Antisweet Natural Products. XIV[1] Structures of Alternosides XI—XIX from *Gymnema alternifolium*

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Received June 18, 1999; accepted August 17, 1999

From the fresh roots of *Gymnema alternifolium*, nine new oleanane-type triterpenoid glycosides, named alternosides XI—XIX (1—9), were isolated, together with two artificial compounds 10 and 11, and two known compounds, sitakisosides X (12) and XVII (13). Their structures were determined on the basis of spectroscopic data and chemical evidence. Compounds 1—3 are 3-*O*-glycosides of chichipegenin having a tigloyl group at C-22, 28 and 16, respectively. Compounds 4—7 are 3,28-*bis*-*O*-glycosides of chichipegenin having a tigloyl group at C-22. Compounds 8 and 9 are 3,28-*bis*-*O*-glycosides of longispinogenin. Compounds 1—7 having an acyl group showed antisweet activity.

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

In the preceding paper[1] of this series, we reported the isolation and structural determination of ten antisweet principles, alternosides I—X, from the roots of *Gymnema alternifolium* (LOUR.) MERR. In this paper, we report the isolation, structural elucidation and antisweet activity of nine additional novel saponins, named alternosides XI—XIX (**1**—**9**), having an oleanene skeleton. Their structures were elucidated by chemical and spectral methods, 2D-NMR techniques being especially helpful.

The EtOH extract obtained from the fresh roots of *Gymnema alternifolium* (LOUR.) MERR. was subjected to Amberlite XAD-2 column chromatography to give a saponin fraction. Repeated separation of the saponin fraction by HPLC gave nine new compounds named alternosides XI (**1**), XII (**2**), XIII (**3**), XIV (**4**), XV (**5**), XVI (**6**), XVII (**7**), XVIII (**8**), and XIX (**9**), besides two artificial compounds **10** and **11**, and two known compounds, sitakisosides X (**12**)[2] and XVII (**13**)[3]. ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY), ^{1}H–^{13}C COSY, 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**—**13**.

Alternoside XI (**1**), one of the main saponins, had the molecular formula $C_{47}H_{74}O_{16}$ (negative FAB-MS *m/z*: 893 $[M-H]^-$) and spectroscopic properties which characterized the carbonyl functions as a carboxyl and an unsaturated acyl function [ν_{max}: 3400 (br), 1730 (br), 1660; δ_C: 172.2 (COOH), 167.5 (enone)]. Methylation of **1** with diazomethane afforded compound **10**, $[\alpha]_D^{25}$ +30.4°, $C_{48}H_{76}O_{16}$ [negative FAB-MS *m/z*: 907 $[M-H]^-$], which was further reduced with $NaBH_4$ for subsequent acid hydrolysis. Acid hydrolysis of the reduction product of **10** afforded chichipegenin (**14**)[1,4] as an aglycone, besides D-glucose[5,6] as the sugar component. Alkaline treatment of **1** gave alternoside VII (**15**)[1] and tiglic acid. The ^{1}H-NMR and ^{13}C-NMR spectra of **1** indicated the presence of one β-D-glucuronopyranosyl unit [H-1: δ 4.99 (d, J=7.0 Hz); C-1: δ 106.7; C-6: δ 172.2], one β-D-glucopyranosyl unit [H-1: δ 5.36 (d, J=8.0 Hz); C-1: δ 105.9] and one tigloyl unit [C-1: δ 167.5; C-2: δ 129.5; C-3: δ 137.2; H-3: δ 7.04 (q, J=7.0 Hz); C-4: δ 14.1; H-4: δ 1.56 (3H, d, J=7.0 Hz); C-5: δ 12.3; H-5: δ 1.87 (3H, s)]. The location of the tigloyl group in **1** was determined by spectral comparison of **1** and **15**, and by an HMBC experiment on **1**. Thus, acylation shifts were observed at the C-22 [+1.07 ppm (H-22), +4.6 ppm (C-22)] and C-21 [−5.1 ppm (C-21)] positions. In the HMBC spectrum, the ester carbon signal of a tigloyl group at δ 167.5 was correlated with the H-22 signal of the aglycone at δ 6.15. Therefore, the structure of **1** was established as chichipegenin 22-*O*-tigloyl-3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucuronopyranoside.

Alternoside XII (**2**), one of the main saponins, had the same molecular formula $C_{47}H_{74}O_{16}$ (negative FAB-MS *m/z*: 893 $[M-H]^-$) as **1**. Methylation of **2** with diazomethane afforded compound **11** $[\alpha]_D^{25}$ +11.8°, $C_{48}H_{76}O_{16}$ [negative FAB-MS *m/z*: 907 $[M-H]^-$], which was further reduced with $NaBH_4$ for subsequent acid hydrolysis. Acid hydrolysis of the reduction product of **11** afforded **14** as an aglycone, besides D-glucose. The ^{1}H-NMR and ^{13}C-NMR spectra indicated that **2** was composed of 1 mol each of chichipegenin, tiglic acid, glucuronic acid, and glucose. Alkaline treatment of **2** gave **15** and tiglic acid. The location of the tigloyl group in **2** was determined in the same way as **1**. Thus, acylation shifts were observed at the C-28 [+0.27 and 0.49 ppm (H_2-28), +3.5 ppm (C-28)] position. In the HMBC spectrum, the ester carbon signal of a tigloyl group at δ 168.1 was correlated with the methylene protons (H_2-28) of the aglycone at δ 4.71 and 5.24. Therefore, the structure of **2** was established as chichipegenin 28-*O*-tigloyl-3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucuronopyranoside.

The NMR data of alternosides XIII—XVIII (**3**—**8**) indicated that they were homologues of **14**[1,4] or longispinogenin (**17**)[1] glycosides, differing in the sugar sequences at C-3 and C-28, and acyl unit at C-16, C-22, and C-28 in the aglycone. To determine the component sugars, compounds **3**—**8** were each treated in the same way as **1** and **2**. D-glucose, D-fucose, L-rhamnose, and D-xylose were detected from each acid hydrolysate of the reduction product of **3**—**8** (see experimental section).

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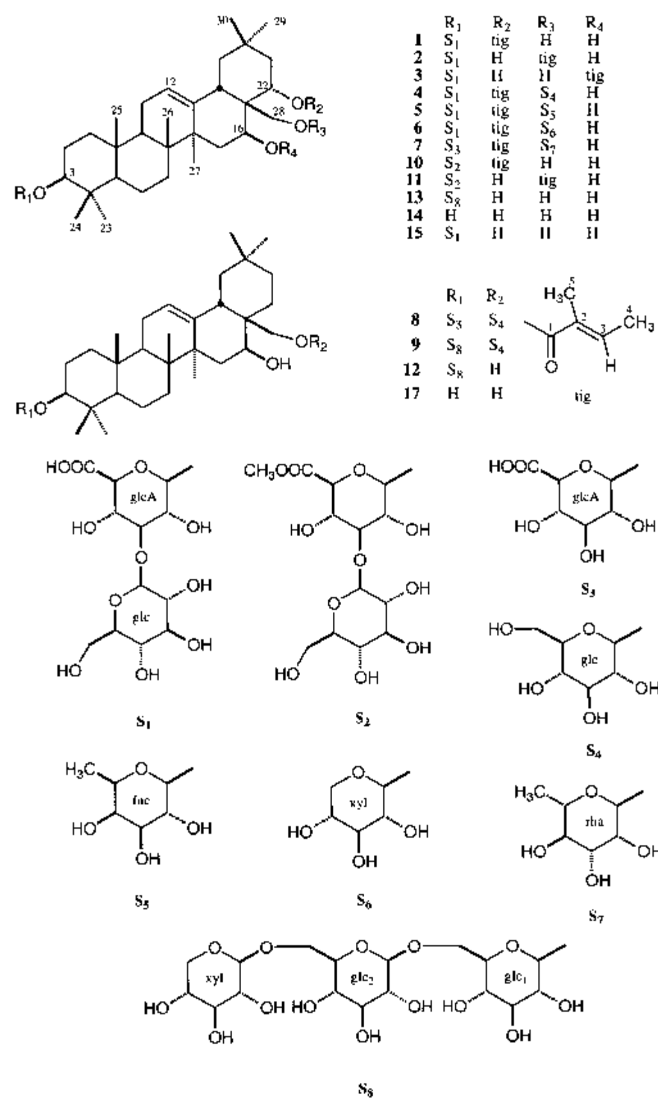

Chart 1

Alternoside XIII (**3**) had the same molecular formula, $C_{47}H_{74}O_{16}$ (negative FAB-MS *m/z*: 893 $[M-H]^-$) as **1**. The ^{1}H-NMR and ^{13}C-NMR spectra indicated that **3** was composed of 1 mol each of chichipegenin, tiglic acid, glucuronic acid, and glucose. Alkaline treatment of **3** gave **15** and tiglic acid. A ^{13}C-NMR spectral comparison of **3** and **15** showed acylation shifts at the C-16 [+1.36 ppm (H-16), +2.1 ppm (C-16)] and C-15 [−2.0 ppm (C-15)] positions, demonstrating that the tigloyl group is located at the C-16-OH. Furthermore, in the HMBC spectrum of **3**, long-range correlation was observed between H-16 (δ 6.47) of the aglycone and C-1 (δ 167.6) of a tigloyl moiety. Therefore, the structure of **3** was established as chichipegenin 16-*O*-tigloyl-3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucuronopyranoside.

Alternoside XIV (**4**) had the molecular formula $C_{53}H_{84}O_{21}$ (negative FAB-MS *m/z*: 1055 $[M-H]^-$), *i.e.*, 162 mass units higher (hexosyl) than that of **1**. The ^{1}H-NMR and ^{13}C-NMR spectra indicated that **4** was composed of 1 mol each of chichipegenin, tiglic acid and β-glucuronic acid [H-1: δ 4.96 (d, J=8.0 Hz); C-1: δ 106.9], and 2 mol of β-glucose [H-1: δ 5.34 (d, J=8.0 Hz); C-1: δ 106.0; H-1: δ 4.74 (d, J=8.0 Hz); C-1: δ 105.3]. A ^{13}C-NMR spectral comparison of **4** with **1** showed that **4** structurally differed from **1** only in its C-28 substitution. In the ^{13}C-NMR spectrum of **4**, the C-28 signal of the aglycone was shifted downfield by +10.4 ppm by the glycosylation shift,[7,8] demonstrating that the glucosyl group is located at C-28-OH. Furthermore, in the HMBC spectrum of **4**, long-range correlation was observed between H-1 (δ 4.74) of the glucose moiety and C-28 (δ 70.4) of the aglycone. Therefore, **4** was formulated as chichipegenin 22-*O*-tigloyl-3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucuronopyranosyl-28-*O*-β-D-glucopyranoside.

Alternoside XV (**5**) had the molecular formula $C_{53}H_{84}O_{20}$ (negative FAB-MS *m/z*: 1039 $[M-H]^-$), *i.e.*, 146 mass units higher (deoxyhexosyl) than that of **1**. The ^{1}H-NMR and ^{13}C-NMR spectra indicated that **5** was composed of 1 mol each of chichipegenin, tiglic acid, glucuronic acid, glucose and β-fucose [H-1: δ 4.55 (d, J=8.5 Hz); C-1: δ 105.4; H-6: δ 1.54 (d, J=6.5 Hz); C-6: δ 17.2]. The ^{1}H-NMR and ^{13}C-NMR spectral data due to the 3-*O*-sugar moiety, the 22-*O*-tigloyl unit and the aglycone moiety, excluding the C-28 position, were almost superimposable on those of **4**, suggesting that a β-fucosyl group is joined at C-28-OH. A ^{13}C-NMR spectral comparison of **5** with **1** disclosed C-28 [+8.7 ppm (C-28)] as the glycosylation site in the former. Furthermore, in the HMBC spectrum of **5**, long-range correlation was observed between H-1 (δ 4.55) of the fucose moiety and C-28 (δ 68.7) of the aglycone moiety. Therefore, **5** was formulated as chichipegenin 22-*O*-tigloyl-3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucuronopyranosyl-28-*O*-β-D-fucopyranoside.

Alternoside XVI (**6**) had the molecular formula $C_{52}H_{82}O_{20}$ (negative FAB-MS *m/z*: 1025 $[M-H]^-$), *i.e.*, 132 mass units higher (pentose) than that of **1**. The ^{1}H-NMR and ^{13}C-NMR spectra indicated that **6** was composed of 1 mol each of chichipegenin, tiglic acid, glucuronic acid, glucose and β-xylose [H-1: δ 4.71(d, J=7.0 Hz); C-1: δ 105.7]. A ^{13}C-NMR spectral comparison of **6** with **1** showed that only the C-28 signal of **6** appeared at a lower field, by +9.6 ppm, because of the glycosylation shift, demonstrating that the β-xylosyl unit is located at C-28-OH of the aglycone moiety. The HMBC spectrum of **6** showed long-range correlation between H-1 (δ 4.71) of the xylose moiety and C-28 (δ 69.6) of the aglycone moiety. Therefore, **6** was formulated as chichipegenin 22-*O*-tigloyl-3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucuronopyranosyl-28-*O*-β-D-xylopyranoside.

Alternoside XVII (**7**) had the molecular formula $C_{47}H_{74}O_{15}$ (negative FAB-MS *m/z*: 877 $[M-H]^-$), *i.e.*, 162 mass units lower than that of alternoside III (**16**).[1] The ^{1}H-NMR and ^{13}C-NMR spectra indicated that **7** was composed of 1 mol each of chichipegenin, tiglic acid, glucuronic acid, and α-rhamnose [H-1: δ 5.23 (br s); C-1: δ 102.3; H-6: δ 1.71 (d, J=6.0 Hz); C-6: δ 19.2]. A ^{13}C-NMR spectral comparison of **7** with **16** showed that **7** structurally differs from **16** only in its 3-*O*-sugar moiety although the tigloyl and rhamnopyranosyl groups are located at the C-22 and C-28 positions, respectively. The HMBC spectrum of **7** showed long-range correlations between H-1 (δ 5.00) of the glucose moiety and C-3 (δ 89.1), H-22 (δ 6.06) and C-1 (δ 167.0) of the tigloyl unit, and H-1 (δ 5.23) of the rhamnose and C-28 (δ 65.5), indicating that a glucosyl unit is located at C-3-OH, a tigloyl unit at C-22-OH and a rhamnosyl unit at C-28-OH. Therefore, **7** was formulated as chichipegenin 22-*O*-tigloyl-3-*O*-β-D-glucuronopyranosyl-28-*O*-α-L-rhamnopyranoside.

Table 1. ^{13}C-NMR Spectral Data for **1**—**9** and **14**—**17** (in Pyridine-d_5, 100 MHz)

C		**1**	**2**	**3**[a]	**4**	**5**[a]	**6**[a]	**7**	**8**[a]	**9**	**14**	**15**	**16**	**17**
	1	38.6	38.6	39.0	38.9	39.0	39.0	39.1	38.9	38.7	39.1	38.7	38.8	39.3
	2	26.5	26.4	26.8	26.9	26.9	26.9	26.9	26.9	26.5	28.1	26.6	26.5	28.1
	3	89.1	89.1	89.1	89.2	89.3	89.3	89.1	89.1	89.0	78.0	89.1	89.0	78.3
	4	39.5	39.4	39.8	39.8	39.8	39.8	39.8	39.7	39.4	39.4	39.6	39.6	39.6
	5	55.5	55.4	55.7	55.8	55.8	55.8	55.9	55.8	55.6	55.6	55.6	55.6	55.9
	6	18.3	18.2	18.7	18.7	18.8	18.7	18.8	18.6	18.5	18.7	18.4	18.5	18.9
	7	33.0	32.7	34.0	33.2	33.3	33.3	33.3	32.9	32.6	33.0	32.9	33.0	33.2
	8	40.1	40.2	40.6	40.5	40.6	40.5	40.6	40.3	40.0	40.2	40.3	40.3	40.3
	9	47.0	46.9	47.2	47.3	47.3	47.3	47.3	47.2	47.0	47.3	47.1	47.1	47.4
	10	36.6	36.6	37.0	37.0	37.0	37.0	37.0	36.9	36.7	37.1	36.7	36.7	37.3
	11	23.7	23.7	24.1	24.1	24.2	24.2	24.4	24.0	23.7	23.9	23.9	24.1	24.1
	12	124.0	124.1	—[b]	—[b]	124.0	—[b]	—[b]	—[b]	—[b]	123.9	—[b]	—[b]	123.9
	13	142.4	142.2	142.2	141.7	141.7	141.9	142.0	143.4	143.3	143.1	143.1	142.0	144.1
	14	42.8	42.5	43.5	43.3	43.2	43.2	43.0	44.1	43.8	42.7	42.7	42.8	44.0
	15	36.1	36.0	34.0	37.0	36.8	36.8	36.5	37.2	37.0	36.0	36.0	36.2	36.8
	16	66.2	66.2	69.1	66.3	66.0	66.1	65.5	66.3	66.1	66.9	66.9	65.3	66.9
	17	45.7	44.2	45.9	45.8	45.8	45.8	45.8	41.5	41.2	45.1	45.1	45.6	41.2
	18	43.7	43.6	44.8	44.6	44.3	44.6	44.5	44.9	44.6	43.1	43.2	44.3	44.6
	19	46.2	46.0	46.9	46.2	46.1	46.3	46.1	46.8	46.7	46.6	46.6	45.9	47.3
	20	32.1	32.0	32.2	32.4	32.5	32.5	32.6	31.2	30.9	32.2	32.2	32.3	31.3
	21	39.1	44.0	43.7	39.5	39.5	39.6	39.6	34.3	34.0	44.3	44.2	39.6	34.4
	22	74.2	69.4	72.0	74.9	74.2	74.7	73.4	26.9	26.5	69.5	69.6	73.2	26.3
	23	27.9	27.9	28.3	28.3	28.4	28.3	28.5	28.3	28.1	28.7	28.1	28.1	28.9
	24	16.8	16.8	17.2	17.3	17.2	17.3	17.3	17.2	16.9	16.5	17.0	17.0	16.7
	25	15.5	15.5	15.9	16.1	15.9	16.0	16.0	16.0	15.7	15.7	15.7	15.7	15.9
	26	16.8	17.0	17.3	17.4	16.9	17.4	17.5	17.3	16.9	17.0	17.0	17.2	17.1
	27	27.3	27.6	27.9	28.3	27.7	27.8	27.9	27.4	27.0	27.6	27.7	27.6	27.3
	28	60.0	62.4	63.6	70.4	68.7	69.6	65.5	78.3	78.3	58.8	58.9	64.9	69.0
	29	33.0	33.2	33.8	33.2	33.3	33.3	33.3	33.6	33.3	33.5	33.6	33.1	33.6
	30	25.5	24.7	25.9	26.1	26.1	25.9	25.5	24.3	24.0	25.0	25.0	25.2	24.3
3-*O*-Glycosyl moiety														
glcA or Inner glc	1	106.7	106.7	106.7	106.9	106.9	106.9	107.3	107.3	106.9		106.6	106.8	
	2	74.4	74.2	74.6	74.5	74.6	74.5	75.7	75.7	74.9		74.4	74.4	
	3	87.5	87.5	87.7	87.7	87.7	87.7	78.4	78.3	78.4		87.5	87.2	
	4	71.5	71.5	71.3	71.8	71.9	71.8	73.7	73.7	71.5		71.6	71.5	
	5	77.4	77.3	77.4	77.7	77.7	77.7	77.2	77.9	76.9		77.4	77.4	
	6	172.2	172.3	172.6	172.1	172.1	172.1	172.2	172.9	70.3		172.2	172.0	
Outer glc	1	105.9	105.8	106.0	106.0	106.0	106.0			105.3		105.9	105.9	
	2	75.6	75.5	75.8	75.8	75.8	75.8			75.5		75.6	75.5	
	3	78.2	78.2	78.4	78.5	78.5	78.5			78.7		78.2	78.2	
	4	71.7	71.7	71.7	71.9	71.8	71.8			71.5		71.8	71.7	
	5	78.7	78.7	78.8	78.7	78.9	78.9			76.9		78.7	78.7	
	6	62.4	62.4	62.6	62.7	62.7	62.7			69.8		62.4	62.4	
xyl	1									106.2				
	2									74.8				
	3									78.0				
	4									71.1				
	5									67.0				
28-*O*-Glycosyl moiety					(glc)	(fuc)	(xyl)	(rha)	(glc)	(glc)			(rha)	
	1				105.3	105.4	105.7	102.3	105.9	105.3			102.2	
	2				75.3	72.3	74.9	72.6	75.2	75.0			72.4	
	3				78.5	75.3	78.1	73.4	78.9	78.5			73.2	
	4				71.9	72.7	71.2	74.0	71.7	71.5			73.8	
	5				78.9	72.0	67.2	70.0	78.9	78.7			69.9	
	6				63.1	17.2		19.2	62.9	62.6			18.9	
tig[c]	1	167.5	168.1	167.6	167.8	167.7	167.7	167.0					167.1	
	2	129.5	129.3	130.2	129.6	129.6	129.6	129.3					129.3	
	3	137.2	137.0	136.7	137.4	137.5	137.3	137.2					137.3	
	4	14.1	14.0	14.5	14.5	14.6	14.5	14.6					14.3	
	5	12.3	12.1	12.7	12.8	12.8	12.8	12.8					12.4	

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

In the negative FAB-MS spectrum, alternoside **8** showed a quasi-molecular ion at *m/z* 795, indicating a molecular formula of $C_{42}H_{68}O_{14}$. Acid hydrolysis of **8** afforded **17**[9,10] as an aglycone. The ^{1}H-NMR and ^{13}C-NMR spectra of **8** indicated the presence of one β-D-glucuronopyranosyl unit [H-1: δ 4.96 (d, J=8.0 Hz); C-1: δ 107.3; C-6: δ 172.9] and one β-D-glucopyranosyl unit [H-1: δ 5.03 (d, J=8.0 Hz); C-1: δ 105.9]. The ^{13}C-NMR spectrum of **8** showed glycosylation shifts of +10.7 ppm at the C-3 signal and +9.3 ppm at the C-28 signal, compared with those of **17**, demonstrating that the

Table 2. ^{1}H-NMR Spectral Data for **1**—**6** (in Pyridine-d_5, 400 MHz)[a]

		1	**2**	**3**[b]	**4**	**5**[b]	**6**[b]
Aglycone	3	3.35 (dd, 11.5, 4.0)	3.34 (dd, 11.5, 4.5)	3.36 (dd, 11.5, 4.0)	3.30 (dd, 11.5, 4.5)	3.35 (dd, 12.0, 4.5)	3.35 (dd, 11.5, 4.0)
	12	5.32 m	5.36 m	5.38 m	5.25 m	5.29 m	5.36 m
	16	5.03 (dd, 11.5, 5.5)	5.13 (dd, 11.0, 5.5)	6.47 (dd, 10.5, 5.0)	4.95 m	5.00 m	4.96 (dd, 11.0, 5.5)
	18	2.88 (dd, 14.0, 4.0)	2.87 (dd, 13.5, 4.5)	2.80 (dd, 13.0, 4.5)	2.66 (dd, 13.0, 4.5)	2.85 (dd, 13.0, 4.0)	2.84 (dd, 13.0, 4.5)
	22	6.15 (dd, 12.5, 4.0)	4.74 (dd, 12.5, 4.0)	4.73 (dd, 12.0, 4.5)	6.01 (dd, 11.0, 4.0)	6.09 (dd, 11.5, 4.0)	6.07 (dd, 11.8, 4.0)
	23	1.30 s	1.29 s	1.28 s	1.25 s	1.30 s	1.29 s
	24	1.01 s	1.06 s	0.99 s	0.96 s	1.00 s	1.00 s
	25	0.81 s	0.79 s	0.80 s	0.78 s	0.85 s	0.83 s
	26	1.19 s	1.13 s	0.92 s	1.01 s	1.10 s	1.07 s
	27	1.43 s	1.43 s	1.50 s	1.36 s	1.41 s	1.42 s
	28	4.34, 4.00 (d, 10.5)	5.24, 4.71 (d, 11.0)	4.74, 4.07 (d, 10.5)	4.32, 4.20 (d, 10.5)	4.35, 4.20 (d, 10.5)	4.34, 4.21 (d, 11.0)
	29	0.95 s	0.97 s	0.98 s	0.94 s	0.97 s	0.98 s
	30	0.99 s	1.03 s	1.04 s	1.09 s	1.10 s	1.15 s
3-*O*-Glycosyl moiety							
glc A	1	4.99 (d, 7.0)	4.98 (d, 8.0)	4.97 (d, 7.5)	4.96 (d, 8.0)	5.00 (d, 8.0)	5.00 (d, 8.0)
glc	1	5.36 (d, 8.0)	5.37 (d, 8.0)	5.37 (d, 8.0)	5.34 (d, 8.0)	5.39(d, 8.0)	5.39 (d, 8.0)
28-*O*-Glycosyl moiety					(glc)	(fuc)	(xyl)
	1				4.74 (d, 8.0)	4.55 (d, 8.5)	4.71 (d, 7.0)
	6					1.54 (d, 6.5)	
tig	3	7.04 (q, 7.0)	6.99 (q, 7.5)	7.13 (q, 7.5)	7.11 (q, 7.0)	7.13 (q, 6.5)	7.13 (q, 7.0)
	4	1.56 (d, 7.0)	1.56 (d, 7.5)	1.56 (d, 7.5)	1.49 (d, 7.0)	1.54 (d, 6.5)	1.54 (d, 7.0)
	5	1.87 s	1.84 s	1.94 s	1.89 s	1.92 s	1.92 s

a) *J* values (in Hz) in parentheses. *b*) 600 MHz.

Table 3. ^{1}H-NMR Spectral Data for **7**—**11** and **15** (in Pyridine-d_5, 400 MHz)[a]

		7	**8**[b]	**9**	**10**	**11**	**15**
Aglycone	3	3.37 (dd, 11.5, 4.0)	3.36 (dd, 11.5, 4.0)	3.30 (dd, 11.5, 4.0)	3.33 (dd, 11.5, 4.0)	3.32 (dd, 11.5, 4.5)	3.34 (dd, 11.5, 4.0)
	12	5.45 m	5.20 m	5.18 m	5.33 m	5.38 m	5.30 m
	16	4.97 (dd, 11.0, 5.5)	4.58 m	4.56 m	5.02 (dd, 11.5, 5.5)	5.12 m	5.11 (dd, 12.0, 5.0)
	18	2.98 (dd, 13.0, 4.5)	2.27 (dd, 13.5, 4.0)	2.28 (dd, 13.0, 4.5)	2.90 (dd, 13.5, 4.0)	2.87 (dd, 13.5, 4.5)	3.08 (dd, 13.0, 4.5)
	22	6.06 (dd, 12.0, 4.5)	2.84 m, 2.25 m	2.82 m, 2.24 m	6.18 (dd, 12.5, 4.0)	4.73 (dd, 11.0, 4.0)	5.08 (dd, 12.0, 5.0)
	23	1.31 s	1.30 s	1.26 s	1.28 s	1.28 s	1.29 s
	24	1.05 s	1.01 s	1.01 s	1.00 s	1.07 s	1.00 s
	25	0.86 s	0.83 s	0.85 s	0.80 s	0.79 s	0.78 s
	26	1.17 s	0.96 s	0.95 s	1.24 s	1.14 s	1.10 s
	27	1.42 s	1.35 s	1.29 s	1.41 s	1.42 s	1.43 s
	28	4.29, 3.92 (d, 10.0)	4.23, 4.06 (d, 10.0)	5.24, 4.71 (d, 11.0)	4.55, 4.08 (d, 10.5)	5.25, 4.72 (d, 11.0)	4.75, 4.44 (d, 9.0)
	29	0.95 s	0.92 s	0.95 s	0.94 s	0.96 s	0.98 s
	30	1.01 s	0.93 s	0.95 s	0.98 s	1.04 s	0.93 s
3-*O*-Glycosyl moiety							
glc A	1	5.00 (d, 7.5)	4.96 (d, 8.0)		4.96 (d, 8.0)	4.94 (d, 8.0)	5.01 (d, 8.0)
COOMe					3.72 s	3.72 s	
Inner glc	1			4.85 (d, 8.0)	5.35 (d, 8.0)	5.35 (d, 8.0)	5.39 (d, 8.0)
Outer glc	1	5.36 (d, 8.0)		5.02 (d, 8.0)			
xyl	1			4.93 (d, 7.0)			
28-*O*-Glycosyl moiety		(rha)	(glc)	(glc)			
	1	5.23 br s	5.03 (d, 8.0)	4.95 (d, 7.0)			
	6	1.71 (d, 6.0)					
tig	3	7.03 (q, 7.0)			7.05 (q, 7.0)	6.99 (q, 7.5)	
	4	1.54 (d, 7.0)			1.53 (d, 7.0)	1.61 (d, 7.5)	
	5	1.84 s			1.87 s	1.84 s	

a) *J* values (in Hz) in parentheses. *b*) 600 MHz.

sugar linkages are located at C-3-OH and C-28-OH. In the HMBC spectrum of **8**, long-range correlations were observed between H-1 (δ 4.96) of the glucuronic acid and C-3 (δ 89.1) of the aglycone, and H-1 (δ 5.03) of the glucose and C-28 (δ 78.3) of the aglycone. Therefore, **8** was determined as longispinogenin 3-*O*-β-D-glucuronopyranosyl-28-*O*-β-D-glucopyranoside.

Alternoside XIX (**9**), one of the main saponins, had the molecular formula $C_{53}H_{88}O_{22}$ (negative FAB-MS *m/z*: 1075 $[M-H]^-$), *i.e.*, 162 mass units higher than that of **12**.[2] Acid hydrolysis of **9** afforded **17**, and D-glucose and D-xylose in a ratio of 3 : 1. The ^{1}H-NMR and ^{13}C-NMR spectra indicated that **9** was composed of 1 mol each of longispinogenin and β-xylose [H-1: δ 4.93 (d, J=7.0 Hz); C-1: δ 106.2], and 3 mol of β-glucose [H-1: δ 4.85 (d, J=8.0 Hz); C-1: δ 106.9; H-1: δ 5.02 (d, J=8.0 Hz); C-1: δ 105.3; H-1: δ 4.95 (d, J=7.0 Hz); C-1: δ 105.3]. The carbon signals due to the sugar moieties at C-3 in **9** are superimposable on those of **12**, indicat-

ing that the sugar moieties are the same (xyl→glc→glc). A ^{13}C-NMR spectral comparison of **9** and **12** showed a glycosylation shift of +9.3 ppm at the C-28 signal, demonstrating that **9** had a glucosyl unit at C-28. Furthermore, in the HMBC spectrum of **9**, long-range correlations were observed between H-1 (δ 4.85) of the glucose (G-1) and C-3 (δ 89.0) of the aglycone, H-1 (δ 5.02) of the glucose (G-2) and C-6 (δ 70.3) of the glucose (G-1), and H-1 (δ 4.93) of the xylose and C-6 (δ 69.8) of the glucose (G-2), and H-1 (δ 4.95) of the glucose and C-28 (δ 78.3) of the aglycone. Hence, **9** was formulated as longispinogenin 3-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl-28-*O*-β-D-glucopyranoside.

A 1 mM solution of any of alternosides XI—XVII led to complete suppression of the sensation of sweetness induced by 0.2 M sucrose but did not suppress the sweetness of 0.4 M sucrose. Alternosides XVIII and XIX, possessing no acyl group, had no activity. The activities of alternosides XI—XVII were half of those of gymnemic acids III, IV and VI.[11] Compounds **10** and **11** could be artifacts occurring during the isolation process.

Experimental

Melting points were measured with a Yanagimoto micromelting point apparatus, without correction. Optical rotations were recorded on a JASCO DIP-140 digital polarimeter. IR spectra were measured with JASCO FT/IR-5300 spectrometer. NMR spectra were recorded on a Varian UNITY 600 or JEOL GSX-400 spectrometer in pyridine-d_5 solution using tetramethylsilane (TMS) as internal standard. NMR experiments included ^{1}H–^{1}H COSY, ^{13}C–^{1}H 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) was measured on a JEOL JMS-HX-100 mass spectrometer. For column chromatography, Kieselgel 60 (230—400 mesh, Merck) was used, and silica gel 60F-254 (Merck) for TLC. HPLC was carried out on a Waters ALC/GPC 244 instrument.

Isolation of Saponins Fresh roots (1.5 kg) of *Gymnema alternifolium* collected in Formosa, in June 1996, and extracted with absolute EtOH at room temperature for 3 weeks. The ethanolic extract (90 g) was chromatographed on Amberlite XAD-2 with 20, 40, 60 and 100% CH_3OH. Alternosides I—X obtained from the 60% CH_3OH fraction (30 g) have already been reported in a previous paper.[1] The 100% CH_3OH fraction (40 g) was subjected to preparative HPLC on octadecyl silica gel (ODS) (Develosil Lop ODS, 80% CH_3OH) to give six fractions (Frs. 1—6). Fr. 1 (2.0 g) was further subjected to HPLC on ODS (60% CH_3OH) to afford three fractions (Frs. 1-1—3). Fr. 1-2 was further purified by HPLC (YMC, ODS S-5, 25—35% CH_3CN) to give alternosides VIII (**8**, 10 mg), and XIX (**9**, 100 mg), and sitakisosides X (35 mg) and XVII (25 mg). Fr. 2 (20 g) was subjected to preparative HPLC on ODS (Develosil Lop ODS, 50% CH_3OH) to give five fractions (Frs. 2-1—5). Fr. 2-1 (74 mg) was further subjected to HPLC on ODS (31% CH_3CN) to afford alternosides XIV (**4**, 8.5 mg). 35—37% CH_3CN elution on ODS of Fr. 2-2 (1.5 g) gave alternosides XIII (**3**, 7.5 mg), XV (**5**, 13.5 mg), XVI (**6**, 6.0 mg) and XVII (**7**, 7.0 mg). 30—35% CH_3CN elution on ODS of Fr. 2-3 (3.5 g) afforded alternosides XI (**1**, 60 mg), XV (**5**, 25 mg) and XVII (**7**, 12 mg). 42—44% CH_3CN elution on ODS of Fr. 2-5 (3.5 g) afforded alternosides XI (**1**, 46 mg), XII (**2**, 75 mg) XIII (**3**, 18 mg), compounds **10** (25 mg) and **11** (15mg).

Alternoside XI (**1**): Colorless needles, mp 203—205 °C, $[\alpha]_D^{25}$ δ 14.9° (c=5.2, MeOH). IR (film) cm^{-1}: 3400 (br), 1730 (br), 1660, 1245, 1160. Negative FAB-MS *m/z*: 893 [M−H]$^-$. *Anal.* Calcd for $C_{47}H_{74}O_{16}\cdot H_2O$: C, 61.82; H, 8.39. Found: C, 61.92; H, 8.70. ^{1}H-NMR and ^{13}C-NMR: Tables 1 and 2.

Alternoside XII (**2**): Colorless needles, mp 204—206 °C, $[\alpha]_D^{25}$ +15.9° (c=3.6, MeOH). IR (film) cm^{-1}: 3400 (br), 1730 (br), 1665, 1240, 1160. Negative FAB-MS *m/z*: 893 [M−H]$^-$. *Anal.* Calcd for $C_{47}H_{74}O_{16}\cdot H_2O$: C, 61.82; H, 8.39. Found: C, 61.85; H, 8.70. ^{1}H-NMR and ^{13}C-NMR: Tables 1 and 2.

Alternoside XIII (**3**): Colorless needles, mp 192—194 °C, $[\alpha]_D^{25}$ +5.4° (c=1.0, MeOH). IR (film) cm^{-1}: 3400 (br), 1730 (br), 1665, 1245, 1165. Negative FAB-MS *m/z*: 893 [M−H]$^-$. *Anal.* Calcd for $C_{47}H_{74}O_{16}\cdot H_2O$: C, 61.82; H, 8.39. Found: C, 61.75; H, 8.65. ^{1}H-NMR and ^{13}C-NMR: Tables 1 and 2.

Alternoside XIV (**4**): Colorless needles, mp 186—188 °C, $[\alpha]_D^{25}$ +2.8° (c=0.6, MeOH). IR (film) cm^{-1}: 3400 (br), 1735 (br), 1660, 1240, 1160. Negative FAB-MS *m/z*: 1055 [M−H]$^-$. *Anal.* Calcd for $C_{53}H_{84}O_{21}\cdot H_2O$: C, 59.20; H, 8.06. Found: C, 59.15; H, 8.20. ^{1}H-NMR and ^{13}C-NMR: Tables 1 and 2.

Alternoside XV (**5**): Colorless needles, mp 192—194 °C, $[\alpha]_D^{25}$ +8.9° (c=1.4, MeOH). IR (film) cm^{-1}: 3460 (br), 1725 (br), 1655, 1240, 1160. Negative FAB-MS *m/z*: 1039 [M−H]$^-$. *Anal.* Calcd for $C_{53}H_{84}O_{20}\cdot H_2O$: C, 60.10; H, 8.18. Found: C, 60.30; H, 8.45. ^{1}H-NMR and ^{13}C-NMR: Tables 1 and 2.

Alternoside XVI (**6**): Colorless needles, mp 180—182 °C, $[\alpha]_D^{25}$ +3.2° (c=0.5, MeOH). IR (film) cm^{-1}: 3400 (br), 1730 (br), 1660, 1240, 1155. Negative FAB-MS *m/z*: 1025 [M−H]$^-$. *Anal.* Calcd for $C_{52}H_{82}O_{20}\cdot 2H_2O$: C, 58.74; H, 8.15. Found: C, 58.55; H, 8.48. ^{1}H-NMR and ^{13}C-NMR: Tables 1 and 2.

Alternoside XVII (**7**): Colorless needles, mp 199—201 °C, $[\alpha]_D^{25}$ +8.6° (c=1.0, MeOH). IR (film) cm^{-1}: 3400 (br), 1735 (br), 1665, 1245, 1160. Negative FAB-MS *m/z*: 877 [M−H]$^-$. *Anal.* Calcd for $C_{47}H_{74}O_{15}\cdot H_2O$: C, 62.93; H, 8.54. Found: C, 63.05; H, 8.68. ^{1}H-NMR and ^{13}C-NMR: Tables 1 and 3.

Alternoside XVIII (**8**): Colorless needles, mp 196—198 °C, $[\alpha]_D^{25}$ −10.7° (c=1.0, MeOH). IR (film) cm^{-1}: 3440, 1730, 1155. Negative FAB-MS *m/z*: 795 [M−H]$^-$. *Anal.* Calcd for $C_{42}H_{68}O_{14}\cdot 2H_2O$: C, 60.56; H, 8.71. Found: C, 60.43; H, 8.95. ^{1}H-NMR and ^{13}C-NMR: Tables 1 and 3.

Alternoside XIX (**9**): Colorless needles, mp 187—189 °C, $[\alpha]_D^{25}$ −23.4° (c=3.1, MeOH). IR (film) cm^{-1}: 3450, 1155. Negative FAB-MS *m/z*: 1075 [M−H]$^-$. *Anal.* Calcd for $C_{53}H_{88}O_{22}\cdot 3H_2O$: C, 56.27; H, 8.37. Found: C, 56.10; H, 8.46. ^{1}H-NMR and ^{13}C-NMR: Tables 1 and 3.

Compound **10**: Colorless needles, mp 173—175 °C, $[\alpha]_D^{25}$ +30.4° (c=0.7, MeOH). IR (film) cm^{-1}: 3420, 1720, 1150. Negative FAB-MS *m/z*: 907 [M−H]$^-$. *Anal.* Calcd for $C_{48}H_{76}O_{16}\cdot 2H_2O$: C, 61.00; H, 8.53. Found: C, 59.95; H, 8.45. ^{1}H-NMR: Table 3.

Compound **11**: Colorless needles, mp 171—173 °C, $[\alpha]_D^{25}$ +11.8° (c=0.8, MeOH). IR (film) cm^{-1}: 3455, 1720, 1155. Negative FAB-MS *m/z*: 907 [M−H]$^-$. *Anal.* Calcd for $C_{48}H_{76}O_{16}\cdot H_2O$: C, 62.18; H, 8.48. Found: C, 62.04; H, 8.25. ^{1}H-NMR: Table 3.

Methylation of 1 A solution of **1** (20 mg) in MeOH (1.0 ml) was added to ethereal CH_2N_2 at room temperature and allowed to stand for 2 h and worked-up as usual to give compound **10** (20 mg).

Reduction of Compound 10 Followed by Acid Hydrolysis of the Reduction Product $NaBH_4$ (40 mg) was added to a stirred solution of **10** (20 mg) in MeOH (3 ml) and the reaction mixture stirred for 2 h at room temperature. After the usual work-up, the reduction product (20 mg) in 5% H_2SO_4 solution was heated at 100 °C for 2 h. The reaction mixture was extracted with EtOAc and the EtOAc layer was purified by HPLC (YMC, ODS S-5, 37% CH_3CN) to provide chichipegenin[4] (**14**, 8.0 mg), mp 315—317 °C, $[\alpha]_D^{25}$ +40.2°, $C_{30}H_{50}O_4$ {negative FAB-MS *m/z*: 473 [M−H]$^-$}, 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 IRA-35 and evaporated to dryness *in vacuo*. The identification and determination of the D or L configuration of a sugar was determined by refraction index (RI) detection (Waters 410) and chiral (Shodex OR-1) detection by HPLC (Shodex RSpak NH2P-50 4D, CH_3CN–H_2O–H_3PO_4, 95 : 5 : 1, 1 ml/min, 47 °C) by comparison with an authentic sample (10 mM of D-glc). The sugar portion gave the following peak: D-(+)-glc 20.70 min.

Alkaline Hydrolysis of 1—3 A solution of **1**—**3** (each 10 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 $CHCl_3$. The $CHCl_3$ layer afforded tiglic acid (3 mg), which was confirmed by co-HPLC with an authentic sample. The H_2O layer was passed through an Amberlite XAD-2 column eluted with MeOH to give prosapogenin (7.0 mg), which was identified as alternoside VII (**15**) by comparison of ^{1}H-NMR data and by co-HPLC.

Methylation of Alternoside XII (2) A solution of **2** (20 mg) in MeOH (2.0 ml) was added to ethereal CH_2N_2 at room temperature for 2 h and work-up as usual to give compound **11** (20 mg).

Reduction of Compound 11 and Acid Hydrolysis of the Reduction Product Compound **11** (20 mg) was processed in the same way as described for the reduction of **10** followed by acid hydrolysis to give

chichipegenin (**14**, 7.0mg) and D-glucose.

Methylation, Reduction and Acid Hydrolysis of 3—7 Compounds **3**—**7** (each *ca*. 3 mg) were treated in the same way as described for the reduction product of **10**. The EtOAc layer gave chichipegenin (**14**, 1—2 mg). The identification and determination of the D or L configuration of the sugar was determined in the same way as **1** using an authentic sample (10 mM each of D-fuc, D-glc, L-rha, and D-xyl). From the H_2O layer of **3** and **4**, D-glc (t_R 20.70 min), from that of **5**, D-fuc (t_R 8.10 min) and D-glc, from that of **6**, D-glc and D-xyl (t_R 9.10 min), and from that of **7**, L-rha (t_R 6.40 min) and D-glc were detected.

Acid Hydrolysis of Alternosides XVIII (8) and XIX (9) Acid hydrolysis of **8** and **9** (each *ca*. 3 mg) was carried out in the same way as for **10**. Each EtOAc layer gave longispinogenin (**17**, 1—2 mg),[9,10] which was confirmed by co-HPLC and co-TLC with an authentic sample. From the H_2O layer of **8**, D-glc, and from that of **9**, D-glc and D-xyl were detected.

Bioassay of Antisweet Activity The antisweet activity of 1 mM solutions of **1**—**9** 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 then tasted 0.2 and 0.4 M sucrose solutions.

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