Antisweet Natural Products. XIII.[1] Structures of Alternosides I—X from *Gymnema alternifolium*

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From the dried roots of *Gymnema alternifolium*, ten new oleanane-type triterpenoid glycosides, named alternosides I—X (1—10), were isolated, as well as two artifactual compounds, 11 and 12. Their structures were determined on the basis of spectroscopic data and chemical evidence. Compounds 1—5, and 6, 8 and 10 are 3,28-*bis*-*O*-glycosides of chichipegenin having an acyl group at position 16 or 18, and no acyl group, respectively. Compound 7 is 3-*O*-glycoside of chichipegenin. Compound 9 consists of the 3,28-*bis*-*O*-glycosides of longispinogenin. Compounds 1—5 and 11, having an acyl group, showed antisweet activity.

Key words *Gymnema alternifolium*; alternoside; antisweet substance; Asclepiadaceae; oleanane triterpene; chichipegenin

The asclepiadaceous plants are rich sources of naturally occurring antisweet substances. Example include gymnemic acids I—XII, gymnemic acids 1 and 2, and gymnemasaponins I—V from *Gymnema sylvestre*[2] and sitakisosides I—XX from *Stephanotis lutchuensis* KOIZ. var. *japonica*.[1,3] As a part of our studies on naturally occurring antisweet substances, we have initiated a chemical study of *Gymnema alternifolium*, which is an evergreen climber growing in the forests in Taiwan and south China. Its roots are used for detoxification, removal of edema and fever.[4] In this paper, we report the isolation, structural elucidation and antisweet activity of twelve novel saponins, alternosides I—X (**1**—**10**) and compounds **11** and **12**, having an oleanene skeleton. Their structures were elucidated by chemical and spectral methods, two dimensional (2D)-NMR techniques having been especially helpful.

The 70% EtOH extract obtained from the dried roots of *Gymnema alternifolium* was subjected to Amberlite XAD-2 column chromatography to give a saponin fraction. Repeated separation of the saponin fraction by HPLC gave ten new compounds named alternosides I (**1**), II (**2**), III (**3**), IV (**4**), V (**5**), VI (**6**), VII (**7**), VIII (**8**), IX (**9**), and X (**10**), as well as two artifacts, **11** and **12**. The ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY), ^{1}H–^{13}C COSY, total correlation spectroscopy (TOCSY), heteronuclear multiple-bond correlation (HMBC) and rotating frame Overhauser enhancement spectroscopy (ROESY) experiments provided sufficient information to enable us to construct the complete structures of **1**—**12**.

Alternoside II (**2**) had the molecular formula $C_{50}H_{80}O_{20}$ (negative FAB-MS, *m/z* 999 $[M-H]^-$) and spectroscopic properties which characterized the carbonyl functions as a carboxyl group and an acetyl group [ν_{max} 3400 (br), 1730 (br), 1245, 1050; δ_C 172.0 (COOH), 170.8, 22.1 (Ac)]. Methylation of **2** with diazomethane afforded compound **11** $[\alpha]_D^{25}$ +1.6°, $C_{51}H_{82}O_{20}$ {negative FAB-MS, *m/z* 1013 $[M-H]^-$}, which was further reduced with $NaBH_4$, follwed by acid hydrolysis to afford chichipegenin (**13**)[5] mp 315—317 °C, $[\alpha]_D^{25}$ +40.0°, $C_{30}H_{50}O_4$ {positive FAB-MS, *m/z* 497 $[M+Na]^+$ as an aglycon, together with D-glucose and L-rhamnose in a molar ratio of 2:1 (confirmed by specific rotation measurement using HPLC with chiral detection)[6] as sugar components. The ^{1}H- and ^{13}C-NMR spectra of **2** indicated the presence of an acetyl group [CH_3: δ 2.12 (3H, s), C-1: δ 170.8, C-2: δ 22.1], one β-D-glucuronopyranosyl unit [H-1: δ 5.00 (d, J=8.5 Hz), C-1: δ 106.7, C-6: δ 172.0], one β-D-glucopyranosyl unit [H-1: δ 5.37 (d, J=7.5 Hz), C-1: δ 105.9], and one α-L-rhamnopyranosyl unit [H-1: δ 5.29 s), C-1: δ 101.8]. Alkaline treatment of **2** gave a prosapogenin.

The prosapogenin revealed a quasi-molecular ion peak $[M-H]^-$ at *m/z* 957 in the negative FAB-MS. A ^{13}C-NMR spectral comparison of the prosapogenin and **13** showed glycosylation shifts[7,8] of +11.2 ppm at the C-3 signal and +5.6 ppm at the C-28 signal, demonstrating the sugar linkages to be located at C-3-OH and C-28-OH. The FAB-MS of the prosapogenin showed a fragment ion peak of *m/z* 795 $[M-162-H]^-$, disclosing a glucosyl group to be located at the terminal. In the ^{13}C-NMR spectrum of the prosapogenin, the C-3 signal of glucuronic acid was shifted to δ 87.6 by the glycosylation shift. Furthermore, in the HMBC spectrum of the prosapogenin, long-range correlations were observed between H-1 (δ 5.37) of glucose and C-3 (δ 87.6) of glucuronic acid, H-1 (δ 4.99) of glucuronic acid and C-3 (δ 89.2) of the aglycon, and H-1 (δ 5.28) of rhamnose and C-28 (δ 64.4) of the aglycon. Therefore, the prosapogenin was formulated as chichipegenin 3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucuronopyranosyl-28-*O*-α-L-rhamnopyranoside. The location of the acetyl group in **2** was determined by spectral comparison of **2** and prosapogenin, and by an HMBC experiment on **2**. Thus, acylation shifts were observed at the C-16 [+1.25 ppm (H-16), +1.3 ppm (C-16)] and C-15 positions [−2.4 ppm (C-15)]. In the HMBC spectrum, the ester carbon signal of the acetyl group at δ 170.8 was correlated with the methine proton (H-16) of the aglycon at δ 6.36. Hence, **2** was formulated as chichipegenin 16-*O*-acetyl-3-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucuronopyranosyl-28-*O*-α-L-rhamnopyranoside.

Alternoside X (**10**), colorless needles, mp 223—225 °C,

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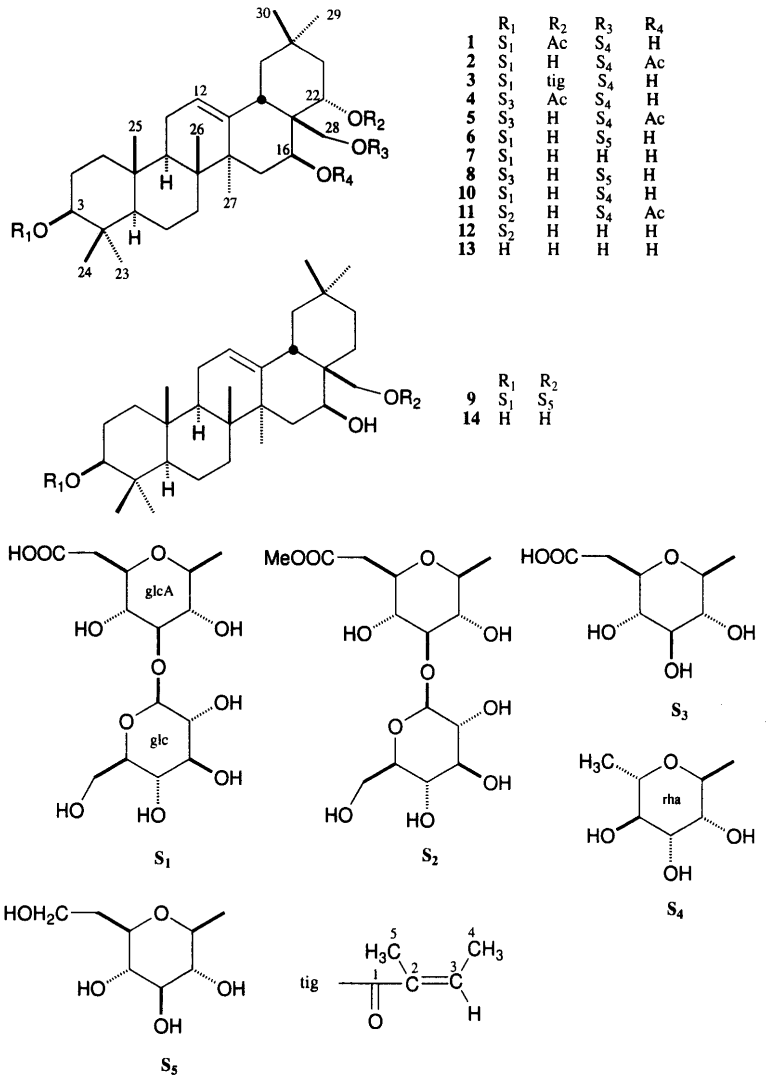

	R_1	R_2	R_3	R_4
1	S_1	Ac	S_4	H
2	S_1	H	S_4	Ac
3	S_1	tig	S_4	H
4	S_3	Ac	S_4	H
5	S_3	H	S_4	Ac
6	S_1	H	S_5	H
7	S_1	H	H	H
8	S_3	H	S_5	H
10	S_1	H	S_4	H
11	S_2	H	S_4	Ac
12	S_2	H	H	H
13	H	H	H	H

	R_1	R_2
9	S_1	S_5
14	H	H

Chart 1

$C_{48}H_{78}O_{19}$, was found to be identical with the prosapogenin by direct comparison.

Alternoside V (**5**) had the molecular formula $C_{44}H_{70}O_{15}$ (negative FAB-MS, *m/z* 837 $[M-H]^-$), *i.e.*, 162 mass units lower than that of **2**. Acid hydrolysis of **5** afforded **13**, and D-glucuronolactone and L-rhamnose. The ^1H- and ^{13}C-NMR spectra indicated that **5** was composed of 1 mol each of chichipegenin, acetic acid, glucuronic acid, and rhamnose. Enzymatic hydrolysis of **2** gave **5**. Therefore, **5** was formulated as chichipegenin 16-*O*-acetyl-3-*O*-*β*-D-glucuronopyranosyl-28-*O*-*α*-L-rhamnopyranoside.

Alternoside I (**1**) had the same molecular formula $C_{50}H_{80}O_{20}$ (negative FAB-MS, *m/z* 999 $[M-H]^-$), as **2**. Acid hydrolysis of **1** provided D-glucuronolactone, D-glucose and rhamnose, and **13**. The ^1H- and ^{13}C-NMR spectra of **1** indicated the presence of an acetyl group [CH_3: δ 1.97 (3H, s), C-1: δ 169.5, C-2: δ 21.1], one *β*-D-glucuronopyranosyl unit [H-1: δ 4.99 (d, J=8.5 Hz), C-1: δ 106.8, C-6: δ 172.3], one *β*-D-glucopyranosyl unit [H-1: δ 5.37 (d, J=7.5 Hz), C-1: δ 105.9], and one *α*-L-rhamnopyranosyl unit [H-1: δ 5.25 s), C-1: δ 102.1]. Alkaline treatment of **1** gave prosapogenin. A ^1H- and ^{13}C-NMR spectral comparison of **1** with prosapogenin disclosed C-22 [+ 0.91 ppm (H-22), +4.0 ppm (C-22)] as the acylation site in the former. Therefore, the structure of **1** was established as chichipegenin 22-*O*-acetyl-3-*O*-*β*-D-glucopyranosyl(1→3)-*β*-D-glucuronopyranosyl-28-*O*-*α*-L-rhamnopyranoside.

Alternoside IV (**4**) had the same molecular formula, $C_{44}H_{70}O_{15}$ (negative FAB-MS, *m/z* 837 $[M-H]^-$), as **5**. Acid hydrolysis of **4** afforded **13**, and D-glucuronolactone and L-rhamnose. The ^1H- and ^{13}C-NMR spectra indicated that **4** was composed of 1 mol each of chichipegenin, acetic acid, glucuronic acid, and rhamnose. Enzymatic hydrolysis of **1** gave **4**. Therefore, **4** was formulated as chichipegenin 22-*O*-acetyl-3-*O*-*β*-D-glucuronopyranosyl-28-*O*-*α*-L-rhamnopyranoside.

Alternoside III (**3**) had the molecular formula $C_{53}H_{84}O_{20}$ (negative FAB-MS, *m/z* 1039 $[M-H]^-$), *i.e.*, 40 mass units high than that of **1**. Acid hydrolysis of **3** afforded **13**, and D-glucuronolactone, D-glucose, and L-rhamnose. Alkaline treatment of **3** gave prosapogenin and tiglic acid. The ^1H- and ^{13}C-NMR spectra indicated that **3** was composed of 1 mol each of chichipegenin, tiglic acid, glucuronic acid, glucose, and rhamnose. A ^1H- and ^{13}C-NMR spectral comparison of **3** with prosapogenin disclosed C-22[+0.97 ppm (H-22), +4.0 ppm (C-22)] as the acylation site in the former. Therefore, the structure

Table 1. ^{13}C-NMR Spectral Data for **1**—**14** (in Pyridine-d_5, 100 MHz)

	C	1[a]	2[a]	3	4	5	6[a]	7	8	9	10	11	12	13	14
	1	38.8	38.8	38.8	38.8	38.8	38.6	38.7	38.8	38.8	38.9	38.7	38.8	39.1	39.3
	2	26.7	26.6	26.5	26.6	26.6	26.6	26.6	26.7	26.6	26.8	26.6	26.7	28.1	28.1
	3	89.1	89.1	89.0	89.0	88.8	89.1	89.1	89.1	89.2	89.2	89.2	89.4	78.0	78.3
	4	39.5	39.6	39.6	39.6	39.5	39.5	39.6	39.6	39.6	39.7	39.6	39.6	39.4	39.6
	5	55.6	55.6	55.6	55.7	55.7	55.5	55.6	55.7	55.7	55.7	55.6	55.7	55.6	55.9
	6	18.4	18.4	18.5	18.4	18.4	18.4	18.4	18.5	18.5	18.6	18.4	18.5	18.7	18.9
	7	33.0	33.1	33.0	33.0	33.0	32.8	32.9	32.9	32.8	33.2	33.0	33.0	33.0	33.2
	8	40.3	40.4	40.3	40.3	40.4	40.3	40.3	40.4	40.1	40.5	40.4	40.4	40.2	40.3
	9	47.1	47.1	47.1	47.1	47.0	47.0	47.1	47.2	47.1	47.2	47.1	47.2	47.3	47.4
	10	36.7	36.8	36.7	36.7	36.7	36.7	36.7	36.8	36.8	36.9	36.8	36.8	37.1	37.3
	11	24.1	24.1	24.1	24.1	24.1	23.8	23.9	23.9	23.8	24.3	24.1	24.0	23.9	24.1
	12	123.2	123.1	—[b]	—[b]	—[b]	123.2	—[b]	—[b]	123.0	—[b]	—[b]	—[b]	—[b]	123.0
	13	142.0	142.0	142.0	142.0	142.0	142.7	143.1	142.7	143.5	142.7	142.0	143.2	143.1	144.1
	14	42.7	43.1	42.8	42.7	43.0	42.6	42.7	42.7	44.0	42.8	43.1	42.8	42.7	44.0
	15	36.1	33.8	36.2	36.1	33.7	36.0	36.0	36.0	37.0	36.2	33.7	36.1	36.0	36.8
	16	65.3	67.8	65.3	65.3	67.4	66.5	66.9	66.6	66.3	66.5	67.5	67.0	66.9	66.9
	17	45.3	45.4	45.6	45.3	45.4	45.2	45.1	45.2	41.3	44.8	45.4	45.2	45.1	41.2
	18	44.0	44.1	44.3	44.0	44.1	43.6	43.2	43.7	44.8	43.7	44.1	43.2	43.1	44.6
	19	46.0	46.7	45.9	45.9	46.6	46.4	46.6	46.5	46.8	46.5	46.6	46.7	46.6	47.3
	20	32.2	31.9	32.3	32.2	31.8	32.2	32.2	32.2	31.1	32.3	31.9	32.3	32.2	31.3
	21	39.5	43.5	39.6	39.6	43.4	44.1	44.2	44.1	34.2	43.3	43.4	44.2	44.3	34.4
	22	73.2	67.4	73.2	73.1	67.8	69.6	69.6	69.7	26.7	69.2	67.8	69.6	69.5	26.3
	23	28.1	28.1	28.1	28.2	28.1	28.0	28.1	28.2	28.1	28.2	28.0	28.2	28.7	28.9
	24	17.0	17.0	17.0	17.0	17.0	16.9	17.0	17.0	17.0	17.1	17.0	17.1	16.5	16.7
	25	15.7	15.7	15.7	15.7	15.7	15.6	15.7	15.7	15.8	15.9	15.7	15.8	15.7	15.9
	26	17.2	17.4	17.2	17.2	17.3	17.1	17.0	17.2	17.1	17.5	17.4	17.1	17.0	17.1
	27	27.5	27.6	27.6	27.5	27.5	27.7	27.7	27.7	27.2	27.7	27.5	27.8	27.6	27.3
	28	65.2	65.2	64.9	64.6	65.1	68.2	58.9	68.3	78.1	64.4	65.1	58.9	58.8	69.0
	29	33.0	33.4	33.1	33.0	33.4	33.5	33.6	33.6	33.5	33.6	33.4	33.7	33.5	33.6
	30	24.9	25.2	25.2	25.0	25.2	25.2	25.0	25.3	24.1	24.9	25.2	25.2	25.0	24.3
3-*O*-glcA	1	106.8	106.7	106.8	107.2	106.8	106.8	106.6	107.2	106.6	106.9	106.7	106.8		
	2	74.4	74.4	74.4	74.5	75.4	74.3	74.4	75.5	74.5	74.5	74.3	74.4		
	3	87.3	87.6	87.2	78.2	78.2	87.6	87.5	78.2	87.5	87.6	87.2	87.3		
	4	71.7	71.8	71.5	73.5	73.5	71.6	71.6	73.5	71.6	71.9	71.5	71.6		
	5	77.4	77.4	77.4	77.6	77.5	77.5	77.4	77.7	77.2	77.6	76.6	76.6		
	6	172.3	172.0	172.0	172.2	172.2	172.1	172.2	172.3	172.1	172.3	170.3	170.4		
3-*O*-glc	1	105.9	105.9	105.9			105.9	105.9		105.9	106.0	105.8	105.8		
	2	75.6	75.6	75.5			75.6	75.6		75.6	75.8	75.7	75.7		
	3	78.2	78.3	78.2			78.2	78.2		78.2	78.4	78.3	78.3		
	4	71.6	71.6	71.7			71.7	71.8		71.7	71.7	71.7	71.7		
	5	78.7	78.7	78.7			78.7	78.7		78.7	78.8	78.7	78.7		
	6	62.4	62.5	62.4			62.5	62.4		62.4	62.6	62.5	62.6		
28-*O*-glc	1	102.1	101.8	102.2	102.1	101.8	105.8		105.8	105.9	101.9	101.9			
or rha	2	72.4	72.6	72.4	72.4	72.5	75.5		75.5	75.1	72.7	72.6			
	3	73.2	73.2	73.2	73.2	73.2	78.7		78.7	78.7	73.4	73.3			
	4	73.8	74.0	73.8	73.8	73.9	71.7		71.8	71.7	74.1	74.0			
	5	69.9	70.0	69.9	69.9	70.0	78.1		78.2	78.7	70.1	70.0			
	6	18.9	19.0	18.9	18.9	19.0	62.7		62.8	62.8	19.2	19.0			
Ac or tig	1	169.5	170.8	167.1	169.5	170.7						170.8			
	2	21.1	22.1	129.3	21.1	22.1						22.1			
	3			137.3											
	4			14.3											
	5			12.4											
COOMe												55.7	55.7		

a) 125 MHz. *b*) Overlapped signal. tig = tigloyl.

of **3** was established as chichipegenin 22-*O*-tigloyl-3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucuronopyranosyl-28-*O*-α-L-rhamnopyranoside.

Alternoside VIII (**8**) had the molecular formula $C_{42}H_{68}O_{15}$ (negative FAB-MS, *m/z* 811 $[M-H]^-$), *i.e.*, 146 mass units less than that of prosapogenin. Its IR spectrum showed the presence of hydroxyl (3455 cm^{-1}) and carboxyl (1720 cm^{-1}) groups. Acid hydrolysis of **8** afforded **13**, and D-glucuronolactone and D-glucose. The ^{1}H- and ^{13}C-NMR spectra of **8** indicated the presence of one β-D-glucuronopyranosyl unit [H-1: δ 5.03 (d, J=8.0 Hz), C-1: δ 107.2, C-6: δ 172.3] and one β-D-glucopyranosyl unit [H-1: δ 5.12 (d, J=8.0 Hz), C-1: δ 105.8]. A ^{13}C-NMR spectral comparison of **8** and **13** showed glycosylation shifts of +11.1 ppm at the C-3 signal and +9.5 ppm at the C-28 signal, demonstrating the sugar linkages to be located at C-3-OH and C-28-OH. Furthermore, in the HMBC spectrum of **8**, long-range correlations were observed between H-1 (δ 5.03) of glucuronic acid and C-3 (δ 89.1) of the aglycon, and H-1

Table 2. ^{1}H-NMR Spectral Data for **1**—**7** (in Pyridine-d_5, 400 MHz)

		1[a]	**2**[a]	**3**	**4**	**5**	**6**[a]	**7**
Aglycone	3	3.34 (11.5, 4.0)	3.36 (11.5, 4.0)	3.34 (11.5, 4.0)	3.36 (11.0, 5.0)	3.38 (11.0, 5.0)	3.34 (11.5, 4.0)	3.34 (11.5, 4.0)
	12	5.43 m	5.44 m	5.45 m	5.44 m	5.44 m	5.36 m	5.30 m
	16	4.95 (11.0, 5.5)	6.36 (11.5, 5.5)	4.93 (11.0, 5.0)	4.96 (11.0, 5.0)	6.37 (11.0, 5.5)	5.08 (11.0, 5.0)	5.11 (12.0, 5.0)
	18	2.95 (13.0, 4.5)	2.98 (13.5, 4.0)	2.95 (13.0, 4.5)	2.95 (13.0, 4.5)	2.98 (13.0, 4.5)	3.03 (13.0, 4.5)	3.08 (13.0, 4.5)
	22	5.99 (12.5, 4.0)	4.66 (12.5, 4.0)	6.05 (12.0, 4.5)	6.00 (12.0, 4.5)	4.68 (12.0, 4.5)	5.12 (12.0, 5.0)	5.08 (12.0, 5.0)
	23	1.29	1.29	1.27	1.31	1.31	1.28	1.29
	24	1.00	1.00	1.04	1.00	1.01	0.97	1.00
	25	0.84	0.85	0.84	0.85	0.86	0.77	0.78
	26	1.19	1.19	1.15	1.15	1.18	1.09	1.10
	27	1.39	1.44	1.41	1.38	1.44	1.41	1.43
	28	4.24, 3.86 (9.0)	4.30 s	—[b]	4.24, 3.86 (10.0)	4.30 s	4.77, 4.44 (10.0)	4.75, 4.44 (9.0)
	29	0.93	0.94	0.98	0.92	0.94	0.97	0.98
	30	1.00	0.92	0.95	0.98	0.92	0.93	0.93
3-glc A	1	4.99 (8.5)	5.00 (8.5)	5.00 (7.5)	5.04 (7.0)	5.05 (7.5)	5.00 (8.0)	5.01 (8.0)
3-glc	1	5.37 (7.5)	5.37 (7.5)	5.37 (7.5)			5.37 (8.5)	5.39 (8.0)
28-rha	1	5.25 s	5.29 s	5.23 s	5.27 s	5.31 s		
	6	1.70 (6.0)	1.71 (6.0)	1.69 (6.0)	1.70 (6.0)	1.72 (6.0)		
28-glc	1						5.13 (8.0)	
Ac	2	1.97	2.12		1.97	2.13		
Tig	3			7.00 (1H, 7.0)		7.00 (1H, 17.0)		
	4			1.53 (3H, 7.0)		1.53 (3H, 7.0)		
	5			1.83 s		1.83 s		

a) 600 MHz. *b*) Overlapped signal.

Table 3. ^{1}H-NMR Spectral Data for **8**—**12** (in Pyridine-d_5, 400 MHz)

		8	**9**	**10**	**11**	**12**
Aglycone	3	3.37 (11.0, 5.0)	3.35 (11.0, 5.0)	3.34 (11.5, 4.0)	3.34 (11.0, 5.0)	3.31 (11.5, 4.0)
	12	5.36 m	5.19 m	5.45 m	5.50 m	5.31 m
	16	5.08 (11.0, 5.5)	4.55 m	5.11 (12.0, 5.0)	6.37 (11.5, 5.0)	5.08 (12.0, 5.0)
	18	3.02 (13.0, 4.5)	2.74 (13.0, 5.0)	3.00 (13.0, 4.5)	3.00 (13.0, 4.0)	3.05 (13.0, 4.5)
	22	5.10 (12.0, 5.5)		5.08 (12.0, 5.0)	0.70 (11.5, 4.5)	5.10 (12.0, 5.0)
	23	1.30	1.28	1.28	1.29	1.28
	24	0.97	0.94	0.94	1.00	1.00
	25	0.78	0.81	0.84	0.85	0.78
	26	1.08	0.99	1.19	1.19	1.10
	27	1.40	1.35	1.42	1.43	1.41
	28	4.75, 4.45 (10.0)	4.05 (2H, s)	4.65 (9.0), —[a]	4.31 (2H, s)	4.75, 4.44 (9.0)
	29	0.97	0.93	0.94	0.94	0.96
	30	0.94	0.91	0.96	0.92	0.93
3-glc A	1	5.03 (8.0)	4.96 (7.5)	4.99 (7.0)	4.97 (8.0)	4.96 (8.0)
3-glc	1		5.39 (8.0)	5.38 (7.0)	5.38 (7.5)	5.35 (8.50)
28-rha	1			5.28	5.33	
	6			1.70 (6.0)	1.73 (6.0)	
28-glc	1	5.12 (8.0)	4.99 (8.0)			
Ac	2				2.11	

a) Overlapped signal.

(δ 5.12) of glucose and C-28 (δ 68.3), indicating a glucuronyl unit to be located at C-3-OH and a glucosyl unit at C-28-OH. Hence, **8** was formulated as chichipegenin 3-*O*-β-D-glucopyranosyl-28-*O*-β-D-glucopyranoside.

Alternoside VI (**6**) had the molecular formula $C_{48}H_{78}O_{20}$ (negative FAB-MS, *m/z* 973 $[M-H]^-$), *i.e.*, 162 mass units greater than that of **8**. Acid hydrolysis of **6** afforded **12**, and D-glucuronolactone and D-glucose. The ^{1}H- and ^{13}C-NMR spectra of **6** indicated the presence of one β-D-glucuronopyranosyl unit [H-1: δ 5.00 (d, J=8.0 Hz), C-1: δ 106.8, C-6: δ 172.1] and two β-D-glucopyranosyl units [H-1: δ 5.13 (d, J=8.0 Hz), C-1: δ 105.8, H-1: δ 5.37 (d, J=8.5 Hz), C-1: δ 105.9]. The C-3 signal of the glucuronic acid in the ^{13}C-NMR spectrum of **6** appeared at lower field by +9.4 ppm than that of **8** because of the glycosylation shift, demonstrating that a β-glucopyranosyl group is located at the C-3-OH of the glucuronic acid. Furthermore, the HMBC spectrum showed couplings between H-1 (δ 5.37) of 3-*O*-glc and C-3 (δ 87.6) of glucuronic acid, H-1 (δ 5.00) of glucuronic acid and C-3 (δ 89.1) of the aglycon, and H-1 (δ 5.13) of 28-*O*-glc and C-28 (δ 68.3) of the aglycon. Therefore, **6** was determined to be chichipegenin 3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucuronopyranosyl-28-*O*-β-D-glucopyranoside.

Alternoside VII (**7**) had the same molecular formula $C_{42}H_{68}O_{15}$ (negative FAB-MS, *m/z* 811 $[M-H]^-$), as **8**. Its IR spectrum showed the presence of hydroxyl

(3460 cm^{-1}) and carboxyl (1720 cm^{-1}) groups. Methylation of **7** with diazomethane afforded compound **12** $[\alpha]_D^{25}$ +7.9°, $C_{43}H_{70}O_{15}$ {negative FAB-MS, *m/z* 825 $[M-H]^-$}, which was further reduced with $NaBH_4$, followed by acid hydrolysis to afford **13** and D-glucose. The ^1H- and ^{13}C-NMR spectra of **7** indicated the presence of one β-D-glucuronopyranosyl unit [H-1: δ 5.01 (d, *J*=8.0 Hz), C-1: δ 106.6, C-6: δ 172.2] and one β-D-glucopyranosyl unit [H-1: δ 5.39 (d, *J*=8.0 Hz), C-1: δ 105.9]. The carbon signals due to the sugar moieties at C-3-OH are superimposable on those of **6**, indicating that the sugar moieties at C-3-OH are the same. Hence, the structure of **7** was established as chichipegenin 3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucuronopyranoside.

In the negative FAB-MS, alternoside IV (**9**) showed a quasi-molecular ion at *m/z* 957, 16 mass units lower than **6**, indicating the molecular formula to be $C_{48}H_{78}O_{19}$. Acid hydrolysis of **9** afforded longispinogenin (**14**)[9,10] mp 240—242 °C, $[\alpha]_D^{25}$ +50.0°, $C_{30}H_{50}O_3$ {positive FAB-MS, *m/z* 481 $[M+Na]^+$} as an aglycon, and D-glucuronolactone acid and D-glucose. The ^1H- and ^{13}C-NMR spectra of **9** indicated the presence of one β-D-glucuronopyranosyl unit [H-1: δ 4.96 (d, *J*=7.5 Hz), C-1: δ 106.6, C-6: δ 172.1] and two β-D-glucopyranosyl units [H-1: δ 4.99 (d, *J*=8.0 Hz), C-1: δ 105.9, H-1: δ 5.39 (d, *J*=8.0 Hz), C-1: δ 105.9]. The carbon signals due to the sugar moieties at C-3 and C-28 in **9** are superimposable on those of **6**, indicating that the sugar moieties are the same. Furthermore, in the HMBC spectrum of **9**, long-range correlations were observed between H-1 (δ 5.39) of glucose and C-3 (δ 87.5) of glucuronic acid, H-1 (δ 4.96) of glucuronic acid and C-3 (δ 89.2) of the aglycon, and H-1 (δ 4.99) of glucose and C-28 (δ 78.1) of the aglycon. Therefore, **9** was determined to be longispinogenin 3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucuronopyranosyl-28-*O*-β-D-glucopyranoside.

A 1 mM solution of any of alternosides I—V, and compound **11** led to complete suppression of the sensation of sweetness induced by 0.2 M sucrose. Alternosides VI—X, with no acyl group, had no activity. The activities of alternosides I—V, and compound **11** were half those of gymnemic acids III and IV. Compounds **11** and **12** could be artifacts generated during the isolation process.

Experimental

Melting points were measured with a Yanagimoto micromelting point apparatus, without correction. Optical rotations were taken on a JASCO DIP-140 digital polarimeter. IR spectra were measured with a JASCO FT/IR-5300. NMR spectra were recorded on a Varian UNITY 600 or JEOL GSX-400 spectrometer in pyridine-d_5 solution using tetramethylsilane (TMS) as an internal standard. NMR experiments included ^1H–^1H COSY, ^{13}C–^1H COSY, distortionless enhancement by polarization transfer (DEPT), HMBC (512×1024 data matrix size, 128 scans, recycle delay = 1.16 s), and ROESY. 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 JEOL JMS-HX-100 mass spectrometers. For column chromatography, Kieselgel 60 (230—400 mesh, Merck), and for TLC, Silica gel 60F-254 (Merck) were used. HPLC was carried out on a Waters ALC/GPC 244 instrument.

Isolation of Saponins The roots (1.5 kg) of *Gymnema alternifolium* collected in Taipei, Formosa, in June 1996, were extracted with 70% EtOH at room temperature for 3 weeks. The ethanolic extract (90 g) was chromatographed on Amberlite XAD-2 with 20, 40, 60 and 100% MeOH to give four fractions (frs. 1—4). Fr. 3 (30.0 g) was subjected to HPLC on octadecy silica (ODS) (Develosil Lop ODS, 40% CH_3OH) to give six fractions (frs. 3-1—6). Fr. 3-2 (9.7 g) was further subjected to HPLC on ODS (25% CH_3CN) to afford eight fractions (frs. 3-2-1—10). Fr. 3-2-4 was purified by preparative HPLC (YMC, ODS S-5, 20% CH_3CN) to give alternosides VI (**6**, 165 mg) and VIII (**8**, 90 mg). From fr. 3-2-6, alternoside IV (**4**, 30 mg), from fr. 3-2-7, alternosides I (**1**, 180 mg), II (**2**, 90 mg), VII (**7**, 470 mg), IX (**9**, 55 mg), X (**10**, 40 mg), and compounds **11** (100 mg), and **12** (25 mg) were obtained. Fr. 3-3 (0.4 g) was purified by preparative HPLC (YMC, ODS S-5, 23% CH_3CN) to give alternosides IV (**4**, 25 mg) and V (**5**, 20 mg).

Alternoside I (**1**): Colorless needles, mp 241—243 °C, $[\alpha]_D^{25}$ +1.2° (*c*=1.3, MeOH). IR (film) cm^{-1}: 3420, 1730, 1660, 1245, 1165. Negative FAB-MS *m/z*: 999 $[M-H]^-$. *Anal.* Calcd for $C_{50}H_{80}O_{20}\cdot 3H_2O$: C, 56.91; H, 8.21. Found: C, 56.74; H, 8.35. ^1H-NMR and ^{13}C-NMR: Tables 1 and 2.

Alternoside II (**2**): Colorless needles, mp 230—232 °C, $[\alpha]_D^{25}$ +2.3° (*c*=2.4, MeOH). IR (film) cm^{-1}: 3400, 1730, 1665, 1240, 1160. Negative FAB-MS *m/z*: 999 $[M-H]^-$. *Anal.* Calcd for $C_{50}H_{80}O_{20}\cdot 3H_2O$: C, 56.91; H, 8.21. Found: C, 56.80; H, 8.41. ^1H-NMR and ^{13}C-NMR: Tables 1 and 2.

Alternoside III (**3**): Colorless needles, mp 205—207 °C, $[\alpha]_D^{25}$ +5.8° (*c*=4.2, MeOH). IR (film) cm^{-1}: 3400, 1720, 1655, 1160. Negative FAB-MS *m/z*: 1039 $[M-H]^-$. *Anal.* Calcd for $C_{53}H_{84}O_{20}\cdot 2H_2O$: C, 59.09; H, 8.23. Found: C, 58.95; H, 8.40. ^1H-NMR and ^{13}C-NMR: Tables 1 and 2.

Alternoside IV (**4**): Colorless needles, mp 242—244 °C, $[\alpha]_D^{25}$ +5.0° (*c*=0.9, MeOH). IR (film) cm^{-1}: 3420, 1730, 1660, 1240, 1155. Negative FAB-MS *m/z*: 837 $[M-H]^-$. *Anal.* Calcd for $C_{44}H_{70}O_{15}\cdot H_2O$: C, 61.52; H, 8.68. Found: C, 61.55; H, 8.53. ^1H-NMR and ^{13}C-NMR: Tables 1 and 2.

Alternoside V (**5**): Colorless needles, mp 246—248 °C, $[\alpha]_D^{25}$ +5.4° (*c*=0.6, MeOH). IR (film) cm^{-1}: 3460, 1710, 1660, 1160. Negative FAB-MS *m/z*: 837 $[M-H]^-$. *Anal.* Calcd for $C_{44}H_{70}O_{15}\cdot 2H_2O$: C, 60.53; H, 8.31. Found: C, 60.32; H, 8.45. ^1H-NMR and ^{13}C-NMR: Tables 1 and 2.

Alternoside VI (**6**): Colorless needles, mp 219—221 °C, $[\alpha]_D^{25}$ +3.5° (*c*=2.0, MeOH). IR (film) cm^{-1}: 3400, 1735, 1655, 1160. Negative FAB-MS *m/z*: 973 $[M-H]^-$. *Anal.* Calcd for $C_{48}H_{78}O_{20}\cdot 4H_2O$: C, 55.05; H, 8.28. Found: C, 54.95.; H, 8.40. ^1H-NMR and ^{13}C-NMR: Tables 1 and 2.

Alternoside VII (**7**): Colorless needles, mp 213—215 °C, $[\alpha]_D^{25}$ +9.1° (*c*=4.5, MeOH). IR (film) cm^{-1}: 3460, 1720, 1655, 1155. Negative FAB-MS *m/z*: 811 $[M-H]^-$. *Anal.* Calcd for $C_{42}H_{68}O_{15}\cdot 2H_2O$: C, 59.42; H, 8.55. Found: C, 59.30; H, 8.45. ^1H-NMR and ^{13}C-NMR: Tables 1 and 2.

Alternoside VIII (**8**): Colorless needles, mp 198—200 °C, $[\alpha]_D^{25}$ +9.5° (*c*=2.8, MeOH). IR (film) cm^{-1}: 3455, 1720, 1660, 1155. Negative FAB-MS *m/z*: 811 $[M-H]^-$. *Anal.* Calcd for $C_{42}H_{68}O_{15}\cdot 3H_2O$: C, 58.18; H, 8.60. Found: C, 58.02; H, 8.55. ^1H-NMR and ^{13}C-NMR: Tables 1 and 3.

Alternoside IX (**9**): Colorless needles, mp 208—210 °C, $[\alpha]_D^{25}$ −8.3° (*c*=3.2, MeOH). IR (film) cm^{-1}: 3450, 1715, 1660, 1155. Negative FAB-MS *m/z*: 957 $[M-H]^-$. *Anal.* Calcd for $C_{48}H_{78}O_{19}\cdot 4H_2O$: C, 55.91; H, 8.41. Found: C, 55.75; H, 8.46. ^1H-NMR and ^{13}C-NMR: Tables 1 and 3.

Alternoside X (**10**): Colorless needles, mp 223—225 °C, $[\alpha]_D^{25}$ +4.1° (*c*=4.1, MeOH). IR (film) cm^{-1}: 3420, 1720, 1660, 1155. Negative FAB-MS *m/z*: 957 $[M-H]^-$. *Anal.* Calcd for $C_{48}H_{78}O_{19}\cdot 3H_2O$: C, 56.90; H, 8.36. Found: C, 56.78; H, 8.45. ^1H-NMR and ^{13}C-NMR: Tables 1 and 3.

Compound **11**: Colorless needles, mp 230—232 °C, $[\alpha]_D^{25}$ +1.6° (*c*=1.2, MeOH). IR (film) cm^{-1}: 3455, 1720, 1660, 1155. Negative FAB-MS *m/z*: 1013 $[M-H]^-$. *Anal.* Calcd for $C_{51}H_{82}O_{20}\cdot H_2O$: C, 59.29; H, 8.19. Found: C, 59.25; H, 8.25. ^1H-NMR and ^{13}C-NMR: Tables 1 and 3.

Compound **12**: Colorless needles, mp 213—215 °C, $[\alpha]_D^{25}$ +7.9° (*c*=5.6, MeOH). IR (film) cm^{-1}: 3455, 1720, 1660, 1155. Negative FAB-MS *m/z*: 825 $[M-H]^-$. *Anal.* Calcd for $C_{43}H_{70}O_{15}\cdot 2H_2O$: C, 59.84; H, 8.64. Found: C, 59.76; H, 8.55. ^1H-NMR and ^{13}C-NMR: Tables 1 and 3.

Methylation of Alternoside II (2) A solution of **2** (30 mg) in MeOH (1.0 ml) was treated with ethereal CH_2N_2 at room temperature for 2 h and worked-up as usual to give compound **11** (30 mg).

Reduction of Compound 11 and Acid Hydrolysis of the Reduction Product $NaBH_4$ (40 mg) was added to a stirred solution of **11** (25 mg)

in MeOH (3 ml) and the reaction mixture was stirred for 2 h at room temperature. Work-up as usual gave the reduction product (20 mg), which was taken up in 5% H_2SO_4 solution and heated at 100 °C for 2 h. The reaction mixture was extracted with EtOAc and the extract was purified by HPLC (YMC, ODS S-5, 37% CH_3CN) to provide chichipegenin[4] (**13**, 10 mg), mp 315—317 °C, $[\alpha]_D^{25}$ +40.0°, $C_{30}H_{50}O_4$ {positive FAB-MS, *m/z* 497 $[M+Na]^+$}, which was confirmed by comparison of ^{1}H-NMR data and by co-HPLC with an authentic sample. The aqueous layer was neutralized with Amberlite IR-35 and evaporated *in vacuo* to dryness. The sugar was determined by HPLC (Shodex RSpak DC-613, 80% CH_3CN, 0.8 ml/min, 70 °C) using RI detection (Waters 410) and chiral detection (Shodex OR-1), in comparison with authentic sugars (10 mM each of D-glc, L-glc and L-rha). The sugar part gave the following peaks: L-(−)-rha; 4.80 min, D-(+)-glucose; 7.38 min.

Alkaline Hydrolysis of Alternoside II (2) A solution of **2** (30 mg) in MeOH (1.0 ml) was treated dropwise with 28% sodium methoxide (0.3 ml) under an N_2 atmosphere. The mixture was stirred for 2 h at room temperature. The reaction mixture obtained after acidification with dilute HCl was passed through an Amberlite XAD-2 column and eluted with MeOH to give the prosapogenin (25 mg), which was identified as alternoside X (**10**) by comparison of ^{1}H-NMR data and by co-HPLC.

Enzymatic Hydrolysis of Alternoside II (2) A solution of **2** (30 mg) and crude cellulase (30 mg, Sigma) in EtOH–H_2O (1:9) and 0.01 M NaH_2PO_4 buffer (pH 4.0), 2 ml each, was incubated for 24 h at 37 °C. Work-up as usual gave compound **5** (20 mg).

Alkaline Hydrolysis of Alternoside I (1) Alkaline hydrolysis of **1** (30 mg) was carried out in the same way as described for **2**. The MeOH eluate gave the prosapogenin (25 mg), which was confirmed by comparison of ^{1}H-NMR data and by co-HPLC with an authentic sample.

Acid Hydrolysis of Alternosides I (1), III—VIII (3—8) and X (10) Acid hydrolysis of **1**, **3**—**8** and **10** (each *ca.* 5 mg) was carried out in the same way as described for the reduction product of **11**. The EtOAc layer gave chichipegenin (**13**, 1—2 mg), which was confirmed by co-HPLC and co-TLC with an authentic sample. The sugar was determined by HPLC (Shodex SUGAR SC1011, H_2O, 1.0 ml/min, 80 °C) using RI detection (Waters 410) and chiral detection (Shodex OR-1), in comparison with authentic sugars (10 mM each of D-glucuronolactone, D-glc, L-glc and L-rha). The sugar part gave the following peaks: D-(+)-glucuronolactone; 5.60 min, D-(+)-glucose; 7.55 min, L-(−)-rha; 8.30 min. D-Glucuronolactone, D-glucose and L-rhamnose were detected in the H_2O layer of **1**, **3**—**5**, and **10**; D-glucuronolactone and D-glucose in that of **6**—**8**.

Alkaline Hydrolysis of Alternoside III (3) A solution of **3** (30 mg) in MeOH (1.0 ml) was treated dropwise with 28% sodium methoxide (0.3 ml) under an N_2 atmosphere. The mixture was stirred for 2 h at room temperature. The reaction mixture was acidified with dilute HCl, and extracted with $CHCl_3$ and then 1-BuOH. The $CHCl_3$ layer afforded tiglic acid (3 mg), which was confirmed by comparison of ^{1}H-NMR data and by co-HPLC with an authentic sample. The 1-BuOH layer was subjected to HPLC (YMC, ODS S-5, 25% CH_3CN) to provide the prosapogenin (22 mg), which was identified as alternoside X (**10**) by comparison of spectral data.

Methylation of Alternoside VII (7) A solution of **7** (30 mg) in MeOH (2.0 ml) was treated with ethereal CH_2N_2 at room temperature for 2 h and worked-up as usual to give compound **12** (30 mg).

Reduction of Compound 12 and Acid Hydrolysis of Reduction Product Compound **12** (25 mg) was treated in the same way as described for **11**. The EtOAc layer provided chichipegenin (**13**, 10 mg), which was confirmed by comparison of ^{1}H-NMR data and by co-HPLC with an authentic sample. D-Glucose was detected in the H_2O layer.

Acid Hydrolysis of Alternosides IX (9) Acid hydrolysis of **9** (25 mg) was carried out in the same way as described for **1**, **3**—**8** and **10**. The EtOAc layer provided longispinogenin[9,10] (**14**, 10 mg), which was confirmed by comparison of ^{1}H-NMR data and by co-HPLC with an authentic sample. D-Glucuronolactone and D-glucose were detected in the H_2O layer.

Bioassay of Antisweet Activity The antisweet activity of 1 mM solutions of **1**—**12** was tested on three volunteers. Each participant held the test solution in the mouth for 3 min, spat, rinsed the mouth with distilled water and tasted a 0.2 M sucrose solution.

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