Antisweet Natural Products. X.[1] Structures of Sitakisosides I—V from *Stephanotis lutchuensis* Koidz. var. *japonica*

Kazuko Yoshikawa,* Hitomi Taninaka, Yukiko Kan, and Shigenobu Arihara

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770, Japan.

Received April 20, 1994; accepted June 10, 1994

From the fresh stem of *Stephanotis lutchuensis* var. *japonica*, we have isolated five new oleane glycosides named sitakisosides I—V (1—5). Their structures were determined on the basis of spectroscopic data and chemical evidence. Sitakisoside V showed the strongest antisweet activity among sitakisosides I—V.

Keywords *Stephanotis lutchuensis* var. *japonica*; Asclepiadaceae; sitakisoside; antisweet substance; *N*-methylanthranilic acid; chichipegenin

As part of our studies on naturally occurring antisweet substances, we have already reported the antisweet principles of *Gymnema sylvestre* R. Br. (Asclepiadaceae).[1,2] Now, we have initiated a chemical study of *Stephanotis lutchuensis* var. *japonica* (Asclepiadaceae), which is an evergreen woody climber growing in the forests near the warm coastal area in Japan. The present paper describes the isolation and structural elucidation of five new oleane glycosides named sitakisosides I—V (**1**—**5**), and their activity.

The EtOH extract obtained from fresh stems of *S. lutchuensis* var. *japonica* (8.5 kg) was subjected to Amberlite XAD-2 column chromatography to give a saponin fraction (143 g). Repeated separation of the saponin fraction by HPLC gave five new compounds named sitakisosides I (**1**), II (**2**), III (**3**), IV (**4**) and V (**5**). ^{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**—**5**, inclusive of the sequence of the sugar moieties and the position of attachment of the acyl moiety and the sugar chains to the aglycone.

Sitakisoside I (**1**), obtained as one of the main saponins, showed an intense blue fluorescence in methanol solution and was deduced to have the molecular formula, $C_{55}H_{85}NO_{19}\cdot H_2O$ based on elemental analysis. The negative FAB-MS of **1** showed ion peaks at *m/z* 1062 $[M-H]^-$, 930 $[M-C_5H_8O_4-H]^-$, and 767 $[M-C_5H_8O_4-C_6H_{10}O_5-H]^-$. On acid hydrolysis with 5% sulfuric acid, **1** afforded D-glucose and D-xylose in a molar ratio of 2 : 1 (confirmed by specific rotation using chiral detection in HPLC). The ^{1}H- and ^{13}C-NMR spectra of **1** indicated the presence of two β-glucopyranosyl units [H-1: δ 4.87 (d, J=7.8 Hz), C-1: δ 106.9, H-1: δ 5.06 (d, J=7.8 Hz), C-1: δ 105.4], one β-xylopyranosyl unit [H-1: δ 4.96 (d, J=7.5 Hz), C-1: δ 106.0] and one *N*-methylanthranilyl group [N-CH$_3$: δ 2.79 (d, J=4.9

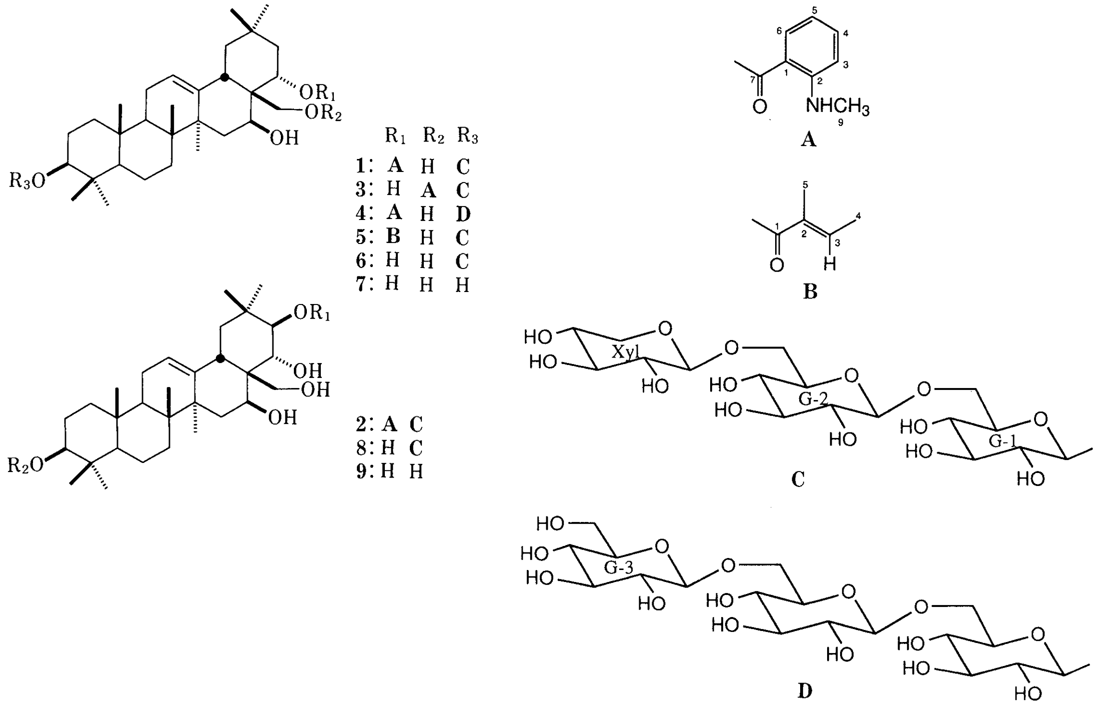

Chart 1

Table I. ^{13}C-NMR Spectral Data for **1**—**9** (in Pyridine-d_5, 125 MHz)

Carbon	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	38.9	38.8	39.0	39.0	39.0	38.9	39.1	38.7	39.1
2	26.7	26.7	26.8	26.8	26.8	26.7	28.1	26.7	28.1
3	89.1	89.0	89.1	89.2	89.1	89.1	78.0	88.9	78.0
4	39.6	39.5	39.6	39.7	39.6	39.6	39.4	39.5	39.4
5	55.8	55.7	55.8	55.9	55.8	55.8	55.6	55.6	55.6
6	18.5	18.4	18.6	18.6	18.7	18.5	18.7	18.4	18.7
7	33.0	32.9	33.1	33.1	32.9	33.0	33.0	32.9	33.0
8	40.4	40.2	40.5	40.5	40.4	40.4	40.2	40.2	40.2
9	47.1	47.0	47.2	47.3	47.2	47.2	47.3	47.0	47.2
10	36.8	36.8	36.9	36.9	36.9	36.8	37.1	36.8	37.1
11	23.9	23.9	24.0	24.1	24.2	24.0	23.9	23.8	23.9
12	124.0	124.0	123.9	123.9	123.9	123.9	123.9	123.9	123.9
13	142.4	142.2	142.1	142.5	142.4	143.0	143.1	142.7	142.8
14	43.1	42.6	42.6	43.2	43.0	42.7	42.7	42.5	42.5
15	37.2	36.2	36.3	37.2	36.2	36.0	36.0	36.0	36.0
16	65.7	68.1	66.5	65.8	66.4	67.0	66.9	68.3	68.3
17	46.0	47.3	44.6	46.1	45.9	45.2	45.1	46.6	46.6
18	44.2	42.0	44.0	44.3	44.0	43.2	43.1	42.1	42.2
19	46.3	46.4	46.3	46.4	46.5	46.7	46.6	46.8	46.8
20	32.4	36.8	32.3	32.6	32.3	32.3	32.2	36.7	36.8
21	39.6	79.6	44.1	39.6	39.4	44.2	44.3	77.3	77.3
22	73.7	71.2	69.9	73.9	74.4	69.6	69.5	73.7	73.7
23	28.3	28.2	28.4	28.4	28.4	28.3	28.7	28.2	28.7
24	17.1	17.1	17.3	17.2	17.2	17.2	16.5	17.0	16.6
25	15.8	15.6	15.9	15.9	15.9	15.8	15.7	15.7	15.7
26	17.0	17.0	17.2	17.1	17.2	17.1	17.0	16.9	17.0
27	27.6	27.4	27.8	27.8	27.6	27.7	27.6	27.4	27.4
28	59.9	58.2	62.6	60.3	60.4	58.9	58.8	58.5	58.6
29	33.3	29.7	33.6	33.4	33.3	33.7	33.5	30.5	30.4
30	25.6	20.1	25.1	25.7	25.8	25.2	25.0	19.1	19.1
3-*O*-Glc (G-1)									
1	106.9	107.0	106.9	107.0	106.9	107.0		107.0	
2	75.0	75.0	75.0	75.1	74.9	75.0		75.0	
3	78.4[a)]	78.4[a)]	78.4[a)]	78.3[a)]	78.3[a)]	78.3[a)]		78.4[a)]	
4	71.6	71.6	71.6	71.7[b)]	71.5	71.5		71.5[a)]	
5	77.0	77.0	77.0	77.1	77.0	77.0		77.0	
6	70.4	70.4	70.4	70.6	70.3	70.4		70.3	
Glc (G-2)									
1	105.4	105.4	105.4	105.4	105.4	105.4		105.4	
2	75.6	75.6	75.5	75.2	75.6	75.6		75.6	
3	78.6[a)]	78.6[a)]	78.5[a)]	78.4[a)]	78.5[a)]	78.5[a)]		78.6[a)]	
4	71.6	71.6	71.6	71.7[b)]	71.5	71.5		71.6[b)]	
5	77.0	77.0	77.0	77.1	77.0	77.0		77.0	
6	69.9	69.9	69.9	70.1	69.9	69.9		69.8	
Xyl or Glc (G-3)									
1	106.0	106.0	105.9	105.4	105.9	106.0		106.1	
2	74.9	74.9	74.9	75.7	74.9	74.9		74.9	
3	78.1	78.1	78.1	78.3[a)]	78.1	78.1		78.2	
4	71.2	71.2	71.2	71.8[b)]	71.2	71.2		71.1	
5	67.1	67.1	67.1	78.6	67.1	67.1		67.1	
6				62.8					
Acyl moieties									
1	111.5	111.2	110.7	111.6	167.5				
2	151.9	152.6	152.5	152.0	129.6				
3	112.2	111.3	111.5	112.2	137.5				
4	134.8	134.8	134.9	134.9	14.5				
5	114.7	114.7	114.8	114.9	12.6				
6	133.0	132.0	131.4	133.1					
7	168.5	169.1	169.0	168.6					
9	29.7	29.5	29.4	29.8					

a, *b*) Assignments may be interchanged in each column.

Hz), δ 29.7, CO: δ 168.5].[3)] The molecular formula, $C_{55}H_{85}NO_{19}$ implied 14 degrees of unsaturation. Five can be assigned to the *N*-methylanthranilyl group, one to an olefinic bond (δ 124.0, 142.4), and three to hemiacetal linkages of the sugar parts. The remaining five are due to the pentacyclic triterpene ring system indicated by the positive Salkowsky reaction.

Alkaline CH_3ONa–MeOH (1 : 3) treatment of **1** released methyl *N*-methylanthranilate, which was identical with an authentic sample (TLC, NMR spectrum), and prosapogenin I (**6**).

Prosapogenin I (**6**) revealed a quasi-molecular ion peak $[M-H]^-$ at *m/z* 929 in the negative FAB-MS. On acid hydrolysis, **6** afforded chichipegenin (**7**),[4] besides D-glucose and D-xylose. A ^{13}C-NMR spectral comparison of **6** with **7** showed a glycosylation shift[5] of 11.1 ppm at the C-3 signal, demonstrating the sugar linkages to be located at the C-3–OH. The sugar sequence was determined as follows. The FAB-MS of **6** showed fragment ion peaks of *m/z* 798 $[M-C_5H_8O_4-H]^-$ and 636 $[M-C_5H_8O_4-C_6H_{10}O_5-H]^-$, disclosing the sugar sequence to be C-3–*O*–Glc–Glc–Xyl. In the ^{13}C-NMR spectrum of **6**, the C-6 signals of glucose were shifted to δ 69.9 and 70.4 by the glycosylation shifts. Furthermore, in the HMBC spectrum of **6**, long-range correlations were observed between H-1 (δ 4.85) of the glucose (G-1) and C-3 (δ 89.1) of the aglycone, H-1 (δ 5.03) of the glucose (G-2) and C-6 (δ 70.4) of the glucose (G-1), and H-1 (δ 4.94) of the xylose and C-6 (δ 69.9) of the glucose (G-2). Hence, **6** was formulated as chichipegenin 3-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside. The location of the *N*-methylanthranilyl group in **1** was determined by spectral comparison of **1** and **6**, and by an HMBC experiment on **1**. Thus, acylation shifts were observed at the C-22 position [+1.27 ppm (H-22), +4.1 ppm (C-22)]. In the HMBC spectrum, the ester carbon signal of *N*-methylanthranilyl group at δ 168.5 was correlated with the methine proton (H-22) at δ 6.35 on the oxygen-bearing carbon (C-22) at δ 73.7, establishing that in **1**, C-22 is acylated. Therefore, **1** was formulated as chichipegenin 22-*O*-*N*-methylanthranilyl-3-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside.

Sitakisoside II (**2**) had the molecular formula $C_{55}H_{85}NO_{20}$ (FAB-MS, *m/z* 1078 $[M-H]^-$), *i.e.*, 16 mass units larger than that of **1**, and gave D-glucose and D-xylose in the ratio of 2 : 1 on acid hydrolysis. The ^{1}H- and ^{13}C-NMR spectra indicated the presence of two β-glucopyranosyl units [H-1: δ 4.89 (d, $J=7.6$ Hz), C-1: δ 107.0, δ 5.05 (d, $J=7.8$ Hz), C-1: δ 105.4] and one β-xylopyranosyl unit [H-1: δ 4.95 (d, $J=7.6$ Hz), C-1: δ 106.0], together with one *N*-methylanthranilyl group. Alkaline hydrolysis of **2** provided prosapogenin II (**8**) and methyl *N*-methylanthranilate.

Prosapogenin II (**8**) showed a quasi-molecular ion peak $[M-H]^-$ at *m/z* 945 in the negative FAB-MS. On acid hydrolysis, **8** afforded marsglobiferin (**9**),[6] besides D-glucose and D-xylose. A ^{13}C-NMR spectral comparison of **8** with **9** showed a glycosylation shift at C-3 signal by 10.9 ppm, demonstrating sugar linkages to be located at C-3–OH. The carbon signals due to the sugar moieties of **8** were superimposable on those of **6**. Consequently, **8** was established as marsglobiferin 3-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside. On comparing the ^{13}C-NMR spectrum of **2** with that of **8** acylation shifts were observed at C-21 (+2.3 ppm) and C-22 (−2.5 ppm) in **2**, indicating the site of acylation in **2** to be *O*-21. The same result was obtained from an HMBC experiment, that is, long-range correlations between CH_3-29 at δ 1.13, CH_3-30 at δ 1.32 and C-21 at δ 79.6, and between H-21 at δ 5.98 and the carbonyl carbon (δ 169.1) of the *N*-methylanthranilyl group were observed. Hence, the structure of sitakisoside II was established as marsglobiferin 21-*O*-*N*-methylanthranilyl-3-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside.

Sitakisoside III (**3**) had the same molecular formula, $C_{55}H_{85}NO_{19}$ (FAB-MS *m/z*, 1062 $[M-H]^-$), as **1**. Acid hydrolysis of **3** provided D-glucose and D-xylose in a ratio of 2 : 1, and **7**. Alkaline treatment of **3** gave **6** and methyl *N*-methylanthranilate. A ^{1}H- and ^{13}C-NMR spectral comparison of **3** with **6** disclosed C_{28} [+0.72, +0.47 ppm (28-H_2), +3.7 ppm (C-28)] as the acylation site in the former. The carbon signals due to the sugar moieties were superimposable on those of **1** and **2** indicating that the sugar moieties are the same. Therefore, the structure of sitakisoside III was established as chichipegenin 28-*O*-*N*-methylanthranilyl-3-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside.

Sitakisoside IV (**4**) had the molecular formula $C_{56}H_{87}NO_{20}$ (FAB-MS, *m/z* 1092 $[M-H]^-$), *i.e.*, 30 mass units higher than that of **1**. The ^{1}H- and ^{13}C-NMR spectra indicated that **4** was composed of 1 mol each of *N*-methylanthranilic acid and **7**, and 3 mol of glucose. On acid hydrolysis, **4** furnished D-glucose and **7**. The ^{1}H- and ^{13}C-NMR spectra due to the aglycone and acyl moiety of **4** were almost superimposable on those of **1** and differed only in signals due to the sugar moieties linked with *O*-3 of the aglycone. In the ^{13}C-NMR spectrum of **4**, two glycosylated C-6 positions of the glucose were observed (δ 70.1, δ 70.6), indicating a gentiotriosyl linkage to be attached at C-3–OH. Hence, the structure of sitakisoside IV (**4**) was established as chichipegenin 22-*O*-*N*-methylanthranilyl-3-*O*-β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside.

To the best of our knowledge, there is only one previous report of a triterpene glycoside having an *N*-methylanthranilyl group as the acyl part.[7]

Sitakisoside V (**5**) had the molecular formula $C_{52}H_{84}O_{19}$ (FAB-MS, *m/z* 1011 $[M-H]^-$) and showed no intense fluorescence in methanol solution. Acid hydrolysis of **5** afforded **7**, and D-glucose and D-xylose in the ratio of 2 : 1. On alkaline hydrolysis, **5** gave **6**. The ^{1}H- and ^{13}C-NMR signals due to the aglycone and sugar moieties of **5** were almost superimposable on those of **3** and differed only in those due to an acyl moiety linked with *O*-22. The NMR signals assigned to the acyl group [δ 1.56 (3H, d, $J=7.2$ Hz), 1.89 (3H, s), 7.04 (1H, q, $J=7.2$ Hz), δ 12.6, 14.5, 129.6, 137.5, 167.5] were in good agreement with those of a tigloyl moiety.[1] Hence, the structure of sitakisoside V was established as chichipegenin 22-*O*-tigloyl-3-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside.

A 1 mM solution of sitakisoside V or one of sitakisosides I—IV led to complete suppression of sweetness induced by 0.4 and 0.2 M sucrose, respectively. The activity of sitakisoside V was half that of gymnemic acids I, II.

Experimental

Melting points were measured with a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were taken on a JASCO DIP-140 digital polarimeter. IR and UV spectra were measured with JASCO FT/IR-5300 and Shimadzu UV-160 instruments. NMR spectra were recorded on Varian UNITY 200 and 600 spectrometers in C_5D_5N 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), TOCSY and ROESY. Coupling constants (J values) are given in hertz (Hz). The high resolution–electron impact (HR-EI)-MS and FAB-MS (Xe gun, 10 kV, *m*-nitrobenzyl alcohol as the matrix) were measured on JEOL JMS-HX-100 and JEOL JMS-PX303 mass spectrometers, respectively. For column chromatography, Kiesel gel 60 (230—400 mesh, Merck), and for TLC, Silica gel 60F-254 (Merck) were used. HPLC was carried out with a Waters ALC/GPC 244 instrument.

Isolation of Saponins The fresh stems (8.5 kg) of *S. lutchuensis* var. *japonica* collected in Tokushima Prefecture, in June 1993, were extracted with absolute EtOH at room temperature for 3 weeks. The ethanolic extract (540 g) was partitioned between H_2O and EtOAc. The water layer was passed through an Amberlite XAD-2 column. The column was washed with water, and the adsorbed materials were eluted with 100% MeOH to get the MeOH eluate (143 g). The MeOH eluate was chromatographed on Bondapak C_{18} with 20—80% MeOH to give four fractions (frs. 1—4). Fraction 4 (22.5 g) was subjected to HPLC on ODS (Develosil Lop ODS, 70% CH_3OH) to give five fractions (frs. 4-1—5). Fraction 4-3 and fr. 4-4 were purified by preparative HPLC (YMC, ODS S-5, 37% CH_3CN) to afford sitakisosides II (**2**, 640 mg), III (**3**, 660 mg) and V (**5**, 1.1 g), respectively. Further, the residue of fr. 4-4 was purified by preparative HPLC (YMC, ODS S-5, 37% CH_3CN) to afford sitakisoside IV (**4**, 80 mg). Sitakisosides I (**1**, 3.2 g) was obtained from fr. 4—5.

Sitakisoside I (**1**): mp 206—208 °C, $[\alpha]_D^{20}$ −12.4° (c = 1.9, MeOH). IR (film) cm^{-1}: 3400, 1680. UV λ_{max}^{EtOH} nm (log ε): 222 (4.36), 255 (3.87), 356 (3.68). Negative FAB-MS m/z: 1062 $[M(C_{55}H_{85}NO_{19})-H]^-$, 930 $[M-H-C_5H_8O_4]^-$, 911 $[M-H-C_8H_9NO_2]^-$, 767 $[M-H-C_5H_8O_4-C_6H_{10}O_5]^-$. *Anal.* Calcd for $C_{55}H_{85}NO_{19}\cdot H_2O$: C, 61.15; H, 7.93; N, 1.30. Found: C, 61.20; H, 8.24; N, 1.22. ^1H-NMR (600 MHz, C_5D_5N) δ: 0.83 (H_3-25), 0.93 (H_3-26), 1.00 (H_3-24), 1.02 (H_3-29), 1.27 (H_3-23), 1.29 (H_3-30), 1.39 (H_3-27), 3.32 (1H, dd, J = 12.0, 4.5 Hz, H-3), 4.07, 4.49 (each 1H, d, J = 10.7 Hz, H_2-28), 5.10 (1H, dd, J = 12.5, 4.5 Hz, H-16), 5.33 (1H, m, H-12), 6.35 (1H, dd, J = 11.5, 4.7 Hz, H-22), 4.87 (1H, d, J = 7.8 Hz, H-1 of Glc), 4.96 (1H, d, J = 7.5 Hz, H-1 of Xyl), 5.06 (1H, d, J = 7.8 Hz, H-1 of Glc). Acyl part: 2.79 (3H, d, J = 4.9 Hz, N-CH_3), 6.64 (1H, ddd, J = 8.4, 8.4, 1.5 Hz, H-5), 6.65 (1H, dd, J = 8.4, 1.5 Hz, H-3), 7.38 (1H, ddd, J = 8.4, 8.4, 1.5 Hz, H-4), 8.45 (1H, dd, J = 8.4, 1.5 Hz, H-6). ^{13}C-NMR: Table I.

Sitakisoside II (**2**): mp 204—206 °C, $[\alpha]_D^{20}$ −8.2° (c = 9.2, MeOH). IR (film) cm^{-1}: 3400, 1680. UV λ_{max}^{EtOH} nm (log ε): 222 (4.33), 253 (3.81), 349 (3.63). Negative FAB-MS m/z: 1078 $[M(C_{55}H_{85}NO_{20})-H]^-$, 946 $[M-H-C_5H_8O_4]^-$, 927 $[M-H-C_8H_9NO_2]^-$. *Anal.* Calcd for $C_{55}H_{85}NO_{20}\cdot 2H_2O$: C, 59.18; H, 8.04; N, 1.25. Found: C, 59.30; H, 7.95; N, 1.40. ^1H-NMR (600 MHz, C_5D_5N) δ: 0.82 (H_3-25), 0.91 (H_3-26), 1.00 (H_3-24), 1.13 (H_3-29), 1.28 (H_3-23), 1.32 (H_3-30), 1.34 (H_3-27), 3.35 (1H, dd, J = 11.5, 4.3 Hz, H-3), 4.13, 4.76 (each 1H, d, J = 10.5 Hz, H_2-28), 5.17 (1H, d, J = 10.8 Hz, H-22), 5.20 (1H, dd, J = 10.5, 4.5 Hz, H-16), 5.33 (1H, m, H-12), 5.98 (1H, dd, J = 10.8, 4.5 Hz, H-21), 4.89 (1H, d, J = 7.6 Hz, H-1 of Glc), 4.95 (1H, d, J = 7.6 Hz, H-1 of Xyl), 5.05 (1H, d, J = 7.8 Hz, H-1 of Glc). Acyl part: 2.70 (3H, d, J = 5.0 Hz, N-CH_3), 6.65 (1H, ddd, J = 7.8, 7.8, 1.5 Hz, H-5), 6.68 (1H, dd, J = 7.8, 1.7 Hz, H-3), 7.43 (1H, ddd, J = 7.8, 7.8, 1.7 Hz, H-4), 8.21 (1H, dd, J = 7.8, 1.7 Hz, H-6). ^{13}C-NMR: Table I.

Sitakisoside III (**3**): mp 200—202 °C, $[\alpha]_D^{20}$ −9.5° (c = 7.7, MeOH). IR (film) cm^{-1}: 3400, 1680. UV λ_{max}^{EtOH} nm (log ε): 222 (3.96), 255 (3.55), 349 (3.17). Negative FAB-MS m/z: 1062 $[M(C_{55}H_{85}NO_{19})-H]^-$. *Anal.* Calcd for $C_{55}H_{85}NO_{19}\cdot H_2O$: C, 61.03; H, 8.10; N, 1.29. Found: C, 61.16; H, 8.23; N, 1.42. ^1H-NMR (600 MHz, C_5D_5N) δ: 0.82 (H_3-25), 0.97 (H_3-24), 1.04 (H_3-29), 1.11 (H_3-26), 1.16 (H_3-30), 1.26 (H_3-23), 1.37 (H_3-27), 3.30 (1H, dd, J = 12.0, 4.5 Hz, H-3), *ca.* 4.83 (H-22), 4.84, 5.32 (each 1H, d, J = 10.7 Hz, H_2-28), 5.14 (1H, dd, J = 12.0, 4.5 Hz, H-16), 5.30 (1H, m, H-12), 4.83 (1H, d, J = 8.1 Hz, H-1 of Glc), 4.91 (1H, d, J = 7.4 Hz, H-1 of Xyl), 5.01 (1H, d, J = 7.6 Hz, H-1 of Glc). Acyl part: 2.67 (3H, s, N-CH_3), 6.70 (1H, ddd, J = 8.0, 8.0, 1.3 Hz, H-5), 6.65 (1H, dd, J = 8.0, 1.3 Hz, H-3), 7.42 (1H, ddd, J = 8.0, 8.0, 1.3 Hz, H-4), 8.22 (1H, dd, J = 8.0, 1.3 Hz, H-6). ^{13}C-NMR: Table I.

Sitakisoside IV (**4**): mp 198—200 °C, $[\alpha]_D^{20}$ −11.5° (c = 2.0, MeOH). IR (KBr) cm^{-1}: 3400, 1680. UV λ_{max}^{EtOH} nm (log ε): 222 (4.06), 255 (3.63), 349 (3.19). Negative FAB-MS m/z: 1092 $[M(C_{56}H_{87}NO_{20})-H]^-$. *Anal.* Calcd for $C_{56}H_{87}NO_{20}\cdot 3/2H_2O$: C, 60.09; H, 8.10; N, 1.25. Found: C, 60.31; H, 8.27; N, 1.31. ^1H-NMR (600 MHz, C_5D_5N) δ: 0.83 (H_3-25), 0.93 (H_3-26), 1.00 (H_3-24), 1.01 (H_3-29), 1.26 (H_3-23), 1.29 (H_3-30), 1.39 (H_3-27), 3.32 (1H, dd, J = 12.0, 4.5 Hz, H-3), 4.06, 4.47 (each 1H, d, J = 10.7 Hz, H_2-28), 5.08 (1H, dd, J = 11.0, 4.8 Hz, H-16), 5.32 (1H, m, H-12), 6.33 (1H, dd, J = 11.0, 4.6 Hz, H-22), 4.86 (1H, d, J = 7.8 Hz, H-1 of Glc), 5.03 (1H, d, J = 8.5 Hz, H-1 of Glc), 5.05 (1H, d, J = 8.3 Hz, H-1 of Glc). Acyl part: 2.78 (3H, d, J = 4.9 Hz, N-CH_3), 6.64 (1H, ddd, J = 8.0, 8.0, 1.2 Hz, H-5), 6.65 (1H, dd, J = 8.0, 1.2 Hz, H-3), 7.38 (1H, ddd, J = 8.0, 8.0, 1.2 Hz, H-4), 8.44 (1H, dd, J = 8.0, 1.2 Hz, H-6). ^{13}C-NMR: Table I.

Sitakisoside V (**5**): mp 202—204 °C, $[\alpha]_D^{20}$ −10.0° (c = 6.6, MeOH). IR (film) cm^{-1}: 3400, 1680. Negative FAB-MS m/z: 1011 $[M(C_{52}H_{84}NO_{19})-H]^-$. *Anal.* Calcd for $C_{52}H_{84}O_{19}$: C, 61.64; H, 8.36. Found: C, 61.58; H, 8.33. ^1H-NMR (600 MHz, C_5D_5N) δ: 0.83 (H_3-25), 0.94 (H_3-26), 0.99 (H_3-24), 1.02 (H_3-29), 1.20 (H_3-30), 1.26 (H_3-23) and 1.36 (H_3-27), 3.32 (1H, dd, J = 12.0, 4.5 Hz, H-3), *ca.* 4.00, 4.34 (each 1H, d, J = 10.7 Hz, H_2-28), 5.01 (1H, dd, J = 11.0, 4.8 Hz, H-16), 5.29 (1H, m, H-12), 6.16 (1H, dd, J = 11.0, 4.6 Hz, H-22), 4.85 (1H, d, J = 7.6 Hz, H-1 of Glc), 4.93 (1H, d, J = 7.3 Hz, H-1 of Xyl), 5.02 (1H, d, J = 7.8 Hz, H-1 of Glc). Acyl part: 1.56 (3H, d, J = 7.2 Hz, CH_3-4), 1.89 (3H, s, CH_3-5), 7.04 (1H, q, J = 7.2 Hz, H-3). ^{13}C-NMR: Table I.

Alkaline Hydrolysis of Sitakisoside I (1) A 28% sodium methylate solution (0.5 ml) was added dropwise to a solution of **1** under an N_2 atmosphere. The mixture was stirred for 4 h at room temperature, then acidified with dilute HCl, and extracted with $CHCl_3$ and then 1-BuOH. From the $CHCl_3$ layer, methyl *N*-methylanthranilate (30 mg) was obtained. Methyl *N*-methylanthranilate, pale yellow oil. UV λ_{max}^{EtOH} nm (log ε): 221 (4.24), 253 (3.72), 349 (3.57). IR (film) cm^{-1}: 3380, 2940, 1680, 1605, 1580, 1440, 1260, 1250. EI-MS m/z: 165 $[M]^+$. ^1H-NMR (200 MHz, C_5D_5N) δ: 2.73 (3H, d, J = 5.0 Hz, N-CH_3), 3.76 (3H, s, $COOCH_3$), 6.67 (1H, ddd, J = 8.1, 8.1, 1.8 Hz, H-5), 6.68 (1H, dd, J = 8.1, 1.8 Hz, H-3), 7.43 (1H, ddd, J = 8.1, 8.1, 1.8 Hz, H-4), 8.00 (1H, dd, J = 8.1, 1.8 Hz, H-6). ^{13}C-NMR (50 MHz, C_5D_5N) δ: 29.5 (N-CH_3), 51.5 ($COOCH_3$), 110.3 (C-1), 111.4 (C-3), 114.8 (C-5), 132.0 (C-6), 135.3 (C-4), 152.5 (C-2), 169.1 (C-7). From the 1-BuOH layer, prosapogenin I (**6**, 140 mg) was obtained. Compound **6**, mp 192—194 °C, $[\alpha]_D^{20}$ −7.0° (c = 9.3, MeOH). IR (film) cm^{-1}: 3400, 3250. Negative FAB-MS m/z: 929 $[M(C_{47}H_{78}O_{18})-H]^-$, 797 $[M-H-C_5H_8O_4]^-$, 635 $[M-H-C_5H_8O_4-C_6H_{10}O_5]^-$. *Anal.* Calcd for $C_{47}H_{78}O_{18}\cdot H_2O$: C, 59.48; H, 8.50. Found: C, 59.21; H, 8.41. ^1H-NMR (600 MHz, C_5D_5N) δ: 0.82 (H_3-25), 0.93 (H_3-26), 0.99 (H_3-24), 1.02 (H_3-29), 1.11 (H_3-30), 1.26 (H_3-23), 1.37 (H_3-27), 3.31 (1H, dd, J = 11.7, 4.4 Hz, H-3), *ca.* 4.12, 4.85 (each 1H, d, J = 10.0 Hz, H_2-28), 5.10 (1H, dd, J = 11.5, 5.0 Hz, H-16), 5.08 (1H, dd, J = 12.0, 4.5 Hz, H-22), 5.29 (1H, m, H-12), 4.85 (1H, d, J = 7.5 Hz, H-1 of Glc), 4.94 (1H, d, J = 7.3 Hz, H-1 of Xyl), 5.03 (1H, d, J = 7.8 Hz, H-1 of Glc). ^{13}C-NMR: Table I.

Acid Hydrolysis of Prosapogenin I (6) A solution of **6** (50 mg) in 5% H_2SO_4 was heated at 100 °C for 3 h. The reaction mixture was extracted with EtOAc to provide the aglycone (**7**, 25 mg). Compound **7**, mp 315—317 °C (MeOH−$CHCl_3$), $[\alpha]_D^{20}$ +35.4° (c = 1.2, $CHCl_3$) was identified as chichipegenin (lit.,[4]) mp 321—323 °C, $[\alpha]_D^{20}$ +43° (c = 1, $CHCl_3$)) by comparison of its spectral data with literature values.[4]) HR-EI-MS Obsd. for $[M-(C_{30}H_{50}O_4)-H_2O]$ 456.3580, Calcd 456.3604. ^1H-NMR (200 MHz, C_5D_5N) δ: 0.90, 1.00, 1.00, 1.05, 1.10, 1.25 and 1.39 (each 3H, s, *tert*-CH_3 × 7), 3.46 (1H, t, J = 8.0 Hz, H-3), *ca.* 4.12, 4.85 (each 1H, d, J = 10.0 Hz, H_2-28), 5.13 (1H, dd, J = 11.5, 5.0 Hz, H-16), 5.42 (1H, m, H-12). ^{13}C-NMR: Table I. The aqueous layer was neutralized with Amberlite IR-45 and evaporated to dryness *in vacuo*. The sugar was determined by using refractive index (RI) detection (Waters 410) and chiral detection (Shodex OR-1) in HPLC (Shodex RSpak DC-613, 80% CH_3CN, 0.8 ml/min, 70 °C), by comparison with authentic sugars (10 mM each of D-Glc, L-Glc and D-Xyl, L-Xyl). The sugar fraction gave positive peaks at 8.80 min (D-Xyl, 8.78 min) and 13.40 min (D-Glc, 13.38 min).

Identification of Component Sugars of 2—5 A solution of each compound **2**—**5** (2—3 mg) was examined in the same way as described for **1**. Compounds **2**, **3**, and **5** gave D-Xyl and D-Glc. Compound **4** gave D-Glc.

Alkaline Hydrolysis of Sitakisoside II (2) Alkaline hydrolysis of **2** (200 mg) was examined in the same way as described for **1** to give methyl *N*-methylanthranilate (30 mg) from the $CHCl_3$ layer. The 1-BuOH layer was subjected to HPLC (YMC, ODS S-5, 20% CH_3CN) to provide prosapogenin II (**8**, 120 mg). Compound **8**, mp 190—192 °C, $[\alpha]_D^{20}$ −17.1°

(c=1.6, MeOH). IR (film) cm^{-1}: 3400, 3250. Negative FAB-MS *m/z*: 945 [M($C_{47}H_{78}O_{19}$)−H]$^-$, 813 [M−H−$C_5H_8O_4$]$^-$, 651 [M−H−$C_5H_8O_4$−$C_6H_{10}O_5$]$^-$. *Anal.* Calcd for $C_{47}H_{78}O_{19}\cdot H_2O$: C, 58.49; H, 8.35. Found: C, 58.61; H, 8.61. ^{1}H-NMR (600 MHz, C_5D_5N) δ: 0.82 (H_3-25), 0.93 (H_3-26), 1.00 (H_3-24), 1.27 (H_3-23), 1.32 (H_3-29), 1.34 (H_3-27), 1.36 (H_3-30), 3.33 (1H, dd, J=11.6, 4.4 Hz, H-3), 4.11, 4.76 (each 1H, d, J=10.5 Hz, H_2-28), *ca.* 4.12 (H-21), 4.93 (1H, d, J=9.5 Hz, H-22), 5.12 (1H, dd, J=11.8, 5.2 Hz, H-16), 5.32 (1H, m, H-12). ^{13}C-NMR: Table I

Acid Hydrolysis of Prosapogenin II (8) A solution of **8** (50 mg) in 5% H_2SO_4 was heated at 100 °C for 3 h and worked-up as usual. The crude product was chromatographed on a silica gel column with CH_2Cl_2–MeOH (50 : 1) giving an aglycone (**9**, 20 mg). Compound **9**, mp 311—313 °C (MeOH), $[\alpha]_D^{20}$ +39.0° (c=1.0, $CHCl_3$) was identified as marsglobiferin (lit.,[6] mp 241—243 °C, $[\alpha]_D^{20}$ +41.8° (c=0.54, $CHCl_3$)) by comparison of its spectral data with literature values.[6] HR-EI-MS Obsd. for [M($C_{30}H_{50}O_5$)−H_2O] 472.3541, Calcd 472.3552. EI-MS *m/z*: 490 [M]$^+$, 472 [M−H_2O]$^+$, 454 [M−2H_2O]$^+$, 282, 264, 246, 215, 197. ^{1}H-NMR (200 MHz, C_5D_5N) δ: 0.90, 0.99, 1.05, 1.24, 1.30, 1.33 and 1.39 (each 3H, s, *tert*-CH_3 × 7), 3.45 (1H, t, J=8.4 Hz, H-3), 4.12, 4.78 (each 1H, d, J=10.2 Hz, H_2-28), 4.12 (1H, d, J=10.2 Hz, H-21), 4.93 (1H, d, J=10.6 Hz, H-22), 5.12 (1H, dd, J=10.5, 6.7 Hz, H-16), 5.39 (1H, m, H-12). ^{13}C-NMR: Table I.

Alkaline Hydrolysis of Sitakisoside III (3) Alkaline hydrolysis of **3** (10 mg) was carried out in the same way as described for **1** to get methyl *N*-methylanthranilate (detected by TLC, hexane: CH_2Cl_2=4 : 6) and **6** [detected by TLC, $CHCl_3$: MeOH : H_2O=65 : 35 : 10 (lower layer) and HPLC (YMC, ODS S-5, 28% CH_3CN)]. *Rf*; 0.67 (methyl *N*-methylanthranilate), 0.29 [prosapogenin I (**6**)], t_R; 12.10 min [prosapogenin I (**6**)].

Acid Hydrolysis of Sitakisoside IV (4) Acid hydrolysis of **4** (50 mg) was carried out in the same way as described for **6** to give **7** (10 mg).

Alkaline Hydrolysis of Sitakisoside V (5) Alkaline hydrolysis of **5** (10 mg) was carried out in the same way as described for **1** to get **6** [detected by TLC, $CHCl_3$: MeOH : H_2O=65 : 35 : 10 (lower layer) and HPLC (YMC, ODS S-5, 28% CH_3CN)]. *Rf*; 0.67 (methyl *N*-methylanthranilate), 0.29 [prosapogenin I (**6**)], t_R; 12.10 min [prosapogenin I (**6**)].

Bioassay of Antisweet Activity The antisweet activity of 1 mM solutions of **1**—**5** was tested on three volunteers. Each participant held a test solution in the mouth for 3 min, spat, rinsed the mouth with distilled water and tasted a sucrose solution (0.2 or 0.4 M).

Acknowledgment This research has been financially supported by Hayashi Memorial Foundation for Female Natural Scientists.

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