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Pregnane Glycosides, Gymnepregosides A—F from the Roots of *Gymnema alternifolium*

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The structural elucidation of six new related polyoxypregnane glycosides, gymnepregosides A (1), B (2), C (3), D (4), E (5) and F (6), together with two known compounds, from the roots of *Gymnema alternifolium* (Asclepiadaceae) was achieved through on a detailed study of ^{1}H- and ^{13}C-NMR spectral data and chemical means. The results obtained for new compounds, 1—6, show that they are (20*S*)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptaol or sarcostin 3-*O*-glycosides, and all the sugars at C-3 are β(1→4)-linked. Some of them possessed benzoyl, cinnamoyl and tigloyl residues as the ester linkages located at C-12 and/or C-20 of the aglycon.

Key words *Gymnema alternifolium*; polyoxypregnane glycoside; gymnepregoside; Asclepiadaceae; 5α-hydroxypregnane; sarcostin

In a previous paper,[1] we have reported the isolation and structural elucidation of ten new oleanane-type triterpenoid glycosides, named alternosides I—X from the dried roots of *Gymnema alternifolium* (Asclepiadaceae). Further investigation on this plant afforded eight oxypregnane-oligoglycosides. The present paper describes the isolation and full structural elucidation of six new oxypregnane-oligoglycosides named gymnepregosides A—F (**1**—**6**).

The 70% EtOH extract obtained from the dried roots of *G. alternifolium* (1.5 kg) was subjected to Amberlite XAD-2 column chromatography eluting with 20—100% MeOH. Separation of the 100% MeOH soluble portion by HPLC on reversed-phase adsorbent provided six new pregnane glycosides, named gymnepregosides A (**1**), B (**2**), C (**3**), D (**4**), E (**5**) and F (**6**), together with two known compounds, **7**[2] and **8**,[3] by comparison of spectral data (NMR, MS) and specific optical rotations with those of

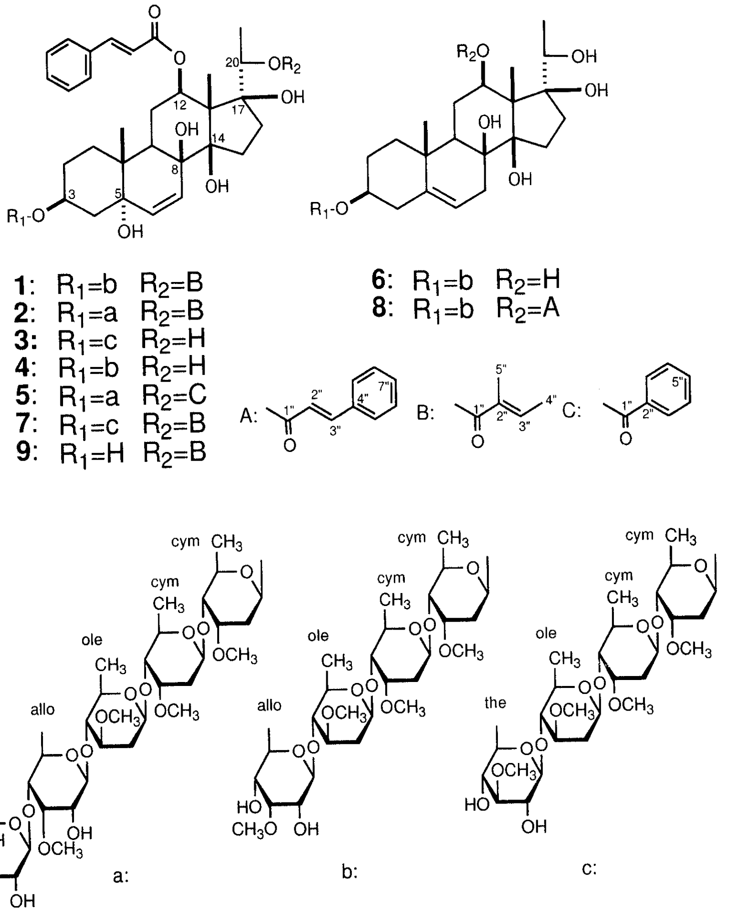

Chart 1

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the literature. The structures of **1**—**6** were determined by spectral methods, especially two dimensional (2D)-NMR experiments and chemical methods.

Gymnepregoside A (**1**) exhibited an $[M-H]^-$ peak at m/z 1201 in the FAB-MS, appropriate for a molecular formula of $C_{63}H_{94}O_{22}$. The IR spectrum of **1** showed absorption bands at 1710, 1640, 1275 cm^{-1} and strong absorption bands at 3460 and 1060 cm^{-1} suggestive of an oligoglycosidic structure. The ^{13}C-NMR spectrum for the aglycon part of **1** exhibited one secondary methyl group at δ 15.5, two tertiary methyl groups at δ 12.1 and 21.4, four tertiary carbinol at δ 74.0, 74.7, 87.8, and 88.1, and one double bond at δ 127.4 and 136.7, besides two ester carbonyls at δ 166.8 and 166.8 and four anomeric carbons appearing at δ 97.7, 100.4, 101.9, and 101.9, suggesting that **1** is a polyoxypregnane glycoside having two acyl groups. On mild acid hydrolysis, **1** afforded the prosapogenin (**9**), namely, 12-*O*-cinnamoyl-20-*O*-(*E*)-2-methyl-2-butenoyl-(20*S*)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptol, which had been obtained on acid hydrolysis of stephanosides K, M and O (**7**),[2] and cymarose, oleandrose, 6-deoxy-3-*O*-methylallose as sugar components detected by TLC. To determine the D- or L-configuration of each sugar of **1**—**6**, a mixture of **1**—**6** was subjected to mild acid hydrolysis with 0.5% H_2SO_4 to give D-cymarose, D-glucose, 6-deoxy-3-*O*-methyl-D-allose, D-oleandrose and D-thevetose whose structures were confirmed by NMR spectral data and specific rotations using chiral detection in HPLC analysis.[4,5]

The anomeric proton signals due to two cymaroses, an oleandrose, and a 6-deoxo-3-*O*-methylallose were observed as expected at δ 5.07 (1H, dd, J=9.5, 1.5 Hz), 5.15 (1H, dd, J=9.3, 1.5 Hz), 4.65 (1H, dd, J=9.3, 1.5 Hz), and δ 5.30 (1H, d, J=7.8 Hz), and their coupling patterns indicated all glycosidic linkages to be β-configuration. The downfield shift of the glycosidic C-3 (δ 74.8) carbon in comparison with that (δ 66.7) of the prosapogenin (**9**) indicated the oligosaccharide chain linkage to be at C-3 in **1**. The chemical shifts for the sugar moieties at C-3 in the NMR spectra of **1** bore close resemblance to those of **8**, indicating that both compounds had a common sugar substitution pattern at C-3. Further, the heteronuclear multiple bond connectivity (HMBC) spectrum showed connectivities between H-1 (δ 5.15) of cymarose (cym_1) and C-3 (δ 74.8) of the aglycone, H-1 (δ 5.07) of cymarose (cym_2) and C-4 (δ 82.9) of cym_1, H-1 (δ 4.65) of oleandrose (ole) and C-4 (δ 83.1) of cym_2, and H-1 (δ 5.30) of 6-deoxy-3-*O*-methylallose (allo) and C-4 (δ 82.8) of ole. Therefore, **1** was formulated as 12-*O*-cinnamoyl-20-*O*-(*E*)-2-methyl-2-butenoyl-(20*S*)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptol 3-*O*-6-deoxy-3-*O*-methyl-β-D-allopyranosyl(1→4)-β-D-oleandropyranosyl(1→4)-β-D-cymaro-

Table 1. ^{13}C-NMR Data for the Aglycon Moieties of Compounds **1**—**9** (in Pyridine-d_5)

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
C- 1	27.6	27.5	27.9	27.9	27.6	38.6	27.6	38.8	27.0
2	26.5	26.4	26.9	26.6	26.6	29.8	26.6	29.8	28.6
3	74.8	74.8	75.0	74.8	74.9	78.0	74.9	77.7	66.7
4	39.1	39.0	39.3	39.0	39.1	39.3	39.0	39.2	41.0
5	74.7	74.8	75.0	74.8	74.8	139.2	74.8	139.1	75.0
6	136.7	136.7	136.3	136.9	136.7	119.8	136.7	119.6	136.9
7	127.4	127.4	127.7	127.6	127.3	34.8	127.4	35.0	127.1
8	74.0	74.0	74.1	73.9	74.0	74.3	74.0	74.1	74.1
9	37.0	37.0	37.1	36.9	36.9	44.7	36.9	44.1	36.4
10	39.6	39.6	39.9	39.7	39.6	37.6	39.6	37.2	39.9
11	23.6	23.6	24.1	23.8	23.7	29.3	23.7	25.6	23.8
12	75.9	75.6	76.1	75.9	75.8	73.4	75.6	74.8	75.7
13	58.0	58.0	58.3	58.0	58.1	58.8	58.0	56.9	58.1
14	88.1	88.2	89.1	88.9	88.3	89.1	88.2	88.8	88.3
15	33.1	33.1	33.6	33.3	33.2	32.4	33.1	32.8	33.2
16	34.2	34.2	33.8	33.6	34.3	34.4	34.3	34.2	34.4
17	87.8	87.8	88.1	87.6	87.7	89.0	87.8	88.6	87.8
18	12.1	12.2	12.9	12.6	12.5	11.7	12.2	11.6	12.4
19	21.4	21.5	21.9	21.6	21.5	18.0	21.5	18.1	21.8
20	74.4	74.5	70.7	70.4	78.4	71.1	74.5	70.9	74.5
21	15.5	15.6	19.9	19.6	15.6	19.1	15.6	19.2	15.6
Cinnamoyl moiety									
1′	166.8	166.8	167.1	167.0	166.8		166.8	167.0	166.8
2′	120.4	120.4	119.8	119.6	120.2		120.5	119.6	120.5
3′	143.8	143.9	145.4	145.3	144.0		143.8	145.3	143.8
4′	134.9	134.9	135.1	135.0	134.9		134.9	135.1	135.1
5′,9′	128.6	128.6	128.7	128.6	128.6		128.6	128.6	128.5
6′,8′	129.3	129.4	129.4	129.2	129.1		129.3	129.2	129.2
7′	130.5	130.6	130.6	130.5	130.4		130.5	130.6	130.5
(*E*)-2-Methyl-2-butenoyl or benzoyl moiety									
1″	166.8	166.8			165.6		166.8		166.8
2″	129.5	129.5			131.2		129.5		129.5
3″	137.7	137.9			130.2		137.7		137.7
4″	14.0	14.1			128.8		14.1		14.1
5″	12.3	12.3			133.2		12.4		12.2
6″					128.8				
7″					130.2				

Table 2. ^{13}C-NMR Data for Sugar Moieties of Compounds **1**—**8** (in Pyridine-d_5)

C-3	Sugar	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
cym_1	1	97.7	97.7	97.8	97.7	97.7	96.5	97.7	96.4
	2	36.6	36.6	37.0	36.7	36.7	37.4	36.7	37.2
	3	77.7	77.7	77.9	77.7	77.7	78.2	77.7	78.0
	4	82.9	82.9	83.3	82.9	82.9	83.2	83.1	83.1
	5	68.9	68.9	69.1	68.9	68.9	69.2	68.9	69.0
	6	18.4	18.4	18.7	18.4	18.5	18.7	18.4	18.5
OMe		58.7	58.8	58.9	58.7	58.8	59.0	58.8	58.8
cym_2	1	100.4	100.4	100.5	100.4	100.4	100.5	100.4	100.4
	2	36.8	36.6	37.0	36.7	36.8	37.2	36.7	36.9
	3	77.9	77.9	78.1	77.8	77.9	78.0	77.9	77.7
	4	83.1	83.1	83.3	83.1	83.1	83.5	83.1	83.3
	5	69.1	69.1	69.3	69.1	69.1	69.1	69.1	68.8
	6	18.5	18.4	18.8	18.6	18.5	18.8	18.5	18.5
OMe		58.9	58.9	59.1	58.9	58.9	59.1	58.9	58.8
ole	1	101.9	101.8	102.1	101.8	101.8	102.0	101.9	101.8
	2	37.4	37.5	37.8	37.5	37.6	37.7	37.6	37.4
	3	79.2	79.3	79.4	79.2	79.2	79.4	79.2	79.2
	4	82.8	82.9	83.1	82.8	82.9	83.0	82.9	82.8
	5	72.0	71.9	72.2	72.0	72.0	72.2	72.0	72.0
	6	18.8	18.8	19.1	18.8	18.8	18.8	18.8	18.8
OMe		57.1	57.4	57.5	57.2	57.4	57.4	57.3	57.1
allo	1	101.9	101.8	104.2	102.0	101.9	102.1	104.1	101.9
or	2	73.2	72.6	75.4	73.2	72.6	73.3	75.2	73.2
the	3	83.9	83.1	88.3	84.0	83.1	84.1	88.2	83.9
	4	74.5	83.3	76.2	74.5	83.3	74.7	76.0	74.5
	5	70.9	69.5	73.0	70.9	69.5	71.0	72.8	70.9
	6	18.4	18.2	18.7	18.4	18.3	18.6	18.4	18.4
OMe		62.0	61.7	61.1	62.0	61.7	62.3	60.9	62.0
glc	1		106.5			106.6			
	2		75.4			75.5			
	3		78.3			78.4			
	4		71.8			71.9			
	5		78.3			78.4			
	6		62.9			63.0			

Table 3. ^{1}H-NMR Data for Compounds **1**—**6** and **9** (in Pyridine-d_5)

	1	**2**	**3**	**4**	**5**	**6**	**9**
Aglycon moiety							
3	4.16 (m)	4.16 (m)	4.20 (m)	4.16 (m)	4.16 (m)	3.82 (m)	4.30 (m)
6	5.92 (10.3 Hz)	5.92 (10.3 Hz)	5.91 (10.3 Hz)	5.91 (10.2 Hz)	5.92 (10.2 Hz)	5.38 (m)	5.97 (10.5 Hz)
7	6.23 (10.3 Hz)	6.23 (10.3 Hz)	6.23 (10.3 Hz)	6.23 (10.2 Hz)	6.23 (10.2 Hz)		6.23 (10.5 Hz)
12	5.33 (10.5, 4.5 Hz)	5.33 (10.5, 4.5 Hz)	5.37 (11.0, 4.5 Hz)	5.33 (10.5, 4.5 Hz)	5.36 (10.5, 4.5 Hz)	3.97 (10.5, 4.5 Hz)	5.38 (10.5, 4.5 Hz)
18	2.14 (s)	2.14 (s)	2.19 (s)	2.19 (s)	2.18 (s)	1.95 (s)	2.15 (s)
19	1.55 (s)	1.54 (s)	1.57 (s)	1.58 (s)	1.51 (s)	1.35 (s)	1.60 (s)
20	5.10 (5.8 Hz)	5.10 (5.9 Hz)	4.16 (6.3 Hz)	4.10 (5.9 Hz)	5.26 (6.0 Hz)	4.44 (5.8 Hz)	5.14 (6.0 Hz)
21	1.46 (5.8 Hz)	1.46 (5.9 Hz)	1.37 (6.3 Hz)	1.37 (5.9 Hz)	1.54 (6.0 Hz)	1.51 (5.8 Hz)	1.50 (6.0 Hz)
Cinnamoyl moiety							
2′	6.72 (16.0 Hz)	6.71 (15.9 Hz)	6.96 (15.8 Hz)	6.94 (15.8 Hz)	6.46 (15.9 Hz)		6.73 (16.0 Hz)
3′	7.90 (16.0 Hz)	7.90 (15.9 Hz)	8.12 (15.8 Hz)	8.12 (15.8 Hz)	7.80 (15.9 Hz)		7.90 (16.0 Hz)
5′,9′	7.63 (m)	7.63 (m)	7.53 (m)	7.51 (m)	7.35 (m)		7.63 (m)
6′,8′	7.36 (m)	7.37 (m)	7.32 (m)	7.31 (m)	7.33 (m)		7.38 (m)
7′	7.34 (m)	7.36 (m)	7.32 (m)	7.30 (m)	7.33 (m)		7.36(m)
(*E*)-2-Methyl-2-butenoyl or benzoyl moiety							
3″,(7″)	6.97 (7.2 Hz)	6.97 (6.8 Hz)			8.19 (7.7 Hz)		6.97 (6.5 Hz)
4″,(6″)	1.48 (7.2 Hz)	1.48 (6.8 Hz)			7.28 (7.7 Hz)		1.48 (6.5 Hz)
5″	1.77 (s)	1.76 (s)			7.49 (7.7 Hz)		1.78
Sugar moiety							
cym$_1$-1	5.15 (9.3, 1.5 Hz)	5.15 (9.3, 1.5 Hz)	5.15 (9.7, 1.5 Hz)	5.17 (9.7, 1.5 Hz)	5.15 (9.5, 1.5 Hz)	5.29 (9.3, 1.5 Hz)	
cym$_1$-6	1.35 (6.5 Hz)	1.34 (5.9 Hz)	1.36 (6.4 Hz)	1.34 (5.9 Hz)	1.34 (6.9 Hz)	1.37 (6.4 Hz)	
OMe	3.55	3.54	3.57	3.55	3.55	3.62	
cym$_2$-1	5.07 (9.5, 1.5 Hz)	5.07 (9.3, 1.5 Hz)	5.10 (9.7, 1.5 Hz)	5.08 (9.7, 1.5 Hz)	5.07 (9.5, 1.5 Hz)	5.10 (9.3, 1.5 Hz)	
cym$_2$-6	1.34 (6.3 Hz)	1.32 (5.9 Hz)	1.36 (6.4 Hz)	1.34 (5.9 Hz)	1.33 (6.9 Hz)	1.39 (5.4 Hz)	
OMe	3.53	3.52	3.57	3.53	3.52	3.55	
ole-1	4.65 (9.3, 1.5 Hz)	4.63 (9.3, 1.5 Hz)	4.70 (9.5, 1.5 Hz)	4.66 (9.3, 1.5 Hz)	4.64 (9.6, 1.5 Hz)	4.68 (10.3, 1.5 Hz)	
ole-6	1.59 (6.0 Hz)	1.57 (5.7 Hz)	1.71 (5.9 Hz)	1.64 (6.3 Hz)	1.59 (5.7 Hz)	1.64 (5.9 Hz)	
OMe	3.52	3.51	3.53	3.52	3.51	3.53	
allo-1	5.30 (7.8 Hz)	5.25 (8.3 Hz)		5.29 (7.8 Hz)	5.25 (7.7 Hz)	5.29 (7.8 Hz)	
allo-6	1.64 (6.4 Hz)	1.65 (6.4 Hz)		1.56 (6.0 Hz)	1.65 (6.3 Hz)	1.56 (6.0 Hz)	
OMe	3.84	3.82		3.84	3.82	3.85	
the-1			4.96 (8.3 Hz)				
the-6			1.60 (5.9 Hz)				
OMe			3.91				
glc-1		4.99 (7.4 Hz)			4.98 (8.0 Hz)		

pyranosyl(1→4)-*β*-D-cymaropyranoside.

In the FAB-MS, gymnepregoside B (**2**) showed a quasi-molecular ion at *m*/*z* 1363, 162 mass units higher than that of **1**, suggesting the presence of an additional hexosyl unit in the molecule. The acid hydrolysis of **2** supported the above observation; that is, in the hydolysate of **2** were detected cymarose, oleandrose, 6-deoxy-3-*O*-methylallose, and in addition, glucose, besides **9**. A ^{13}C-NMR spectral comparison of **2** with **1** showed a glycosylation shift[6,7] of +8.8 ppm at the C-4 (δ 83.3) position of the 6-deoxy-3-*O*-methylallose in **2**, indicating that the O-4 should be glucosylated. Further, the remaining sugar sequences at C-3 were corroborated by an HMBC experiment. The long-range correlations between C-3 (δ 74.8) of the aglycon and H-1 (δ 5.15) of cym$_1$, C-4 (δ 82.9) of cym$_1$ and H-1 (δ 5.07) of cym$_2$, C-4 (δ 83.1) of cym$_2$ and H-1 (δ 4.63) of ole, and C-4 (δ 82.9) of ole and H-1 (δ 5.25) of allo, and C-4 (δ 83.3) of allo and H-1 (δ 4.99) of glucose (glc) were all definitely observed. Consequently, the structure of 2 was concluded to be 12-*O*-cinnamoyl-20-*O*-(*E*)-2-methyl-2-butenoyl-(20*S*)-pregn-6-ene-3*β*,5*α*,8*β*,12*β*,14*β*,17*β*,20-heptol 3-*O*-*β*-D-glucopyranosyl(1→4)-6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl(1→4)-*β*-D-oleandropyranosyl(1→4)-*β*-D-cymaropyranosyl(1→4)-*β*-D-cymaropyranoside.

Gymnepregoside C (**3**) gave in the FAB-MS an $[M-H]^-$ peak at *m*/*z* 1119, 82 mass units lower than that of **7**, suggesting the absence of a tigloyl (2-methyl-2-butenoyl) unit in the molecule. Alkaline treatment of **3** gave cinnamic acid. On acid hydrolysis, **3** gave cymarose, oleandrose, and thevetose. The ^{1}H- and ^{13}C-NMR spectra showed two *β*-cymaropyranosyl units [H-1, δ 5.10 (dd, $J=9.7$, 1.5 Hz); C-1, δ 100.5; H-1, δ 5.15 (dd, $J=9.7$, 1.5 Hz); C-1, δ 97.9], one *β*-oleandropyranosyl unit [H-1, δ 4.70 (dd, $J=9.8$, 1.5 Hz); C-1, δ 102.1], and one thevetopyranosyl unit [H-1, δ 4.96 (d, $J=8.3$ Hz); C-1, δ 104.2], and a cinnamoyl unit [C-1′, δ 167.1]. The location of the cinnamoyl group in **3** was deduced to be the C-12 position based on the significant downfield shift at δ 5.37 (dd, $J=11.0$, 4.5 Hz) as compared with gymnepregoside F (**6**) described later.

The carbon signals due to the oligosaccharide moiety and the aglycon part except for C-20 and C-21 are similar with those of **7**, indicating that both compounds had a common sugar substitution pattern at C-3, and a cinnamoyl moiety at C-12. Therefore, **3** was formulated as 12-*O*-cinnamoyl-(20*S*)-pregn-6-ene-3*β*,5*α*,8*β*,12*β*,14*β*,17*β*,20-heptol 3-*O*-*β*-D-thevetopyranosyl(1→4)-*β*-D-oleandropyranosyl(1→4)-*β*-D-cymaropyranosyl(1→4)-*β*-D-cymaropyranoside.

In the FAB-MS, gymnepregoside D (**4**) showed a quasi-molecular ion at *m*/*z* 1119, the same as **3** and alkaline treatment gave cinnamic acid. Acid hydrolysis of **4** afforded cymarose, oleandrose, and 6-deoxy-3-*O*-methylallose. The hydrolysis results combined with the MS data suggested that gymnepregoside D (**4**) differed from gymnepregoside C (**3**) only by the replacement of thevetose with 6-deoxy-3-*O*-methylallose in the C-3-linked sugar units. Indeed, the carbon signals due to the oligosaccharide moiety and the aglycon moiety are superimposable on those of **1** and **3**, respectively. Hence, the structure of **4** was concluded to be 12-*O*-cinnamoyl-(20*S*)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptol 3-*O*-6-deoxy-3-*O*-methyl-β-D-allopyranosyl(1→4)-β-D-oleandropyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-cymaropyranoside.

Gymnepregoside E (**5**) exhibited in the FAB-MS a peak at *m*/*z* 1385 $[\mathrm{M-H}]^-$, consistent with the molecular formula $C_{71}H_{102}O_{27}$ and afforded cymarose, oleandrose, 6-deoxy-3-*O*-methylallose, and glucose as the sugar moieties on acid hydrolysis. Alkaline treatment of **5** gave cinnamic acid and benzoic acid. The NMR spectra of **5** showed the presence of one each of cinnamic acid, benzoic acid, oleandrose, 6-deoxy-3-*O*-methylallose and glucose, and of two cymaroses. The chemical shifts for the sugar moieties at C-3, and C-12 carbon in the ^{13}C-NMR spectrum of **5** bore close resemblance to those of **2**, indicating that both compounds had a common sugar substitution pattern at C-3 and a cinnamoyl group at C-12. Again, the acylated signals for H-20 and C-20 were observed at δ 5.26 (q, J=6.0 Hz) and C-20 (δ 78.4), suggesting that a benzoyl group was located at C-20 position. Further, the locations of two acyl moieties were confirmed by HMBC experiment which exhibited significant correlations between H-12 (δ 5.36) of the aglycon and the carbonyl carbon (δ 166.8) of the cinnamoyl unit, and between H-20 (δ 5.26) of aglycon and the carbonyl carbon (δ 165.6) of the benzoyl unit. Consequently, the structure of **5** has been established as 12-*O*-cinnamoyl-20-*O*-benzoyl-(20*S*)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptol 3-*O*-β-D-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl(1→4)-β-D-oleandropyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-cymaropyranoside.

Gymnepregoside F (**6**) showed in the FAB-MS an $[\mathrm{M-H}]^-$ peak at *m*/*z* 973, 130 mass unit lower than that of **8**, suggesting the absence of a cinnamoyl unit in the molecule. The acid hydrolysis of **6** allowed the identification of the same sugar components as **8**, that is, cymarose, 6-deoxy-3-*O*-methylallose and oleandrose on TLC. A ^{13}C-NMR spectral comparison of **6** with **8** showed that **6** was a deacylate of **8**. Thus, **6** was formulated as sarcostin 3-*O*-6-deoxy-3-*O*-methyl-β-D-allopyranosyl(1→4)-β-D-oleandropyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-cymaropyranoside.

In conclusion, we have isolated six new oxypregnane-oligoglycosides, named gymnepregosides A (**1**)—F (**6**), having a very rare 5α-hydroxypregnane[2] or sarcostin as the aglycone, from the roots of *Gymnema alternifolium* (Asclepiadaceae).

Experimental

General Procedure Melting points were measured with a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were taken on a JASCO DIP-1000 digital polarimeter. IR and UV spectra were measured with JASCO FT/IR-5300 and Shimadzu UV-160 instruments. NMR spectra were recorded on JEOL JNM-GX-400FT and Varian UNITY 600 spectrometers 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, HMBC (512×1024 data matrix size, 128 scans, recycle delay=1.16 s), total correlation spectroscopy (TOCSY) and rotating frame nuclear Overhauser effect spectroscopy (ROESY). Coupling constants (J values) are given in 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 TLC, Silica gel 60F-254 (Merck) were used. HPLC was carried out with a Waters ALC/GPC 244 instrument.

Extraction and Isolation of Compounds 1—8 The dried roots (1.5 kg) of *G. alternifolium* collected in Taiwan, in June 1996, were extracted with 70% EtOH at room temperature for 3 weeks. The ethanolic extract was passed through an Amberlite XAD-2 column. The column was washed with water, and the adsorbed materials were eluted with 20—100% MeOH to obtain the 20%, 40%, 60% and 100% MeOH eluate. The 100% MeOH eluate (64 g) was chromatographed on Bondapack C_{18} with 80% MeOH to give six fractions (Frs. 1—6). Fr. 1 (2.0 g) was subjected to HPLC on octadecyl silica (ODS) (Develosil Lop ODS, 60% CH_3OH) to give three fractions (Frs. 1-1—3). Fr. 1-2 was purified by preparative HPLC (YMC, ODS S-5, 28—35% CH_3CN) to afford gymnepregoside F (**6**, 20 mg). Fr. 3 (2.9 g) was subjected to HPLC on ODS (Develosil Lop ODS, 48—55% CH_3CN) to give four fractions (Frs. 3-1—4). Fr. 3-3 were purified by preparative HPLC (YMC, ODS S-5, 46% CH_3CN) to afford gymnepregosides B (**2**, 160 mg) and C (**3**, 30 mg) from Fr. 3-3. Fr. 3-4 was purified by preparative HPLC (YMC, ODS S-5, 47% CH_3CN) to afford gymnepregoside D (**4**, 55 mg). Fr. 4 (5.0 g) was subjected to HPLC on ODS (Develosil Lop ODS, 55% CH_3CN) to give six fractions (Frs. 4-1—6). Fr. 4-3 was purified by preparative HPLC (YMC, ODS S-5, 46% CH_3CN) to afford gymnepregoside E (**5**, 80 mg). Fr. 6 (1.8 g) was purified by preparative HPLC (YMC, ODS S-5, 55% CH_3CN) to afford gymnepregoside A (**1**, 100 mg), sitakisoside O (**7**, 70 mg) and compound 20 (**8**, 75 mg).

Gymnepregoside A (**1**): mp 131—133 °C, $[\alpha]_D^{25}$ +76.3° (c=1.6, $CHCl_3$). IR (film) cm^{-1}: 3460, 1710, 1640, 1275, 1060. UV λ_{max} (MeOH) nm (log ε): 206 (4.27), 217 (4.28), 280 (4.17). Negative FAB-MS *m*/*z*: 1201 $[\mathrm{M-H}]^-$. *Anal.* Calcd for $C_{63}H_{94}O_{22}$: C, 62.88; H, 7.87. Found: C, 63.19; H, 7.92. ^{1}H-NMR and ^{13}C-NMR: Tables 1—3.

Gymnepregoside B (**2**): mp 172—174 °C, $[\alpha]_D^{25}$ +79.9° (c=2.7, $CHCl_3$). IR (film) cm^{-1}: 3420, 1710, 1640, 1110. UV λ_{max} (MeOH) nm (log ε): 206 (4.24), 217 (4.27), 280 (4.16). Negative FAB-MS *m*/*z*: 1363 $[\mathrm{M-H}]^-$. *Anal.* Calcd for $C_{69}H_{104}O_{27}$: C, 60.69; H, 7.68. Found: C, 60.69; H, 7.76. ^{1}H-NMR and ^{13}C-NMR: Tables 1—3.

Gymnepregoside C (**3**): mp 153—154 °C, $[\alpha]_D^{25}$ +25.2° (c=2.6, $CHCl_3$). IR (film) cm^{-1}: 3460, 1710, 1630, 1165, 1100, 1005. UV λ_{max} (MeOH) nm (log ε): 205 (4.18), 217 (4.18), 279 (4.33). Negative FAB-MS *m*/*z*: 1119 $[\mathrm{M-H}]^-$. *Anal.* Calcd for $C_{58}H_{88}O_{21}\cdot 2H_2O$: C, 60.19; H, 8.01. Found: C, 60.38; H, 8.05. ^{1}H-NMR and ^{13}C-NMR: Tables 1—3.

Gymnepregoside D (**4**): mp 154—155 °C, $[\alpha]_D^{25}$ +22.1° (c=2.7, $CHCl_3$). IR (film) cm^{-1}: 3480, 1710, 1640, 1165, 1090, 1005. UV λ_{max} (MeOH) nm (log ε): 203.5 (4.37), 217 (4.25), 279.5 (4.36). Negative FAB-MS *m*/*z*: 1119 $[\mathrm{M-H}]^-$. *Anal.* Calcd for $C_{58}H_{88}O_{21}\cdot 3H_2O$: C, 59.27; H, 8.06. Found: C, 59.45; H, 7.81. ^{1}H-NMR and ^{13}C-NMR: Tables 1—3.

Gymnepregoside E (**5**): mp 169—170 °C, $[\alpha]_D^{25}$ +51.3° (c=2.1, $CHCl_3$). IR (film) cm^{-1}: 3440, 1715, 1640, 1280, 1110. UV λ_{max} (MeOH) nm (log ε): 222 (4.34), 236 (4.17), 278 (4.32). Negative FAB-MS *m*/*z*: 1385 $[\mathrm{M-H}]^-$. *Anal.* Calcd for $C_{71}H_{102}O_{27}\cdot 3H_2O$: C, 59.15; H, 7.55. Found: C, 59.05; H, 7.80. ^{1}H-NMR and ^{13}C-NMR: Tables 1—3.

Gymnepregoside F (**6**): mp 112—114 °C, $[\alpha]_D^{25}$ +12.8° (c=1.1, $CHCl_3$–MeOH=1:1). IR (film) cm^{-1}: 3480, 1100, 1040. Negative FAB-MS *m*/*z*: 973 $[\mathrm{M-H}]^-$. *Anal.* Calcd for $C_{49}H_{82}O_{19}\cdot H_2O$: C, 59.26; H, 8.52. Found: C, 59.82; H, 8.70. ^{1}H-NMR and ^{13}C-NMR: Tables 1—3.

Mild Acid Hydrolysis of 1—6 A solution of **1**—**6** (600 mg) in 4 ml of dioxane was treated with 2 ml of 1% H_2SO_4 with stirring at 100 for 60 min. After cooling, the reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. The CH_2Cl_2 layer was evaporated under reduced pressure to afford the CH_2Cl_2 extract (120 mg). The CH_2Cl_2 extract was separated by HPLC (YMC, ODS, 38% CH_3CN) to afford prosapogenin (**9**, 10 mg).

Prosapogenin (**9**): A white amorphous powder, $[\alpha]_D^{25}$ +13.4° (c=1.0, MeOH). IR (film) cm^{-1}: 3480, 1710, 1640, 1170. Positive FAB-MS *m/z*: 649 $[M+K]^+$. High-resolution CI-MS *m/z*: 611.3235 (Calcd for $[C_{35}H_{46}O_9+H]^+$: 611.3220). ^{1}H-NMR and ^{13}C-NMR: Table 1 and 3.

The H_2O layer was neutralized with Amberlite IRA-45 and evaporated under reduced pressure to give the sugar portion (160 mg). This was purified on silica gel column eluting with CH_2Cl_2–MeOH–H_2O (30 : 1 : 0—25 : 6 : 0.1) to afford cymarose (15 mg), glucose (15 mg), oleandrose (15 mg), a mixture of 6-deoxy-3-*O*-methylallose and thevetose (9 : 1, 30 mg), which were identified by NMR spectra. ^{13}C-NMR (400 MHz, C_5D_5N) δ: β-cymarose 19.2 (C-6), 37.2 (C-2), 57.8 (OMe), 70.9 (C-5), 74.4 (C-4), 79.1 (C-3), 92.6 (C-1). β-glucose 63.0 (C-6), 72.1 (C-4), 76.9 (C-2), 78.6 (C-3), 78.7 (C-5), 98.9 (C-1). β-oleandrose 19.1 (C-6), 39.1 (C-2), 57.0 (OMe), 73.0 (C-5), 76.6 (C-4), 82.0 (C-3), 94.9 (C-1). β-6-deoxy-3-*O*-methylallose 18.8 (C-6), 62.0 (OMe), 70.8 (C-5), 74.7 (C-2 and C-4), 84.0 (C-3), 96.0 (C-1). β-thevetose 18.4 (C-6), 60.8 (OMe), 72.6 (C-5), 76.2 (C-4), 76.7 (C-2), 88.0 (C-3), 98.5 (C-1). The D or L configuration of each sugar was determined by using refractive index (RI) detection (Waters 410) and chiral detection (Shodex OR-1) by HPLC (Shodex SC-1011, H_2O, 0.5 ml/min, 70 °C) by comparison with an authentic sugar (D-cymarose, D-glucose, L-oleandrose, 6-deoxy-3-*O*-D-methylallose and L-thevetose). The sugar portion gave the following peaks: D-(+)-glc 14.00 min; D-(+)-the 14.20 min; D-(+)-ole 15.60 min; D-(+)-allo 17.60 min and D-(+)-cym 17.80 min.

Identification of Sugar Components of Compounds 1—6 Each compound (5 mg) was hydrolyzed in the same way mentioned above. Each solution was analyzed on TLC. TLC solvent 1 [CH_2Cl_2–EtOH (9 : 1)] *Rf*: cymarose, 0.47; oleandrose, 0.36; 6-deoxy-3-*O*-methylallose, 0.20; thevetose, 0.20. TLC solvent 2 [CH_2Cl_2–MeOH–H_2O (25 : 8 : 0.1)] *Rf*: allo, 0.73; the, 0.73; glc, 0.12. Cym, glc, ole and allo from **2** and **5**, cym, ole and allo from **1**, **4** and **6**, cym, ole and the from **3** were detected, respectively.

Alkaline Hydrolysis of Compounds 3—5 Each compound (5 mg) was heated in 28% sodium methylate and methanol (1 : 1) at 37 °C for 2 h. The reaction mixture was diluted with H_2O, and passed through a column of Amberlyst 15. The methanol eluate gave (*E*)-cinnamic acid from **3** and **4**, and (*E*)-cinnamic acid and benzoic acid from **5**, which were confirmed by HPLC (YMC-pack C_8, 22% CH_3CN) with authentic samples. t_R: 12.40 min (benzoic acid), 14.0 min [(*E*)-cinnamic acid].

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