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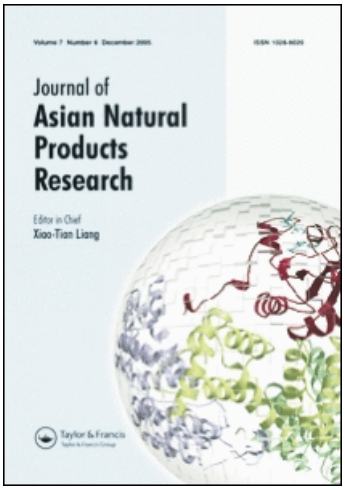

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Further polyoxypregnane glycosides from *Marsdenia tenacissima*

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ORIGINAL ARTICLE

Further polyoxypregnane glycosides from *Marsdenia tenacissima*

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Three new polyoxypregnane glycosides, tenacigenosides F–H (**1**–**3**), were isolated from the stems of *Marsdenia tenacissima*. The structures of these new compounds were elucidated from 1D and 2D NMR spectra, as well as from HR-MS and acid hydrolysis.

Keywords: *Marsdenia tenacissima*; Asclepiadaceae; polyoxypregnane glycoside; tenacigenosides F–H

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1. Introduction

Marsdenia tenacissima (ROXB.) WIGHT et ARN. (Asclepiadaceae), popularly known as 'Tong-guang-teng', is widely employed to treat inflammation, asthma, and cancer in southwest China [1]. Clinically, a preparation from the aqueous extract of the stems of *M. tenacissima*, Tong-guang-teng tablet, has been used for the treatment of chronic bronchitis and tracheitis, and Xiao-ai-ping preparations (including injection, syrup, and tablet) are effective for the treatment of gastric carcinoma and liver cancer [2]. Thirty-nine pregnane glycosides and 18 pregnane genins have been isolated from this plant [3–14], and some of them have been assayed for bioactivity [6,8]. As part of our continuing investigation of this plant, three new polyoxypregnane glycosides, namely tenacigenoside F (**1**), tenacigenoside G (**2**), and tenacigenoside H (**3**), were also isolated. This paper deals with the isolation and structural determination of these new compounds (Figure 1).

2. Results and discussion

Tenacigenoside F (**1**) was obtained as an amorphous hygroscopic white powder. The molecular formula was determined to be $C_{59}H_{88}O_{24}$ by HR-ESI-MS at *m/z* 1203.5552 $[M + Na]^+$. The IR spectrum of **1** showed absorption bands at 3443 and 1718 cm^{-1} for the hydroxyl and carbonyl groups. The isolate gave Liebermann–Buchard, Keller–Kiliani, and xanthydrol positive tests, indicating that **1** was a steroidal glycoside with a 2-deoxy sugar moiety [10]. The carbon and proton signals of **1** were assigned by extensive studies of HSQC, HMBC, and NOESY spectra (Table 1). In the ^1H and ^{13}C NMR spectra of **1**, signals of four anomeric protons (δ_H 4.58, t, $J = 8.3$ Hz; 4.57, d, $J = 8.0$ Hz; 4.26, d, $J = 7.9$ Hz; 4.29, d, $J = 7.8$ Hz) (labeled A–D) and carbons (δ_C 96.9, 101.1, 103.6, and 104.9) indicated the presence of four sugars in compound **1**. Interpretation of the HSQC and HMBC spectra revealed that two of the sugar units belonged to 6-deoxy sugars (A and B).

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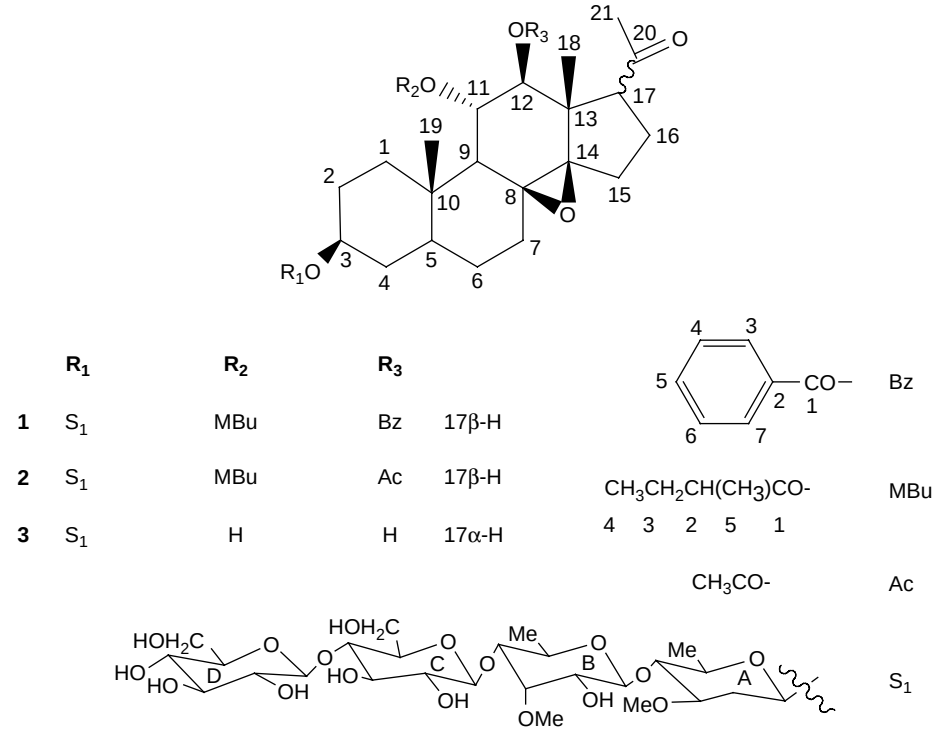

	R_1	R_2	R_3	
1	S_1	MBu	Bz	17β-H
2	S_1	MBu	Ac	17β-H
3	S_1	H	H	17α-H

Figure 1. Structures of compounds **1**–**3**.

In addition, the ^{1}H NMR and HSQC spectra showed two methyl doublets at δ_H 1.25 (3H, d, $J = 5.8$ Hz) and 1.18 (3H, d, $J = 5.8$ Hz) corresponding to carbon signals at δ_C 18.8 and 18.0, respectively, and two methoxyl groups at δ_H 3.29 (3H, s) and 3.46 (3H, s) corresponding to carbon signals at δ_C 56.9 and 61.3, respectively. In the HMBC spectrum, the signals of H–C correlations (OMe-Allo-3/Allo-C-3 and Me-Allo-6/Allo-C-5 for A; OMe-Ole-3/Ole-C-3 and Me-Ole-6/Ole-C-5 for B) suggested that the methyl groups should be linked at C-5 and the methoxyl groups at C-3 in each of the two sugar units. Thus, the B and A sugar units were identified as 6-deoxy-3-*O*-methyl-D-allose and 2,6-dideoxy-3-*O*-methyl pyranose (oleandrose), respectively. Furthermore, the ^{13}C NMR and DEPT spectra displayed two methylene groups at δ_C 61.5 and 61.2. By combining the information from HMBC and ^{13}C NMR spectra, the C and D sugar units were identified as glucopyranoses. All of the monosaccharides were in β-configuration as inferred from the *J* values (7.8–8.3 Hz) of the anomeric protons [3]. This was confirmed by comparing the ^{13}C NMR spectra of sugar units with literatures [3,15]. In the HMBC spectrum of **1** (Figure 2), cross-peaks (H-Allo-1/Ole-C-4, H-Glc-1/Allo-C-4, H-Glc′-1/Glc-C-4) were observed. Furthermore, acid hydrolysis of **1** gave D-oleandrose (Ole), 6-deoxy-3-*O*-methyl-D-allose (Allo), and D-glucose (Glc), which confirmed that the sugars of **1** were oleandrose (A), allose (B), and glucose (C and D). Therefore, the sugar moiety was β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1 → 4)-β-D-oleandropyranosyl.

^{1}H NMR signals of the aglycone part of **1** at δ_H 7.85 (2H, d, $J = 7.5$ Hz, CH-3″ and

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Table 1. ^{1}H NMR spectral data of **1**–**3** at 600 MHz in DMSO-d_6 (δ in ppm, J in Hz).

	Aglycone		
Position	**1**	**2**	**3**
H-1α	1.29 m	1.25 m	1.59 m
H-1β	1.56 m	1.51 m	2.81 dt, $J = 14.0, 8.0$
H-2α	1.31 m	1.22 m	2.10 m
H-2β	1.93 m	1.90 m	1.76 m
H-3α	3.46 m	3.47 m	3.51 m
H-4α	1.35 m	1.32 m	1.43 m
H-4β	1.75 m	1.71 m	1.90 m
H-5α	1.37 m	1.33 m	1.32 m
H-6α	1.66 m	1.63 m	1.71 m
H-6β	2.03 m	2.00 m	1.91 m
H-7α	1.51 m	1.48 m	1.35 m
H-7β	1.81 m	1.78 m	1.64 m
H-9α	1.96 d, $J = 9.4$	1.88 d, $J = 9.8$	1.43 d, $J = 8.9$
H-11β	5.36 t, $J = 10.0$	5.18 t, $J = 10.1$	3.26 t, $J = 9.2$
H-12α	5.19 d, $J = 10.0$	4.91 d, $J = 10.1$	3.23 d, $J = 9.2$
H-15α	1.44 m	1.36 m	1.27 m
H-15β	1.62 m	1.59 m	1.77 m
H-16α	1.87 m	1.84 m	1.66 m
H-16β	1.96 m	1.93 m	2.32 m
H-17β	2.91 d, $J = 7.5$	2.94 d, $J = 7.0$	2.90 dd, $J = 11.2, 6.0$
18-CH_3	1.07 s	0.97 s	0.71 s
19-CH_3	0.96 s	0.92 s	0.91 s
H-21	2.13 s	2.08 s	2.24 s
H-4′	0.47 t, $J = 7.0$	0.83 t, $J = 7.2$	
H-5′	0.78 d, $J = 7.0$	0.99 d, $J = 7.2$	
2″-Me		1.93 s	
H-3″			
H-7″	7.85 d, $J = 7.5$		
H-4″			
H-6″	7.52 t, $J = 7.5$		
H-5″	7.66 d, $J = 7.5$		
Ole-H-1	4.58 t, $J = 8.3$	4.60 t, $J = 8.3$	4.59 t, $J = 8.3$
Ole-H-6	1.25 d, $J = 5.8$	1.80 d, $J = 6.0$	1.25 d, $J = 5.8$
Ole-3-OMe	3.29 s	3.29 s	3.29 s
Allo-H-1	4.57 d, $J = 8.0$	4.57 d, $J = 8.2$	4.57 d, $J = 8.2$
Allo-H-6	1.18 d, $J = 5.8$	1.25 d, $J = 5.4$	1.18 d, $J = 6.0$
Allo-3-OMe	3.46 s	3.47 s	3.46 s
Glc-H-1	4.26 d, $J = 7.9$	4.26 d, $J = 8.3$	4.26 d, $J = 8.0$
Glc′-H-1	4.29 d, $J = 7.8$	4.29 d, $J = 8.2$	4.29 d, $J = 8.0$

7″), 7.52 (2H, t, $J = 7.5$ Hz, CH-4″ and 6″), and 7.66 (1H, t, $J = 7.5$ Hz, CH-5″) were consistent with a benzoyl group (Bz) [3], and those at δ_H 0.47 (3H, t, $J = 7.0$ Hz, Me-4′) and 0.78 (3H, d, $J = 7.0$ Hz, Me-5′) were consistent with a 2-methylbutyryl group (MBu) [3]. This was further supported by carbonyl signals at δ_C 175.2 (C-1′) and 165.7 (C-1″). The ^{1}H and ^{13}C NMR spectra of the aglycone exhibited the pattern of a C_{21} steroid skeleton. The ^{13}C NMR spectra (Table 1) were similar to those of the known polyoxypregnane 11α-*O*-2-methylbutyryl-12β-*O*-benzoyltenacigenin B [8]. The HMBC spectrum displayed correlations between aglycone-H-11/2-methylbutyryl-C-1 and aglycone-H-12/benzoyl-C-1, indicating that the 2-methylbutyryl group was at position 11 and the benzoyl group was at position 12.

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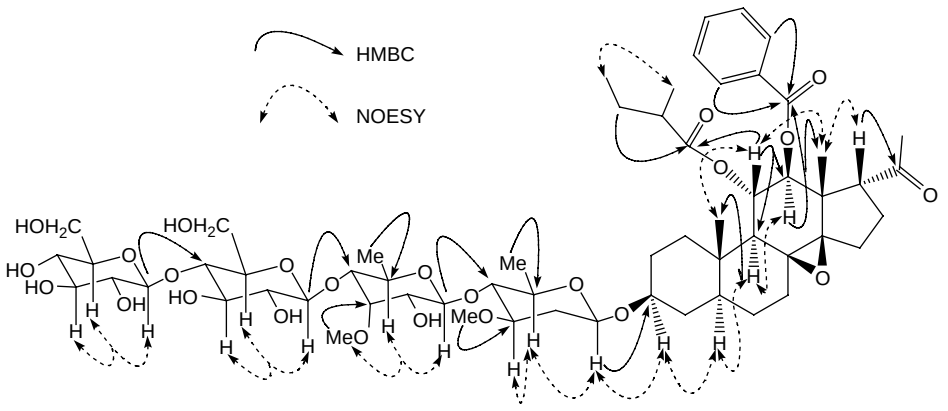

Figure 2. Key HMBC and NOESY correlations of compound **1**.

Thus, the aglycone of **1** was determined to be 11α-*O*-2-methylbutyryl-12β-*O*-benzoyltenacigenin B. Glycosidation shifts in **1** relative to the literature values of 11α-*O*-2-methylbutyryl-12β-*O*-benzoyltenacigenin B [8] were observed for C-2 ($\Delta\delta$ −2.4 ppm), C-3 (+7.2 ppm), and C-4 (−4.8 ppm), indicating that the sugar moiety was attached at the C-3 position [7]. This was further supported by the HMBC correlation between Ole-H-1/aglycone-C-3. Therefore, the structure of tenacigenoside F (Figure 1) was elucidated as 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1 → 4)-β-D-oleandropyranosyl-11α-*O*-2-methylbutyryl-12β-*O*-benzoyl-tenacigenin B.

The ^{1}H, ^{13}C NMR, NOESY, and HMBC spectra of the sugar moieties of **2** and **3** were in agreement with those of **1** (Table 1). It was therefore concluded that they contain the same sugar moiety as **1**. The same glycosidation shifts were also observed in compounds **2** and **3** as in **1**, which indicated that they all bear the sugar moiety at the C-3 position. The main difference of compounds **2** and **3** from **1** was due to the substitute groups in the aglycone moiety (Table 1).

Tenacigenoside G (**2**) was obtained as an amorphous hygroscopic white powder. Its molecular formula was determined to be $C_{54}H_{86}O_{24}$ by HR-ESI-MS at *m/z* 1141.5415 $[M + Na]^+$, ^{13}C NMR, and DEPT spectra. The ^{1}H NMR spectrum showed proton signals at δ_H 0.83 (3H, t, $J = 7.2$ Hz, Me-4′) and 0.99 (3H, d, $J = 7.2$ Hz, Me-5′), consistent with a 2-methylbutyryl group, and 1.93 (3H, s, Me-2″), consistent with an acetyl group [3]. This was further supported by carbonyl signals at δ_C 175.2 (C-1′) and 170.6 (C-1″). The HMBC spectrum displayed correlations between aglycone-H-11/2-methylbutyryl-C-1 and aglycone-H-12/acetyl-C-1, indicating that the 2-methylbutyryl group was at position 11 and the acetyl group at position 12 (Figure 3). Consequently, tenacigenoside G was established as 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1 → 4)-β-D-oleandropyranosyl-11α-*O*-2-methylbutyryl-12β-*O*-acetyltenacigenin B.

The molecular formula of tenacigenoside H (**3**) was found to be $C_{47}H_{76}O_{22}$ from the HR-ESI-MS peak at *m/z* 1015.4702 $[M + Na]^+$. The ^{1}H and ^{13}C NMR spectra (Table 1) of the genin of **3** were in good agreement with those of 17β-tenacigenin B [4,7]. The cross-peaks between the protons at δ_H 2.90 (H-17) and 3.23 (H-12α) in the NOESY spectrum (Figure 4) indicated that the aglycone of **3** was 17β-tenacigenin B. Furthermore, acid hydrolysis of **3** gave a

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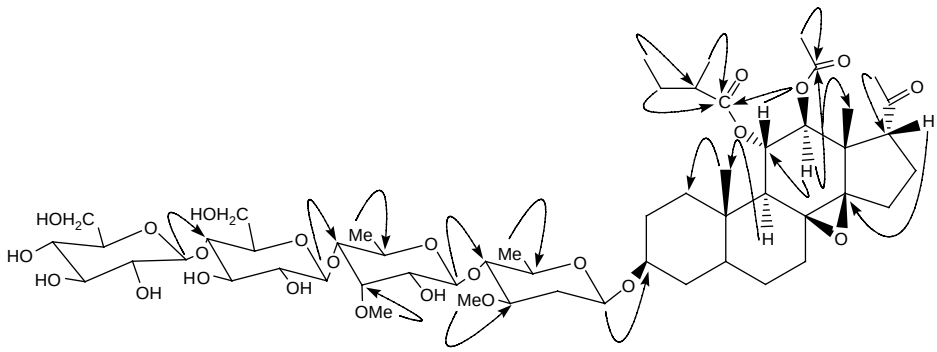

Figure 3. Key HMBC correlations of compound **2**.

genin **3a** whose ^{1}H NMR spectral data were the same as those of 17β-tenacigenin B [4]. Therefore, the structure of tenacigenoside H was established as 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1 → 4)-β-D-oleandropyranosyl-17β-tenacigenin B.

Compounds **1**–**3** were applied to cytotoxic tests against A549 and MCF-7 cell lines, but no significant activity was observed.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a JASCO P-1020 digital polarimeter. The IR spectrum was recorded on a Perkin-Elmer-341 polarimeter at 589 nm. The UV spectra were recorded on 756 MC UV–vis spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 600 spectrometer (600 MHz). Chemical shifts (δ) are reported in ppm relative to an internal TMS standard, and the coupling constant (J) in Hz. ^{1}H and ^{13}C NMR assignments were supported by ^{1}H–^{1}H COSY, HMQC, and HMBC experiments. ESI-MS and HR-ESI-MS were recorded on a Finnigan LCQ^{DECA} spectrometer in *m/z*. Gas chromatography was done on a Varian CP-3800 gas chromatograph equipped with an FID and a CP-sil 5 CB capillary column (30 m, 0.25 mm i.d., 0.25 μm). Column chromatography (CC) was performed on a commercial silica gel (200–300 mesh; Qingdao Marine Chemical Group Co., Qingdao, China), octadecylsilyl silica gel (45–60 μm; YMC, Kyoto, Japan), and Sephadex LH-20 (Amersham Biosciences, Piscataway, NJ, USA). L-Cysteine methyl ester hydrochloride, trimethylchlorosilane,

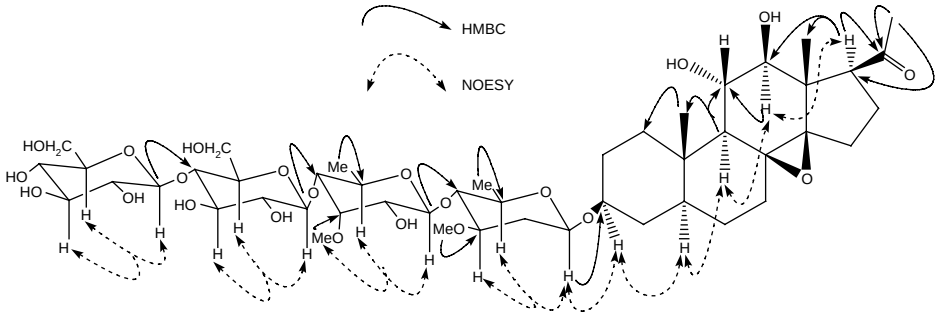

Figure 4. Key HMBC and NOESY correlations of compound **3**.

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and the authentic standard compounds D-oleandrose (Ole), 6-deoxy-3-*O*-methyl-D-allose (Allo), and D-glucose (Glc) were purchased from Sigma-Aldrich (Shanghai, China).

3.2 Plant material

The stems of *M. tenacissima* were collected in September 2005 from Wenshan, Yunnan Province, China, and identified by Prof. Zuo-Cheng Zhao (Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, China). A voucher specimen (No. W2289) has been deposited in the Herbarium of the Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, China.

3.3 Extraction and isolation

Air-dried and powdered stems of *M. tenacissima* (10 kg) were boiled in water (80 liters) for 2 h. After concentration, the aqueous phase (1.3 kg) was extracted with EtOAc (4 × 5 liters) and *n*-BuOH (4 × 5 liters). The EtOAc extract (286 g) was chromatographed on a silica gel (160–200 mesh, 2.0 kg) column using $CHCl_3$ to $CHCl_3$/MeOH mixtures of increasing polarity. This furnished 12 fractions (A–L) according to TLC analysis. Fraction H (5.1 g) was subjected to CC on ODS (50 g) and eluted with CH_3OH/H_2O (45–70% gradient system) and yielded **1** (82 mg). The *n*-BuOH extract (230 g) was chromatographed on a silica gel (160–200 mesh, 2.0 kg) column using $CHCl_3$ and $CHCl_3$/MeOH mixtures of increasing polarity (50:1–1:1 gradient system), and 10 fractions (A–J) were furnished according to TLC analysis. Fraction E (7.2 g) was subjected to CC on a silica gel (200–300 mesh, 200 g) using $CHCl_3$/MeOH mixtures of increasing polarity (20:1–2:1 gradient system) to afford eight fractions (E1–E8). Fraction e5 (3.2 g) was subjected to CC on ODS and eluted with MeOH/H_2O (50–70% gradient system) to afford five fractions (E5a–E5f). Fractions E5d (102 mg) and E5e (63 mg) were also subjected to CC and eluted with $CHCl_3$–MeOH–H_2O (20:6:1) to furnish **2** (52 mg) and **3** (33 mg), respectively.

3.3.1 Tenacigenoside F (*1*)

An amorphous hygroscopic white powder (MeOH, 82 mg). $[\alpha]_D^{25}$ + 16 ($c = 0.1$, MeOH). UV $\lambda_{max}^{CH_3OH}$ nm ($\log \varepsilon$): 220 (4.07). IR ν_{max} cm^{-1} (KBr): 3433, 2933, 1718, 1635, 1452, 1366, 1279, 1160, 1127, 1072, 711, 618. ^1H NMR (DMSO-d_6) spectral data: see Table 1. ^{13}C NMR spectral data: see Table 2. HR-ESI-MS: *m/z* 1203.5552 $[M + Na]^+$ (calcd for $C_{59}H_{88}NaO_{24}$, 1203.5563).

3.3.2 Tenacigenoside G (*2*)

An amorphous hygroscopic white powder (MeOH, 52 mg). $[\alpha]_D^{25}$ −13 ($c = 0.1$, MeOH). IR ν_{max} (KBr): 3419, 2934, 1712, 1651, 1372, 1269, 1160, 1074, 733, 624 cm^{-1}. ^1H NMR (DMSO-d_6) spectral data: see Table 1. ^{13}C NMR spectral data: see Table 2. HR-ESI-MS: *m/z* 1141.5415 $[M + Na]^+$ (calcd for $C_{54}H_{86}NaO_{24}$, 1141.5401).

3.3.3 Tenacigenoside H (*3*)

An amorphous hygroscopic white powder (MeOH, 33 mg). $[\alpha]_D^{25}$ −9 ($c = 0.1$, MeOH). IR ν_{max} (KBr): 3429, 2932, 1693, 1452, 1380, 1161, 1074, 617 cm^{-1}. ^1H NMR spectral data: see Table 1. ^{13}C NMR spectral data: see Table 2. HR-ESI-MS: *m/z* 1015.4702 $[M + Na]^+$ (calcd for $C_{47}H_{76}NaO_{22}$, 1015.4720).

3.4 Acid hydrolysis of 1–3 and determination of the absolute configuration of monosaccharides

Compounds **1**–**3** (5 mg) were heated with 1.5 M HCl–CH_3OH (10 ml) under reflux for 7 h, respectively. The reaction mixture was diluted with water and extracted with

Table 2. ^{13}C NMR spectral data of compounds **1**–**3** at 600 MHz in DMSO-d_6 (δ in ppm)[a].

	Aglycone				Sugar moieties		
Position	**1**	**2**	**3**	Position	**1**	**2**	**3**
C-1	37.5	37.4	38.3	Ole			
C-2	29.1	29.1	29.4	C-1	96.9	96.9	96.9
C-3	75.4	75.5	76.1	C-2	37.2	37.2	37.2
C-4	34.9	34.9	34.8	C-3	79.1	79.1	79.1
C-5	43.3	43.4	44.3	C-4	82.9	82.9	82.9
C-6	26.9	26.8	27.7	C-5	70.5	70.5	70.5
C-7	31.7	31.7	32.4	C-6	18.8	18.8	18.8
C-8	66.4	66.3	65.9	3-OMe	56.9	56.9	56.8
C-9	51.1	51.2	53.6	Allo			
C-10	39.0	38.9	39.1	C-1	101.1	101.1	101.1
C-11	68.6	68.3	67.5	C-2	71.7	71.7	71.7
C-12	74.2	74.1	80.0	C-3	82.1	82.2	82.1
C-13	46.0	45.7	48.0	C-4	81.0	81.0	80.9
C-14	71.6	71.4	71.5	C-5	68.5	68.7	68.7
C-15	24.9	24.8	27.3	C-6	18.0	18.0	18.0
C-16	26.8	26.7	25.0	3-OMe	61.3	61.3	61.3
C-17	59.4	59.4	62.0	Glc			
C-18	17.0	16.9	11.2	C-1	103.6	103.6	103.6
C-19	13.0	12.9	13.1	C-2	75.4	75.4	75.3
C-20	210.4	210.2	210.5	C-3	77.3	77.3	77.2
C-21	31.7	31.1	32.7	C-4	82.6	82.6	82.6
	MBu	MBu		C-5	74.1	74.0	74.0
C-1′	175.2	175.2		C-6	61.5	61.5	61.5
C-2′	40.9	40.9		Glc′			
C-3′	25.9	26.1		C-1′	104.9	104.9	104.9
C-4′	11.5	11.8		C-2′	75.2	75.2	75.2
C-5′	15.6	15.7		C-3′	77.0	77.0	76.9
	Bz	Ac		C-4′	71.0	71.0	71.0
C-1″	165.7	170.6		C-5′	73.7	73.7	73.7
C-2″	129.5	21.0		C-6′	61.2	61.2	61.2
C-3″	129.7						
C-4″	129.2						
C-5″	134.1						
C-6″	129.2						
C-7″	129.7						

Note: [a] The assignments were based on DEPT, HSQC, HMBC, and NOESY experiments.

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$CHCl_3$. The H_2O layer was neutralized with K_2CO_3 and concentrated. After the inorganic precipitate was filtered off, the filtrate was concentrated and these residues were dissolved in pyridine (each 2 ml). L-Cysteine methyl ester hydrochloride (2 mg each) was added to pyridine solutions subsequently. The resulting mixtures were heated at 60°C for 2 h, then trimethylchlorosilane (0.5 ml each) was added, and the mixture was heated at 60°C for 30 min. The reaction solutions were then concentrated to dryness, and the residues were suspended in water (1 ml each) and extracted with *n*-hexane (1 ml × 3). The supernatant was subjected to GC-MS analysis under the following conditions: capillary column (30 m, 0.25 mm i.d., 0.25 μm); detection, FID; detector temperature, 280°C; injection temperature, 250°C; initial temperature was maintained at 100°C for 2 min and then raised to 280°C at the rate of 10°C/min, and final temperature was maintained for 5 min; and carrier gas, N_2.

In the acid hydrolysate of **1**–**3**, 6-deoxy-3-*O*-methyl-D-allose, D-oleandrose, and D-glucose were confirmed by comparison of the retention times of their derivatives with those of 6-deoxy-3-*O*-methyl-D-allose, D-oleandrose, and D-glucose derivatives prepared in a similar way, which showed retention times of 20.13, 22.01, and 22.35 min, respectively.

The organic layers were washed with H_2O (4 × 10 ml), dried using Na_2SO_4, and evaporated to dryness. The resulting residues were identified as tenacigenin B [16] in **1** and **2**, and 17β-tenacigenin B [4,7] in **3** by their ^1H NMR spectral data.

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