GYMNEMIC ACID V, VI AND VII FROM GUR-MA, THE LEAVES OF GYMNEMA SYLVESTRE R. BR.[1)]

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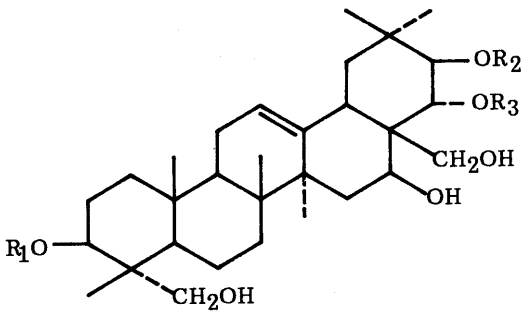
From the leaves of Gymnema sylvestre were isolated three new saponins named gymnemic acid V (**1**), VI (**2**) and VII (**3**), besides the known saponins gypenoside II, V, XLIII, XLV, XLVII LXXIV and gynosaponin TN-2. Their structures were elucidated on the basis of spectral and chemical evidence. **1** and **2** of the three new saponins had antisweet activity.

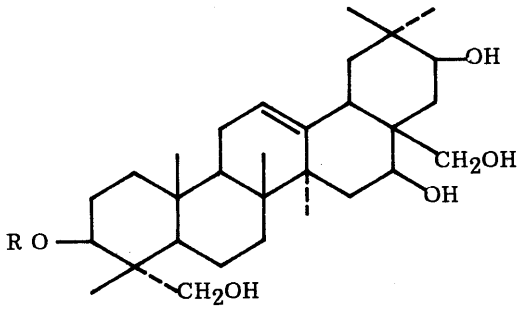
KEYWORDS Gymnema sylvestre ; Gur-ma ; Asclepiadaceae ; gymnemic acid V ; gymnemic acid VI ; gymnemic acid VII ; antisweetener ; acylated saponin ; gymnemagenin ; gymnestrogenin

Leaves of Gymnema sylvestre R. Br.(Asclepiadaceae), known as "Gur-ma" in Indian folklore, one of the traditional medicines used as a stomachic, diuretic, cough remedy, etc., have the unique property of inhibiting the ability to taste sweet substances.[2)] A number of investigators[3a-f)] isolated gymnemic acids (mixture) as active principles. However, their chemical structure had not yet become clear. Therefore, we started to isolate gymnemic acid and elucidate the structure. We have already isolated four main active principles named gymnemic acid I, II, III and IV.[4)] The present paper deals with the isolation and structure determination of the three novel saponins, named gymnemic acid V, VI and VII.

Hot water extraction of the dry leaves (1.5 kg) of G. sylvestre R.Br. followed by treatment with Amberlite XAD-2 column chromatography gave a saponin fraction. Repeated separation of the saponin fraction by reversed-phase and ordinary-phase SiO_2 column chromatography furnished three new saponins, gymnemic acid V (**1**, 80 mg), VI (**2**, 15 mg) and VII (**3**, 500 mg), besides gypenoside II, V,[5a)] XLIII, XLV,[5b)] XLVII,[5c)] LXXIV and gynosaponin TN-2.[5d)]



	R_1	R_2	R_3
1	-glcUA	-tig	-tig
2	-glcUA	-tig	-H
4	-glc(3)UA	-tig	-H
5	-glcUA	-H	-H
6	-H	-H	-H



	R_1
3	-glcUA
7	-H

Gymnemic acid V (**1**), mp 202-203°C, $C_{46}H_{70}O_{14}$, $[\alpha]_D$ +2.2°(c=3.6, MeOH) revealed a quasi-molecular peak at m/z 892(M+2Na)$^+$ in the positive FAB-MS spectrum. Mild acid hydrolysis of **1** furnished glucuronic acid (glcUA) and gymnemagenin (**6**)[6], mp 313-314°C , $[\alpha]_D$ +53.5°(c=1.9, MeOH), $C_{30}H_{50}O_6$[FAB-MS m/z 530(M+Na+H)$^+$]. Alkaline hydrolysis of **1** furnished prosapogenin (**5**), mp 230-231°C, $[\alpha]_D$ + 8.4°(c=1.8, MeOH), $C_{36}H_{58}O_{12}$[FAB-MS m/z 728(M+2Na)$^+$], which, when subjected to acid hydrolysis, provided **6** and glcUA.

By comparison of the ^{13}C-NMR spectrum of **5** with that of **6** a glycosylation shift[7] of + 8.2 ppm was observed at C_3, disclosing the site glycosylation in **5**. Hence, the structure of **5** is 3-O-β-D-glucuronopyranosyl gymnemagenin.[8] The ^{1}H-NMR spectrum of **1** suggested that **1** was composed of one mol each of **6**, glcUA and two mols of tiglic acid. Tiglic acid obtained by mild alkaline hydrolysis of **1** was identified as p-nitrobenzyl ester [HPLC, YMAC-PackC_8, 6φ, 15 cm, 60% MeOH]. In comparing the ^{1}H-NMR spectra of **1** and **5**, two acylation shifts were observed at the 21-H(+1.43 ppm) and 22-H(+1.73 ppm). Therefore, in **1**, the O-21 and O-22 of **1** must be acylated. The structure of **1** is thus 3-O-β-D-glucuronopyranosyl-21,22-bis-O-tigloyl gymnemagenin.

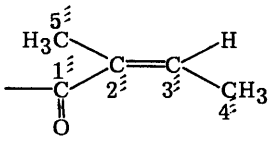
Table I. ^{1}H- and ^{13}C-NMR Data for **1-7** (C_5D_5N, Me_4SiO=0)

	H-16	H-21	H-22	H_2-23	H_2-28	Tigloyl moiety			Anomeric H	
1	5.04dd (J=11.0 5.0)	6.33d (J=11.5)	5.81d (J=11.5)	3.71,4.35d (J=11.0)	4.02,4.23d (J=11.0)	1.61d, (J=7.5) 7.01q, (J=7.5)	1.47d, (J=7.5) 7.01q (J=7.5)	1.87s, 1.81s	5.26d (J=7.5)	
2	5.12dd (J=11.0 5.0)	5.79d (J=10.5)	5.03d (J=10.5)	3.72d,* (J=10.5)	*,4.72d (J=11.0)	1.63d, (J=7.5)	1.89s,	7.02q (J=7.5)	5.26d, (J=8.0)	5.33d (J=8.0)
3	4.68dd (J=11.0 5.0)	4.14dd (J=13.0 4.5)	2.08dd (J=13.0 13.0) 3.24dd (J=13.0,4.5)	3.70,4.35d, (J=11.0)	3.74,4.41d (J=10.5)				5.25d (J=7.5)	
4	5.12dd (J=11.5 5.0)	5.79d (J=10.5)	5.03d (J=10.5)	3.71,4.35d (J=10.0)	4.08,4.70d (J=10.0)	1.64d, (J=7.5)	1.90s,	7.02q (J=7.5)	5.27d (J=8.0)	
5	5.08dd (J=11.0 5.0)	4.90d (J=10.5)	4.08d (J=10.5)	3.71,4.37d (J=11.0)	4.09,4.74d (J=10.5)				5.25d (J=7.5)	
6	5.07dd (J=11.0 5.0)	4.89d (J=10.5)	4.07d (J=10.5)	3.71,4.18d (J=11.0)	4.12,4.74d (J=10.5)					
7	4.70dd (J=11.0 5.0)	4.14d (J=13.0 4.5)	2.11dd (J=13.0 13.0) 3.27dd (J=13.0,4.5)	3.72,4.18d (J=10.0)	3.78,4.41d (J=10.0)					

		1	**2**	**3**	**4**	**5**	**6**	**7**
Aglycone moiety	C-2	26.1	26.1	26.2	26.1	26.1	27.7	27.7
	3	82.0	81.9	82.1	81.9	82.0	73.8	73.4
	15	36.7	36.3	36.9	36.3	35.8	36.0	36.8
	16	67.1	68.0	67.8	68.1	68.4	67.8	67.7
	17	48.1	47.2	43.7	47.2	46.6	46.6	43.7
	20	36.7	36.7	36.9	36.8	36.7	36.8	37.0
	21	76.6	79.7	72.9	79.7	77.4	77.3	72.7
	22	74.6	71.2	35.1	71.3	73.5	73.3	35.1
	23	64.4	64.0	64.5	64.4	64.4	68.3	67.9
	24	13.7	13.6	13.7	13.7	13.7	13.1	13.1
	28	59.9	58.1	68.5	58.2	58.6	58.6	68.5

		1	**2**	**3**	**4**	**5**	**6**
3-O- -D- Glucurono- pyranosyl moiety	C-1'	106.3	106.0	106.3	106.3	106.2	
	2'	75.5	74.3	75.5	75.5	75.5	
	3'	78.1	87.6	78.2	78.1	78.2	
	4'	73.5	71.7	73.5	73.5	73.8	
	5'	77.9	77.5	77.9	77.9	77.9	
	6'	172.9	172.1	172.9	172.9	173.0	
3'-O- -D- Glucopyra- nosyl moiety	C-1"		106.0				
	2"		75.6				
	3"		78.8				
	4"		71.6				
	5"		78.3				
	6"		62.5				

		1	**2**	**4**
Tigloyl moiety	C-1"'	167.7,	167.9	168.2	168.3
	2"'	129.0,	129.0	129.7	129.7
	3"'	137.8,	138.1	136.5	136.6
	4"'	12.3,	12.4	12.4	12.5
	5"'	14.3	14.3	14.2	14.3



Gymnemic acid VI (**2**), mp 225-226 ℃, $[\alpha]_D$ + 11.7 °(c=1.1, MeOH), $C_{47}H_{74}O_{18}$, [FAB-MS m/z 972$(M+2Na)^+$], gave glucose, glcUA and **6** on mild acid hydrolysis. A cellulase treatment of **2** furnished gymnemic acid IV (**4**)[9] and glucose. The ^{1}H-NMR spectrum of **2** suggested that **2** was composed of one mol each of **6**, tiglic acid, glucose and glcUA. Comparison of the ^{13}C-NMR spectrum of **2** with that of **4** disclosed C_3, (+ 9.5 ppm) of glcUA as the glycosylation site in the former. Hence the structure of **2** is 3-O-[β-D-glucopyranosyl(1→3)-β-D-glucuronopyranosyl]-21-O-tigloyl gymnemagenin.

Mild acid hydrolysis of gymnemic acid VII (**3**), mp 222-223°C, $[\alpha]_D$ + 9.6°(c=5.7, MeOH), $C_{36}H_{58}O_{11}\cdot 3/2H_2O$[FAB-MS m/z 712$(M+2Na)^+$], furnished glcUA and gymnestrogenin (**7**)[3e], mp 290-291°C, $[\alpha]_D$ + 53.1° (c=2.4, MeOH).

In comparing the ^{13}C-NMR spectra of **3** and **7** a glycosylation shift of + 8.7 ppm, appeared at C_3, disclosing the site of the glycosylation in **3**. Hence the structure of **3** is 3-O-β-D-glucuronopyranosyl gymnestrogenin.

A solution of gymnemic acid V and VI, 1 mM each, led to a complete suppression of sweetness induced by 0.4 mM sucrose. Gymnemic acid VII and **5** were not active at all.

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8) The assignment for the proton in the ^{1}H-NMR spectrum of **1-7** were made by ^{1}H/^{1}H spin decoupling and also especially by 2D cosy spectra(^{1}H/^{1}H, ^{13}C/^{1}H). The anomeric configuration of glcUA was confirmed as β based on the coupling constant of its anomeric proton (5.25d, J=7.5 Hz) as well as the ^{13}C-NMR spectrum
9) Independent of our work, gymnemic acid IV has been isolated from the same source by Kurihara et al., (56th National Meeting of the Chemical Society of Japan, Tokyo, April, 1988, Abstr. II, p.1200).

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