Four New Pregnane Glycosides from the Stems of *Marsdenia tenacissima*

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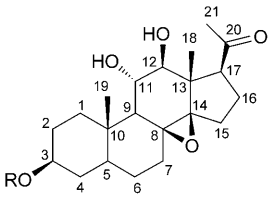
Four new pregnane glycosides, tenacigenosides A–D (**1**–**4**), along with six known pregnane aglycones and five known pregnane glycosides, were isolated from the stems of *Marsdenia tenacissima* (ROXB.) WIGHT et ARN. (Asclepiadaceae). The chemical structures of the new compounds were established by 1D- and 2D-NMR as well as HR-MS analyses. The absolute configuration of **1** was confirmed by X-ray crystallography.

Introduction. – *Marsdenia tenacissima* (ROXB.) WIGHT et ARN. (Asclepiadaceae), distributed in Southwest China, is known to have anti-inflammation, anti-asthmatic, and anti-cancer properties [1]. Previous chemical investigations on this plant showed the presence of pregnanes and aromatic acids [2–20]. Some pregnane glycosides showed cytotoxicity against KB cells [7].

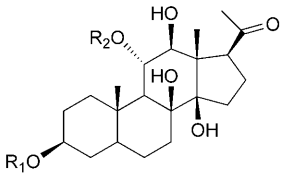
Herein, we report four new pregnane glycosides, tenacigenosides A–D (**1**–**4**), which were isolated from the stems of *M. tenacissima*, together with eleven known pregnanes: tenacigenin A (**5**) [6][14], tenacigenin B (**6**) [6][9], 17β-tenacigenin B (**7**) [15], tenacissoside A (**8**) [11][14], tenacissoside F (**9**) [11], tenacissoside G (**10**) [10][11], marsdenoside H (**11**) [14], 11α-*O*-(2-methylbutanoyl)-12β-*O*-acetyl-tenacigenin B (**12**) [7], 11α-*O*-tigloyl-12β-*O*-acetyl-tenacigenin B (**13**) [7], marsdenoside D (**14**) [14], and tenacigenin C (**15**) [6][15].

Results and Discussion. – The four new compounds **1**–**4** gave rise to positive *Liebermann–Burchard*, *Keller–Kiliani*, and xanthydrol reactions, indicating that they were all steroidal glycosides containing 2-deoxy sugar moieties [14][16]. Compound **1** was obtained as colorless prisms. Its molecular formula, $C_{35}H_{56}O_{12}$, was deduced by HR-ESI-MS (*m*/*z* 691.3661 ($[M+Na]^+$; calc 691.3669). Its IR spectrum showed absorptions at 3403 (OH) and 1712 (C=O) cm^{-1}. The structure of **1** was established by ^{1}H- and ^{13}C-NMR spectroscopy (*Tables 1* and *2*, resp.), including DEPT, HSQC, HMBC, and NOESY experiments, as well as by X-ray single-crystal diffraction (see *Fig. 1* below; see also *Exper. Part*).

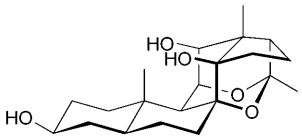
The ^{1}H-NMR spectrum of **1** showed the presence of two anomeric signals at δ(H) 4.83 (*dd*, $J=10$, 2 Hz) and 5.32 (br. *d*, $J=8$ Hz), with the corresponding ^{13}C-NMR signals at δ(C) 97.3 and 101.8, respectively, suggesting that **1** was a disaccharide glycoside. The glycosidic linkages were in β-orientation, as deduced from the coupling constants of the two anomeric signals. The NMR data of the sugar moiety was in good agreement



1 R = Pac
7 R = H



	R^1	R^2
3	Pac	Tig
4	Pac	MBu
15	H	H



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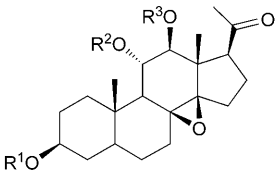
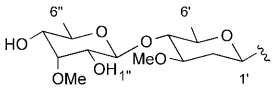
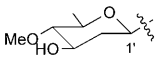


	R^1	R^2	R^3
2	S^1	MBu	Ac
6	H	H	H
8	S^2	Tig	Ac
9	Pac	H	H
10	Pac	Tig	Ac
11	S^2	MBu	Ac
12	H	MBu	Ac
13	H	Tig	Ac
14	Pac	MBu	H

Pac =



S^1 =



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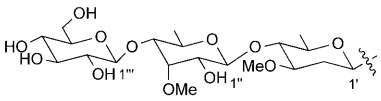

Abbreviations:
Ac = acetyl, MBu = 2-methylbutanoyl, Tig = tigloyl (= (*E*)-2-methylbut-2-enoyl), Pac = pachybiosyl (= 2,6-dideoxy-4-*O*-(6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl)-3-*O*-methyl-*β*-D-*arabino*-hexopyranosyl)

with those of tenacissoside F (**9**) [11], indicating that the sugar moiety of **1** was also pachybiose (=2,6-dideoxy-4-*O*-(6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl)-3-*O*-methyl-*β*-D-*arabino*-hexopyranose). This was confirmed by mild acid hydrolysis of **1**, which gave pachybiose exclusively, as identified by co-TLC with an authentic sample.

The ^{13}C-NMR data of the aglycone part of **1** (*Table 2*) showed signals of a C_{21}-steroidal skeleton resembling 17*β*-tenacigenin B (**7**) [14]. Glycosidation shifts in **1** relative to **7** were observed for C(2) ($\Delta\delta$ −0.5), C(3) (+5.4), and C(4) (−3.0), indicating that the sugar moiety in **1** was linked at the 3-O-atom of the aglycone [21]. This was confirmed by an HMBC correlation between δ(H) 4.83 (H−C(1′)) and δ(C) 76.3 (C(3)). Thus, the structure of **1** was identified as 3-*O*-pachybiosyl-17*β*-tenacigenin B, and named *tenacigenoside A*[1]). The structure of **1** was also unequivocally confirmed by X-ray single-crystal diffraction (*Fig. 1*).

Compound **2** was obtained as colorless needles. The molecular formula, $C_{35}H_{54}O_{10}$, was established by HR-ESI-MS (*m*/*z* 657.3595, ([*M*+Na]$^+$; calc. 657.3615). The IR spectrum showed OH absorption bands at 3457 cm^{-1}, and C=O absorptions at 1707, 1713, and 1738 cm^{-1}. The ^{1}H-NMR spectrum of **2** (*Table 1*) showed one anomeric signal at δ(H) 4.63 (*d*, *J*=8 Hz), with δ(C) 97.1. The glycosidic linkage was *β*-oriented, based

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

Table 1. *^{1}H-NMR Data of the Sugar Moieties of* **1**–**4**. At 600 MHz in $CDCl_3$, unless noted otherwise; δ in ppm, J in Hz. For resonances of the aglycones, see *Exper. Part.*

Position	**1**[a])	**2**	**3**	**4**
1′	4.83 (*dd*, *J* = 10, 2)	4.63 (*d*, *J* = 8)	4.56 (*d*, *J* = 9)	4.56 (*d*, *J* = 9)
2′	1.79–1.81 (*m*), 2.45–2.46 (*m*)	1.46–1.48 (*m*), 2.28–2.30 (*m*)	1.47–1.49 (*m*), 2.30–2.31 (*m*)	1.48–1.49 (*m*), 2.30–2.32 (*m*)
3′	3.65–3.67 (*m*)	3.16–3.18 (*m*)	3.34–3.37 (*m*)	3.39–3.41 (*m*)
3′-MeO[b])	3.53 (*s*)	3.39 (*s*)	3.37 (*s*)	3.37 (*s*)
4′	3.62–3.63 (*m*)	3.19–3.21 (*m*)	3.33–3.34 (*m*)	3.34–3.35 (*m*)
5′	3.63–3.64 (*m*)	3.31–3.34 (*m*)	3.54–3.57 (*m*)	3.56–3.57 (*m*)
6′	1.69 (*d*, *J* = 5)	1.36 (*d*, *J* = 6)	1.36 (*s*)	1.36 (*d*, *J* = 5)
1″	5.32 (*d*, *J* = 8)		4.79 (*d*, *J* = 8)	4.80 (*d*, *J* = 8)
2″	3.88–3.90 (*m*)		3.48–3.49 (*m*)	3.47–3.48 (*m*)
3″	4.07 (*t*, *J* = 2.8)		3.79 (*t*, *J* = 4)	3.79 (br. *s*)
3″-MeO	3.83 (*s*)		3.66 (*s*)	3.66 (*s*)
4″	3.62 (*d*, *J* = 2.8)		3.19 (*t*, *J* = 8)	3.19 (*d*, *J* = 3)
5″	4.14–4.17 (*m*)		3.33–3.34 (*m*)	3.34–3.35 (*m*)
6″	1.54 (*d*, *J* = 6)		1.26 (*d*, *J* = 6)	1.26 (*d*, *J* = 6)

[a]) In C_5D_5N. [b]) 4′-MeO for **2**.

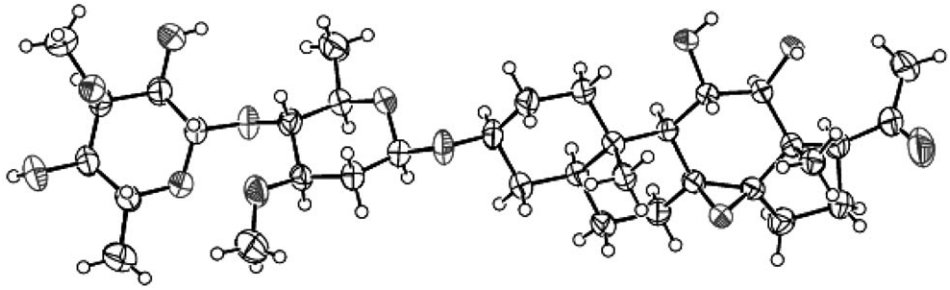

Fig. 1. *X-Ray crystal structure of* **1**

on the *J* value of 8 Hz of the anomeric H-atom. The sugar resonances at δ(H) 1.44–1.46 and 2.27–2.28 (2*m*, 2×1 H), at 3.16–3.17 (*m*, 1 H), and at 3.19–3.21 (*m*, 1 H) were assigned to CH_2(2′), H–C(3′), and H–C(4′), respectively, based on HSQC analysis.

Interpretation of the HSQC, HMBC, and NOSEY spectra of **2** (*Figs. 2* and *3*) revealed that the Me signal at δ(H) 1.36 (*d*, *J* = 6 Hz, Me(6′)) was linked at C(5′), and that the MeO group at δ(H) 3.39 (*s*) was in 4′-position of the sugar unit. From the NOSEY signals, it was concluded that H–C(3′) and H–C(4′) were in axial positions, indicating a 2,6-dideoxy-β-D-*arabino*-hexopyranosyl (= olivomosyl) residue.

The ^{13}C-NMR spectrum of the aglycone of **2** (*Table 2*) showed signals of a C_{21} steroid resembling those of 11α-*O*-(2-methylbutanoyl)-12β-*O*-acetyl-tenacigenin B (**12**) [7]. Glycosidation shifts of **2** compared to **12** were observed for C(2) ($\Delta\delta$ −1.4), C(3) (+4.9), and C(4) (−4.2), which indicated that the sugar moiety was linked at the 3-O-atom of the aglycone. HMBC and NOESY correlations (*Figs. 2* and *3*) further

Table 2. *^{13}C-NMR Data of* **1**–**4**. At 150 MHz in $CDCl_3$, unless noted otherwise; δ in ppm.

Position	Aglycone				Position	Sugar moieties			
	1[a])	**2**	**3**	**4**		**1**[a])	**2**	**3**	**4**
1	38.9	37.6	38.9	38.9	1′	97.3		96.8	96.9
2	29.7	31.7	29.1	28.9	2′	37.6		36.1	36.1
3	76.3	76.1	76.3	76.3	3′	79.4		78.8	78.8
4	35.0	34.6	35.2	35.1	4′	83.0		79.1	79.2
5	44.7	43.9	45.1	45.1	5′	71.8		71.3	71.3
6	27.9	26.5	28.7	28.7	6′	18.9		18.6	18.6
7	32.8	28.9	34.5	34.5	3′-MeO	56.9		55.6	55.6
8	65.9	66.8	77.6	77.6					
9	54.2	51.1	49.8	49.6	1″	101.8	97.1	99.2	99.2
10	39.4	39.1	37.3	37.5	2″	73.1	35.7	71.8	71.8
11	67.9	68.4	74.1	74.0	3″	83.8	75.5	81.0	81.0
12	81.0	75.1	76.9	76.8	4″	74.4	80.8	72.8	72.9
13	48.2	45.8	52.9	52.9	5″	70.8	71.3	71.3	71.3
14	71.5	71.4	87.2	87.0	6″	18.4	17.9	17.8	17.9
15	27.4	26.8	24.7	24.6	3″-MeO[b])	61.8	56.2	61.9	61.9
16	25.2	24.9	23.1	23.2					
17	62.8	60.6	59.6	59.6					
18	11.1	16.8	14.1	14.1					
19	13.0	12.7	13.0	12.9					
20	209.9	210.7	213.3	213.6					
21	32.1	29.8	31.1	31.1					
Ac		170.8							
		20.9							
MBu or Tig:									
O=C(1)		175.6	168.4	177.3					
C(2)		41.3	129.0	41.8					
C(3)		26.2	138.0	26.8					
C(4)		11.8	12.2	11.6					
2-Me		15.3	14.5	15.8					

[a]) in C_5D_5N. [b]) 4′-MeO for **2**.

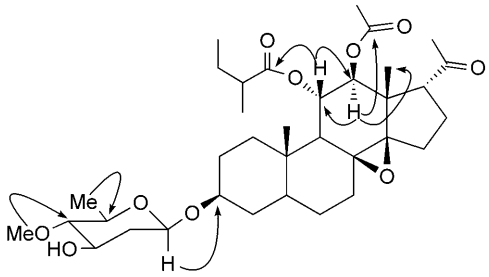

Fig. 2. *Key HMBC correlations for* **2**

supported this assumption. Thus, the structure of **2** was elucidated as 3-*O*-olivomosyl-11-*O*-(2-methylbutanoyl)-12β-*O*-acetyl-tenacigenin B, and named *tenacigenoside B.*

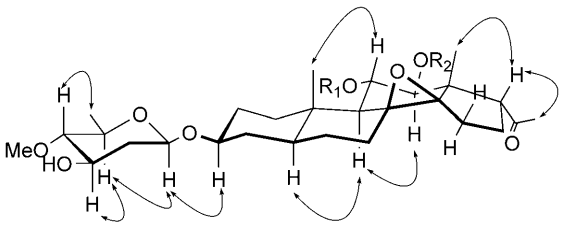

Fig. 3. *Key NOESY correlations for* **2**

The NMR data due to the sugar moieties of **3** and **4** were identical to those of **1**. This was confirmed by mild acid hydrolysis of **3** and **4**, which gave only pachybiose according to TLC comparison. The same glycosidation shifts were observed in compounds **3** and **4**, the sugar moiety thus being attached at the 3-O-atom of the aglycone.

Compound **3** was obtained as colorless needles. The formula $C_{40}H_{64}O_{14}$ was confirmed by HR-ESI-MS (*m*/*z* 791.4216 ($[M+Na]^+$; calc. 791.4194). The IR spectrum showed absorption bands for OH (3446) and C=O (1691 cm^{-1}) groups. In the ^{1}H-NMR spectrum, there were signals for one tigloyl (=(*E*)-2-methylbut-2-enoyl; Tig) group at δ(H) 1.86 (*s*, Me), 1.79 (*d*, $J=6$, Me), and 6.90 (br. *d*, $J=7$, =CH). The ^{13}C-NMR spectrum also displayed typical Tig resonances (*Table 2*).

The ^{1}H-NMR spectrum of the aglycone moiety of **3** resembled that of tenacigenin C (**15**) [6] [15], except for the Tig group of **3**. The HMBC spectrum of **3** displayed correlations between the signal at δ(H) 5.63 (*t*, $J=10$ Hz, H_β–C(11)) and the Tig carbonyl group at δ(C) 168.4, indicating that the Tig was linked to the O-atom at C(11). The NOESY correlations between δ(H) 2.15 (H–C(9)) and 4.10 (H–C(12)), and between δ(H) 3.37 (H–C(17)) and 4.10 (H–C(12)) clearly showed that H–C(17) was α-oriented. Consequently, the structure of **3** was elucidated as 3-*O*-pachybiosyl-11α-*O*-tigloyl-tenacigenin C, and named *tenacigenoside C*.

Compound **4** was obtained as colorless needles. The formula $C_{40}H_{66}O_{14}$ was deduced by HR-ESI-MS (*m*/*z* 793.4379, ($[M+Na]^+$; calc. 793.4350). The IR spectrum showed absorption bands for OH (3436) and C=O (1708 cm^{-1}) groups. In the ^{1}H-NMR spectrum of **4**, there were signals for one 2-methylbutanoyl (MBu) group at δ(H) 1.14 (*d*, $J=7$ Hz, Me), 0.92 (*t*, $J=7$ Hz, Me), 1.46/1.75 ($2m_c$, CH_2), and 2.35 (m_c, 1 H). The ^{13}C-NMR spectrum (*Table 2*) of **4** also displayed MBu resonances. The HMBC correlation between δ(H) 5.55 (*dd*, $J=10.0$, 9.0, H_β–C(11)) and δ(C) 177.3 of the MBu unit indicated that the MBu residue was attached at the O-atom in 11-position of the aglycone. Thus, the structure of **4** was elucidated as 3-*O*-pachybiosyl-11α-*O*-(2-methylbutanoyl)-tenacigenin C, and named *tenacigenoside D*.

Finally, the eleven known compounds were identified by comparison of their ORD, IR, ^{1}H- and ^{13}C-NMR, and MS data with literature values. Compounds **8**, **10** and **12** were the main constituents isolated from the stems of *M. tenacissima*.

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Experimental Part

General. TLC: Silica gel GF_{254} plates (0.5 mm; *Qingdao Haiyang Chemical Group Co*). Column chromatography (CC): silica gel (160–200 or 200–300 mesh; *Qingdao Marine Chemical Factory*), *ODS* (300–400 mesh; *Fuji Sylisia Chemical, Ltd.*), or *Sephadex LH-20* (*Pharmacia*). Optical rotations: *Perkin-Elmer-341* polarimeter, at 589 nm. IR Spectra: *Perkin-Elmer* FT-IR apparatus; in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker Advance 600* instrument; δ in ppm rel. to Me_4Si, J in Hz. ESI-MS: *Finnigan* LCQ^{DECA} spectrometer. HR-ESI-MS: *Bruker BioTOF-Q* spectrometer; in m/z. X-Ray crystallography: *Siemens-P4* four-circle diffractometer.

Plant Material. The stems of *M. tenacissima* were collected in Sept. 2005 in Yunnan Province, P. R. China, and identified by *Prof. Zuo-Cheng Zhao*, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041. A voucher specimen (No. W2289) was deposited at the Herbarium of the Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, P. R. China.

Extraction and Isolation. The air-dried, powdered roots of *M. tenacissima* (11 kg) were boiled for 2 h in H_2O. The solvent was evaporated *in vacuo*, the resulting deep-brown syrup (*ca*. 1.0 kg) was suspended in H_2O and extracted with petroleum ether (PE) and then with AcOEt. The AcOEt-soluble fraction (286 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 98:2 → 50:50) to afford nine fractions (Fr.) according to TLC: *Fr. 1* (1.5 g), *Fr. 2* (3.2 g), *Fr. 3* (6.5 g), *Fr. 4* (2.8 g), *Fr. 5* (2.6 g), *Fr. 6* (3.5 g), *Fr. 7* (5.6 g), *Fr. 8* (8.9 g), and *Fr. 9* (19 g). Compound **5** (0.5 g) was obtained from *Fr. 1* by recrystallization from MeOH. *Fr. 2* was subjected to reverse-phase CC (*ODS*; MeOH/H_2O 1:2) to afford **1** (0.14 g), **2** (0.10 g) and **15** (15 mg). Compound **9** (4.0 g) was obtained from *Fr. 3* by recrystallization from MeOH. Further purification of the mother liquor by CC (*ODS*; MeOH/H_2O 2:3; then *Sephadex LH-20*, MeOH) gave **10** (1.12 g) and **11** (15 mg). *Fr. 4* was subjected to CC (*ODS*; MeOH/H_2O 1:2) to afford **3** (0.11 g) and **4** (0.15 g). *Fr. 5* was also purified by CC (*ODS*; MeOH/H_2O 1:3; then *Sephadex LH-20*; MeOH) to provide **6** (0.95 g) and **7** (0.16 g). Compound **8** (1.5 g) was separated from *Fr. 6* by CC (*ODS*; MeOH/H_2O 1:3). *Fr. 7* was further purified by CC (*ODS*; MeOH/H_2O 1:2) to give **12** (3.7 g) and **14** (0.1 g). *Fr. 8* was subjected to CC (*ODS*; MeOH/H_2O 1:3; then *Sephadex LH-20*, MeOH) to afford **13** (0.11 g).

Tenacigenoside A (= *(3β,5α,11α,12β,14β)-3-{[2,6-Dideoxy-4*-O-*(6-deoxy-3*-O-*methyl-β*-D-*allopyranosyl)-3*-O-*methyl-β*-D-arabino-*hexopyranosyl]oxy}-11,12-dihydroxy-8,14-epoxypregnan-20-one*; **1**). Yield: 140 mg. Colorless prisms. M.p. 144–146° (MeOH). $[\alpha]_D^{25} = -4$ ($c = 0.1$, MeOH). IR (KBr): 3403, 2971, 1712, 1160, 1102, 1098. ^{1}H-NMR (C_5D_5N; aglycone): 1.21 (*s*, Me(19)); 1.26 (*s*, Me(18)); 1.74 (*d*, $J = 10.0$, H–C(9)); 2.48 (*s*, Me(21)); 2.93 (*dd*, $J = 12.0$, 6.0, H_α–C(17)); 3.88–3.91 (*m*, H–C(3)); 3.62 (*d*, $J = 10.0$, H_α–C(12)); 3.90 (*d*, $J = 10.0$, H_β–C(11)); for sugar resonances, see *Table 1*. ^{13}C-NMR: see *Table 2*. HR-ESI-MS: 691.3661 ($[M+Na]^+$, $C_{35}H_{56}NaO_{12}^+$; calc. 691.3669). X-Ray crystal structure: see *Fig. 1*.

Tenacigenoside B (= *(3β,5α,11α,12β,14β)-12-Acetoxy-3-[(2,6-dideoxy-4*-O-*methyl-β*-D-arabino-*hexopyranosyl)oxy]-20-oxo-8,14-epoxypregnan-11-yl 2-Methylbutanoate*; **2**). Yield: 100 mg. Colorless needles. M.p. 166–167° (MeOH). $[\alpha]_D^{25} = -9$ ($c = 0.1$, MeOH). IR (KBr): 3457, 2971, 1738, 1713, 1707, 1255, 1141, 1103, 1070. ^{1}H-NMR ($CDCl_3$; aglycone): 0.70 (*t*, $J = 7.0$, Me of MBu); 1.05 (*d*, $J = 7.0$, Me of MBu); 1.07 (*s*, Me(19)); 1.10 (*s*, Me(18)); 1.99 (*s*, Ac); 2.03 (*d*, $J = 9.0$, H–C(9)); 2.22 (*s*, Me(21)); 2.94–2.95 (*m*, H_α–C(17)); 3.65–3.67 (*m*, H–C(3)); 5.00 (*d*, $J = 10.0$, H_α–C(12)); 5.37 (*dd*, $J = 10.0$, 3, H_β–C(11)); for sugar resonances, see *Table 1*. ^{13}C-NMR: see *Table 2*. HR-ESI-MS: 657.3595 ($[M+Na]^+$, $C_{35}H_{54}NaO_{10}^+$; calc. 657.3615).

Tenacigenoside C (= *(3β,5α,11α,12β,14β)-3-{[2,6-Dideoxy-4*-O-*(6-deoxy-3*-O-*methyl-β*-D-*allopyranosyl)-3*-O-*methyl-β*-D-arabino-*hexopyranosyl]oxy}-8,12,14-trihydroxy-20-oxopregnan-11-yl (2*E*)-2-Methylbut-2-enoate*; **3**). Yield: 110 mg. Colorless needles. M.p. 127–129° (MeOH). $[\alpha]_D^{25} = +31$ ($c = 0.1$, MeOH). IR (KBr): 3446, 2932, 1691, 1273, 1162, 1127, 1071. ^{1}H-NMR ($CDCl_3$; aglycone): 1.09 (*s*, Me(19)); 1.13 (*s*, Me(18)); 1.79 (*d*, $J = 6.0$, Me of Tig); 1.86 (*s*, Me of Tig); 2.15 (*d*, $J = 11.0$, H–C(9)); 2.23 (*s*, Me(21)); 3.34–3.37 (*m*, H_α–C(17)); 3.61–3.62 (*m*, H–C(3)); 4.10 (*dd*, $J = 10.0$, 3, H_α–C(12)); 5.63 (*t*, $J = 10.0$, H_β–C(11)); 6.90 (*d*, $J = 7.0$, 1 H of Tig); for sugar resonances, see *Table 1*. ^{13}C-NMR: see *Table 2*. HR-ESI-MS: 791.4216 ($[M+Na]^+$, $C_{40}H_{64}NaO_{14}^+$; calc. 791.4194).

Tenacigenoside D (= *(3β,5α,11α,12β,14β)-3-{[2,6-Dideoxy-4*-O-*(6-deoxy-3*-O-*methyl-β*-D-*allopyranosyl)-3*-O-*methyl-β*-D-arabino-*hexopyranosyl]oxy}-8,12,14-trihydroxy-20-oxopregnan-11-yl 2-Methyl-*

butanoate; **4**). Yield: 150 mg. Colorless needles. M.p. 114–116° (MeOH). $[\alpha]_D^{25} = +30$ ($c = 0.1$, MeOH). IR (KBr): 3436, 2933, 1708, 1377, 1160, 1128, 1082. ^{1}H-NMR ($CDCl_3$; aglycone): 1.09 (*s*, Me(19)); 1.13 (*s*, Me(18)); 0.92 (*t*, $J = 7.0$, Me of MBu); 1.14 (*d*, $J = 7.0$, Me of MBu); 1.46, 1.75 ($2m_c$, CH_2 of MBu); 2.08 (*d*, $J = 11.0$, H–C(9)); 2.25 (*s*, Me(21)); 2.35 (m_c, CH of MBu); 3.35–3.37 (*m*, H_α–C(17)); 3.62–3.63 (*m*, H–C(3)); 4.04 (*d*, $J = 9$, H_α–C(12)); 5.55 (*dd*, $J = 10.0, 9.0$, H_β–C(11)); for sugar resonances, see *Table 1*. ^{13}C-NMR: see *Table 2*. HR-ESI-MS: 793.4379 ($[M+Na]^+$, $C_{40}H_{66}NaO_{14}^+$; calc. 793.4350).

Acid Hydrolysis. A soln. of the appropriate compound (5 mg of **1**, **3**, or **4**) 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 (Pac), was analyzed by co-TLC (SiO_2; $CHCl_3$/MeOH 9:1) with an authentic sample.

X-Ray Crystallography and Structure Refinement of **1**[2]). The analysis was performed with a single crystal (0.56×0.38×0.34 mm) at 298 K. Formula $C_{35}H_{56}O_{12}$, M_r 668.80; monoclinic, space group *P* 21, $a = 12.555(2)$, $b = 6.435(1)$, $c = 21.828(4)$ Å, $\alpha = \gamma = 90.00°$, $\beta = 90.17(15)°$; $V = 1763.5(6)$ Å^3, $Z = 2$; $\rho = 1.260$ Mg/m^3, $\mu = 0.094$ mm^{-1}, $F(000) = 724$. Intensity data were collected on a *Siemens-P4* four-circle diffractometer with a graphite monochromator (MoK_α radiation, $\lambda = 0.71073$ Å). A total of 4,521 unique reflections were collected, of which 4,186 were observed. The structure was solved by direct methods using SHELXS-97, and refined by full-matrix least-squares calculations. The final *R* indices for $I > 2\sigma(I)$ were $R_1 = 0.0705$ and $wR_2 = 0.0768$.

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[2]) The crystallographic data of **1** were deposited with the *Cambridge Crystallographic Data Centre* as deposition No. CCDC-606857. Copies of the data can be obtained, free of charge, at http://www.ccdc.cam.ac.uk/data_request/cif.