -4.8 (*c* 2.0, MeOH); IR (KBr) *v* (cm^{-1}): 3532, 3451, 2933, 1694, 1446, 1368, 1300, 1277, 1251, 1164, 1129, 1067, 1023, 983; HR-ESI-MS *m/z* 725.3513 (calcd. 725.3515 for [$C_{35}H_{58}O_{13}$+K]$^+$); ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 0.93 (*s*, 3H, 18-CH_3), 1.04 (*s*, 3H, 19-CH_3), 1.26 (*d*, 3H, *J*= 6.1 Hz, Allo-6-CH_3), 1.37 (*d*, 3H, *J*= 5.7 Hz, Ole-6-CH_3), 1.47 (*d*, 1H, *J*=9.3 Hz, 9-H), 1.49 (*m*, 1H, Ole-2-H_a), 2.26 (*s*, 3H, 21-CH_3), 2.31 (*dd*, 1H, *J*= 10.2, 3.3 Hz, Ole-2-H_e), 2.58 (*dd*, 1H, *J*=11.8, 7.4 Hz, 17-H), 3.18 (*dt*, 1H, *J*= 6.8, 2.2 Hz, Allo-4-H), 3.25 (*d*, 1H, *J*=9.3 Hz, 12-H), 3.34 (*m*, 1H, Ole-5-H), 3.36 (*t*, 1H, *J*= 6.4 Hz, Ole-4-H), 3.38 (*s*, 3H, Ole-3-OCH_3), 3.39 (*m*, 1H, Ole-3-H), 3.48 (br *d*, 1H, *J*= 6.1 Hz, Allo-2-H), 3.56 (*m*, 1H, Allo-5-H), 3.58 (*t*, 1H, *J*=9.3 Hz, 11-H), 3.66 (*s*, 3H, Allo-3-OCH_3), 3.67 (*m*, 1H, 3-H), 3.80 (*t*, 1H, *J*= 2.7 Hz, Allo-3-H), 4.59 (*dd*, 1H, *J*= 9.7, 1.8 Hz, Ole-1-H), 4.80 (*d*, 1H, *J*= 8.5 Hz, Allo-1-H); ^{13}C NMR data: **Table 1**.

Compound **3a**: mp 121.5-123.0℃; [α]$_D^{22}$ -42.4 (*c* 0.3, MeOH); IR (KBr) *v* (cm^{-1}): 3523, 3443, 1696, 1449, 1109, 1047, 1032, 934, 865, 830, 769; HR-ESI-MS *m/z* 405.2248 (calcd. 405.2253 for [$C_{21}H_{34}O_6$+Na]$^+$); ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 0.93 (*s*, 3H, 18-CH_3), 1.05 (*s*, 3H, 19-CH_3), 1.45 (*d*, 1H, *J*= 9.5 Hz, 9-H), 2.27 (*s*, 3H, 21-CH_3), 2.55 (*dd*, 1H, *J*= 12.1, 6.4 Hz, 17-H), 3.25 (*d*, 1H, *J*= 9.5 Hz, 12α-H), 3.60 (*t*, 1H, *J*= 9.5 Hz, 11β-H), 3.66 (*m*, 1H, 3-H); ^{1}H NMR (400 MHz, C_5D_5N, δ ppm): 1.28 (*s*, 6H, 18, 19-CH_3), 1.79 (*d*, 1H, *J*= 9.4 Hz, 9-H), 2.46 (*s*, 3H, 21-CH_3), 2.93 (*dd*, 1H, *J*= 11.7, 6.2 Hz, 17-H), 3.65 (*d*, 1H, *J*= 9.4 Hz, 12-H), 3.90 (*m*, 1H, 3-H), 3.93 (*t*, 1H, *J*= 9.4 Hz, 11-H); ^{13}C NMR: **Table 1**.

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