Antisweet Natural Products. V.[1] Structures of Gymnemic Acids VIII—XII from *Gymnema sylvestre* R. Br.

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Five oleanane-type triterpenoid saponins, gymnemic acids VIII—XII as antisweet principles were isolated from the leaves of *Gymnema sylvestre* (Asclepiadaceae). Their structures were established on the basis of spectral and chemical evidence. They were characterized as glucosideuronic acid derivatives of gymnemagenin acylated with acetyl, tigloyl and/or 2-methylbutyroyl moieties.

Keywords *Gymnema sylvestre*; Asclepiadaceae; antisweet substance; oleanane; acylsaponin; gymnemic acid; gymnemagenin; tiglic acid; 2-methylbutyric acid; acetic acid

In the previous paper,[2] we reported the isolation and structure elucidation of five oleanane glucosides named gymnemasaponins I—VII as antisweet principles in the title plant. Further separation of the saponin fraction afforded five new compounds, gymnemic acids VIII—XII. All of them possess one or two acyl groups (acetyl, 2-methylbutyroyl or tigloyl group) in the aglycone and show antisweet activity. This paper deals with the isolation and elucidation of their structures and activities. The EtOH extract of the leaves (6 kg) of *G. sylvestre* was successively chromatographed on Amberlite XAD-2 and Toyopearl HW-40 to give a saponin fraction (150 g). The crude saponin was further separated by ordinary-phase SiO_2 and reversed-phase octadodecyl silica (ODS) column chromatography to furnish five new saponins, gymnemic acids VIII (**1**, 80 mg), IX (**2**, 20 mg), X (**3**, 30 mg), XI (**4**, 180 mg) and XII (**5**, 40 mg).

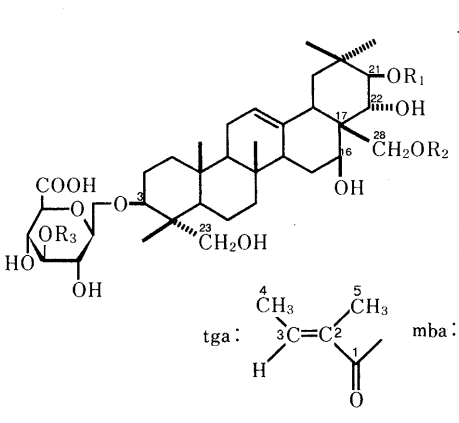
On mild acid hydrolysis, gymnemic acids VIII—XII (**1**—**5**) afforded gymnemagenin (**6**),[3] mp 313—314 °C, $[\alpha]_D$ +53.5° (c=1.8, MeOH), $C_{30}H_{50}O_6$ {positive fast atom bombardment spectrum (FAB-MS) m/z: 529 $[M+Na]^+$} as the common aglycone and only D-glucuronic acid (D-glcA) as a sugar component. Alkaline treatment of **1**—**4** gave 3-*O*-*β*-D-glucuronopyranosyl gymnemagenin (**7**),[3] mp 230—231 °C, $[\alpha]_D$ +8.4° (c=1.8, MeOH), $C_{36}H_{58}O_{12}$ {FAB-MS m/z: 705 $[M+Na]^+$}, which, on acid hydrolysis, provided **6** and glcA.

Gymnemic acid VIII (**1**), amorphous powder, mp 185—187 °C, $[\alpha]_D$ +21.5° (c=3.5, MeOH), has the molecular formula $C_{41}H_{66}O_{13}\cdot H_2O$ based on the elemental analysis. The ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra (Tables I, II and III) of **1** suggested that **1** was composed of one mol of 2-methylbutyric acid and **7**. 2-Methylbutyric acid obtained by mild alkaline hydrolysis of **1** was identified as its *p*-nitrobenzyl ester in high pressure liquid chromatography (HPLC). The absolute configuration of 2-methylbutyric acid was determined as *S* by the optical rotation {$[\alpha]_D$ +16.3° (c=0.3, 50% MeOH)}.[4] Comparison of the NMR spectra of **1** with those of **7** showed acylation shifts[5] for the 28-proton and carbon signals [+0.58 ppm, from δ 4.09 to 4.67 and +0.35 ppm, from δ 4.74 to 5.09 (28-H_2), +3.7 ppm, from δ 58.6 to 62.3 (C-28)], demonstrating that the 2-methylbutyroyl group was located at C_{28}-OH. Therefore, **1** was formulated as 3-*O*-*β*-D-glucuronopyranosyl-28-*O*-2*S*-methylbutyroyl gymnemagenin.

Gymnemic acid IX (**2**), amorphous powder, mp 194—196 °C, $[\alpha]_D$ +7.6° (c=1.8, MeOH), $C_{41}H_{64}O_{13}$ revealed a quasi-molecular peak $[M-H]^-$ at m/z 763 in the negative FAB-MS. The ^{1}H- and ^{13}C-NMR spectra (Tables I, II and III) of **2** suggested that **2** was composed of one mol each of tiglic aid and **7**. Tiglic acid obtained by mild alkaline hydrolysis of **2** was identified as in the case of **1**. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **2** with those of **7** showed the 28 postion to be the acylation site in the former (+0.57 ppm, from δ 4.09 to 4.66 and +0.35 ppm, from δ 4.74 to 5.09 (28-H_2), +3.8 ppm, from δ 58.6 to 62.4 (C-28)]. Accordingly, **2** was formulated as 3-*O*-*β*-D-glucuronopyranosyl-28-*O*-tigloyl gymnemagenin.

Gymnemic acid X (**3**), amorphous powder, mp 210—212 °C, $[\alpha]_D$ +14.9° (c=2.3, MeOH), $C_{38}H_{60}O_{13}\cdot H_2O$ furnished acetic acid on alkaline hydrolysis. The ^{1}H- and ^{13}C-NMR spectra of **3** suggested that **3** was composed of **7** and one mol of acetic acid, which was identified as in the case of **1**. The ^{1}H- and ^{13}C-NMR spectra of the aglycone moiety of **3** were almost superimposable on those of **1** and **2**. The acetyl group should therefore be present at C-28-OH. So, **3** was formulated as 3-*O*-*β*-D-glucuronopyranosyl-28-*O*-acetyl gymnemagenin.

Gymnemic acid XI (**4**), amorphous powder, mp 190—192 °C, $[\alpha]_D$ +1.7° (c=5.3, MeOH), $C_{46}H_{70}O_{14}$ {negative FAB-MS m/z: 845 $[M-H]^-$} furnished tiglic acid and **7** on mild alkaline hydrolysis. The ^{1}H- and ^{13}C-NMR spectra of **4** suggested that **4** was composed of two mol of



	R_1	R_2	R_3
1	-H	-mba	-H
2	-H	-tga	-H
3	-H	-Ac	-H
4	-tga	-tga	-H
5	-tga	-Ac	-glc
7	-H	-H	-H
8	-tga	-Ac	-H
9	-mba	-Ac	-H
10	-mba	-H	-H
11	-tga	-H	-H

Chart 1

TABLE I. ^{1}H-NMR Spectral Data for **1**—**8** (in Pyridine-d_5, 400 MHz, δ-Values)

	1	**2**	**3**	**5**	**4**	**6**	**7**	**8**
H-16	5.06 dd (12.4, 5.0)	5.08 dd (12.4, 5.0)	5.05 dd (12.4, 5.0)	5.13 dd (12.4, 5.0)	5.15 dd (12.4, 5.0)	5.08 dd (11.5, 5.0)	5.07 dd (11.5, 5.0)	5.14 dd (11.5, 5.0)
H-21	4.04 d (J=10.2)	4.07 d (J=10.3)	4.03 d (J=10.0)	5.78 d (J=10.2)	5.80 d (J=10.2)	4.08 d (J=10.5)	4.07 d (J=10.5)	5.78 d (J=10.5)
H-22	4.46 d (J=10.2)	4.51 d (J=10.3)	4.45 d (J=10.0)	4.60 d (J=10.2)	4.65 d (J=10.2)	4.90 d (J=10.5)	4.89 d (J=10.5)	4.59 d (J=10.5)
H-23	3.70 d 4.35 d (J=11.0)	3.70 d 4.37 d (J=11.0)	3.70 d 4.36 d (J=10.3)	3.71 d 4.34 d (J=11.0)	3.70 d 4.35 d (J=10.2)	3.72 d 4.18 d (J=10.5)	3.71 d 4.37 d (J=11.0)	3.71 d 4.37 d (J=10.5)
H-28	4.67 d 5.09 d (J=11.0)	4.66 d 5.09 d (J=11.0)	4.62 d 5.15 d (J=11.0)	4.63 d 5.05 d (J=11.0)	4.65 d 5.20 d (J=11.0)	4.12 d 4.75 d (J=10.5)	4.09 d 4.74 d (J=10.5)	4.65 d 5.08 d (J=11.0)
Tigloyl or 2-methylbutyroyl					H_{21}			
	0.83 t (J=7.0) 1.07 d (J=7.0) 1.44 q (J=7.0) 1.67 q (J=7.0) 2.39 sex (J=7.0)	1.51 d (J=7.0) 1.78 s 6.97 q (J=7.0)		1.61 d (J=7.0) 1.88 s 7.05 q (J=7.0)	1.61 d (J=7.0) 1.88 s 7.05 q (J=7.0) H_{28} 1.59 q (J=7.0) 1.82 s 6.97 q (J=7.0)			1.64 d (J=6.5) 1.91 s 7.07 q (J=6.5)
Acetyl			1.94 s	2.02 s				2.06 s
Anomeric H	5.24 d (J=8.0)	5.26 d (J=8.0)	5.25 d (J=7.3)	5.23 d (J=7.3) 5.31 d (J=7.3)	5.26 d (J=7.4)		5.28 d (J=8.0)	5.29 d (J=7.5)

TABLE II. ^{13}C-NMR Spectral Data of Aglycone Moieties for **1**—**8** (in Pyridine-d_5, 100 MHz, δ-Values)

	1	**2**	**3**	**5**	**4**	**6**	**7**	**8**
C- 1	38.9	38.6	38.8	38.8	38.8	38.9	38.8	39.0
C- 2	26.1	25.8	26.1	26.1	26.1	27.7	26.1	26.3
C- 3	81.6	81.6	81.9	81.7	81.9	73.8	82.0	82.3
C- 4	43.5	43.4	43.5	43.5	43.6	42.9	43.6	43.7
C- 5	47.4	47.3	47.4	47.2	47.4	48.5	47.5	47.5
C- 6	18.1	18.1	18.1	18.0	18.0	18.5	18.1	18.2
C- 7	32.6	32.3	32.6	32.5	32.5	32.7	32.7	32.7
C- 8	40.3	40.3	40.3	40.2	40.3	40.3	40.3	40.5
C- 9	47.2	47.2	47.2	47.1	47.2	47.3	47.3	47.3
C-10	36.6	36.6	36.6	36.5	36.7	37.0	36.8	36.9
C-11	24.0	23.7	24.0	24.0	24.0	24.0	24.0	24.2
C-12	124.0	124.0	124.2	124.6	124.2	123.9	123.9	124.8
C-13	141.9	141.9	141.9	141.2	141.4	142.8	142.8	141.5
C-14	42.5	42.5	42.8	42.4	42.6	42.7	42.7	42.8
C-15	36.2	36.0	36.1	36.3	36.4	36.0	35.8	36.4
C-16	67.8	67.7	67.7	67.4	67.6	67.8	68.4	67.7
C-17	45.5	45.6	45.3	46.7	46.1	46.6	46.6	45.9
C-18	42.7	43.0	42.5	42.6	42.7	42.2	42.2	42.7
C-19	46.2	46.2	46.2	45.7	45.8	46.7	46.7	45.8
C-20	36.8	36.7	36.6	36.6	36.7	36.8	36.7	36.9
C-21	76.9	76.7	76.8	79.0	78.9	77.3	77.4	79.1
C-22	74.0	73.9	73.8	71.6	71.6	73.3	73.5	71.7
C-23	64.4	64.0	64.4	63.8	64.3	68.3	64.4	64.4
C-24	13.6	13.6	13.6	13.6	13.7	13.1	13.7	13.9
C-25	16.3	16.2	16.3	16.2	16.3	16.1	16.3	16.5
C-26	17.2	17.2	17.1	17.0	17.2	17.1	17.1	17.3
C-27	27.5	27.5	27.5	27.4	27.5	27.4	27.5	27.7
C-28	62.3	62.4	62.6	62.4	62.5	58.6	58.6	62.6
C-29	30,2	30.2	30.2	29.3	29.5	30.4	30.4	29.6
C-30	19.0	18.9	19.0	19.2	19.8	19.1	19.1	20.0

TABLE III. ^{13}C-NMR Spectral Data of Sugar and Acyl Moieties for **1**—**5**, **7** and **8** (in Pyridine-d_5, 100 MHz, δ-Values)

	1	**2**	**3**	**5**	**4**		**7**	**8**
3-*O*-GlcA								
C-1	106.3	106.3	106.3	105.9[a]	106.4		106.2	106.3
C-2	75.4	75.1	75.5	74.2	75.5		75.5	75.5
C-3	78.1	78.0	78.1	87.5	78.1		78.2	78.1
C-4	73.4	73.2	73.4	71.7[b]	73.5		73.5	73.5
C-5	77.9	78.0	77.9	77.3	78.0		77.9	77.8
C-6	173.0	173.0	172.9	172.9	172.9		173.0	173.1
Glc (1→3)								
C-1				105.8[a]				
C-2				75.6				
C-3				78.2				
C-4				71.6[b]				
C-5				78.8				
C-6				62.3				
21-*O*- or 28-*O*-Tigloyl or 2-methylbutyroyl					C_{21}	C_{28}		
C-1	176.2	168.0		168.5	168.2	168.0		168.5
C-2	41.8	129.2		129.7	129.6	129.2		129.7
C-3	27.1	136.9		137.3	137.4	137.0		137.3
C-4	11.9	14.1		14.6	14.3	14.3		14.6
C-5	17.0	12.2		12.7	12.6	12.4		12.7
28-*O*-Acetyl								
C-1			20.7	20.7				21.1
C-2			171.0	170.9				171.4

a, b) May be interchanged within the same column.

tiglic acid and **7**. In a comparison of the ^{1}H-NMR spectra of **4** and **7**, two acylation shifts were observed for the signals due to the 21-H (+0.91 ppm, from δ 4.89 to 5.80) and 28-H_2 (+0.56 ppm, from δ 4.09 to 4.65 and +0.46 ppm, from δ 4.74 to 5.20). Therefore, in **4**, the *O*-21 and

O-28 of **4** must be acylated. Furthermore aglycone carbon signals in the ^{13}C-NMR spectrum of **4** were in good agreement with those of gymnemic acid I (**8**), one of the main antisweet substances contained in this plant.[3] Hence, **4** was formulated as 3-*O*-*β*-D-glucuronopyranosyl-21, 28-bis-*O*-tigloyl gymnemagenin.

Gymnemic acid XII (**5**), amorphous powder, mp 209—211 °C, $[\alpha]_D$ +11.7° (*c*=3.6, MeOH), $C_{49}H_{76}O_{19}$ [negative FAB-MS *m/z*: 967 $(M-H)^-$], gave **6** as the aglycone with D-glucose and D-glcA in the ratio 1:1 as sugar components on acid hydrolysis. The ^{1}H- and ^{13}C-NMR spectra of **5** indicated the presence of one *β*-glucopyranosyl unit [anomeric H: *δ* 5.31 (d, *J*=7.3 Hz), anomeric C: *δ* 105.8], one *β*-glucuronopyranosyl unit [anomeric H: *δ* 5.23 (d, *J*=7.3 Hz), anomeric C: *δ* 105.9], one acetyl unit [*δ* 2.02s, 20.7, 170.9] and one tigloyl unit [*δ* 12.7, 14.6, 129.7, 137.3, 168.5]. On alkaline hydrolysis, **5** furnished acetic acid and 2-methylbutyric acid as acyl components. In the ^{1}H-NMR spectrum of **5**, two acylation shifts (**5** *vs.* **7**) were observed for the 21-H (+0.89 ppm, from *δ* 4.89 to 5.78) and 28-H_2 (+0.54 ppm, from *δ* 4.09 to 4.63 and +0.31 ppm, from *δ* 4.74 to 5.05), as in **4**. Therefore, in **5**, *O*-21 and *O*-28 should be acylated. A long-range selective proton decoupling (LSPD)[6] experiment revealed that 21-H (*δ* 5.78) coupled to carbonyl carbon of tiglic acid (*δ* 168.5) and 28-H_2 (*δ* 4.63, 5.05) coupled to carbonyl carbon of acetic acid (*δ* 170.9), establishing the existence of a tigloyl group at C_{21} and an acetyl group at C_{28}. Cellulase treatment of **5** furnished **8** as a prosapogenin. Comparison of the ^{13}C-NMR spectrum of **5** with that of **8** disclosed C_3 (+9.4 ppm, from *δ* 78.1 to 87.5) of glcA as a glycosylation site in the former.[7] Hence, **5** was formulated as 3-*O*-*β*-D-glucopyranosyl(1→3)-*O*-*β*-D-glucuronopyranosyl-21-*O*-tigloyl-28-*O*-acetyl gymnemagenin.

Application of a 0.5 mM solution of each of **1**—**3** led to complete suppression of the sweet taste of 0.2 M sucrose. This is similar to the activity of gymnemic acid III (**10**) and IV (**11**)[3] having one acyl moiety at C_{21}. Compounds **4** and **5** suppressed the sweet taste of 0.4 M sucrose. This activity was the same as that of **8** and gymnemic acid II (**9**).[3] These results suggested that the antisweet activity of the saponins increases with increasing number of acyl groups.

Experimental

Melting points were measured with a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were taken on a JASCO DIP-140 digital polarimeter. Infrared (IR) spectra were taken on a Hitachi IR-27G. NMR spectra were recorded on a JEOL GX-400 spectrometer in C_5D_5N solution using tetramethylsilane (TMS) as an internal standard. NMR experiments included ^{1}H–^{1}H-correlation spectroscopy (COSY), ^{13}C–^{1}H-COSY, DEPT, LSPD and heteronuclear multiple bond connectivity (HMBC) (512×1024 data matrix size, 128 scans, recycle delay=1.16 s). Coupling constants (*J* values) are given in hertz (Hz). The FAB-MS (Xe gun, 10 kV, *m*-nitrobenzyl alcohol as the matrix) were measured on a JEOL JMS-PX303 mass spectrometer. For column chromatography, Kiesel gel 60 (230—400 mesh, Merck), and for thin layer chromatography (TLC), Silica gel 60F-254 (Merck) were used. HPLC were carried out with a Waters ALC/GPC 244 instrument.

Isolation of Saponins The dried leaves (6 kg) of *Glymnema sylvestre*, supplied by Teikoku Seiyaku Co., were extracted with 60% EtOH at 60 °C for 2 weeks. The EtOH extract obtained after evaporation of the solvent *in vacuo* was passed through an Amberlite XAD-2 column then a Toyopearl HW-40 column to give the crude saponin (150 g). It was further chromatographed on Servachrome XAD-2 (40—70% MeOH) to give four fractions, fr. 1—4 in order of elution. Fraction 1 was repeatedly chromatographed on silica gel with AcOEt:MeOH:H_2O (10:3:1), $CHCl_3$:MeOH:H_2O (65:35:10, lower layer) and then purified by HPLC (Nomura, ODS, 25% CH_3CN) to afford gymnemic acid X (**3**, 30 mg). Fr. 2 and 3 were each subjected to chromatography on silica gel column with CH_3Cl:MeOH:H_2O (65:35:10, lower phase) and purified by HPLC (Nomura, ODS, 23—40% CH_3CN) to isolate gymnemic acids VIII (**1**, 80 mg) and IX (**2**, 30 mg) from fr. 2, and gymnemic acids XI (**4**, 180 mg) and XII (**5**, 50 mg) from fr. 3.

Gymnemic Acid VIII (1) An amorphous white powder, mp 185—187 °C, $[\alpha]_D$ +21.5° (*c*=3.5, MeOH). IR (KBr) cm^{-1}: 3400 (OH), 1720 (C=O), 1600 (C=C), 1040 (OH). Negative FAB-MS *m/z*:765 $[(M-H)^-]$. *Anal.* Calcd for $C_{41}H_{66}O_{13}\cdot H_2O$: C, 62.73; H, 8.73. Found: C, 62.46; H, 8.41. For NMR data, see Tables I, II and III.

Gymnemic Acid IX (2) An amorphous white powder, mp 194—196 °C $[\alpha]_D$ +7.6° (*c*=1.8, MeOH). IR (KBr) cm^{-1}: 3380 (OH), 1705 (C=O), 1605 (C=C), 1060 (OH). Negative FAB-MS *m/z*: 763 $[(M-H)^-]$. *Anal.* Calcd for $C_{41}H_{64}O_{13}\cdot 2H_2O$: C, 61.48; H, 8.56. Found: C, 61.55; H, 8.70. For NMR data, see Tables I, II and III.

Gymnemic Acid X (3) An amorphous white powder, mp 210—212 °C, $[\alpha]_D$ +14.9° (*c*=2.3, MeOH). IR (KBr) cm^{-1}: 3400 (OH), 1740 (C=O), 1610 (C=C), 1040 (OH). Negative FAB-MS *m/z*: 723 $[(M-H)^-]$. *Anal.* Calcd for $C_{38}H_{60}O_{13}\cdot H_2O$: C, 61.44; H, 8.41. Found: C, 61.22; H, 8.89. For NMR data, see Tables I, II and III.

Gymnemic Acid XI (4) An amorphous white powder, mp 190—192 °C, $[\alpha]_D$ +1.7° (*c*=5.3, MeOH). IR (KBr) cm^{-1}: 3400 (OH), 1740 (C=O), 1600 (C=C), 1040 (OH). Negative FAB-MS *m/z*: 845 $[(M-H)^-]$. *Anal.* Calcd for $C_{46}H_{70}O_{14}\cdot 2H_2O$: C, 62.57; H, 8.45. Found: C, 62.14; H, 8.22. For NMR data, see Tables I, II and III.

Gymnemic Acid XII (5) An amorphous powder, mp 209—211 °C, $[\alpha]_D$ +11.7° (*c*=3.6, MeOH). IR (KBr) cm^{-1}: 3400 (OH), 1740 (C=O), 1720 (C=O), 1610 (C=C), 1040 (OH). Negative FAB-MS *m/z*: 967 $[(M-H)^-]$. *Anal.* Calcd for $C_{49}H_{76}O_{19}\cdot 3H_2O$: C, 57.52; H, 8.08. Found: C, 57.84; H, 7.73. For NMR data, see Tables I, II and III.

Acid Hydrolysis of Gymnemic Acid XII (5) A solution of **5** (30 mg) in 5% H_2SO_4 in 50% EtOH (2 ml) was heated at 100 °C for 3 h. The reaction mixture was extracted with ether. The organic layer was subjected to silica gel column chromatography with CH_3Cl:MeOH=50:1 to give gymnemagenin (**6**, 8 mg). **6**: mp 313—314 °C, $[\alpha]_D$ +53.5° (*c*=0.8, MeOH), positive FAB-MS *m/z* 529 $[(M+Na)^+]$. For NMR data, see Tables I and II. The aqueous layer was neutralized with Amberlite IR-45 and evaported *in vacuo* to dryness. The sugar was checked by using refraction index (RI) detection (Waters 410) and chiral detection (Shodex OR-1) in HPLC (Shodex RSpak DC-613, 4.8 mm i.d.×15 cm, 75% CH_3CN, 1 ml/min, 70 °C) by comparison with authentic sugars as standards. These sugars gave the following peaks. t_R: D(+)-glucuronolactone; 2.4 min, D(+)-glc; 7.38 min.

Acid Hydrolysis of Gymnemic Acids VIII—XI (1—4) Acid hydrolysis of **1**—**4** (each 5 mg) was carried out in the same was as for **5** and the products were analyzed by TLC (CH_3Cl:MeOH=10:1) and HPLC (C_8, 40% CH_3CN) with authentic gymnemagenin as a standard. The aqueous layer was treated as described in the case of **5**. t_R: D(+)-glucuronolactone; 2.4 min.

Alkaline Hydrolysis of Gymnemic Acid VIII (1) Gymnemic acid VIII (**1**) (60 mg) was dissolved in 50% 1,4-dioxane (5 ml) and 10% KOH (1 ml), and heated at 37 °C for 1 h. The reaction mixture was adjusted to pH 4.0 with 5% HCl, and extracted with ethylenechloride to give 2-methylbutyric acid (8 mg), oil, $[\alpha]_D$ +16.3° (*c*=0.3, 50% MeOH) [lit. $[\alpha]_D$ +19.2° (*c*=1.0, EtOH)]. Its *p*-nitrobenzyl ester was compared by HPLC (YMC-pack C_8, 6 mm i.d.×15 cm, 60% MeOH, 1 ml/min) with an authentic sample. t_R: 2-methylbutyric acid; 16 min. The aqueous layer was further extracted with *n*-BuOH to give the prosapogenin (**7**, 12 mg), mp 230—231 °C, $[\alpha]_D$ +8.4° (*c*=1.0, MeOH), positive FAB-MS *m/z*: 705 $[(M+Na)^+]$. For NMR data, see Tables I, II and III.

Alkaline Hydrolysis of Gymnemic Acid IX—XI (2—4) Alkaline hydrolysis of **2**—**4** (each 5 mg) was carried out in the same way as for **1** to give the acyl component from the organic layer. The organic acid peaks were as follows: acetic acid, 7.8 min; tiglic acid, 14 min; 2-methylbutyric acid, 16 min. Each aqueous layer gave **7**, which was analyzed by TLC (CH_3Cl:MeOH:H_2O=25:4:0.5) and HPLC (C_8, 25% CH_3CN).

Enzymatic Hydrolysis of Gymnemic Acid XII (5) Gymnemic acid XII (**5**) (35 mg) was dissolved in EtOH–H_2O (1:9) and 0.01 M NaH_2PO_4 buffer (pH 4.0), 2 ml each, and incubated with cellulase (30 mg, Tanabe) for 8 h at 37 °C, then worked up as usual. The crude sapogenin was subjected to HPLC (ODS, 35% CH_3CN) to give gymnemic acid I (**8**,

5 mg).

Bioassay of Antisweet Activity The antisweet activity of 0.5 mM solution of **1**—**5** was tested on three volunteers. Each participant held the tests solutions in the mouth for 3 min, spat, rinsed with distilled water and tasted a sucrose solution (0.2 and 0.4 M).

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