Antisweet Natural Products. XI.[1] Structures of Sitakisosides VI—X 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 June 27, 1994; accepted August 1, 1994

From the fresh stem of *Stephanotis lutchuensis* var. *japonica*, five new oleanane-type triterpenoid glycosides named sitakisosides VI—X (1—5) were isolated. Their structures were determined on the basis of spectroscopic data and chemical evidence. Sitakisosides VI and VII are 3-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosido-21-*O*-(6-*N*-methylanthranilyl)-β-D-glucopyranosyl and 3-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosido-21-*O*-(4-*N*-methylanthranilyl)-β-D-glucopyranosyl sitakisogenin, respectively. Sitakisoside VIII is 3-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosido-21-*O*-*N*-methylanthranilyl-3β,16β,21β,28-tetrahydroxyolean-12-ene-22-one. Sitakisoside IX is 3-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosido-21-*O*-(6-*N*-methylanthranilyl)-β-D-glucopyranosyl gymnestrogenin. Sitakisoside X is 3-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl longispinogenin.

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

In the preceding paper[1] of this series, we reported the isolation and structure determination of five antisweet principles named sitakisosides I—V, from the stem of *S. lutchuensis* var. *japonica* (Asclepiadaceae). Sitakisosides I, III, IV and V are 3-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosido-22-*O*-*N*-methylanthranilyl, 3-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosido-28-*O*-*N*-methylanthranilyl, 3-*O*-β-gentiotriosido 22-*O*-*N*-methylanthranilyl and 3-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosido-22-*O*-tigloyl chichipegenin, respectively. Sitakisoside II is 3-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosido-21-*O*-*N*-methylanthranilyl marsglobiferin. In this paper, we report the isolation and structural elucidation of five additional novel saponins, sitakisosides VI—X (**1**—**5**), having an oleanene skeleton. Their structures were elucidated by chemical and spectral methods, two dimensional NMR (2D-NMR) techniques being especially helpful.

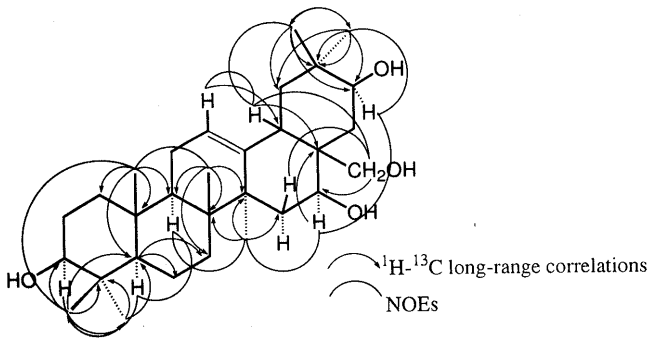

Fig. 1. Significant Correlations Observed in HMBC and NOEs of Sitakisogenin (**7**)

The EtOH extract obtained from the fresh stem of *S. lutchuensis* var. japonica was subjected to Amberlite XAD-2 column chromatography to give a saponin fraction. Repeated separation of the saponin fraction by HPLC gave five new compounds named sitakisosides VI (**1**), VII (**2**), VIII (**3**), IX (**4**) and X (**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 positions of attachment of the acyl moiety and the sugar chains to the aglycone.

Sitakisosides VI (**1**) and VII (**2**) showed an intense blue fluorescence in methanol solution. An alkaline treatment with CH_3ONa–MeOH (1 : 3) of **1** and **2** released a methyl

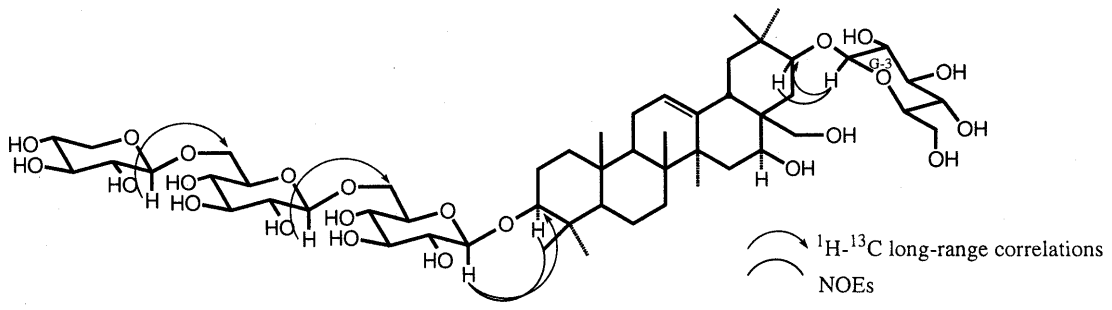

Fig. 2. Most Significant Correlations Observed in HMBC and NOEs of Compound **6**

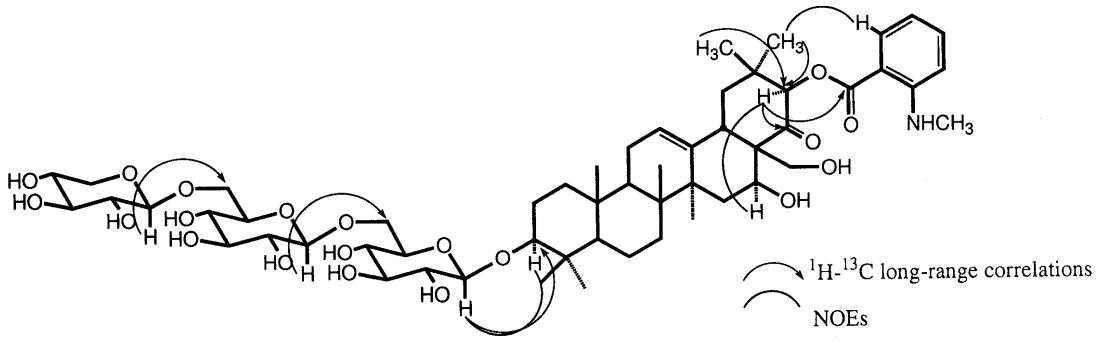

Fig. 3. Most Significant Correlations Observed in HMBC and NOEs of Shitakisoside VIII (**3**)

N-methylanthranilate, which was shown to be identical with an authentic sample by TLC and NMR spectroscopy, and prosapogenin I (**6**). On acid hydrolysis, **6** afforded sitakisogenin (**7**) as an aglycone, besides D-glucose and D-xylose in molar ratio of 3 : 1, confirmed by specific rotation measurement using HPLC with chiral detection.

Sitakisogenin (**7**) showed an $[M-H_2O]^+$ ion peak at *m/z* 456.3628 in the HR-MS, suggesting the molecular formula to be $C_{30}H_{50}O_4$. The ^{13}C-NMR (DEPT) and ^{1}H-NMR (D_2O exchange) spectra indicated the presence of seven methyls, eight methylenes, three methines, six quaternary carbons, one oxygen-bearing methylene, three oxygen-bearing methines and one double bond. The ^{1}H–^{1}H COSY and TOCSY spectra of **7** revealed isolated spin systems (H-1—3, H-5—7, H-9—12, H-15—16, H-18—19, H-21—22). The gross structure of **7** was determined by analysis of NMR data including ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, HMBC and ROESY experiments (Fig. 1), and by referring to the data for gymnestrogenin (**8**).[2,3] Thus, **7** is shown to be olean-12-ene-3β,16β,21β,28-tetrol.

Prosapogenin I (**6**) was deduced to have the molecular formula $C_{53}H_{88}O_{23}$ based on elemental analysis. The ^{1}H- and ^{13}C-NMR spectra of **6** indicated the presence of three β-glucopyranosyl units [H-1: δ 4.86 (d, J=7.8 Hz), C-1: δ 107.0, H-1: d 5.05 (d, J=7.6 Hz), C-1: δ 105.4, H-1: δ 5.08 (d, J=7.6 Hz), C-1: δ 106.5], and one β-xylopyranosyl unit [H-1: δ 4.94 (d, J=7.3 Hz), C-1: δ 106.0]. A ^{13}C-NMR spectral comparison of **6** with **7** showed glycosylation shifts[4,5] of +10.6 ppm at the C-3 signal and 9.8 ppm at the C-21 signal, demonstrating the sugar linkages to be located at C-3-OH and C-21-OH. The sugar sequences were determined as follows. The negative FAB-MS of **6** showed the fragment ion peaks *m/z* 959 $[M-C_5H_8O_4-H]^-$ and 797 $[M-C_5H_8O_4-C_6H_{10}O_5-H]^-$, disclosing a xylose to be terminal. In the ^{13}C-NMR spectrum of **6**, the C-6 positions of each of two glucoses were shifted to δ 70.0 and 70.4 by the glycosylation shifts, showing that the sugar sequences were Xyl^{6}Glc^{6}Glc–*O* and Glc–*O*, or Glc^{6}Glc–*O*, and Xyl^{6}Glc1–*O*. In the HMBC experiment on **6**, long-range correlations were observed between H-1 (δ 4.86) of the glucose (G-1) and C-3 (δ 89.0) of the aglycone, H-1 (δ 5.05) 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 70.0) of the glucose (G-2), and H-1 (δ 5.08) of the glucose (G-3) and C-21 (δ 82.9) of the aglycone. Furthermore, NOEs were observed between H-1 (δ 4.86) of the glucose (G-1) and C-3-H (δ 3.34) of the aglycone, and H-1 (δ 5.08) of the glucose (G-3) and C-21-H (δ 4.16) of the aglycone (Fig. 2). Hence, **6** was formulated as 3-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosido-21-*O*-β-D-glucopyranosyl sitakisogenin.

Sitakisoside VI (**1**) had the molecular formula $C_{61}H_{95}NO_{24}$ based on elemental analysis. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **1** with those of **6** showed the **6** position of glucose joined to C-21-OH to be the acylation site in the former [+0.77 ppm, from δ 4.10 to 4.87 and +0.56 ppm, from δ 4.61 to 5.17 (C6-H2), +0.6 ppm, from δ 64.0 to 64.6 (C-6), −2.9 ppm, from δ 78.2 to 75.3 (C-5)]. Accordingly, **1** was formulated as 3-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosido-21-*O*-(6-*N*-methylanthranilyl)-β-D-glucopyranosyl sitakisogenin.

Sitakisoside VII (**2**) had the same molecular formula, $C_{61}H_{95}NO_{24}$ (positive FAB-MS *m/z* 1226 $[M+H]^+$), as **1**. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **2** with those of **6** showed the **4** position of glucose joined to C-21-OH to be the acylation site in the former [+1.46 ppm, from δ 4.20 to 5.66 and +0.42 ppm (C_4-H), +0.3 ppm, from δ 72.6 to 72.9 (C-4), −2.3 ppm, from δ 78.5 to 76.2 (C-3), −1.8 ppm, from δ 78.2 to 76.4 (C-5)]. This was further confirmed by an HMBC experiment. A long-range correlation was seen between H-4 (δ 5.66) of the glucose (Glc-3) and the carbonyl carbon (δ 168.3) of the *N*-methylanthranilyl group in the HMBC spectrum. Hence, **2** was formulated as 3-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosido-21-*O*-(4-*N*-methylanthranilyl)-β-D-glucopyranosyl sitakisogenin.

Sitakisoside VIII (**3**) had the molecular formula $C_{55}H_{83}NO_{20}$ (negative FAB-MS, *m/z* 1076 $[M-H]^-$), *i.e.*, 2 H less than that of sitakisoside II (**9**). On acid hydrolysis, **3** afforded several unresolved aglycone components, besides D-glucose and D-xylose in the ratio 2 : 1, confirmed by HPLC with chiral detection. The ^{1}H- and ^{13}C-NMR spectra indicated that **3** was composed of 1 mol each of the aglycone, *N*-methylanthranilic acid and xylose, and 2 mol of glucose. A ^{13}C-NMR spectral comparison of **3** with **9**, showed that **3** differs structurally from **9** only in its E ring, though the same sugar units and the same acyl unit are affixed to the C-3 and E ring, respectively. The long-range correlation between a singlet signal at δ 6.20 and C-29/C-30 in the HMBC spectrum,

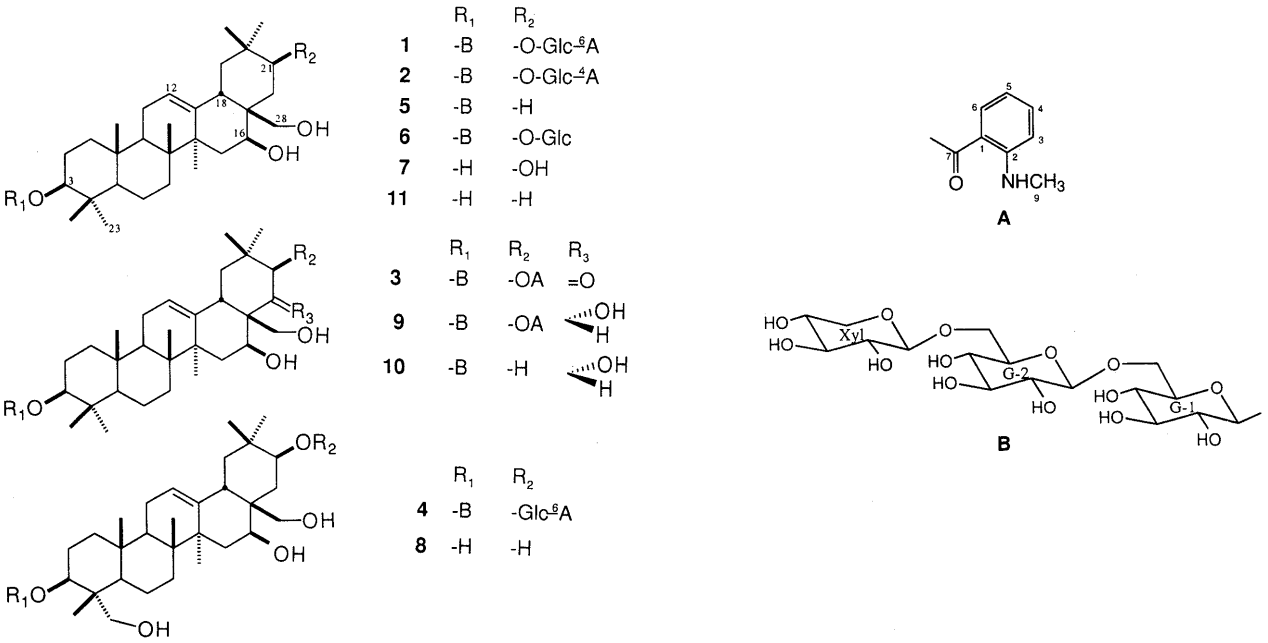

Chart 1

and an NOE between the former and H-29 at δ 1.09 in the ROESY spectrum of **3** (Fig. 3), indicated the presence of 21β-OH. A long-range correlation between H-21 and carbonyl carbon at δ 206.6 established the site of the carbonyl at C-22. Hence, the aglycone of **3** was formulated as 3β,16β,21β,28-tetrahydroxyolean-12-ene-22-one, which has not been reported before. The HMBC experiment of **3** revealed that H-21 was coupled to the carbonyl of the acyl group, indicating the acyl group to be at C-21-OH. Further, reduction of **3** with lithium borohydride gave sitakisoside II (**9**). Accordingly, **3** was formulated as 3-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosido-21-*O*-*N*-methylanthranilyl-3β,16β,21β,28-tetrahydroxyolean-12-ene-22-one.

Sitakisoside IX (**4**) had the molecular formula $C_{61}H_{95}NO_{25}$ (positive FAB-MS, *m/z* 1242 $[M+H]^+$), *i.e.*, one oxygen atom less than that of **1**. The ^{1}H- and ^{13}C-NMR spectra indicated that **4** was composed of 1 mol each of the aglycone, *N*-methylanthranilic acid and xylose, and 3 mol of glucose. On acid hydrolysis, 4 afforded gymnestrogenin (**8**), besides D-glucose and D-xylose in the ratio of 3 : 1, confirmed by HPLC with chiral detection. The C-3 and C-21 signals in the ^{13}C-NMR spectrum of **4** appeared at lower field by 9.5 ppm and 11.4 ppm, respectively, than those of **8** because of the glycosylation shifts, demonstrating that sugar units are located at C-3-OH and C-21-OH of the aglycone. The carbon signals due to the sugar moieties and *N*-methylanthranilyl group are superimposable on those of **1**, indicating that the sugar moieties are the same. Hence, the structure of sitakisoside IX (**4**) was established as 3-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosido-21-*O*-(6-*N*-methylanthranilyl)-β-D-glucopyranosyl gymnestrogenin.

Sitakisoside X (**5**) had the molecular formula $C_{47}H_{78}O_{17}$ (FAB-MS, *m/z* 915 $[M+H]^+$), *i.e.*, one oxygen atom less than that of prosapogenin II (**10**) derived from sitakisoside I and showed no intense fluorescence in methanol solution. The ^{1}H- and ^{13}C-NMR spectra indicated that **5** was composed of 1 mol each of the aglycone and xylose, and 2 mol of glucose. On acid hydrolysis, **5** afforded longispinogenin (**11**),[6,7] besides D-glucose and D-xylose in the ratio of 2 : 1, confirmed by HPLC with chiral detection. A ^{13}C-NMR spectral comparison of **5** with **11** showed a glycosylation shift of +9.8 ppm at C-3, demonstrating sugar moieties to be located at C-3-OH. The carbon signals due to the sugar moieties are superimposable on those of **1**, indicating that the sugar moieties are the same. Hence, the structure of sitakisoside X (**5**) was established as 3-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl longispinogenin.

A 1 mM solution of any of sitakisosides VI—IX led to complete suppression of the sensation of sweetness induced by 0.2 M sucrose. Compound **5** was not active at all. The activities of sitakisosides VI—IX were half those of gymnemic acids III and IV.

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 and UV spectra were measured with JASCO FT/IR-5300 and Shimadzu UV-160. NMR spectra were recorded on Varian UNITY 200 and 600 spectrometers in C_5D_5N solution using TMS as an internal standard. NMR experiments included ^{1}H–^{1}H-COSY, ^{13}C–^{1}H-COSY, 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 import MS (HR-EI-MS) and the 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 on 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

TABLE I. ^{13}C-NMR Spectral Data for **1**—**11** (in Pyridine-d_5, 150 MHz)

Carbon	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	38.9	38.8	38.8	38.9	39.0	38.8	39.3	39.0	38.8	38.9	39.3
2	26.8	26.7	26.7	26.1	26.9	26.8	28.1	27.7	26.7	26.7	28.1
3	89.1	88.9	89.0	82.9	89.1	89.0	78.4	73.4	89.0	89.1	78.3
4	39.6	39.5	39.6	43.5	39.7	39.6	39.6	42.9	39.5	39.6	39.6
5	55.8	55.7	55.8	47.7	55.9	55.8	56.0	48.6	55.7	55.8	55.9
6	18.5	18.4	18.4	18.3	18.6	18.4	18.9	18.6	18.4	18.5	18.9
7	33.0	32.8	32.9	32.8	33.1	33.0	33.2	32.8	32.9	33.0	33.2
8	40.2	40.1	40.1	40.2	40.3	40.2	40.3	40.2	40.2	40.4	40.3
9	47.2	47.0	47.0	47.2	47.2	47.1	47.4	47.3	47.0	47.2	47.4
10	36.9	36.8	36.8	36.8	36.9	36.9	37.4	36.9	36.8	36.8	37.3
11	24.0	23.9	23.9	24.0	24.0	24.0	24.1	24.0	23.9	24.0	24.1
12	124.0	123.9	123.9	124.0	123.9	123.9	123.1	123.1	124.0	123.9	122.8
13	143.1	142.7	142.2	143.1	143.9	142.8	143.4	143.2	142.2	143.0	144.1
14	43.9	43.8	43.7	43.9	43.9	43.8	44.0	43.9	42.6	42.7	44.0
15	36.8	36.2	36.5	36.8	36.9	36.4	36.8	36.8	36.2	36.0	36.8
16	67.6	67.9	68.6	67.5	66.9	68.0	67.9	67.7	68.1	67.0	66.9
17	43.8	43.9	59.3	43.8	41.1	44.0	43.9	43.7	47.3	45.2	41.2
18	43.6	43.5	44.0	43.6	44.6	43.6	44.0	44.0	42.0	43.2	44.6
19	48.0	47.7	46.0	47.9	47.2	48.0	48.0	47.7	46.4	46.7	47.3
20	37.0	36.7	39.2	37.0	31.3	36.9	37.1	37.0	36.8	32.3	31.3
21	84.1	83.0	81.3	84.1	34.5	82.9	73.1	72.7	79.6	44.2	34.4
22	33.5	33.9	206.6	33.3	26.4	34.0	35.0	35.1	71.2	69.6	26.3
23	28.3	28.2	28.2	65.0	28.4	28.3	29.0	67.9	28.2	28.3	28.9
24	17.2	17.1	17.1	13.8	17.2	17.2	16.8	13.1	17.1	17.2	16.7
25	15.8	15.7	15.7	16.4	15.9	15.8	16.0	16.2	15.6	15.8	15.9
26	17.0	16.9	16.9	17.1	17.1	17.0	17.2	17.0	17.0	17.1	17.1
27	27.2	27.0	26.8	27.2	27.3	27.2	27.3	27.1	27.4	27.7	27.3
28	68.2	67.9	60.3	68.2	69.1	68.2	68.6	68.5	58.2	58.9	69.0
29	29.9	29.2	28.5	29.8	33.7	29.4	30.2	30.0	29.7	33.7	33.6
30	18.9	18.4	20.1	18.8	24.3	18.6	18.2	17.9	20.1	25.2	24.3
3-*O*-Glc (G-1)											
1	107.0	107.0	106.9	106.1	107.0	107.0			107.0	107.0	
2	75.0	75.0	75.0	75.0	75.0	75.0			75.0	75.0	
3	78.4[a]	78.4[a]	78.4[a]	78.4[a]	78.3[a]	78.3[a]			78.4[a]	78.3[a]	
4	71.5	71.5[b]	71.6	71.5	71.5	71.5			71.6	71.5	
5	77.1	77.0	77.0	77.1	77.0	77.1			77.0	77.0	
6	70.4	70.4	70.4	70.12	70.4	70.4			70.4	70.4	
Glc (G-2)											
1	105.4	105.4	105.4	105.4	105.4	105.4			105.4	105.4	
2	75.6	75.6	75.6	75.7	75.6	75.6			75.6	75.6	
3	78.6[a]	78.6[a]	78.5[a]	78.6[a]	78.6[a]	78.5[a]			78.6[a]	78.5[a]	
4	71.5	71.6[b]	71.6	71.5	71.5	71.5			71.6	71.5	
5	77.1	77.0	77.0	77.1	77.0	77.1			77.0	77.0	
6	69.9	69.8	69.9	79.9	69.9	70.0			69.9	69.9	
Xyl											
1	106.1	106.1	106.0	106.0	106.0	106.0			106.0	106.0	
2	74.9	74.9	74.9	74.9	74.9	74.9			74.9	74.9	
3	78.2	78.2	78.1	78.2	78.1	78.1			78.1	78.1	
4	71.1	71.1	71.2	71.2	71.2	71.2			71.2	71.2	
5	67.1	67.1	46.1	67.1	67.1	67.1			67.1	67.1	
21-*O*-Glc (G-3)											
1	106.5	106.5		106.4		106.5					
2	75.8	75.8		75.4		75.9					
3	78.6[a]	76.2		78.6		78.5[a]					
4	71.5	72.9		71.5		72.6					
5	75.3	76.4		75.8		78.2					
6	64.6	63.2		64.7		64.0					
Acyl moieties											
1	110.7	110.2	110.5	110.7					111.2		
2	152.3	152.6	152.6	152.3					152.6		
3	111.2	111.2	111.4	111.1					111.3		
4	135.1	135.7	135.3	135.1					134.8		
5	115.0	114.6	114.8	114.9					114.7		
6	132.6	132.0	132.2	132.6					132.0		
7	168.8	168.3	168.0	168.8					169.0		
9	29.6	29.4	29.4	29.6					29.5		

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

washed with water, and the adsorbed materials were eluted with 100% MeOH to obtain 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 3 (20.0 g) was subjected to HPLC on ODS (Develosil Lop ODS, 60% CH_3OH) to give four fractions (frs. 3-1—4). Fraction 3-3 and fr. 3-4 were purified by preparative HPLC (YMC, ODS S-5, 27% CH_3CN) to afford sitakisosides VI (**1**, 480 mg), VII (**2**, 540 mg), IX (**4**, 150 mg) and X (**5**, 300 mg). 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 was purified by preparative HPLC (YMC, ODS S-5, 37% CH_3CN) to afford sitakisoside VIII (**3**, 210 mg). Fraction 4-2 was purified by preparative HPLC (YMC, ODS S-5, 27% CH_3CN) to afford **1** (140 mg), **2** (430 mg), **4** (75 mg) and **5** (460 mg). Sitakisosides I—V had been obtained from fr. 4-4 and fr. 4-5.

Sitakisoside VI (**1**). An amorphous powder, $[\alpha]_D^{20}$ −30.1° (c=7.7, MeOH). IR (film) cm^{-1}: 3460, 1680, 1090. UV λ_{max}^{EtOH} nm (log ε): 204 (4.11), 222 (4.27), 255 (3.86), 353 (3.61). Positive FAB-MS m/z: 1226 $[M(C_{61}H_{95}NO_{24})+H]^+$, 1094 $[M+H-C_8H_8NO_2]^+$. *Anal.* Calcd for $C_{61}H_{95}NO_{24}$: C, 59.74; H, 7.81; N, 1.14. Found: C, 60.00; H, 7.81; N, 1.54. ^{1}H-NMR (600 MHz, C_5D_5N) δ: 0.84 (H_3-25), 0.97 (H_3-26), 0.99 (H_3-24), 1.22 (H_3-30), 1.28 (H_3-23), 1.34 (H_3-27), 1.43 (H_3-29), 1.72 (1H, dd, J=12.5, 4.3 Hz, H_α-15), 2.06 (1H, dd, J=12.5, 12.5 Hz, H_β-22), 2.21 (1H, dd, J=12.5, 11.0 Hz, H_β-15), 2.52 (1H, dd, J=11.5, 4.0 Hz, H-18), 3.33 (1H, dd, J=11.5, 4.5 Hz, H-3), 3.56 (1H, dd, J=12.5, 4.5 Hz, H_α-22), 3.69, 4.34 (each 1H, d, J=11.0 Hz, H_2-28), *ca.* 4.18 (1H, m, H-21), 4.67 (1H, dd, J=11.0, 4.3 Hz, H-16), 5.24 (1H, m, H-12), *ca.* 4.30, 4.77 (each 1H, br d, J=10.5 Hz, H-6 of Glc-2), *ca.* 4.30, 4.89 (each 1H, br d, J=11.0 Hz, H-6 of Glc-1), 4.86 (1H, d, J=7.8 Hz, H-1 of Glc-1), 4.87, 5.17 (each 1H, br d, J=10.5 Hz, H-6 of Glc-3), 4.94 (1H, d, J=7.6 Hz, H-1 of Xyl), 5.04 (1H, d, J=7.6 Hz, H-1 of Glc-2), 5.09 (1H, d, J=7.8 Hz, H-1 of Glc-3). Acyl part: 2.67 (3H, d, J=4.1 Hz, N–CH_3), 6.56 (1H, dd, J=8.0, 1.2 Hz, H-3), 6.67 (1H, ddd, J=8.0, 8.0, 1.2 Hz, H-5), 7.33 (1H, ddd, J=8.0, 8.0, 1.2 Hz, H-4), 7.82 (1H, q, J=4.1 Hz, NH), 8.29 (1H, dd, J=8.0, 1.2 Hz, H-6). ^{13}C-NMR: Table I.

Sitakisoside VII (**2**). Colorless needles from MeOH, mp 214—216°C $[\alpha]_D^{20}$ −36.0° (c=6.5, MeOH). IR (film) cm^{-1}: 3460, 1680, 1090. UV λ_{max}^{EtOH} nm (log ε): 222 (4.34), 254 (3.86), 356 (3.70). Positive FAB-MS m/z: 1226 $[M(C_{61}H_{95}NO_{24})]+H]^+$, 1094 $[M-C_8H_8NO_2+H]^+$. *Anal.* Calcd for $C_{61}H_{95}NO_{24}$: C, 59.74; H, 7.81; N, 1.14. Found: C, 59.58; H, 8.10; N, 1.27. ^{1}H-NMR (600 MHz, C_5D_5N) δ: 0.84 (H3-25), 0.94 (H_3-26), 0.99 (H_3-24), 1.20 (H_3-30), 1.24 (H_3-27), 1.26 (H_3-23), 1.40 (H_3-29), 1.67 (1H, dd, J=12.1, 4.3 Hz, H_α-15), 2.09 (1H, dd, J=12.8, 12.8 Hz, H_β-22), 2.16 (1H, dd, J=12.1, 12.1 Hz, H_β-15), 2.53 (1H, dd, J=11.4, 4.3 Hz, H-18), 3.34 (1H, dd, J=11.4, 4.3 Hz, H-3), 3.54 (1H, dd, J=12.8, 4.0 Hz, H_α-22), 3.73, 4.33 (each 1H, d, J=10.3 Hz, H_2-28), *ca.* 4.20 (1H, m, H-21), *ca.* 4.31, 4.79 (each 1H, br d, J=10.0 Hz, H-6 of Glc-2), *ca.* 4.31, 4.91 (each 1H, brd, J=11.0 Hz, H-6 of Glc-1), 4.48 (1H, dd, J=9.5, 9.0 Hz, H-4 of Glc-3), 4.66 (1H, dd, J=12.1, 4.3 Hz, H-16), 5.22 (1H, m, H-12), 4.88 (1H, d, J=7.5 Hz, H-1 of Glc-1), 4.96 (1H, d, J=7.3 Hz, H-1 of Xyl), 5.06 (1H, d, J=7.8 Hz, H-1 of Glc-2), 5.17 (1H, d, J=7.8 Hz, H-1 of Glc-3), 5.66 (1H, dd, J=9.5, 9.5 Hz, H-4 of Glc-3). Acyl part: 2.65 (3H, d, J=4.9 Hz, N-CH_3), 6.59 (1H, ddd, J=8.0, 8.0, 1.5 Hz, H-5), 6.61 (1H, dd, J =8.0, 1.5 Hz, H-3), 7.38 (1H, ddd, J=8.0, 8.0, 1.5 Hz, H-4), 7.81 (1H, q, J=4.9 Hz, NH), 8.11 (1H, dd, J=8.0, 1.5 Hz, H-6). ^{13}C-NMR: Table I.

Sitakisoside VIII (**3**). An amorphous powder, $[\alpha]_D^{20}$ −8.0° (c=2.5, MeOH). IR (film) cm^{-1}: 3400, 1700, 1680. UV λ_{max}^{EtOH} nm (log ε): 224 (4.41), 254 (3.92), 349 (3.70). Negative FAB-MS m/z: 1076 $[M(C_{55}H_{83}NO_{20})-H]^-$. *Anal.* Calcd for $C_{55}H_{83}NO_{20}$: C, 58.81; H,7.90; N, 1.25. Found: C, 58.78; H, 7.95; N, 1.53. ^{1}H-NMR (600 MHz, C_5D_5N) δ: 0.86 (H_3-25), 0.95 (H_3-26), 1.01 (H_3-24), 1.22 (H_3-29), 1.24 (H_3-30), 1.30 (H_3-23), 1.42 (H_3-27), 3.36 (1H, dd, J=12.0, 5.0 Hz, H-18), 3.38 (1H, dd, J=12.0, 4.5 Hz, H-3), 4.08, 4.75 (each 1H, d, J=11.0 Hz, H_2-28), 5.16 (1H, dd, J=11.0, 4.8 Hz, H-16), 5.44 (1H, m, H-12), 6.20 (1H, s, H-21), 4.89 (1H, d, J=7.8 Hz, H-1 of Glc-1), 4.96 (1H, d, J=7.8 Hz, H-1 of Xyl), 5.07 (1H, d, J=7.8 Hz, H-1 of Glc-2). Acyl part: 2.66 (3H, d, J=5.4 Hz, N–CH_3), 6.68 (1H, dd, J=7.8, 1.7 Hz, H-3), 6.74 (1H, ddd, J=7.8, 7.8, 1.7 Hz, H-5), 7.44 (1H, ddd, J=7.8, 7.8, 1.7 Hz, H-4), 7.87 (1H, q, J=5.4 Hz, NH), 8.35 (1H, dd, J=7.8, 1.7 Hz, H-6). ^{13}C-NMR: Table I.

Sitakisoside IX (**4**). Colorless needles from MeOH, mp 209—211 °C, $[\alpha]_D^{20}$ −25.1° (c=0.8, MeOH). IR (film) cm^{-1}: 3400, 1680. UV λ_{max}^{EtOH} nm (log ε): 203 (4.15), 222 (4.23), 255 (3.99), 349 (3.54). Positive FAB-MS m/z: 1242 $[M(C_{61}H_{95}NO_{25})+H]^+$, negative FAB-MS m/z: 1240 $[M-H]^-$, 1108 $[M-H-C_5H_8O_4]^-$. *Anal.* Calcd for $C_{61}H_{95}NO_{25}\cdot 3/2H_2O$: C, 57.72; H, 7.78; N, 1.10. Found: C, 57.96; H, 7.97; N, 1.11. ^{1}H-NMR (600 MHz, C_5D_5N) δ: 0.93 (H_3-25), 0.98 (H_3-24), 1.00 (H_3-26), 1.20 (H_3-30), 1.25 (H_3-27), 1.38 (H_3-29), 2.52 (1H, dd, J=11.5, 4.0 Hz, H-18), *ca.* 2.06 (1H, m, H_β-22), 3.54 (1H, dd, J=12.0, 4.5 Hz, H_α-22), 3.68, 4.32 (each 1H, d, J=10.5 Hz, H_2-28), 3.70, 4.35 (each 1H, d, J=10.8 Hz, H_2-23), *ca.* 4.14 (1H, m, H-21), *ca.* 4.24 (1H, m, H-3), *ca.* 4.30, 4.78 (each 1H, br d, J=10.5 Hz, H-6 of Glc-2), *ca.* 4.30, 4.81 (each 1H, br d, J=10.5 Hz, H-6 of Glc-1), 4.62 (1H, dd, J=11.0, 4.5 Hz, H-16), 4.89 (1H, dd, J=10.3, 4.5 Hz, H-6 of Glc-3), 5.24 (1H, m, H-12), 4.93 (1H, d, J=7.4 Hz, H-1 of Xyl), 5.02 (1H, d, J=7.8 Hz, H-1 of Glc-2), 5.08 (1H, d, J=7.6 Hz, H-1 of Glc-1), 5.08 (1H, d, J=7.6 Hz, H-1 of Glc-3), 5.17 (1H, br d, J=10.3 Hz, H-6 of Glc-3). Acyl part: 2.67 (3H, d, J=4.2 Hz, N-CH3), 6.56 (1H, dd, J=8.0, 1.2 Hz, H-3), 6.65 (1H, ddd, J=8.0, 8.0, 1.2 Hz, H-5), 7.32 (1H, ddd, J=8.0, 8.0, 1.2 Hz, H-4), 7.82 (1H, q, J=4.2 Hz, NH), 8.28 (1H, dd, J=8.0, 1.2 Hz, H-6). ^{13}C-NMR: Table I.

Sitakisoside X (**5**). Colorless needles from MeOH, mp 213—215 °C, $[\alpha]_D^{20}$ −16.7° (c=4.8, MeOH). IR (film) cm^{-1}: 3400, 1680. Positive FAB-MS m/z: 915 $[M(C_{47}H_{78}O_{17})+H]^+$, 897 $[M+H-H_2O]^+$, 765 $[M+H-H_2O-C_5H_8O_4]^+$, 603 $[M+H-H_2O-C_5H_8O_4-C_6H_{10}O_5]^+$. *Anal.* Calcd for $C_{47}H_{78}O_{17}\cdot 9/2H_2O$: C, 56.67; H, 8.80. Found: C, 56.51; H, 8.64. ^{1}H-NMR (600 MHz, C_5D_5N) δ: 0.85 (H_3-25), 0.96 (H_3-26), 1.00 (H_3-24), 1.00 (H_3-29), 1.02 (H_3-30), 1.27 (H_3-23), 1.33 (H_3-27), *ca.* 1.75 (1H, m, H_α-15), 2.24 (1H, dd, J=12.2, 10.5 Hz, H_β-15), 2.81 (1H, dd, J=11.5, 4.0 Hz, H-18), 3.32 (1H, dd, J=11.5, 4.3 Hz, H-3), 3.68, 4.42 (each 1H, d, J=10.4 Hz, H_2-28), 4.66 (1H, dd, J=10.5, 4.5 Hz, H-16), 5.23 (1H, m, H-12), 4.87 (1H, d, J=7.5 Hz, H-1 of Glc-1), 4.95 (1H, d, J=7.5 Hz, H-1 of Xyl), 5.05 (1H, d, J=7.8 Hz, H-1 of Glc-2). ^{13}C-NMR: Table I.

Alkaline Hydrolysis of Sitakisoside VI (1) To a solution of **1** (100 mg) in MeOH (1.5 ml) was added dropwise 28% sodium methoxide (0.5 ml), under an N_2 atmosphere. The mixture was stirred for 4 h at room temperature. The reaction mixture was acidified with dilute HCl, and extracted with $CHCl_3$ and then 1-BuOH. From the $CHCl_3$ layer, methyl *N*-methylanthranilate (15 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 $[M]^+$. ^{1}H-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). The 1-BuOH layer was subjected to HPLC (YMC, ODS S-5, 20% CH_3CN) to provide **6** (60 mg). Compound **6**, an amorphous powder, $[\alpha]_D^{20}$ −16.4° (c=5.3, MeOH). IR (film) cm^{-1}: 3400, 3250. Negative FAB-MS m/z: 1091 $[M(C_{53}H_{88}O_{23})-H]^-$, 959 $[M-H-C_5H_8O_4]^-$, 797 $[M-H-C_5H_8O_4-C_6H_{10}O_5]^-$. *Anal.* Calcd for $C_{53}H_{88}O_{23}\cdot 3/2H_2O$: C, 57.75; H, 8.14. Found: C, 57.94; H, 8.37. ^{1}H-NMR (600 MHz, C_5D_5N) δ: 0.84 (H_3-25), 0.95 (H_3-26), 0.99 (H_3-24), 1.20 (H_3-30), 1.26 (H_3-23), 1.26 (H_3-27), 1.36 (H_3-29), 1.68 (1H, dd, J=12.5, 4.3 Hz, H_α-15), *ca.* 2.06 (1H, m, H_β-22), 2.17 (1H, dd, J=12.5, 12.5 Hz, H_β-15), 2.52 (1H, dd, J=11.5, 4.0 Hz, H-18), 3.34 (1H, dd, J=11.5, 4.5 Hz, H-3), 3.53 (1H, dd, J=12.5, 4.5 Hz, H_α-22), 3.73, 4.34 (each 1H, d, J=11.0 Hz, H_2-28), 4.68 (1H, dd, J=12.5, 4.3 Hz, H-16), 5.24 (1H, m, H-12), ca 4.10 (1H, br d, J =9.5 Hz, H-6 of Glc-3), ca 4.30 (2H, br d, J =11.0 Hz, H-6 of Glc-1 and Glc-2), 4.61 (1H, br d, J=9.5 Hz, H-6 of Glc-3), 4.78 (1H, br d, J=11.0 Hz, H-6 of Glc-2), 4.86 (1H, d, J=7.8 Hz, H-1 of Glc-1), 4.90 (1H, br d, J=11.0 Hz, H-6 of Glc-1), 4.94 (1H, d, J =7.3 Hz, H-1 of Xyl), 5.05 (1H, d, J =7.6 Hz, H-1 of Glc-2), 5.08 (1H, d, J=7.6 Hz, H-1 of Glc-3). ^{13}C-NMR: Table I.

Acid Hydrolysis of Prosapogenin I (6) A solution of **6** (150 mg) in 5% H_2SO_4 was heated at 100 °C for 8 h. The reaction mixture was extracted with EtOAc and purified by HPLC (YMC, ODS S-5, 37% CH_3CN) to provide sitakisogenin (**7**, 25 mg). Compound **7**, colorless needles from MeOH, mp 333—335 °C, $[\alpha]_D^{20}$ +57.0° (c=0.9, $CHCl_3$: MeOH = 1 : 1). HR-EI-MS Obsd for $[M(C_{30}H_{50}O_4)-H_2O]^+$ 456.3628, Calcd 456.3604. ^{1}H-NMR (400 MHz, C_5D_5N) δ: 0.93 (H_3-25), 1.04 (H_3-24), 1.06 (H_3-26), 1.25 (H_3-23), 1.29 (H_3-29), 1.29 (H_3-30), 1.35 (H_3-27), 2.13 (1H, dd, J=13.2, 13.2 Hz, H_β-22), 2.64 (1H, dd, J=13.4, 4.1 Hz, H-18), 3.28 (1H, dd, J=13.2, 4.2 Hz, H_α-22), 3.48 (1H, dd, J=8.2, 8.2 Hz, H-3), 3.80, 4.43 (each 1H, d, J=10.6 Hz, H_2-28), 4.18 (1H, dd, J=13.2, 4.2 Hz, H-21), 4.74 (1H, dd, J=11.8, 4.8 Hz, H-16), 5.38 (1H,

m, H-12). For ^{13}C-NMR: Table I. The aqueous layer was neutralized with Amberlite IR-35 and evaporated *in vacuo* to dryness. 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 part gave positive peaks at 8.80 min (D-Xyl, 8.78 min) and 13.40 min (D-Glc; 13.38 min).

Alkaline Hydrolysis of Sitakisoside VI (2) Alkaline hydrolysis of **2** (100 mg) was carried out in the same way as described for **1** to give methyl *N*-methylanthranilate (13 mg) from the $CHCl_3$ layer, and **6** (60 mg) from the 1-BuOH layer.

Identification of Component Sugars of Sitakisoside VIII (3) A solution of compound **3** (3 mg) was examined in the same way as described for **6**. The sugar part gave D-Xyl and D-Glc.

$LiBH_4$ Reduction of Sitakisoside VIII (3) A solution of **3** (50 mg) and $LiBH_4$ (50 mg) in MeOH (5 ml) was stirred for 1h at room temperature and worked up as usual. The reaction mixture (50 mg) was purified by HPLC (YMC, ODS S-5, 37% CH_3CN) to afford **8** (25 mg) and **3** (10 mg).

Acid Hydrolysis of Sitakisoside IX (4) A solution of **4** (100 mg) in 5% H_2SO_4 (10 ml) was heated at 100 °C for 2 h. The reaction mixture was extracted with EtOAc to provide an aglycone (**8**, 25 mg). Compound **8**, colorless needles from MeOH, mp 290—291 °C $[\alpha]_D^{20}$ +53.1° (c=2.4, MeOH) was identified as gymnestrogenin (lit[2] mp 288—289 °C, $[\alpha]_D^{20}$ +53.5° (c=0.71, MeOH)) by comparison of spectral data with literature values.[3] EI-MS m/z 490 $[M]^+$ *Anal.* Calcd for $C_{30}H_{50}O_5 \cdot H_2O$: C, 70.83; H, 10.30. Found: C, 70.49; H, 10.12. ^{1}H-NMR (200 MHz, C_5D_5N) δ: 1.00 (H_3-25), 1.06 (H_3-24), 1.06 (H_3-26), 1.25 (H_3-29), 1.27 (H_3-30), 1.31 (H_3-27), 2.03 (1H, dd, J=14.0, 14.0 Hz, H_α-19), 2.11 (1H, dd, J=13.0, 13.0 Hz, H_β-22), 2.62 (1H, dd, J=14.0, 4.5 Hz, H-18), 3.27 (1H, dd, J=13.0, 4.5 Hz, H_α-22), 3.71, 4.18 (each 1H, d, J=10.2 Hz, H_2-23), 3.78, 4.41 (each 1H, d, J=10.2 Hz, H_2-28), 4.14 (1H, dd, J=13.0, 4.5 Hz, H-21), *ca.* 4.20 (1H, m, H-3), 4.70 (1H, dd, J=10.5, 5.5 Hz, H-16), 5.36 (1H, m, H-12). ^{13}C-NMR: Table I. The detection of the component sugars was carried out in the same way as described for **6**. The sugar part gave D-Xyl and D-Glc.

Acid Hydrolysis of Sitakisoside X (5) A solution of **5** (100 mg) in 5% H_2SO_4 (10 ml) was heated at 100 °C for 2 h. The reaction mixture was extracted with EtOAc to provide an aglycone (**11**, 25 mg). Compound **11**, colorless needles from MeOH, mp 216—218 °C, $[\alpha]_D^{20}$ +38.7° (c=2.5, $CHCl_3$) was identified as longispinogenin (lit.[7] mp 218—220 °C, $[\alpha]_D^{20}$ +51° ($CHCl_3$)) by comparison of spectral data with literature values.[7,8] EI-MS m/z 458 $[M]^+$. HR-EI-MS Obsd for $C_{30}H_{50}O_3$ 458.3755, Calcd 458.3760. ^{1}H-NMR (200 MHz, C_5D_5N) δ: 0.95 (H_3-25), 0.95 (H_3-30), 1.03 (H_3-24), 1.06 (H_3-26), 1.06 (H_3-29), 1.25 (H_3-23), 1.36 (H_3-27), 3.47 (1H, dd, J=8.0, 8.0 Hz, H-3), 3.71, 4.44 (each 1H, d, J=10.3 Hz, H_2-28), 4.68 (1H, dd, J=12.0, 4.5 Hz, H-16), 5.33 (1H, m, H-12). ^{13}C-NMR: Table I. The detection of the component sugars was carried out in the same way as described for **6**. The sugar part gave D-Xyl and D-Glc.

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

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

References

1) Part X: K. Yoshikawa, H. Taninaka, Y. Kann, S. Arihara, *Chem. Pharm. Bull.*, **42**, 1750 (1994).
2) W. Stœcklin, *Helv. Chim. Acta.*, **51**, 1235 (1968).
3) K. Yoshikawa, K. Amimoto, S. Arihara, K. Matsuura, *Chem. Pharm. Bull.*, **37**, 852 (1989).
4) K. Tori, Y. Yoshimura, H. Arita, Y. Tomita, *Tetahedron Lett.*, **1979**, 179.
5) R. Kasai, M. Ogihara, J. Asakawa, K. Mizutani, O. Tanaka, *Tetrahedron*, **35**, 1427 (1979).
6) S. B. Mahato, B. C. Pal, *J. Chem. Soc., Perkin Tans. 1*, **1987**, 629.
7) K. Kazuo, Y. Yoshimura, S. Seo, K. Sakurai, Y. Tomita, H. Ishii, *Tetahedron Lett.*, **1976**, 4163.