Pregnane Glycosides, Gymnepregosides G—Q from the Roots of *Gymnema alternifolium*

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The structural elucidation of eleven new related polyoxypregnane glycosides, gymnepregosides G (1), H (2), I (3), J (4), K (5), L (6), M (7), N (8), O (9), P (10) and Q (11), from the roots of *Gymnema alternifolium* (Asclepiadaceae) was achieved by a detailed study of ^{1}H- and ^{13}C-NMR spectral data and chemical means. The results obtained for new compounds, 1—11, show that they are (20*S*)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptaol 3-*O*-glycosides, and all the sugars at C-3 are β(1→4)-linked. Some of them possess benzoyl, (*E*)- and (*Z*)-cinnamoyl, and tigloyl residues as the ester linkages located at C-12 and/or C-20 of the aglycone.

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

In a previous paper,[1)] we reported the isolation and structural elucidation of six new oxypregnane-oligoglycosides, gymnepregosides A—F, from the dried roots of *Gymnema alternifolium* (Asclepiadaceae). Further investigation of this plant afforded eleven oxypregnane-oligoglycosides. The present paper describes the isolation and full structural elucidation of eleven new oxypregnane-oligoglycosides, named gymnepregosides G—Q (**1**—**11**).

The 70% EtOH extract of the dried roots of *G. alternifolium* (1.5 kg) was subjected to Amberlite XAD-2 column chromatography eluting with 20—100% MeOH. Further separation of the 100% MeOH soluble portion by HPLC on reversed-phase adsorbent provided eleven new pregnane glycosides, named gymnepregosides G (**1**), H (**2**), I (**3**), J (**4**), K (**5**), L (**6**), M (**7**), N (**8**), O (**9**), P (**10**) and Q (**11**), together with three known compounds, stephanoside I (**12**),[2)] compounds 24 (**13**) and 30 (**14**),[3)] identified by comparison of spectral data (NMR, MS) and specific optical rotations with literature data. The structures of **1**—**11** were determined by various NMR spectral methods, especially rotating frame Overhauser enhancement spectroscopy (ROESY) and heteronuclear multiple bond connectivity (HMBC) experiments, and chemical methods.

The NMR data of gymnepregosides G—Q (**1**—**11**) indicated that they were homologues of (20*S*)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptaol[1)] 3-*O*-glycosides, and differing in the sugar sequences at C-3 and the acyl units at C-12 and/or C-20 positions in the aglycones. To determine the component sugars, a mixture of the glycosides was subjected to acid hydrolysis and subsequently purified by silica-gel column chromatography. Cymarose, 6-deoxy-3-*O*-methylallose (abbreviated alm), glucose and oleandrose were identified based on comparison with authentic sugars using TLC and NMR spectral data. Unfortunately, canarose could not be detected in this experiment because of its extremely low concentration. The D- or L-configuration of each sugar was confirmed by specific rotation using chiral detection during HPLC analysis.[1)] All detected sugar components had the D-configuration.

Gymnepregosides G (**1**) and H (**2**) showed the same quasi-molecular ions at *m/z* 989, which corresponded to a loss of a cinnamoyl moiety from gymnepregosides C and D[1)] in the negative FAB-MS. The ^{13}C-NMR spectra of **1**, on comparison with that of gymnepregoside C, exhibited deacylation shifts at C-11 (δ 27.1 *vs*. 24.1), C-12 (δ 72.7 *vs*. 76.1) and C-13 (δ 59.7 *vs*. 58.3), and the remaining signals were very similar in both compounds. Therefore, the structure of **1** was determined as a deacylated gymnepregoside C. Using a similar strategy, the structure of **2** was determined to be a deacylated gymnepregoside D.

The molecular formula of gymnepregoside I (**3**) was deduced as $C_{64}H_{98}O_{26}$ from a $[M-H]^-$ peak observed at *m/z* 1281 in the FAB-MS and from its ^{13}C-NMR data. The IR spectrum showed carbonyl group (1710 cm^{-1}) and hydroxy group (3460 cm^{-1}). The ^{13}C-NMR spectrum of **3** exhibited the characteristic features of a (20*S*)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptaol 3-*O*-pentaglycoside, which consisted of two cymaroses, each one a 6-deoxy-3-*O*-methylallose, glucose and oleandrose, having an (*E*)-cinnamoyl group at C-12. The five anomeric proton signals due to two cymaroses, each one a 6-deoxy-3-*O*-methylallose, glucose and oleandrose were observed as expected at δ 5.18 (dd, *J*=9.2, 1.5 Hz), 5.09 (dd, *J*=9.3, 1.5 Hz), 5.26 (d, *J*=8.2 Hz), 4.97 (d, *J*=7.2 Hz), and δ 4.64 (dd, *J*=9.6, 1.5 Hz), indicating that all glycosidic linkages had the β-equatorial configuration. The downfield shift of the glycosidic C-3 (δ 75.0) carbon indicated the oligosaccharide chain linkage to be at C-3. A ^{13}C-NMR spectral comparison of the sugar moieties of **3** with that of **2** showed a glycosylation shift[4,5)] of +8.7 ppm at the C-4 (δ 83.5) position of the 6-deoxy-3-*O*-methylallose in **3**, indicating that the *O*-4 was glucosylated. The (*E*)-cinnamoyl group at the C-12 position was deduced from the acylation shifts for H-12 [δ 5.34 (dd, *J*=11.0, 4.4 Hz)] and C-12 (δ 76.2). Therefore, **3** was formulated as 12-*O*-(*E*)-cinnamoyl-(20*S*)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptaol 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.

In the FAB-MS, gymnepregoside J (**4**) showed a quasi-molecular ion at *m/z* 1281, which was the same as **3**. A ^{13}C-

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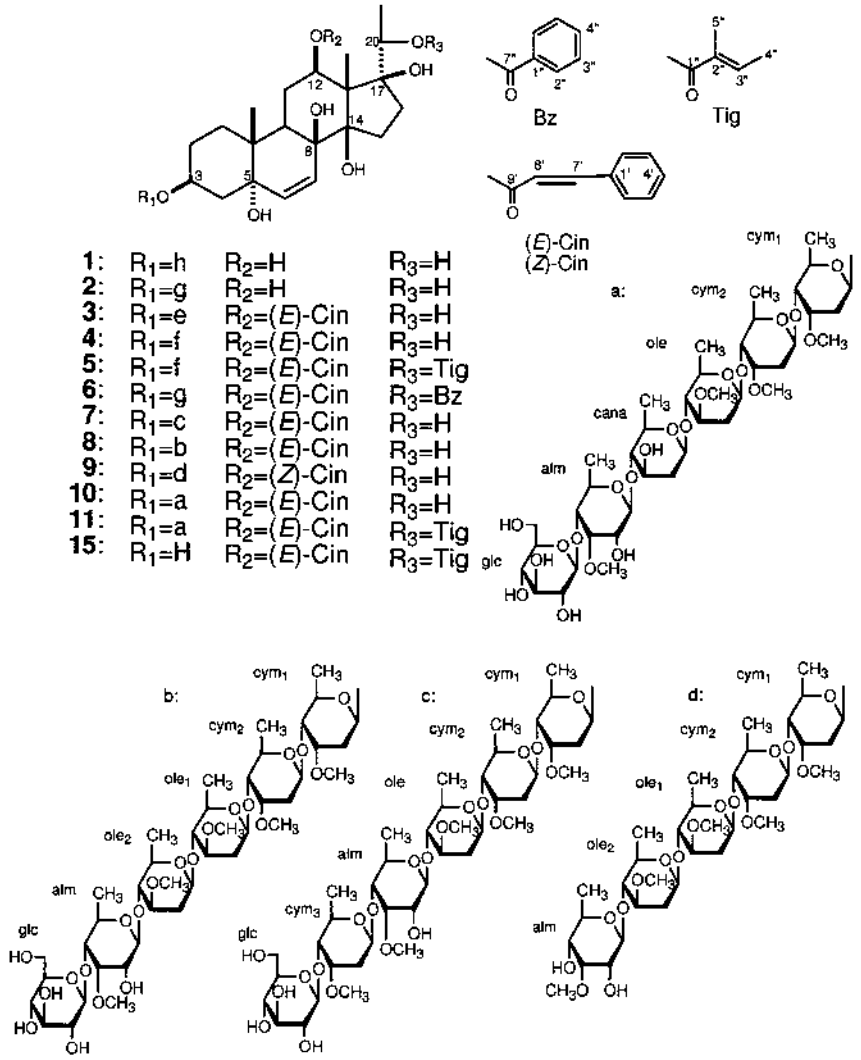

Chart 1

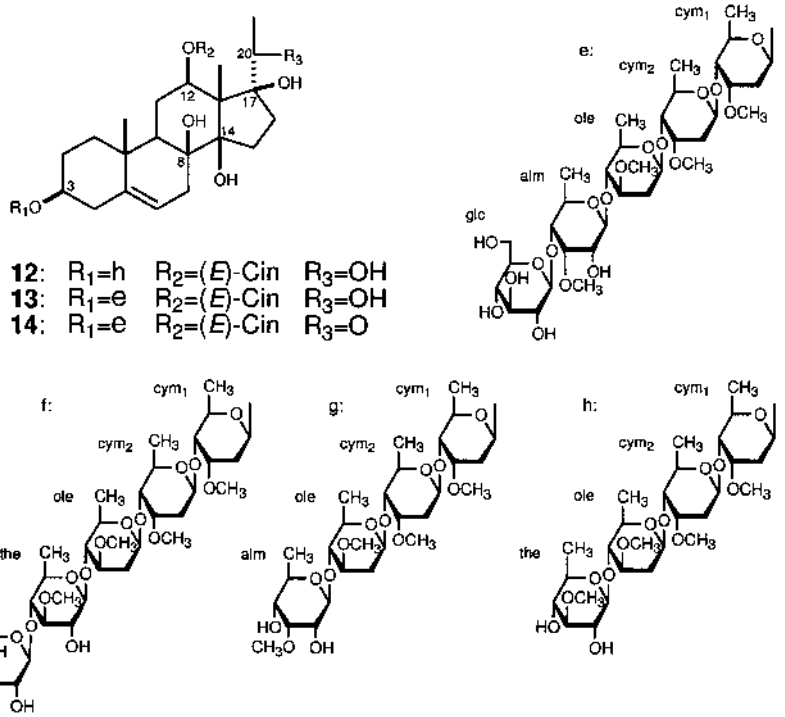

Chart 2

NMR spectral comparison of **4** and **3** showed that **4** had an (*E*)-cinnamoyl group at C-12 and differed structurally from **3** only in its saccharide moieties: a β-thevetopyranosyl group in **4** instead of a 6-deoxy-3-*O*-methylallose group in **3**. The sugar linkages at C-3 were determined by means of the glycosylation shift rule in the same way as for **3**. A glycosylation shift of +7.3 ppm at C-4 position of thevetose in **4** compared with that of **1** indicated the site of glycosylation in **4**. Hence, the structure of **4** was concluded to be 12-*O*-(*E*)-cinnamoyl-(20*S*)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptaol 3-*O*-β-D-glucopyranosyl(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-cymaropyranoside.

In the FAB-MS, gymnepregoside K (**5**) gave an $[M-H]^-$ peak at *m/z* 1363, which was 82 mass units higher than that of **4**, consistent with the molecular formula $C_{69}H_{104}O_{27}$. The ^{1}H- and ^{13}C-NMR spectral data showed the presence of an (*E*)-cinnamoyl and a tigloyl residue, together with five sugar units which were consisted with one β-oleandropyranosyl and one β-thevetopyranosyl group, and two β-cymaropyranosyl groups as found in **4** (Tables 1, 2, 4, 5). A ^{13}C-NMR spectral comparison of **5** with that of **4** showed that a common sugar substitution located at C-3 and two acyl groups located at C-12 and C-20 positions. The downfield shift at δ 5.10 (q, J=6.0 Hz) can be explained satisfactorily by acylation at C-20. Mild acid hydrolysis of **5** afforded a prosapogenin (**15**)[2] namely, 12-*O*-(*E*)-cinnamoyl-20-*O*-tigloyl-(20*S*)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptaol. Consequently, the structure of **5** was concluded to be 3-*O*-β-D-glucopyranosyl(1→4)-β-D-thevetopyranosyl(1→4)-β-D-oleandropyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-cymaropyranoside of **15**.

Table 1. ^{1}H-NMR Data for the Aglycone and Ester Parts of Gymnepregosides G (**1**), K (**5**)—M (**7**) and O (**9**) (400 MHz in Pyridine-d_5)

	1[a]	**5**[b]	**6**	**7**[c]	**9**[d]
Aglycone moiety					
3	4.20 (1H, m)	4.18 (1H, m)	4.15 (1H, m)	4.19 (1H, m)	4.15 (1H, m)
6	5.87 (1H, d, J=10.5 Hz)	5.93 (1H, d, J=10.2 Hz)	5.92 (1H, d, J=10.4 Hz)	5.91 (1H, d, J=10.3 Hz)	5.89 (1H, d, J=10.2 Hz)
7	6.21 (1H, d, J=10.5 Hz)	6.24 (1H, d, J=10.2 Hz)	6.24 (1H, d, J=10.4 Hz)	6.23 (1H, d, J=10.3 Hz)	6.20 (1H, d, J=10.2 Hz)
12	4.11 (1H, dd, J=10.5, 4.5 Hz)	5.34 (1H, dd, J=11.0, 4.6 Hz)	5.37 (1H, dd, J=10.1, 4.1 Hz)	5.34 (1H, dd, J=11.0, 4.6 Hz)	5.25 (1H, dd, J=11.2, 4.2 Hz)
18	2.01 (3H, s)	2.14 (3H, s)	2.19 (3H, s)	2.19 (3H, s)	2.02 (3H, s)
19	1.56 (3H, s)	1.54 (3H, s)	1.52 (3H, s)	1.58 (3H, s)	1.57 (3H, s)
20	4.44 (1H, q, J=6.1 Hz)	5.10 (1H, q, J=5.9 Hz)	5.27 (1H, q, J=6.1 Hz)	4.10 (1H, q, J=6.3 Hz)	4.06 (1H, q, J=6.3 Hz)
21	1.51 (3H, d, J=6.1 Hz)	1.47 (3H, d, J=5.9 Hz)	1.54 (3H, d, J=6.1 Hz)	1.34 (3H, d, J=6.3 Hz)	1.36 (3H, d, J=6.3 Hz)
(*E*) or (*Z*)-Cinnamoyl moiety					
2′,6′		7.63 (2H, dd, J=7.2, 1.5 Hz)	7.36 (2H, dd, J=7.2, 1.5 Hz)	7.51 (2H, dd, J=8.0, 1.6 Hz)	7.88 (2H, dd, J=7.5, 1.5 Hz)
3′,5′		7.41 (2H, t, J=7.2, 6.7 Hz)	7.34 (2H, t, J=7.2, 6.7 Hz)	7.31 (2H, t, J=8.0 Hz)	7.36 (2H, t, J=7.5 Hz)
4′		7.39 (1H, dt, J=7.2, 6.7, 1.5 Hz)	7.34 (1H, dt, J=7.2, 6.7, 1.6 Hz)	7.29 (1H, dt, J=8.0, 8.0, 1.5 Hz)	7.31 (1H, dt, J=7.5, 7.5, 1.5 Hz)
7′		7.89 (1H, d, J=15.9 Hz)	7.80 (1H, d, J=15.8 Hz)	8.12 (1H, d, J=15.9 Hz)	7.00 (1H, d, J=12.8 Hz)
8′		6.71 (1H, d, J=15.9 Hz)	6.49 (1H, d, J=15.8 Hz)	6.94 (1H, d, J=15.9 Hz)	6.36 (1H, d, J=12.8 Hz)
Tigloyl or benzoyl moiety					
2″			8.19 (2H, d, J=8.0 Hz)		
3″		7.00 (1H, q, J=6.8 Hz)	7.28 (2H, t, J=8.0 Hz)		
4″		1.51 (1H, d, J=6.8 Hz)	7.49 (1H, t, J=8.0 Hz)		
5″		1.78 (3H, s)			

a)—*c*) The deviations of the chemical shifts for *a*) **1** and **2**, *b*) **5** and **11**, *c*) **3**, **4**, **7**, **8** and **10**, were within the range ±0.02 ppm, only the data for *a*) **1**, *b*) **5**, and *c*) **7** were given. *d*) Measured at 600 MHz.

Table 2. ^{1}H-NMR Data for Gymnepregosides G (**1**)—I (**3**), K (**5**) and O (**9**) (400 MHz, in Pyridine-d_5)

	1	**2**[a]	**3**	**5**[b]	**9**[c]
cym_1-1	5.15 (1H, dd, J=9.2,1.5 Hz)	5.21 (1H, dd, J=9.6,1.5 Hz)	5.18 (1H, dd, J=9.2,1.5 Hz)	5.17 (1H, dd, J=9.2,1.5 Hz)	5.14 (1H, dd, J=9.1,1.5 Hz)
cym_1-4					3.45 (1H, m)
cym_1-6	1.35 (3H, d, J=7.0 Hz)	1.35 (3H, d, J=6.7 Hz)	1.37 (3H, d, J=6.3 Hz)	1.37 (3H, d, J=6.3 Hz)	1.35 (3H, d, J=6.6 Hz)
OMe	3.57 (3H, s)	3.56 (3H, s)	3.56 (3H, s)	3.56 (3H, s)	3.55 (3H, s)
cym_2-1	5.10 (1H, dd, J=9.2,1.5 Hz)	5.07 (1H, dd, J=9.3,1.5 Hz)	5.09 (1H, dd, J=9.3,1.5 Hz)	5.08 (1H, dd, J=9.7,1.5 Hz)	5.10 (1H, dd, J=9.5,1.5 Hz)
cym_2-4					3.43 (1H, dd, J=9.8,2.6 Hz)
cym_2-6	1.37 (3H, d, J=6.9 Hz)	1.37 (3H, d, J=6.7 Hz)	1.38 (3H, d, J=6.3 Hz)	1.37 (3H, d, J=6.3 Hz)	1.35 (3H, d, J=6.9 Hz)
OMe	3.57 (3H, s)	3.55 (3H, s)	3.54 (3H, s)	3.56 (3H, s)	3.55 (3H, s)
ole_1-1	4.70 (1H, dd, J=9.5,1.5 Hz)	4.68 (1H, dd, J=9.3,1.5 Hz)	4.64 (1H, dd, J=9.6,1.5 Hz)	4.67 (1H, dd, J=9.6,1.5 Hz)	4.67 (1H, dd, J=9.6,1.5 Hz)
ole_1-4					3.47 (1H, m)
ole_1-6	1.71 (3H, d, J=6.0 Hz)	1.57 (3H, d, J=6.0 Hz)	1.60 (3H, d, J=6.0 Hz)	1.75 (3H, d, J=6.0 Hz)	1.42 (3H, d, J=5.7 Hz)
OMe	3.53 (3H, s)	3.53 (3H, s)	3.51 (3H, s)	3.50 (3H, s)	3.42 (3H, s)
ole_2-1					4.87 (1H, dd, J=9.8,1.5 Hz)
ole_2-4					3.62 (1H, m)
ole_2-6					1.68 (3H, d, J=5.7 Hz)
OMe					3.53 (3H, s)
alm-1		5.30 (1H, d, J=8.2 Hz)	5.26 (1H, d, J=8.2 Hz)		5.30 (1H, d, J=7.6 Hz)
alm-6		1.65 (3H, d, J=6.3 Hz)	1.65 (3H, d, J=6.0 Hz)		1.54 (3H, d, J=5.5 Hz)
OMe		3.85 (3H, s)	3.84 (3H, s)		3.82 (3H, s)
the-1	4.98 (1H, d, J=8.0 Hz)			4.87 (1H, d, J=7.7 Hz)	
the-6	1.58 (3H, d, J=6.0 Hz)			1.66 (3H, d, J=6.0 Hz)	
OMe	3.94 (3H, s)			3.94 (3H, s)	
glc-1			4.97 (1H, d, J=7.2 Hz)	4.99 (1H, d, J=7.4 Hz)	

a), *b*) The deviations of the chemical shifts for *a*) **2** and **6**, and *b*) **4** and **5** were within the range ±0.02 ppm, only the data for *a*) **2** and *b*) **5** were given. *c*) Measured at 600 MHz.

Table 3. ^{1}H-NMR Data for Gymnepregosides M (**7**), N (**8**) and P (**10**) (600 MHz in Pyridine-d_5)

	7	**8**	**10**[a)]
cym_1-1	5.17 (1H, dd, J=9.3, 1.5 Hz)	5.18 (1H, dd, J=10.0, 1.5 Hz)	5.18 (1H, dd, J=10.0, 1.5 Hz)
cym_1-4	3.45 (1H, dd, J=9.6, 2.7 Hz)	3.46 (1H, dd, J=9.6, 2.7 Hz)	3.45 (1H, dd, J=9.6, 2.7 Hz)
cym_1-6	1.34 (3H, d, J=6.0 Hz)	1.36 (3H, d, J=6.3 Hz)	1.35 (3H, d, J=6.0 Hz)
OMe	3.55 (3H, s)	3.56 (3H, s)	3.56 (3H, s)
cym_2-1	5.08 (1H, dd, J=9.3, 1.5 Hz)	5.10 (1H, dd, J=9.9, 1.5 Hz)	5.10 (1H, dd, J=10.0, 1.5 Hz)
cym_2-4	3.39 (1H, dd, J=9.9, 2.2 Hz)	3.43 (1H, dd, J=9.6, 2.7 Hz)	3.43 (1H, dd, J=9.5, 2.6 Hz)
cym_2-6	1.35 (3H, d, J=5.3 Hz)	1.37 (3H, d, J=6.3 Hz)	1.36 (3H, d, J=6.0 Hz)
OMe	3.53 (3H, s)	3.56 (3H, s)	3.55 (3H, s)
ole_1-1	4.64 (1H, dd, J=9.3, 1.5 Hz)	4.66 (1H, dd, J=9.3, 1.5 Hz)	4.66 (1H, dd, J=10.0, 1.5 Hz)
ole_1-4	3.55 (1H, m)	3.45 (1H, m)	3.45 (1H, m)
ole_1-6	1.60 (3H, d, J=5.0 Hz)	1.41 (3H, d, J=6.0 Hz)	1.38 (3H, d, J=6.0 Hz)
OMe	3.52 (3H, s)	3.45 (3H, s)	3.46 (3H, s)
ole_2-1		4.85 (1H, dd, J=9.9, 1.5 Hz)	
ole_2-4		3.57 (1H, m)	
ole_2-6		1.62 (3H, d, J=5.8 Hz)	
OMe		3.53 (3H, s)	
cana-1			4.92 (1H, dd, J=9.8,1.5 Hz)
cana-2			1.92 (1H, ddd, J=10.0, 9.8, 9.1 Hz)
			2.57 (1H, ddd, J=10.0, 4.5, 1.5 Hz)
cana-3			3.96 (1H, ddd, J=9.8, 9.1, 4.5 Hz)
cana-4			3.30 (1H, dd, J=9.1, 8.8 Hz)
cana-5			3.54 (1H, m)
cana-6			1.55 (3H, d, J=6.0 Hz)
alm-1	5.24 (1H, d, J=8.0 Hz)	5.27 (1H, d, J=8.0 Hz)	5.09 (1H, d, J=8.0 Hz)
alm-4	3.48 (1H, dd, J=9.6, 1.6 Hz)	3.74 (1H, dd, J=9.7, 2.3 Hz)	3.74 (1H, dd, J=9.6, 2.2 Hz)
alm-6	1.34 (3H, d, J=6.0 Hz)	1.65 (3H, d, J=6.0 Hz)	1.60 (3H, d, J=6.3 Hz)
OMe	3.87 (3H, s)	3.82 (3H, s)	3.81 (3H, s)
cym_3-1	5.11 (1H, dd, J=10.0, 1.5 Hz)		
cym_3-4	3.65 (1H, dd, J=9.8, 2.6 Hz)		
cym_3-6	1.56 (3H, d, J=5.0 Hz)		
OMe	3.54 (3H, s)		
glc-1	4.93 (1H, d, J=7.7 Hz)	4.98 (1H, d, J=7.4 Hz)	4.96 (1H, d, J=8.0 Hz)

a) The deviations of the chemical shifts for **10** and **11** were within the range ±0.02 ppm, only the data for **10** are given.

In the FAB-MS, gymnepregoside L (**6**) gave an $[M-H]^-$ peak at *m/z* 1223, consistent with the molecular formula $C_{65}H_{92}O_{22}$. The ^{1}H- and ^{13}C-NMR spectral data showed benzoyl and (*E*)-cinnamoyl residues, four sugar units whose carbon signals were similar to those of **2**. A ^{13}C-NMR spectral comparison of **6** with that of **2** indicated that both compounds had a common sugar substitution pattern at C-3, and differing only in the signals ascribable to esterfied C-12 at δ 76.0, together with C-11 and C-13, and C-20 at δ 75.5, together with C-17 and C-21 of the aglycone. The locations of two acyl groups were established by a HMBC connectivity experiment, in which, the significant correlations between H-12 (δ 5.37) and the C-9′ (δ 166.8) of the cinnamoyl unit, and between H-20 (δ 5.27) and the C-7″ (δ 165.6) of the benzoyl unit were clearly observed. Therefore, the structure of **6** was concluded to be 12-*O*-(*E*)-cinnamoyl-20-*O*-benzoyl-(20*S*)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptaol-6-deoxy-3-*O*-methyl-β-D-allopyranosyl(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl(1→4)-β-D-cymaropyranoside.

In the FAB-MS, gymnepregoside M (**7**) gave an $[M-H]^-$ peak at *m/z* 1425, 144 mass units higher than that of **3**, suggesting the presence of an additional deoxyhexosyl group in the molecule. Indeed, the NMR spectrum of **7** was very close to that of **3**, except for the signals of a 2-deoxyhexosyl residue. The extensive NMR experiments conducted in this investigation allowed assignment of the sugar moieties at the C-3 position (Tables 3, 5): the inserted sugar was a β-cymaropyranosyl group. Complete evidence for the sugar sequence and their linkage sites was obtained from the results of a ROESY experiment which showed significant correlation peaks between anomeric protons and protons linked to glycosylated carbons. Namely, nuclear Overhauser effect (NOE) peaks between the H-3 (δ 4.19) of the aglycone and H-1 (δ 5.17) of cymarose$_1$, the H-4 (δ 3.45) of cymarose$_1$ and H-1 (δ 5.08) of cymarose$_2$, the H-4 (δ 3.39) of cymarose$_2$ and H-1 (δ 4.64) of oleandrose, the H-4 (δ 3.55) of oleandrose and H-1 (δ 5.24) of 6-deoxy-3-*O*-methylallose, the H-4 (δ 3.48) of 6-deoxy-3-*O*-methylallose and H-1 (δ 5.11) of cymarose$_3$, and the H-4 (δ 3.65) of cymarose$_3$ and H-1 (δ 4.93) of glucose were clearly observed. Based on the above evidence, the structure of **7** was established as 12-*O*-(*E*)-cinnamoyl (20*S*)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptaol 3-*O*-β-D-glucopyranosyl(1→4)-β-D-cymaropyranosyl(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.

In the FAB-MS, gymnepregoside N (**8**) gave an $[M-H]^-$ peak at *m/z* 1425, which was the same as **7**. Comparison of ^{13}C-NMR spectra of **7** and **8** revealed that **8** differed from **7** only by the replacement of the third cymarose (cymarose$_3$) with an oleandrose (oleandrose$_2$) in the C-3-linked sugar units. Indeed, in the ROESY experiment of **8**, significant cross peaks between H-3 (δ 4.20) of the aglycone and H-1 (δ 5.18) of cymarose$_1$, and H-4 (δ 3.46) of cymarose$_1$ and H-1 (δ 5.10) of cymarose$_2$, and H-4 (δ 3.43) of cymarose$_2$ and H-1 (δ 4.66) of oleandrose$_1$, and H-4 (δ 3.45) of oleandrose$_1$ and H-1 (δ 4.85) of oleandrose$_2$, and H-4 (δ 3.57) of oleandrose$_2$ and H-1 (δ 5.27) of 6-deoxy-3-*O*-methylallose, and

Table 4. ^{13}C-NMR Data for the Aglycone Moieties of Gymnepregosides G (**1**)—Q (**11**) (100 MHz in Pyridine-d_5)

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**[a]	**8**[a]	**9**[a]	**10**[a]	**11**
C-1	27.6	28.1	27.9	27.7	27.9	27.9	27.6	27.7	27.6	28.0	27.8
2	26.6	27.1	27.0	26.7	26.9	26.9	26.6	27.0	26.6	27.0	26.8
3	74.7	74.6	75.0	75.0	75.1	75.1	74.8	74.8	74.2	75.0	75.0
4	38.9	39.3	39.4	39.1	39.3	39.4	39.0	39.1	39.1	39.4	39.2
5	75.1	75.1	75.0	74.8	75.0	75.0	74.8	74.8	74.7	75.0	74.9
6	136.2	136.2	136.2	136.2	136.2	136.2	136.2	136.2	136.2	136.2	136.2
7	127.7	128.0	127.7	127.6	127.5	127.4	127.6	127.6	127.7	127.7	127.4
8	73.7	74.1	74.1	73.9	74.3	74.2	73.8	73.9	73.8	74.1	74.1
9	36.8	37.3	37.3	37.0	37.1	37.3	37.0	37.0	37.0	37.2	37.1
10	39.5	40.0	40.0	39.8	39.9	39.9	39.7	39.7	39.7	40.0	39.8
11	27.1	27.7	24.2	23.9	24.0	24.0	23.8	23.9	23.7	24.2	23.9
12	72.7	73.1	76.2	76.0	76.0	76.0	75.9	76.0	75.9	76.1	75.8
13	59.7	60.2	58.3	58.1	58.3	58.4	58.0	58.1	57.9	58.3	58.2
14	88.1	88.5	89.1	88.9	88.4	88.4	88.9	88.9	88.8	89.1	88.3
15	33.5	34.0	33.6	33.4	33.4	33.5	33.3	33.3	33.4	33.6	33.3
16	34.5	35.1	33.8	33.6	34.6	34.6	33.5	33.5	33.4	33.9	34.5
17	89.0	89.4	88.1	87.9	88.0	87.9	87.9	87.9	87.9	88.1	87.9
18	12.2	12.9	13.1	12.7	12.5	12.8	12.7	12.7	12.6	13.1	12.6
19	21.4	22.0	22.0	21.6	21.8	21.9	21.6	21.6	21.6	22.0	21.7
20	72.7	73.1	70.7	70.5	74.7	75.5	70.5	70.5	70.7	70.7	74.6
21	17.8	18.3	20.0	19.6	15.9	16.0	19.6	19.6	19.4	20.0	15.8
(*E*) or (*Z*)-Cinnamoyl moiety											
1′			135.0	135.3	135.3	135.0	135.0	135.0	134.9	135.0	135.3
2′, 6′			128.7	128.6	128.7	128.6	128.6	128.6	130.4	128.7	128.6
3′, 5′			129.3	128.9	129.4	129.2	129.2	129.1	128.3	129.3	129.3
4′			130.6	130.5	130.7	130.5	130.5	130.5	129.3	130.6	130.5
7′			145.3	145.3	143.9	144.0	145.3	145.3	143.4	145.3	143.8
8′			119.7	119.7	120.5	120.3	119.6	119.6	121.0	119.7	120.1
9′			167.0	167.0	166.9	166.8	167.0	167.0	166.2	167.0	166.7
Tigloyl or benzoyl moiety											
1″					166.9	131.3					166.7
2″					129.6	130.2					129.5
3″					137.9	128.8					137.8
4″					14.4	133.2					14.3
5″					12.7	128.8					12.4
6″						130.2					
7″						165.6					

a) Measured at 125 MHz.

H-4 (δ 3.74) of 6-deoxy-3-*O*-methylallose and H-1 (δ 4.98) of glucose were all clearly observed. Consequently, the structure of **8** was established as 12-*O*-(*E*)-cinnamoyl (20*S*)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptaol 3-*O*-β-D-glucopyranosyl(1→4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl(1→4)-β-D-oleandropyranosyl(1→4)-β-D-oleandropyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-cymaropyranoside.

In the FAB-MS, gymnepregoside O (**9**) showed an $[M-H]^-$ peak at *m/z* 1263, 162 mass units lower than that of **8**, suggesting the loss of a glucosyl unit in the molecule. The NMR spectral data of **9** were very close to those of **8**, except for the signals assigned to a terminal glucopyranosyl group and to the cinnamoyl residue at C-12. The ^{1}H-NMR data of **9** showed signals assignable to a (*Z*)-cinnamoyl group at δ 6.35 and 7.00 (each 1H, d, J=12.5 Hz). Furthermore, the sugar sequences at C-3 were corroborated by an ROESY experiment. Thus, **9** was formulated as 12-*O*-(*Z*)-cinnamoyl (20*S*)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptaol 3-*O*-6-deoxy-3-*O*-methyl-β-D-allopyranosyl(1→4)-β-D-oleandropyranosyl(1→4)-β-D-oleandropyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-cymaropyranoside.

In the FAB-MS, gymnepregoside P (**10**) gave an $[M-H]^-$ peak at *m/z* 1411, 130 mass units higher than that of **3**. The ^{13}C-NMR chemical shifts of **10** were in good agreement with **3**, the only difference being the additional six carbon signals due to a 2-deoxy sugar at δ 100.4, 88.3, 71.6, 70.4, 40.2, and 18.5 in **10** (Table 5). The ^{1}H–^{1}H correlation spectroscopy (COSY), ^{13}C–^{1}H COSY and ROESY experiments achieved the construction of these signals and led to the β-canaropyranosyl unit.[6,7] The sugar sequence was confirmed by the same procedure for **7**—**9**. NOEs were detected between the H-3 (δ 4.19) of aglycone and H-1 (δ 5.18) of cymarose$_1$, the H-4 (δ 3.45) of cymarose$_1$ and H-1 (δ 5.10) of cymarose$_2$, the H-4 (δ 3.43) of cymarose$_2$ and H-1 (δ 4.66) of oleandrose, the H-4 (δ 3.45) of oleandrose and H-1 (δ 4.92) of canarose, the H-4 (δ 3.30, t, J=9.0 Hz)] of canarose and H-1 (δ 5.09) of 6-deoxy-3-*O*-methylallose, and the H-4 (δ 3.74, dd, J=9.6, 2.2 Hz)] of 6-deoxy-3-*O*-methylallose and H-1 (δ 4.96) of glucose, confirming the sugar sequence at C-3. Moreover, in the HMBC spectrum, the long-range correlations between C-3 (δ 75.0) of the aglycone and H-1 (δ 5.18) of cymarose$_1$, and H-4 (δ 3.45) of cymarose$_1$ and C-1 (δ 100.5) of cymarose$_2$, and H-4 (δ 3.43) of cymarose$_2$ and C-1 (δ 102.0) of oleandrose, and H-4 (δ 3.45) of oleandrose and C-1 (δ 100.4) of canarose, and H-4 (δ 3.30) of canarose and C-1 (δ 103.1) of 6-deoxy-3-*O*-methylallose, and H-4 (δ 3.74) of 6-deoxy-3-*O*-methylallose and C-1 (δ 106.6) of glucose, were all clearly

Table 5. ^{13}C-NMR Data for Sugar Moieties of Gymnepregosides G (**1**)—Q (**11**) (100 MHz in Pyridine-d_5)

C-3	Sugar	**1**	**2**	**3**	**4**	**5**	**6**	**7**[a]	**8**[a]	**9**[a]	**10**[a]	**11**
cym$_1$	1	97.2	97.5	97.8	97.7	97.9	97.8	97.7	97.7	97.7	97.8	97.8
	2	36.8	37.2	37.1	36.8	37.0	37.1	36.8	36.8	36.8	37.1	36.9
	3	77.7	78.0	78.1	78.0	78.1	77.9	78.0	77.9	77.9	78.1	78.0
	4	82.9	83.2	83.1	83.1	83.2	83.1	83.1	83.1	83.0	83.1	83.0
	5	69.0	69.2	69.3	69.1	69.4	69.3	69.1	69.1	69.1	69.3	69.2
	6	18.3	18.9	18.8	18.5	18.7	18.8	18.4	18.5	18.4	18.8	18.6
OMe		58.6	59.0	59.1	58.8	59.0	59.0	58.8	58.8	58.7	59.0	58.9
cym$_2$	1	100.2	100.6	100.5	100.4	100.5	100.5	100.4	100.4	100.4	100.5	100.5
	2	36.6	37.1	37.1	36.8	36.9	37.0	36.8	36.8	36.8	37.1	36.8
	3	77.6	78.1	77.9	77.8	77.9	78.1	78.0	77.8	77.7	77.9	77.8
	4	82.9	83.3	83.3	83.1	83.2	83.2	83.1	83.1	83.1	83.3	83.2
	5	68.8	69.4	68.8	69.0	69.1	69.1	68.9	68.9	68.9	69.1	69.0
	6	18.3	18.9	18.8	18.5	18.7	18.8	18.4	18.5	18.4	18.8	18.6
OMe		58.8	59.2	59.2	58.9	59.1	59.2	58.9	58.9	58.9	59.1	59.0
ole$_1$	1	101.7	102.0	102.0	101.9	102.4	102.0	101.8	101.9	101.9	102.0	102.0
	2	37.4	37.9	37.9	37.6	37.8	37.8	37.6	37.6	37.6	37.9	37.8
	3	79.1	79.5	79.3	79.3	79.4	79.4	79.3	79.0	79.0	79.2	79.1
	4	82.8	83.1	83.1	83.0	83.1	83.0	83.1	82.7	82.7	83.1	83.0
	5	71.9	72.3	72.2	72.0	75.1	72.2	72.0	71.6	71.6	71.8	71.9
	6	18.7	19.0	19.2	18.9	19.1	19.0	18.8	18.7	18.6	18.9	19.0
OMe		57.1	57.5	57.7	57.4	57.6	57.5	57.4	57.4	57.3	57.7	57.5
ole$_2$	1								100.0	100.0		
	2								37.7	37.6		
	3								79.6	79.5		
	4								83.0	82.9		
	5								71.9	72.1		
	6								18.9	19.0		
OMe									57.3	57.2		
cana	1										100.4	100.3
	2										40.2	40.1
	3										70.4	70.2
	4										88.3	88.2
	5										71.6	71.5
	6										18.5	18.3
alm or	1	103.9	102.2	102.0	104.0	104.1	102.2	102.0	101.9	102.0	103.1	103.1
the	2	75.1	73.5	72.9	74.8	75.0	73.4	72.6	72.7	73.3	72.2	72.2
	3	88.0	84.3	83.3	86.3	86.5	84.2	83.1	83.1	84.0	83.1	83.0
	4	75.9	74.8	83.5	83.2	83.4	74.8	83.3	83.3	74.6	82.8	82.8
	5	72.7	71.2	69.7	72.0	72.1	71.2	69.1	69.5	71.0	70.0	69.9
	6	18.3	19.3	18.4	18.7	18.9	19.2	18.0	18.3	18.6	18.2	18.0
OMe		60.8	62.4	62.0	60.6	61.0	62.3	61.7	61.7	62.0	62.0	61.9
cym$_3$	1							100.4				
	2							37.1				
	3							77.7				
	4							82.9				
	5							69.4				
	6							18.4				
OMe								58.7				
glc	1			106.7	104.8	104.9		106.6	106.5		106.6	106.6
	2			75.7	75.8	75.9		75.4	75.5		75.6	75.5
	3			78.6	78.7	78.5		78.5	78.3		78.7	78.6
	4			72.1	72.2	72.2		71.8	72.1		72.0	71.6
	5			78.6	78.1	78.8		78.9	78.3		78.5	78.4
	6			63.2	63.2	63.3		63.0	63.0		63.2	63.1

alm=6-deoxy-3-*O*-methylallose. *a*) Measured at 125 MHz.

observed. Therefore, the structure of **10** was determined as 12-*O*-(*E*)-cinnamoyl-(20*S*)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptaol 3-*O*-β-D-glucopyranosyl(1→4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl(1→4)-β-canaropyranosyl-(1→4)-β-D-oleandropyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-cymaropyranoside.

In the FAB-MS gymnepregoside Q (**11**) showed an $[M-H]^-$ peak at *m/z* 1493, 82 mass units higher than that of **10**, suggesting an additional tigloyl unit in the molecule. A detailed ^{13}C-NMR spectral comparison of **11** with **10** showed that the chemical shifts for the six sugar residues of both compounds were superimposable, the only difference was the signal ascribable to esterified C-20 (δ 74.6), with C-17 and C-21 for the aglycone. Additionally, in the ^{1}H-NMR spectrum, the marked downfield shift of the H-12 [δ 5.10 (dd, J= 9.7, 1.5 Hz)] and H-20 (δ 5.26 (q, J=6.0 Hz) were identical with those observed in **5** and **15**, whose C-12 and C-20 positions were esterified with (*E*)-cinnammoyl and tigloyl residues, respectively. Thus, **11** was formulated as 12-*O*-(*E*)-cinnamoyl-20-*O*-tigloyl-(20*S*)-pregn-6-ene-3β,5α,8β,12β,14β,17β,20-heptaol 3-*O*-β-D-glucopyranosyl(1→4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl(1→4)-β-canaropyranosyl-

(1→4)-β-D-oleandropyranosyl(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

In conclusion, we have isolated from the roots of *Gymnema alternifolium* (Asclepiadaceae), eleven new oxypregnane-oligoglycosides, named gymnepregosides G (**1**)—Q (**11**), having a very rare 5α-hydroxypregnane[2] as the aglycone.

Experimental

General Procedure Melting points were measured with a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were recorded 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 using tetramethylsilane (TMS) as an internal standard. NMR experiments included ^1H–^1H COSY, ^{13}C–^1H COSY, HMBC (512×1024 data matrix size, 128 scans, recycle delay=1.16 s), total correlation spectroscopy (TOCSY) and 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) was used and for TLC, Silica-gel 60F-254 (Merck). HPLC was carried out on a Waters ALC/GPC 244 instrument.

Extraction and Isolation of Compounds 1—14 The dried roots (1.5 kg) of *G. alternifolium* collected in Taipei, Formosa, 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 then washed with water, and the adsorbed materials were eluted with 20—100% MeOH to obtain the 20%, 40%, 60% and 100% MeOH eluates. The 100% MeOH eluate (64 g) was chromatographed on Bondapack C_{18} with 80% MeOH to give six fractions (frs. 1—6). Fraction 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). Fraction 1-1 was purified by preparative HPLC (YMC, ODS S-5, 24% CH_3CN) to afford gymnepregosides G (**1**, 15 mg) and H (**2**, 15 mg). Fraction 2 (20 g) was subjected to HPLC on ODS (Develosil Lop ODS, 50% CH_3CN) to give five fractions (frs.2-1—5). Fractions 2-2 and 2-3 were purified by preparative HPLC (YMC, ODS S-5, 34—35% CH_3CN) to afford gymnepregosides I (**3**, 25 mg) and J (**4**, 25 mg). Fraction 3 (2.9 g) was subjected to HPLC on ODS (Develosil Lop ODS, 55% CH_3CN) to give four fractions (frs.3-1—4). Fractions 3-1 and 3-3 were purified by preparative HPLC (YMC, ODS S-5, 45—46% CH_3CN) to afford gymnepregosides M (**7**, 60 mg), N (**8**, 40 mg) and P (**10**, 25 mg) from fr. 3-1, and K (**5**, 40 mg) and compound 24 (**13**, 130 mg) from fr.3-3. Fraction 4 (5.0 g) was subjected to HPLC on ODS (Develosil Lop ODS, 55% CH_3CN) to give six fractions (frs. 4-1—6). Fractions 4-2 and 4-3 were purified by preparative HPLC (YMC, ODS S-5, 44—46% CH_3CN) to afford compound 24 (**13**, 10 mg) from fr. 4-2, and gymnepregoside Q (**11**, 25mg) from fr. 4-3. Fraction 5 (1.8 g) was purified by preparative HPLC (YMC, ODS S-5, 32% CH_3CN) to afford compound 30 (**14**, 5 mg). Fraction 6 (1.8 g) was purified by preparative HPLC (YMC, ODS S-5, 55—57% CH_3CN) to afford gymnepregosides L (**6**, 10 mg), O (**9**, 10 mg) and stephanoisde I (**12**, 20 mg).

Gymnepregoside G (**1**): mp 140—142 °C, $[\alpha]_D^{25}$ +10.3° (*c*=0.9, $CHCl_3$). Negative FAB-MS *m/z*: 989 $[M-H]^-$. *Anal*. Calcd for $C_{49}H_{82}O_{20}\cdot H_2O$: C, 58.32; H, 8.39. Found: C, 58.45; H, 8.61. ^1H-NMR and ^{13}C-NMR: Tables 1, 2, 4 and 5.

Gymnepregoside H (**2**): mp 146—148 °C, $[\alpha]_D^{25}$ +16.7° (*c*=1.4, $CHCl_3$). Negative FAB-MS *m/z*: 989 $[M-H]^-$. *Anal*. Calcd for $C_{49}H_{82}O_{20}\cdot H_2O$: C, 58.32; H, 8.39. Found: C, 58.38; H, 8.05. ^1H-NMR and ^{13}C-NMR: Tables 2, 4 and 5.

Gymnepregoside I (**3**): mp 173—175 °C, $[\alpha]_D^{25}$ +10.5 ° (*c*=1.9, $CHCl_3$). 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*: 1281 $[M-H]^-$. *Anal*. Calcd for $C_{64}H_{98}O_{26}\cdot 3H_2O$: C, 57.47; H, 7.84. Found: C, 57.46; H, 8.14. ^1H-NMR and ^{13}C-NMR: Tables 2, 4 and 5.

Gymnepregoside J (**4**): mp 169—171 °C, $[\alpha]_D^{25}$ +11.0° (*c*=1.9, $CHCl_3$). IR (film) cm^{-1}: 3480, 1710, 1640, 1165, 1090, 1005. UV λ_{max} (MeOH) nm (log ε): 204 (4.37), 217 (4.15), 280 (4.36). Negative FAB-MS *m/z*: 1281 $[M-H]^-$. Anal. Calcd for $C_{64}H_{98}O_{26}\cdot 2H_2O$: C, 58.26; H, 7.79. Found: C, 58.13; H, 8.02. ^1H-NMR and ^{13}C-NMR: Tables 4 and 5.

Gymnepregoside K (**5**): mp 166.2—167.3 °C, $[\alpha]_D^{25}$ +10.4° (*c*=0.5, $CHCl_3$). IR (film) cm^{-1}: 3460, 1710, 1640, 1075, 1060. UV λ_{max} (MeOH) nm (log ε): 206 (4.27), 217 (4.28), 280 (4.17). Negative FAB-MS *m/z*: 1363 $[M-H]^-$. *Anal*. Calcd for $C_{69}H_{104}O_{27}$: C, 60.69; H, 7.68. Found: C, 60.69; H, 7.76. ^1H-NMR and ^{13}C-NMR: Tables 1, 2, 4 and 5.

Gymnepregoside L (**6**): mp 146—148 °C, $[\alpha]_D^{25}$ +93.5° (*c*=0.7, $CHCl_3$). IR (film) cm^{-1}: 3440, 1715, 1640, 1110. UV λ_{max} (MeOH) nm (log ε): 222 (4.34), 236 (4.17), 278 (4.32). Negative FAB-MS *m/z*: 1223 $[M-H]^-$. *Anal.* Calcd for $C_{65}H_{92}O_{22}\cdot H_2O$: C, 61.32; H, 7.90. Found: C, 61.62; H, 7.70. ^1H-NMR and ^{13}C-NMR: Tables 1, 4 and 5.

Gymnepregoside M (**7**): mp 166.2—167.3 °C, $[\alpha]_D^{25}$ +10.4° (*c*=2.1, $CHCl_3$). IR (film) cm^{-1}: 3460, 1705, 1635, 1580, 1120. UV λ_{max} (MeOH) nm (log ε): 205 (4.18), 217 (4.18), 279 (4.33). Negative FAB-MS *m/z*: 1425 $[M-H]^-$. *Anal*. Calcd for $C_{71}H_{110}O_{29}\cdot 2H_2O$: C, 58.26; H, 7.85. Found: C, 58.05; H, 7.80. ^1H-NMR and ^{13}C-NMR: Tables 1 and 3—5.

Gymnepregoside N (**8**): mp 165—167 °C, $[\alpha]_D^{25}$ +14.0° (*c*=3.4, $CHCl_3$). IR (film) cm^{-1}: 3460, 1710, 1630, 1165, 1100, 1005. UV λ_{max} (MeOH) nm (log ε): 205 (4.18), 217 (4.18), 280 (4.33). Negative FAB-MS *m/z*: 1425 $[M-H]^-$. *Anal*. Calcd for $C_{71}H_{110}O_{29}\cdot 2H_2O$: C, 58.26; H, 7.85. Found: C, 58.42; H, 8.10. ^1H-NMR and ^{13}C-NMR: Tables 3—5.

Gymnepregoside O (**9**): mp 112—114 °C, $[\alpha]_D^{25}$ +12.8° (*c*=1.1, $CHCl_3$). IR (film) cm^{-1}: 3450, 1705, 1635, 1585, 1120. UV λ_{max} (MeOH) nm (log ε): 200 (4.50), 276 (4.11). Negative FAB-MS *m/z*: 1263 $[M-H]^-$. *Anal*. Calcd for $C_{65}H_{100}O_{24}\cdot 2H_2O$: C, 57.42; H, 8.43. Found: C, 57.62; H, 8.70. ^1H-NMR and ^{13}C-NMR: Tables 1, 2, 4 and 5.

Gymnepregoside P (**10**): mp 175—176 °C, $[\alpha]_D^{25}$ +10.6° (*c*=2.6, $CHCl_3$). IR (film) cm^{-1}: 3480, 1710, 1640, 1165, 1090, 1005. UV λ_{max} (MeOH) nm (log ε): 203.5 (4.37), 217 (4.25), 280 (4.36). Negative FAB-MS *m/z*: 1411 $[M-H]^-$. *Anal*. Calcd for $C_{70}H_{108}O_{29}\cdot H_2O$: C, 58.73; H, 7.74. Found: C, 58.52; H, 8.00. ^1H-NMR and ^{13}C-NMR: Tables 3—5.

Gymnepregoside Q (**11**): mp 151—153 °C, $[\alpha]_D^{25}$ +60.6° (*c*=2.0, $CHCl_3$). 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*: 1493 $[M-H]^-$. *Anal.* Calcd for $C_{75}H_{114}O_{30}\cdot H_2O$: C, 59.51; H, 7.72. Found: C, 59.82; H, 7.60. ^1H-NMR and ^{13}C-NMR: Tables 4 and 5.

Hydrolysis of Crude Glycosides A solution of MeOH eluate (500 mg) in 4 ml dioxane was treated with 2 ml 1% H_2SO_4 with stirring at 100 °C for 60 min. After cooling, the reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. The H_2O layer was neutralized with Amberlite IRA-45 and evaporated under reduced pressure to give the sugar fraction (160 mg). This was purified on a silica-gel column eluting with CH_2C1_2–MeOH–H_2O (30 : 1 : 0—25 : 6 : 0.1) to afford D-cymarose (15 mg, $[\alpha]_D^{25}$ +47.4° (*c*=0.5, 24 h after dissolution in H_2O), D-glucose (15 mg, $[\alpha]_D^{25}$ +56.8° (*c*=0.3, 24 h after dissolution in H_2O), D-oleandrose (15 mg, $[\alpha]_D^{25}$ −4.94° (*c*=0.2, 24 h after dissolution in H_2O), a mixture of 6-deoxy-3-*O*-methylallose and thevetose (9 : 1, 30 mg), which were identified by their NMR spectra.[1] However, canarose could not be isolated. The D or L configuration of each sugar was determined by refractive index (RI) detection (Waters 410) and chiral detection (Shodex OR-1) during 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*-methyl-D-allose and L-thevetose). The sugar gave the following peaks: D-(+)-glc 14.00 min; D-(+)-the 14.20 min; D-(+)-ole 15.60 min; D-(+)-alm 17.60 min and D-(+)-cym 17.80 min.

Mild Acid Hydrolysis of Compound 5 A solution of **5** (15 mg) in 1 ml dioxane was treated with 0.5 ml 1% H_2SO_4 with stirring at 100 °C for 60 min. After cooling, the reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. The CH_2Cl_2 extract was subjected to HPLC (YMC, ODS, 40% CH_3CN) and TLC (CH_2Cl_2 : MeOH=10 : 1) and identified as a prosapogenin (**15**, 2 mg).

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