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Antisweet Natural Products. IX.[1] Structures of Gymnemic Acids XV—XVIII from *Gymnema sylvestre* R. Br. V[2]

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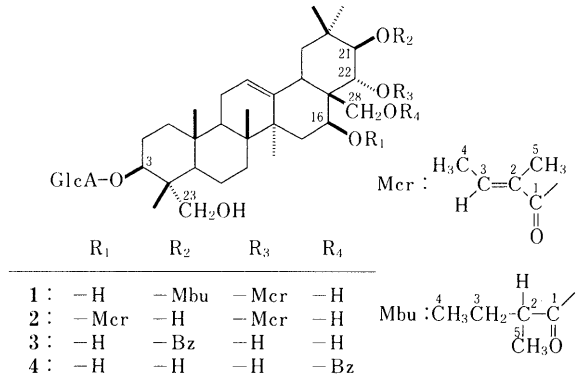
Systematic separation of the saponin fraction of the leaves of *Gymnema sylvestre* has led to the isolation of four new saponins, gymnemic acids XV—XVIII, as antisweet substances. The structures were elucidated by spectral and chemical studies. Gymnemic acids XV—XVIII, are 21-*O*-2-methylbutyryl-22-*O*-2-methylcrotonoyl, 16,22-*O*-bis-2-methylcrotonoyl, 21-*O*-benzoyl, and 28-*O*-benzoyl gymnemagenin 3-*O*-gluculonides, respectively. Gymnemic acids VIII and IX that we had named in the previous paper were renamed gymnemic acids XV and XVI, respectively.

Keywords *Gymnema sylvestre*; Asclepiadaceae; gymnemic acid; gymnemagenin; antisweet substance; name change

In the preceding paper[2] of this series, we reported the isolation and structure determination of five antisweet principles named gymnemic acids VIII—XII, from the leaves of *Gymnema sylvestre* (Asclepiadaceae). Tsuda *et al.*[3] also then reported compounds designated gymnemic acids VIII and IX from the title plant. We here change the name of our gymnemic acid VIII to gymnemic acid XIII, and gymnemic acid IX to XIV. Our continuing study of *G. sylvestre* has led to the isolation of four new compounds, named gymnemic acids XV—XVIII (**1**—**4**). All of them possess one or two acyl groups (*i.e.*, 2-methylbutyryl, 2-methylcrotonoyl and benzoyl groups) in the aglycone and show strong antisweet activity. This paper describes 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 repeatedly separated by ordinary-phase SiO_2 and reversed-phase SiO_2 (octadecyl silica (ODS)) column chromatographies to furnish four new saponins, gymnemic acids XV (**1**) and XVI (**2**), XVII (**3**) and XVIII (**4**).

Gymnemic acid XV (**1**), an amorphous powder, exhibited a prominent quasi-molecular peak at *m/z* 847 $[M(C_{46}H_{72}O_{14})-H]^-$ and fragment peaks at *m/z* 747 $[M-H-C_5H_8O_2]^-$, *m/z* 745 $[M-H-C_5H_{10}O_2]^-$ and *m/z* 645 $[M-H-C_5H_8O_2-C_5H_{10}O_2]^-$ in the negative FAB-MS. Mild acid hydrolysis of **1** furnished glucuronic acid and gymnemagenin (**5**).[2] The ^1H- and ^{13}C-NMR spectra of **1** suggested that **1** was composed of 1 mol each of 2-methylbutyric acid, 2-methylcrotonic acid, glucuronic acid and **5** (Tables I and II). Alkaline treatment of **1** gave 3-*O*-β-D-glucuronopyranosyl gymnemagenin (**6**)[2] as a prosapogenin, and 2-methylbutyric acid and 2-methylcrotonic acid. These acids were identified as their *p*-nitrobenzyl esters by HPLC analysis. A ^1H-NMR spectral comparison of **1** with **6** revealed acylation shifts[4] at the H-21 and H-22 signals [H-21, +1.67 ppm (δ 4.07→5.74); H-22, +1.39 ppm (δ 4.89→6.28)], indicating that the C-21-OH and C-22-OH should be acylated. In the heteronuclear multiple bond connectivity (HMBC) experiment on **1**, cross peaks were observed between H-21 (δ 5.74) and the carbonyl (δ 176.2) of the 2-methylbutyryl group, and H-22 (δ 6.28) and that of 2-methylcrotonoyl group (δ 167.4), indicating the 2-methylbutyryl group to be attached at C-21-OH, and the 2-methylcrotonyl group at C-22-OH. Therefore, **1** was formulated as 3-*O*-β-D-glucuronopyranosyl-21-*O*-2-methylbutyryl-22-*O*-2-methylcrotonoyl gymnemagenin.



	R_1	R_2	R_3	R_4
1 :	−H	−Mbu	−Mcr	−H
2 :	−Mcr	−H	−Mcr	−H
3 :	−H	−Bz	−H	−H
4 :	−H	−H	−H	−Bz
6 :	−H	−H	−H	−H

Fig. 1

Gymnemic acid XVI (**2**), $C_{46}H_{70}O_{14}$ was obtained as colorless needles. The FAB-MS of **2** showed a deprotonated molecular ion $[M-H]^-$ at *m/z* 845, *i.e.*, 2 mass units less than that of **1**, and fragment ions at *m/z* 745 $[M-H-C_5H_8O_2]^-$, and *m/z* 645 $[M-H-2C_5H_8O_2]^-$ corresponding to successive loss of 2-methylcrotonic acid. The ^1H- and ^{13}C-NMR spectra of **2** suggested that **2** was composed of 1 mol each of glucuronic acid and **5**, and 2 mol of 2-methylcrotonic acid. Alkaline treatment of **2** gave **6** as a prosapogenin, and 2-methylcrotonic acid. In the ^1H-NMR spectrum of **2**, the H-16 and H-22 signals were shifted downfield by 1.36 ppm (δ 5.07→6.43), and 0.95 ppm (δ 4.89→5.84), respectively, compared with those of **6**, confirming the presence of 2-methylcrotonoyl groups at C-16 and C-22. Moreover, one proton signal of H_2-28 was shifted upfield by 0.69 ppm (δ 4.74→4.05), compared with that of **6** owing to the shielding effect of the carbonyl group at C-16. Accordingly, **2** was formulated as 3-*O*-β-D-glucuronopyranosyl-16,22-*O*-bis-2-methylcrotonoyl gymnemagenin.

Gymnemic acid XVII (**3**), $C_{43}H_{62}O_{13}$ was obtained as colorless needles and the relative molecular mass (M_r) was proposed to be 789, as the deprotonated molecular ion was apparent at *m/z* 785 in the negative FAB-MS. The ^1H- and

TABLE I. ^{1}H-NMR Spectral Data for **1**—**4** and **6** (in Pyridine-d_5, 400 MHz)

H	1	2	3
H-16	5.00 (dd, 11.3, 5.2)	6.43 (dd, 11.4, 5.7)	5.16 (dd, 11.5, 5.0)
H-21	5.74 (d, 11.3)	4.34 (d, 10.8)	5.98 (d, 10.5)
H-22	6.28 (d, 11.3)	5.84 (d, 10.8)	5.15 (d, 10.5)
H_2-23	3.71 (d, 10.8)	3.70 (d, 10.8)	3.74 (d, 10.5)
	4.36 (d, 10.8)	4.36 (d, 10.8)	4.35 (d, 10.5)
H_2-28	4.01 (d, 11.0)	4.05 (2H, s)	4.08 (d, 10.0)
	4.21 (d, 11.0)		4.75 (d, 10.0)
21-*O*-Mbu or 16-*O*-Mcr or 21-*O*-Bz			
H-2	2.42 (tq, 7.0, 7.0)		8.28 (dd, 8.0, 1.0)
H-3	1.43 (m)	6.96 (q, 7.0)	7.44 (dt, 1.0, 8.0)
H-3	1.75 (m)		
H-4	0.89 (t, 7.0)	1.64 (d, 7.0)	7.56 (tt, 8.0, 1.0)
H-5	1.13 (d, 7.0)	1.84 (s)	
22-*O*-Mcr			
H-3	7.12 (q, 7.0)	6.79 (q, 7.0)	
H-4	1.53 (d, 7.0)	1.41 (d, 7.0)	
H-5	1.89 (s)	1.77 (s)	
3-*O*-GlcA			
H-1	5.25 (d, 8.0)	5.25 (d, 8.0)	5.30 (d, 8.0)

H	4	6
H-16	5.13 (dd, 12.4, 5.0)	5.07 (dd, 11.0, 5.0)
H-21	4.10 (d, 10.2)	4.07 (d, 10.5)
H-22	4.66 (d, 10.2)	4.89 (d, 10.5)
H_2-23	3.70 (d, 11.0)	3.71 (d, 11.0)
	4.39 (d, 11.0)	4.37 (d, 11.0)
H_2-28	4.92 (d, 11.0)	4.09 (d, 10.5)
	5.40 (d, 11.0)	4.74 (d, 10.5)
28-*O*-Bz		
H-2	8.18 (dd, 8.0, 1.0)	
H-3	7.36 (dt, 1.0, 8.0)	
H-4	7.50 (tt, 8.0, 1.0)	
3-*O*-GlcA		
H-1	5.27 (d, 8.0)	5.28 (d, 8.0)

Mbu = 2-methylbutyryl, Mcr = 2-methylcrotonoyl, Bz = benzoyl.

TABLE II. ^{13}C-NMR Spectral Data for **1**—**4** and **6** (in Pyridine-d_5, 100 MHz)

C	1	2	3	4	6
1	38.8	38.8	38.8	38.8	38.8
2	26.1	26.0	26.1	26.0	26.1
3	81.7	81.8	81.7	81.8	82.0
4	43.5	43.5	43.5	43.5	43.6
5	47.4	47.4	47.4	47.4	47.5
6	18.1	18.0	18.1	18.1	18.1
7	32.5	32.5	32.6	32.6	32.7
8	40.2	40.4	40.3	40.3	40.3
9	47.1	47.1	47.2	47.2	47.3
10	36.7	36.6	36.7	36.6	36.8
11	23.9	23.9	24.0	24.0	24.0
12	124.2	124.4	124.2	124.2	123.9
13	141.5	141.1	142.2	141.8	142.8
14	42.7	42.8	42.6	42.6	42.7
15	36.6	33.9	36.3	36.1	35.8
16	66.9	68.9	68.0	67.8	68.4
17	48.1	47.9	47.2	45.8	46.6
18	42.7	42.6	42.0	43.1	42.2
19	45.8	46.2	46.2	46.2	46.7
20	36.8	37.3	36.7	36.8	36.7
21	76.1	74.1	80.7	76.8	77.4
22	74.4	75.6	71.2	74.1	73.5
23	64.4	64.4	64.4	64.4	64.4
24	13.6	13.6	13.6	13.6	13.7
25	16.2	16.2	16.3	16.2	16.3
26	16.9	17.0	17.0	17.2	17.1
27	27.4	27.5	27.4	27.5	27.5
28	60.1	59.0	58.1	63.4	58.6
29	29.2	29.9	29.5	30.2	30.4
30	19.8	18.9	19.9	19.1	19.1
3-*O*-GlcA					
1	106.1	106.1	106.1	106.1	106.2
2	75.4	75.4	75.4	75.4	75.5
3	78.2	78.1	78.1	78.1	78.2
4	73.4	73.4	73.4	73.5	73.5
5	77.8	77.8	77.8	77.8	77.9
6	172.9	172.9	173.0	173.0	173.0
21-*O*-Mbu or 16-*O*-Mcr or 21-*O*-Bz					
1	176.2	167.0	131.8		
2	41.6	129.6	130.0		
3	27.0	137.4	128.8		
4	11.8	14.3	133.1		
5	16.6	12.2			
α			167.0		
22-*O*-Mcr or 28-*O*-Bz					
1	167.4	170.1		131.1	
2	129.0	128.7		129.6	
3	138.4	138.0		128.9	
4	14.2	14.0		133.2	
5	12.3	12.2			
α				166.7	

Mbu = 2-methylbutyryl, Mcr = 2-methylcrotonoyl, Bz = benzoyl.

^{13}C-NMR spectra of **3** suggested that **3** was composed of 1 mol each of glucuronic acid, **5** and benzoic acid. Alkaline treatment of **3** gave **6** as a prosapogenin, and benzoic acid, which was identified by HPLC analysis. In comparing the ^{1}H- and ^{13}C-NMR spectra of **3** and **6**, acylation shifts were observed at C-21 [+1.91 ppm (H-21), +3.3 ppm (C-21)] and C-22 [+0.26 ppm (H-22), −2.3 ppm (C-22)], establishing the existence of the benzoyl group at the C-21 position. Hence, **3** was formulated as 3-*O*-β-D-glucuronopyranosyl-21-*O*-benzoyl gymnemagenin.

Gymnemic acid XVIII (**4**), $C_{43}H_{62}O_{13}$ was obtained as colorless needles. The FAB-MS of **4** showed a deprotonated molecular ion $[M-H]^-$ at *m/z* 785, as did **3**, and fragment ions at *m/z* 663 ($[M-H-C_7H_6O_2]^-$ loss of a benzoic acid), indicating that **4** is isomeric to **3**. Alkaline hydrolysis of **4** afforded benzoic acid and **6**. A ^{1}H- and ^{13}C-NMR spectral comparison of **4** with **6** revealed acylation shifts at C-28 [+0.83 and +0.66 ppm (H_2-28), +4.8 ppm (C-28)], indicating that the C-28-OH should be acylated. Hence, **4** was formulated as 3-*O*-β-D-glucuronopyranosyl-28-*O*-benzoyl gymnemagenin.

Gymnemic acids XVII and XVIII are the first examples of antisweet saponins acylated with a benzoyl moiety from *G. sylvestre*. Although Imoto *et al.*[5] reported that a saponin in *G. sylvestre* possessed benzoic acid as an acyl component, based on MS analysis of the saponin mixture, they have not isolated it.

A 0.5 mM solution of each of **1**—**4** completely suppressed the sweet taste of 0.4 M sucrose, showing the same activity as the main active substances, gymnemic acids I and II.[6]

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. IR spectra were taken on a Hitachi IR-27G as KBr disks. 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 ^1H–^1H-COSY, ^{13}C–^1H-COSY and HMBC (512 × 1024 data matrix size, 128 scans, recycle delay time = 1.16 s). Coupling constants (*J* values) are given in hertz (Hz). The FAB-MS (Xe gun, 10 kV, triethylene glycol as the matrix) were measured on a JEOL JMS-AX500 mass spectrometer. For column chromatography, Kiesel gel 60 (230-400 mesh, Merck), and for TLC, Silica gel 60F-254 (Merck) were used. HPLC was carried out with a Waters ALC/GPC 244 (column Develosil ODS, Develosil PhA) instrument.

Isolation of Saponins The dried leaves (6.0 kg) of *Gymnema 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 (MeOH) column and a Toyopearl HW-40 (MeOH) column to give the crude saponin (150 g). It was further chromatographed on Servachrome XAD-2 (40—70% MeOH) to give four fractions, frs. 1—4, in order of elution. Fraction 2 was repeatedly chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower layer) and then purified by HPLC (Develosil ODS, 23—28% CH_3CN, Develosil PhA, 20% CH_3CN) to afford gymnemic acids XVII (**3**, 30 mg) and XVIII (**4**, 50 mg). Fraction 3 was subjected to chromatography on a silica gel column with $CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower phase) and purified by HPLC (Develosil ODS, 25—30% CH_3CN) to give gymnemic acids XV (**1**, 30 mg) and XVI (**2**, 30 mg).

Gymnemic Acid XV (**1**): An amorphous white powder, $[\alpha]_D$ +7.2° (*c* = 1.52, MeOH). IR ν_{max} cm^{-1}: 3400 (OH), 1740, 1720 (C=O), 1610 (C=C), 1040 (OH). *Anal.* Calcd for $C_{46}H_{72}O_{14}\cdot 2H_2O$: C, 62.42; H, 8.65. Found: C, 62.42; H, 8.44. Negative FAB-MS *m/z*: 847 $[M(C_{46}H_{72}O_{14})-H]^-$, 747 $[M-H-C_5H_8O_2]^-$, 745 $[M-H-C_5H_{10}O_2]^-$, 645 $[M-H-C_5H_8O_2-C_5H_{10}O_2]^-$. For NMR data, see Tables I and II.

Gymnemic Acid XVI (**2**): mp 203—205 °C (colorless needles from MeOH), $[\alpha]_D$ −6.8° (*c* = 2.96, MeOH). IR ν_{max} cm^{-1}: 3380 (OH), 1740, 1720 (C=O), 1605 (C=C), 1060 (OH). *Anal.* Calcd for $C_{46}H_{70}O_{14}\cdot 2H_2O$: C, 62.56; H, 8.45. Found: C, 62.26; H, 8.18. Negative FAB-MS *m/z*: 845 $[M(C_{46}H_{70}O_{14})-H]^-$, 745 $[M-H-C_5H_8O_2]^-$, 645 $[M-H-2C_5H_8O_2]^-$. For NMR data, see Tables I and II.

Gymnemic Acid XVII (**3**): mp 211—213 °C (colorless needles from MeOH), $[\alpha]_D$ +7.1° (*c* = 2.96, MeOH). IR ν_{max} cm^{-1}: 3450 (OH), 1700 (C=O), 1650 (C=C), 1050 (OH). UV λ_{max}^{MeOH} nm: 230, 273. *Anal.* Calcd for $C_{43}H_{62}O_{13}\cdot H_2O$: C, 64.16; H, 8.01. Found: C, 63.94; H, 8.05. Negative FAB-MS *m/z*: 785 $[M(C_{43}H_{62}O_{13})-H]^-$, 663 $[M-H-C_7H_6O_2]^-$. For NMR data, see Tables I and II.

Gymnemic Acid XVIII (**4**): mp 201—203 °C (colorless needles from MeOH), $[\alpha]_D$ +6.4° (*c* = 1.71, MeOH). IR ν_{max} cm^{-1}: 3400 (OH), 1700 (C=O), 1650 (C=C), 1040 (OH). UV λ_{max}^{MeOH} nm: 230, 273. *Anal.* Calcd for $C_{43}H_{62}O_{13}\cdot H_2O$: C, 64.16; H, 8.01. Found: C, 64.10; H, 7.95. Negative FAB-MS *m/z*:785 $[M(C_{43}H_{62}O_{13})-H]^-$, 633 $[M-H-C_7H_6O_2]^-$. For NMR data, see Tables I and II.

Acid Hydrolysis of Gymnemic Acids XV—XVIII (1—4) A solution of each compound (2 mg) in 5% H_2SO_4 in 50% ethanol was heated in boiling water for 1 h. The reaction mixture was passed through a column of Amberlite IR-45 on Mitsubishi Daiaion HP-20. From the water eluate, D(+)-glucuronolactone was detected by using RI detection (Waters 410) and chiral detection (Shodex OR-1) in HPLC (Shodex RSpak DC-613, 75% CH_3CN, 1 ml/min, 70 °C). From the methanol eluate, gymnemagenin (**5**) was detected by HPLC (Develosil ODS, 98% CH_3CN). t_R: 2.40 min [D(+)-glucuronolactone], 7.90 min (gymnemagenin).

Alkaline Hydrolysis of Gymnemic Acids XV—XVIII (1—4) A solution of each compound (2 mg) was dissolved in 50% 1,4-dioxane (2 ml) and 10% KOH (0.2 ml), and heated at 37 °C for 1 h. The reaction mixture was passed through a column of Amberlite IR-120 on Mitsubishi Daiaion HP-20. From the methanol eluate of **1**, 3-*O*-*β*-D-glucuronopyranosyl gymnemagenin (**6**) was detected by TLC ($CHCl_3$–MeOH–H_2O = 25 : 8 : 0.5) and HPLC (YMC-pack C_8, 22% CH_3CN), and 2-methylcrotonic acid and 2-methylbutyric acid were detected as their *p*-nitrobenzyl esters by HPLC (YMC-pack C_8, 60% MeOH). From that of **2**, **6** and 2-methylcrotonic acid, and from those of **3** and **4**, **6** and benzoic acid were detected by HPLC (YMC-pack C_8, 22% CH_3CN). *Rf*: 0.46 (3-*O*-*β*-D-glucuronopyranosyl gymnemagenin). t_R: 12.10 min (3-*O*-*β*-D-glucuronopyranosyl gymnemagenin), 12.40 min (benzoic acid), 14.0 min (2-methylcrotonic acid), 16.0 min (2-methylbutyric acid).

Bioassay of Antisweet Activity The antisweet activity of 0.5 mM solution of **1**—**4** was tested on three volunteers. Each participant held the test solutions in the mouth for 3 min, spat, rinsed with distilled water and tasted a 0.4 M sucrose solution.

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