Three New Polyoxypregnane Glycosides from *Marsdenia tenacissima*

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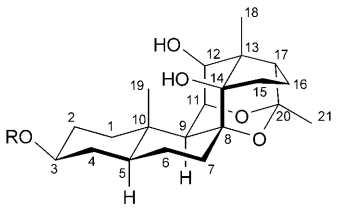
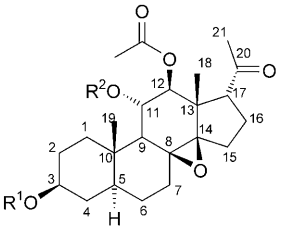
Three new polyoxypregnane glycosides, marsdenosides I–K (**1**–**3**), were isolated from the stem of *Marsdenia tenacissima*. The structures were elucidated on the basis of in-depth spectroscopic analyses, and by means of chemical evidence. Marsdenoside I (=(3β,5α,11α,12β,14β,17α,20*R*)-3-[(2,6-dideoxy-4-O-(6-deoxy-3-O-methyl-β-D-allopyranosyl)-3-O-methyl-β-D-arabino-hexopyranosyl)oxy]-8,20:11,20-diepoxypregnane-12,14-diol; **1**) is the first C_{21} steroidal glycoside with a rigid cage. Also, the isolation of **1** demonstrated that tenacigenin A (**1a**) is a true natural product as well, rather than an artifact.

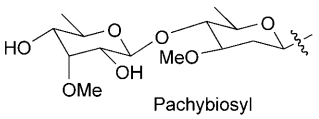
Introduction. – *Marsdenia tenacissima* (ROXB.) WIGHT. et ARN. (Asclepiadaceae) is a perennial climber widespread from tropical to subtropical Asia. Its stem, known as *'tong-guang-san'*, has long been used for the treatment of asthma, cancer, trachitis, tonsillitis, pharyngitis, cystitis, and pneumonia in Chinese folk medicine [1]. The crude $CHCl_3$ extract from the stem of *M. tenacissima* was reported to exhibit mild anti-asthmatic properties [2]. The phytochemical investigations of *M. tenacissima* have focused on polyoxypregnane constituents because of their antitumor activities and high contents in the plant [2–10]. We have previously reported some polyoxypregnane glycosides from *M. tenacissima* [11][12]. In this paper, we report the isolation and character-



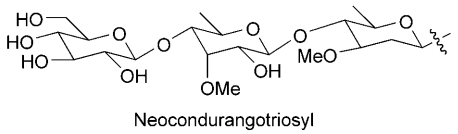


	R^1	R^2
1	R = Pachybiosyl	
1a	R = H (Tenacigenin A)	
2	Pachybiosyl	HPA
3	Neocondurangotriosyl	Bz
3a	Pachybiosyl	Bz

Bz = Benzoyl
HPA = (4-hydroxyphenyl)acetyl



Pachybiosyl



Neocondurangotriosyl

ization of three new such polyoxypregnane glycosides, marsdenosides I–K (**1**–**3**), and of two known glycosides.

Results and Discussion. – Compounds **1**–**3** gave rise to positive *Liebermann–Burchard*, *Keller–Kiliani*, and xanthydrol reactions, indicating that they were all steroidal glycosides containing 2-deoxy sugar moieties [13–15].

Marsdenoside I (**1**) had the molecular formula $C_{35}H_{56}O_{12}$ based on the quasi-molecular ion at *m/z* 707.3414 ($[M+K]^+$) in its HR-ESI mass spectrum. The IR spectrum of **1** displayed absorption bands for OH (3480 and 3440 cm^{-1}) and C–O moieties (1028, 1058 cm^{-1}), but not for C=O groups.

The ^{1}H-NMR spectrum of **1** (see *Table 1* in the *Exper. Part*) displayed two anomeric signals at δ(H) 4.80 (*d*, *J* = 8.3, 1 H) and 4.60 (*dd*, *J* = 9.7, 1.7 Hz, 1 H), with corresponding ^{13}C-NMR signals at δ(C) 98.9 and 96.9, respectively, suggesting that **1** was a disaccharide glycoside. Both glycosidic linkages were β-oriented, as deduced from the coupling constants (8.3 and 9.7 Hz) of the two anomeric signals. The ^{13}C-NMR spectroscopic data ascribed to the sugar moiety of **1** (see *Table 2* in the *Exper. Part*) were the same as those reported for pachybiose[1]) [16], which was further confirmed by examination of the corresponding TOCSY, NOESY, HMQC, and HMBC spectra (*Figs. 1* and *2*). Mild acid hydrolysis of **1** gave only one sugar fragment, which was identified as pachybiose by paper chromatography and TLC comparison with an authentic sample.

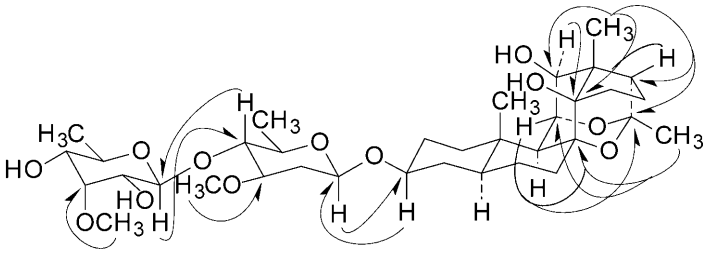


Fig. 1. *Key HMBC correlations for* **1**

For the aglycone moiety of **1**, 21 C-atoms were left, suggesting a C_{21} steroid. This was supported by the three Me *singlet*s at δ(H) 1.14, 1.18, and 1.22 in the ^{1}H-NMR spectrum. The ^{1}H- and ^{13}C-NMR data of the aglycone were in good agreement with those of tenacigenin A (**1a**) [2][3][17], except C(2), C(3), and C(4), which were shifted by $\Delta\delta$ −2.2, +5.0, and −2.9 ppm, respectively, due to glycosylation. The resonance at δ(H) 1.18 (*s*, Me(21)) indicated that C(20) was quaternary. The HMBC correlations of δ(C) 99.3 with both δ(H) 1.18 (Me(21)) and 1.86 (H–C(17)) indicated that this ^{13}C-NMR resonance was due to C(20). Consequently, C(20) was linked to two O-atoms [17]. In the ^{1}H,^{1}H-COSY spectrum, the signal at δ(H) 4.28 (br. *d*, *J* = 2.6 Hz, 1 H) was coupled with those at 3.98 (*d*, *J* = 2.2 Hz, 1 H) and 2.43 (br. *s*, H–C(9)), while the signal at δ(H) 3.98 was correlated only with that at δ(H) 4.28, which substantiated

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[1]) Pachybiose = 2,6-dideoxy-4-*O*-(6-deoxy-3-*O*-methyl-β-D-allopyranosyl)-3-*O*-methyl-β-D-*arabino*-hexopyranose.

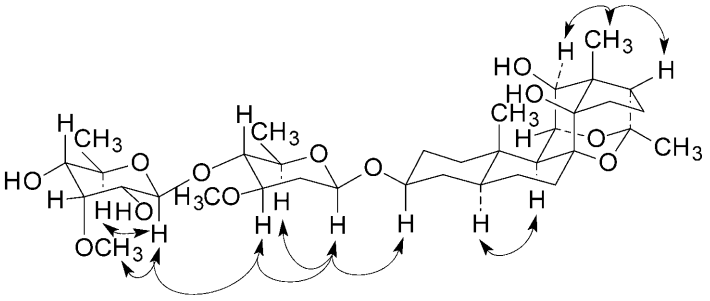

Fig. 2. *Key NOESY correlations for* **1**

that the signal at δ(H) 4.28 was due to H–C(11), and that the one at δ(H) 3.98 was due to H–C(12).

The HMBC correlations between δ(H) 4.28 (H–C(11)) and δ(C) 99.3 (C(20)), between δ(H) 4.28 and δ(C) 24.6 (C(21)), and between δ(H) 1.18 (Me(21)) and δ(C) 71.3 (C(11)) indicated that there was an O-atom between C(20) and C(11). From the HMBC correlations between δ(H) 1.86 (H–C(17)) and δ(C) 82.0, between δ(H) 1.22 (Me(18)) and δ(C) 82.0, and between δ(H) 3.98 (H–C(12)) and δ(C) 82.0, the resonance at δ(C) 82.0 was ascribed to C(14). Similarly, from the correlations between δ(H) 4.28 (H–C(11)) and δ(C) 77.9, and between δ(H) 2.43 (H–C(9)) and δ(C) 77.9, the resonance at δ(C) 77.9 was assigned to C(8). The HMBC correlation between δ(H) 1.18 (Me(21)) and δ(C) 77.9 (C(8)) supported that C(20) was also linked to C(8) *via* an oxy bridge (*Fig. 1*). Finally, the NOESY correlation between δ(H) 1.22 (Me(18)) and 1.86 (H–C(17)) confirmed that C(17) was α-configured (*Fig. 2*). From these data, the aglycone of **1** was identified as tenacigenin A (**1a**).

Mild acid hydrolysis of **1** yielded an aglycone, whose molecular weight and ^{1}H- and ^{13}C-NMR spectroscopic data were identical with those reported for tenacigenin A (**1a**) [17]. The observed shifts at C(2), C(3), and C(4) (see above) indicated that the oligosaccharide chain was linked to the 3-OH group of the aglycone [18], which was supported by HMBC correlations between δ(H) 4.60 (H–C(1)$_{Ole}$[2]) and δ(C) 77.1 (C(3)), and between δ(H) 3.66–3.71 (H–C(3)) and δ(C) 96.9 (C(1)$_{Ole}$) (see *Fig. 1*).

From the above data, the structure of marsdenoside I (**1**) was established as 3-*O*-pachybiosyl-tenacigenin A[3]). Compound **1** is the first reported glycoside of tenacigenin A (**1a**). The isolation of **1** demonstrates that tenacigenin A is a true natural product, and not, as proposed earlier [4], an artifact formed under acidic or basic conditions.

The HR-ESI mass spectrum of marsdenoside J (**2**) showed a quasi-molecular ion peak at *m/z* 883.3889 ($[M+K]^+$), in accord with the molecular formula $C_{45}H_{64}O_{15}$. The ^{1}H- and ^{13}C-NMR spectroscopic data of the sugar moiety of **2** were in good agreement with those of **1** (see *Tables 1* and *2* in the *Exper. Part*), so it also contained a pachybiosyl residue. The ^{13}C-NMR data of the aglycone of **2** (*Table 2*) resembled those of the 11,12-diester of tenacigenin B previously reported [5]. The ^{1}H-NMR signals at δ(H) 5.31 (t, $J=10.1$, H$_\beta$–C(11)) and 4.95 (d, $J=10.1$, H$_\alpha$–C(12)) indicated two ester-bearing

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[2]) Sugar abbreviations: Allo = allose, Ole = oleandrose, Glc = glucose.

[3]) For systematic names, see the *Exper. Part*.

methine groups. The signals at δ(H) 1.64 (*s*, 3 H) and δ(C) 20.2 suggested the presence of an acetyl (Ac) group. The signals at δ(H) 6.75 and 7.09 (2*d*, $J = 8.5$ Hz each, 2×1 H) and the ^{13}C-NMR *singlet* at δ(C) 155.1 indicated that **2** contained a 4-hydroxyphenyl group. In the HMBC spectrum, the aromatic resonances at δ(C) 125.4 and 130.7 were correlated with the signal at δ(H) 3.39 (*s*, 2 H), and δ(H) 7.09 correlated with the δ(C) 41.4 (*Fig. 3*). Also, the HMQC spectrum displayed a cross-peak between δ(H) 3.39 and δ(C) 41.4, and the HMBC spectrum showed a correlation between δ(H) 3.39 and δ(C) 168.4. Therefore, a CH_2 group (δ(H) 3.39, δ(C) 41.4) was located between the 4-hydroxyphenyl moiety and the C=O group (δ(C) 168.4). Hence, **2** contained a (4-hydroxyphenyl)acetyl (HPA) group.

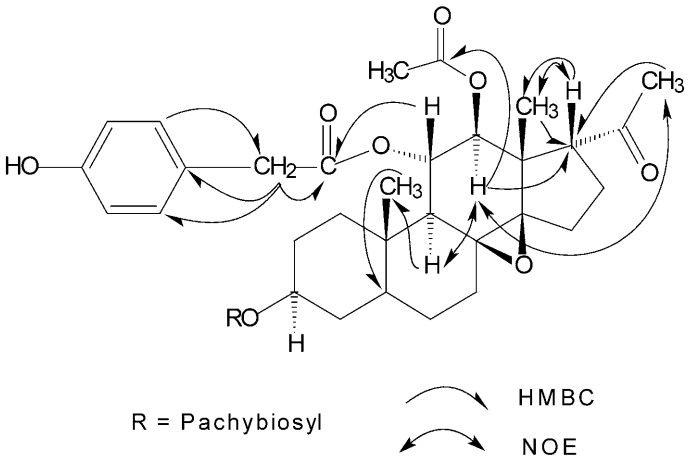

Fig. 3. *Key HMBC and NOESY correlations for* **2**

As shown in *Fig.3*, the HMBC correlations between δ(H) 5.31 (H_β–C(11)) and δ(C) 168.4 ($C(1')_{HPA}$) and between δ(H) 4.95 (H_α–C(12)) and δ(C) 170.7 ($C(1'')_{Ac}$) supported that the HPA and Ac groups were at C(11) and C(12), respectively. Coupling constants of 10.1 Hz for H–C(9), H–C(11) and H–C(12) indicated that these H-atoms were all in an axial positions. The NOESY correlations between δ(H) 1.05 (Me(18)) and 2.90 (H–C(17)), and between δ(H) 2.19 (Me(21)) and 4.95 (H_α–C(12)) confirmed that the C(17) side chain was in an α-orientation (*Fig. 3*). The glycosyl-induced shifts at C(2), C(3), and C(4) of the aglycone indicated that the oligosaccharide chain was attached at the 3-OH group [18], which was confirmed by the HMBC correlation between δ(H) 4.58 ($H–C(1)_{ole}$) and δ(C) 76.2 (C(3)). From the above data, the structure of **2** was determined as 12-*O*-acetyl-11-*O*-[(4-hydroxyphenyl)acetyl]-3-*O*-pachybiosyl-tenacigenin B[3]).

Marsdenoside K (**3**) had the molecular formula $C_{50}H_{72}O_{19}$ on the basis of its quasi-molecular ion at *m/z* 1015.4309 ($[M+K]^+$) in the HR-ESI mass spectrum. Its ^{1}H-NMR spectrum exhibited three anomeric signals at δ(H) 4.36 (*d*, $J = 6.7$, 1 H), 4.53 (*dd*, $J = 9.8$, 1.6, 1 H), and 4.78 (*d*, $J = 6.7$ Hz, 1 H), which were correlated with δ(C) 104.3, 97.1, and 100.1, respectively, in the HMQC spectrum, thus, indicating three sugar units. The unit with an anomeric double *doublet* was a 2-deoxypyranose. The two Me signals at δ(H)

1.26 (*d*, *J* = 5.8 Hz, 3 H) and 1.31 (*d*, *J* = 5.6 Hz, 3 H), and the two MeO groups at δ(H) 3.35 and 3.57 (*s*, 3 H each) suggested that two of the three sugar units were 6-deoxy-3-*O*-methyl-pyranoses [19]. The signals in the ^{1}H-NMR, ^{13}C-NMR, ^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY spectra of **3** due to these two 6-deoxy-3-*O*-methyl-pyranose units were identical with those in the spectra of **1**, except for the chemical shifts of C(3), C(4), and C(5) of the 6-deoxy-3-*O*-methyl-allose, with $\Delta\delta$ values of -0.6, $+7.8$, and -2.2 ppm, respectively. The other sugar unit was identified as a glucopyranose on the basis of its characteristic coupling pattern of the all-axial ring H-atoms and CH_2(6) (δ(H) 3.61–3.74 and 3.86–3.93, both *ABM* spin systems) [20].

When **3** was exposed to β-glucosidase, enzymolysis yielded only one sugar fragment of glucose, in accord with the above experiments. The β-linkages of each of the sugars were evident from the ^{1}H-NMR coupling constants of the anomeric 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 between the following pairs: H−C(1)$_{Glc}$/C(4)$_{Allo}$, H−C(1)$_{Allo}$/C(4)$_{Ole}$, and H−C(1)$_{Ole}$/C(3)$_{Steroid}$ in the HMBC spectrum. Thus, the sugar moiety of **3** was identified as neocondurangotriose[4]), as further supported by the fact that the ^{13}C-NMR data of the glycone of **3** (in (D_5)pyridine; *Table 2*) coincided well with those reported in the literature [21].

The ^{1}H- and ^{13}C-NMR spectroscopic data of the aglycone of **3** matched those of **2**, except that the signals of the HPA group of **2** were replaced with those of a benzoyl (Bz) group in **3**. The Bz group was identified from δ(H) 7.42 (*t*, *J* = 7.7, H−C(4′,6′)), 7.55 (*t*, *J* = 7.4, H−C(5′)), and 7.95 (*d*, *J* = 7.2 Hz, H−C(3′,7′)). HMBC Correlations between δ(H) 5.60 (*t*, *J* = 10.1, H$_\beta$−C(11)) and δ(C) 166.0 (C(1′)$_{Bz}$), and between δ(H) 5.12 (*d*, *J* = 10.1 Hz, H$_\alpha$−C(12)) and δ(C) 170.7 (C(1″)$_{Ac}$) indicated that the Bz and Ac groups were at C(11) and C(12), respectively. The HMBC correlation between δ(H) 4.53 (H−C(1)$_{ole}$) and δ(C) 76.5 (C(3)) suggested that the oligosaccharide chain was attached to the 3-OH group of the steroid. Thus, the structure of **3** was established as 12-*O*-acetyl-3-*O*-neocondurangotriosyl-tenacigenin B[3]). The selective enzymatic degradation of the neocondurangotriosyl moiety of **3** with β-glucosidase furnished the corresponding pachybiosyl conjugate **3a**, known as tenacissoside I (**3a**) [8], which further corroborated the structure of **3**.

Finally, the two known compounds were identified as tenacissoside F and tenacissoside D by comparing their ORD, UV, IR, ^{1}H-NMR, ^{13}C-NMR, and MS data with those reported in the literature [8][5].

The authors are grateful to Prof. *Jinlan Yuan*, Department of Pharmaceutical Chemistry, School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology, P. R. China, for kindly providing an authentic sample of pachybiose.

Experimental Part

General. Anal. TLC was performed on precoated silica gel F_{254} plates (0.15 mm; *Yantai Institute of Chemical Technology*) and *RP-18* F_{254} *S* plates (*Merck*). For prep. TLC, precoated silica gel F_{254} plates (0.5 mm; *Yantai Institute of Chemical Technology*) were used. Spots were visualized by spraying with 0.5% vanillin in conc.

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[4]) Systematic name: β-D-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-β-D-*arabino*-hexopyranose

Table 1. *^{1}H-NMR Data of the Sugar Moieties of* **1**–**3**. At 500 MHz, in $CDCl_3$; δ in ppm, J in Hz. Abbreviations: Allo, allosyl; Ole, oleandrosyl; Glc, glucosyl.

	1	**2**	**3**
Ole:			
H–C(1)	4.60 (*dd*, *J* = 9.5, 1.6)	4.58 (*dd*, *J* = 8.2, 2.9)	4.53 (*dd*, *J* = 9.8, 1.6)
CH_2(2)	1.42–1.51 (*m*),	1.46–1.56 (*m*),	1.38–1.50 (*m*),
	2.33 (*dd*, *J* = 11.6, 3.7)	2.31 (*ddd*, *J* = 12.1, 4.8, 1.7)	2.25 (br. *d*, *J* = 8.7)
H–C(3)	3.38–3.43 (*m*)	3.38–3.44 (*m*)	3.27–3.35 (*m*)
H–C(4)	3.36 (*t*, *J* = 7.4)	3.37 (*t*, *J* = 7.3)	3.26 (*t*, *J* = 8.4)
H–C(5)	3.32–3.38 (*m*)	3.34–3.38 (*m*)	3.23–3.31 (*m*)
Me(6)	1.37 (*d*, *J* = 5.0)	1.40 (*d*, *J* = 6.2)	1.31 (*d*, *J* = 5.6)
3-MeO	3.38 (*s*)	3.39 (*s*)	3.35 (*s*)
Allo:			
H–C(1)	4.80 (*d*, *J* = 8.3)	4.80 (*d*, *J* = 8.3)	4.78 (*d*, *J* = 6.7)
H–C(2)	3.49 (br. *d*, *J* = 8.2)	3.49 (*dd*, *J* = 8.2, 2.8)	3.44 (br. *d*, *J* = 7.7)
H–C(3)	3.80 (*t*, *J* = 2.7)	3.80 (*t*, *J* = 2.9)	3.88 (br. *s*)
H–C(4)	3.19 (br. *d*, *J* = 8.7)	3.20 (br. *d*, *J* = 7.0)	3.25 (br. *d*, *J* = 8.4)
H–C(5)	3.52–3.59 (*m*)	3.53–3.60 (*m*)	3.84–3.88 (*m*)
Me(6)	1.26 (*d*, *J* = 6.2)	1.27 (*d*, *J* = 6.2)	1.26 (*d*, *J* = 5.8)
3-MeO	3.67 (*s*)	3.67 (*s*)	3.57 (*s*)
Glu:			
H–C(1)			4.36 (*d*, *J* = 6.7)
H–C(2)			3.33 (*t*, *J* = 7.2)
H–C(3)			3.52 (*t*, *J* = 7.2)
H–C(4)			3.59 (*t*, *J* = 7.3)
H–C(5)			3.25–3.33 (*m*)
CH_2(6)			3.61–3.74 (*m*),
			3.86–3.93 (*m*)

H_2SO_4, followed by heating at 100°. For paper chromatography (PC), *Xinhua* filter paper (middle rate) was used, and spots were visualized with vanillin/perchloric acid. Column chromatography (CC): silica gel (200–300 or 300–400 mesh; *Qingdao Marine Chemical Factory*) and *Cosmosil 140 C_{18}-OPN* (*Nacalai Tesque*). UV Spectra: *Shimadzu UV-260* spectrophotometer, in anh. MeOH; λ_{max} (log ε) in nm. Optical rotations (ORD): *JASCO P-1020* spectropolarimeter. IR Spectra: *Avatar 360 ESP-TM* FT-IR spectrophotometer (*Thermo Nicolet*), as KBr pellets; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker AV-500* spectrometer, in $CDCl_3$; δ in ppm rel. to Me_4Si (= 0 ppm), J in Hz. ESI- and HR-ESI-MS: *Q-TOF Micro* mass spectrometer; in *m*/*z*.

Plant Material. The stems of *Marsdenia tenacissima* were collected in August 2001 in Yunnan Province, P. R. China, and identified by Dr. *Daofeng Chen*. A voucher specimen (DFC-TGS0108) was deposited at the Herbarium of Materia Medica, Department of Pharmacognosy, School of Pharmacy, Fudan University, Shanghai, P. R. China.

Extraction and Isolation. The dried and ground stems of *M. tenacissima* (5 kg) were repeatedly percolated with 95% EtOH at r.t. The solvent was evaporated *in vacuo* to give a dark-brown extract (436 g), which was partitioned between petroleum ether (PE) and H_2O, followed by $CHCl_3$ and H_2O. A sample (100 g) of the $CHCl_3$-soluble part (314 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 100 : 0 → 85 : 15) to give *Fr. 1* (13 g), *Fr. 2* (22 g), *Fr. 3* (17 g), *Fr. 4* (21 g), and *Fr. 5* (19 g). *Fr. 4* was applied to repeated CC (SiO_2; 1. $CHCl_3$/MeOH 90 : 10, 2. PE/acetone 60 : 40) to afford **1** (13 mg) and fraction *Fr. 4.1* (2.3 g). The latter was subjected to prep. TLC (SiO_2; $CHCl_3$/acetone 80 : 20) to yield tenacissoside F (14 mg) and *Fr. 4.2*. The latter was further purified by prep. HPLC (MeOH/H_2O 60 : 40) to afford **2** (1 mg). *Fr. 5* was applied to CC (1. SiO_2, $CHCl_3$/MeOH 85 : 15; 2. *Cosmosil*, MeOH/H_2O 60 : 40) to afford tenacissoside D (8 mg) and *Fr. 5.1*. The latter was subjected to prep. HPLC (MeOH/H_2O 60 : 40) to afford **3** (30 mg).

Marsdenoside 1 (= *(3β,5α,11α,12β,14β,17α,20*R*)-3-[(2,6-Dideoxy-4-*O*-(6-deoxy-3-*O*-methyl-β-*D*-allopyranosyl)-3-*O*-methyl-β-*D*-*arabino*-hexopyranosyl)oxy]-8,20 : 11,20-diepoxypregnane-12,14-diol*; **1**). Colorless amorphous powder. $[\alpha]_D^{22} = -15.3$ ($c = 1.2$, MeOH). IR: 3455, 2929, 1455, 1382, 1301, 1292, 1244, 1206, 1175, 1130, 1070, 1026, 1008. ^{1}H-NMR (500 MHz, $CDCl_3$; aglycone): 1.14 (*s*, Me(19)); 1.18 (*s*, Me(21)); 1.22 (*s*,

Table 2. *^{13}C-NMR Data* of **1**–**3** *and* **1a**. In $CDCl_3$ (unless noted otherwise); δ in ppm. Abbreviations: Ac, acetyl; Bz, benzoyl; HPA, (4-hydroxyphenyl)acetyl; Allo, allosyl; Ole, oleandrosyl; Glu, glucosyl.

Position	Aglycone				Position	Sugar moieties			
	1	**1a**	**2**	**3**		**1**	**2**	**3**	**3**[a])
C(1)	38.4	38.3	37.4	37.4	Ole:				
C(2)	28.7	30.9	29.1	29.1	C(1)	97.1	97.1	97.1	97.3
C(3)	77.1	72.1	76.2	76.5	C(2)	36.1	36.1	36.2	38.5
C(4)	34.5	37.4	34.8	34.8	C(3)	78.8	78.9	79.1	79.8
C(5)	46.0	46.2	44.0	44.0	C(4)	79.1	79.4	82.5	83.3
C(6)	28.0	27.9	26.6	26.8	C(5)	71.3	71.4	71.4	72.0
C(7)	33.8	34.2	31.7	31.8	C(6)	18.6	18.6	18.5	18.9
C(8)	77.9	77.9	66.7	66.9	3-MeO	55.6	55.9	55.9	57.5
C(9)	58.0	58.1	51.1	51.3	Allo:				
C(10)	35.9	35.8	39.0	39.1	C(1)	99.1	99.3	100.1	102.0
C(11)	71.3	71.3	69.0	69.7	C(2)	71.8	72.0	70.9	72.9
C(12)	71.2	71.2	74.9	75.2	C(3)	81.0	81.0	80.4	83.4
C(13)	44.7	44.8	45.8	45.9	C(4)	72.8	72.9	80.6	83.5
C(14)	82.0	82.1	71.4	71.2	C(5)	71.4	71.3	69.2	69.9
C(15)	34.1	34.5	26.8	26.6	C(6)	17.9	17.9	18.0	19.7
C(16)	23.7	23.7	24.9	25.0	3-MeO	61.9	62.7	61.2	62.0
C(17)	53.7	53.8	60.1	59.9	Glc:				
C(18)	17.4	17.4	16.7	16.6	C(1)			104.3	106.6
C(19)	16.0	16.1	12.7	12.7	C(2)			73.8	75.7
C(20)	99.3	99.3	210.7	210.8	C(3)			76.1	78.5
C(21)	24.6	24.6	29.9	30.1	C(4)			70.1	72.2
HPA or Bz:					C(5)			75.5	78.6
C(1′)			168.4	166.0	C(6)			62.1	63.2
C(2′)			41.4	130.1					
C(3′)			125.4	129.6					
C(4′)			130.7	128.5					
C(5′)			115.6	133.2					
C(6′)			155.1	–					
Ac:									
Me*C*O			170.7	170.7					
*Me*CO			20.2	20.4					

[a]) In C_5D_5N

Me(18)); 1.86 (br. *s*, H_β–C(17)); 2.43 (br. *s*, H–C(9)); 3.66–3.71 (*m*, H–C(3)); 3.98 (*dd*, *J* = 8.5, 4.0, H_α–C(12)); 4.28 (br. *d*, *J* = 2.6, H_β–C(11)); for sugar resonances, see *Table 1*. ^{13}C-NMR: see *Table 2*. HR-ESI-MS: 707.3414 ($[M+K]^+$, $C_{35}H_{56}KO_{12}^+$; calc. 707.3409).

Marsdenoside J (= *(3β,5α,11α,12β,14β,17α)-12-Acetoxy-3-[(2,6-dideoxy-4-*O*-(6-deoxy-3-*O*-methyl-β-*D*-allo-pyranosyl)-3-*O*-methyl-β-*D*-*arabino*-hexopyranosyl)oxy]-20-oxo-8,14-epoxypregnan-11-yl (4-Hydroxyphenyl)-acetate*; **2**). Colorless amorphous powder. $[\alpha]_D^{22} = +10.2$ (*c* = 0.15, MeOH). UV (MeOH): 212 (3.52), 276 (3.27). IR: 3443, 2928, 1739, 1723, 1630, 1517, 1469, 1372, 1250, 1162, 1131, 1098, 1069, 1034, 1005, 996, 952, 934. ^{1}H-NMR (500 MHz, $CDCl_3$; aglycone): 1.00 (*s*, Me(19)); 1.05 (*s*, Me(18)); 1.64 (*s*, Me(2″)); 1.96 (*d*, *J* = 10.0, H–C(9)); 2.19 (*s*, Me(21)); 2.90 (br. *d*, *J* = 7.2, H_β–C(17)); 3.53–3.61 (*m*, H–C(3)); 4.95 (*d*, *J* = 10.1, H_α–C(12)); 5.31 (*t*, *J* = 10.1, H_β–C(11)); 6.75 (*d*, *J* = 8.5, H–C(5′,7′)); 7.09 (*d*, *J* = 8.5, H–C(4′,8′)); for sugar resonances, see *Table 1*. ^{13}C-NMR: see *Table 2*. HR-ESI-MS: 883.3889 ($[M+K]^+$, $C_{45}H_{70}KO_{14}^+$; calc. 883.3882).

Marsdenoside K (= *(3β,5α,11α,12β,14β,17α)-12-Acetoxy-3-[(β-*D*-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-β-*D*-allopyranosyl-(1 → 4)-2,6-dideoxy-3-*O*-methyl-β-*D*-*arabino*-hexopyranosyl)oxy]-20-oxo-8,14-epoxy-pregnan-11-yl Benzoate*; **3**). Colorless amorphous powder. $[\alpha]_D^{22} = -5.0$ (*c* = 2.2, MeOH). UV (MeOH): 280 (2.89), 275 (3.04), 230 (4.08). IR: 3439, 2934, 1725, 1646, 1600, 1446, 1372, 1314, 1276, 1214, 1163, 1070, 992, 930, 923, 714. ^{1}H-NMR (500 MHz, $CDCl_3$; aglycone): 1.12 (*s*, Me(19)); 1.14 (*s*, Me(18)); 1.60 (*s*, Me(2″));

2.17 (*d*, *J* = 10.3, H–C(9)); 2.20 (*s*, Me(21)); 2.94 (br. *d*, *J* = 7.2, H_β–C(17)); 3.52–3.62 (*m*, H–C(3)); 5.12 (*d*, *J* = 10.1, H_α–C(12)); 5.60 (*t*, *J* = 10.1, H_β–C(11)); 7.42 (*t*, *J* = 7.7, H–C(4′,6′)); 7.55 (*t*, *J* = 7.4, H–C(5′)); 7.95 (*d*, *J* = 7.2, H–C(3′,7′)); for sugar resonances, see *Table 1*. ^{13}C-NMR: see *Table 2*. HR-ESI-MS: 1015.4309 ($[M+K]^+$, $C_{47}H_{68}KO_{14}^+$; calc. 1015.4305).

Acid Hydrolysis of **1**. A soln. of **1** (5 mg) in MeOH (3 ml) and 0.1M H_2SO_4 (1 ml) was kept at 60° for 30 min. Then, H_2O (3 ml) was added, the mixture was concentrated to a volume of *ca*. 4 ml, and kept at 60° for another 30 min, before cooled to r.t. The soln. was extracted with Et_2O (3×5 ml), the org. layer was washed with H_2O (4×5 ml), dried (Na_2SO_4), and evaporated to dryness. The resulting residue was recrystallized from PE/AcOEt. The aq. acidic layer of the hydrolysate was neutralized with 5% aq. $Ba(OH)_2$ soln. The precipitate was filtered, and the filtrate was evaporated. The residue, pachybiose, was compared with an authentic sample by paper chromatography (upper layer of BuOH/AcOH/H_2O 4 : 1 : 5) and TLC ($CHCl_3$/MeOH 9 : 1).

Data of Tenacigenin A (**1a**). Colorless amorphous powder. ^{1}H-NMR (500 MHz, $CDCl_3$): 1.15 (*s*, Me(19)); 1.18 (*s*, Me(21)); 1.22 (*s*, Me(18)); 1.86 (br. *s*, H–C(17)); 2.43 (br. *s*, H–C(9)); 3.61–3.67 (*m*, H–C(3)); 3.98 (*d*, *J* = 2.4, H_α–C(12)); 4.30 (br. *d*, *J* = 2.6, H_β–C(11)). ^{13}C-NMR: see *Table 2*. ESI-MS: 387.4 ($[M+Na]^+$, $C_{21}H_{32}NaO_5^+$).

Enzymatic Hydrolysis of **3**. A soln. of 0.1M AcOH/AcONa (pH 4.6) buffer (1 ml) and β-glucosidase (30 µl; 250 U/ml; *Sigma*) was added to **3** (2 mg). The mixture was incubated at 60° for 30 min, and then extracted with $CHCl_3$ (3×5 ml). The $CHCl_3$ layer was evaporated under reduced pressure, and the residue was identified as tenacissoside I [8] by TLC comparison with an authentic sample (visualization with $CHCl_3$/MeOH 9 : 1). The aq. layer contained only one monosaccharide identified as glucose by paper chromatography (upper layer of BuOH/AcOH/H_2O 4 : 1 : 5).

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