Five New Spirostanol Glycosides from the Subterranean Parts of Smilax sieboldii

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FIVE NEW SPIROSTANOL GLYCOSIDES FROM THE SUBTERRANEAN PARTS OF *SMILAX SIEBOLDII*

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ABSTRACT.—Five new steroidal saponins designated as smilaxin A [**1**], smilaxin B [**2**], smilaxin C [**3**], sieboldiin A [**4**], and sieboldiin B [**5**] were elucidated as laxogenin 3-*O*-α-L-arabinopyranosyl(1↦6)-β-D-glucopyranoside [**1**], laxogenin 3-*O*-β-D-glucopyranosyl(1↦4)-[α-L-arabinopyranosyl(1↦6)]-β-D-glucopyranoside [**2**], tigogenin 3-*O*-β-D-glucopyranosyl(1↦4)-[α-L-arabinopyranosyl(1↦6)]-β-D-glucopyranoside [**3**], sieboldogenin 3-*O*-β-D-glucopyranosyl(1↦4)-[α-L-arabinopyranosyl(1↦6)-β-D-glucopyranoside [**4**], and sieboldogenin 3-*O*-α-L-arabinopyranosyl(1↦6)]-β-D-glucopyranoside [**5**], on the basis of chemical and spectral data. The common aglycone of **4** and **5** is a new steroidal sapogenin, sieboldogenin, 5α,25(*S*)-spirostan-6-one-3β,27-diol [**10**].

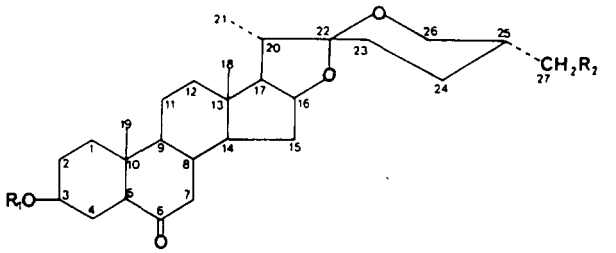
Smilax sieboldii Miq. (Liliaceae) is a climbing shrub which grows in Korea, Japan, and China. Its young leaves are used for food. The subterranean parts have been used in traditional Chinese medicine for arthritis, tumors, and lumbago (1). Previous reports on this plant were the identification of laxogenin together with tigogenin and neotigogenin (2,3). This paper describes the isolation and structural characterization of five new steroidal saponins from the subterranean parts of this plant.

RESULTS AND DISCUSSION

The MeOH extract of the subterranean parts of *S. sieboldii*, on repeated Si gel chromatographic separation, gave five new spirostanol glycosides named smilaxins A [**1**], B [**2**], and C [**3**], and sieboldiins A [**4**] and B [**5**].

Compound **1**, mp 265–266°, gave a positive Liebermann-Burchard test and showed characteristic absorptions of the 25(*R*)-spiroketal moiety in addition to strong absorptions of hydroxyl and carbonyl groups in its ir spectrum. Acid hydrolysis of **1** yielded glucose, arabinose, and an aglycone **6**. Compound **6** was identified as laxogenin on the basis of spectroscopic (ms, ^{1}H nmr and ^{13}C nmr) evidence (4). The fabms of **1** exhibited a cationized molecular ion $[M + Na]^+$ at *m/z* 747 and an ion $[genin + H]^+$ at *m/z* 431, suggesting that **1** was a laxogenin disaccharide. The ^{1}H-nmr spectrum of **1** showed two doublet signals at δ 4.78 (J = 10.7 Hz) and 4.95 (J = 6.6 Hz) ascribable to anomeric protons, suggesting that the anomeric linkages are β for glucose and α for arabinose. Partial hydrolysis of **1** with mild acid afforded prosapogenin **7** and laxogenin. Compound **7** was established as laxogenin 3-*O*-β-D-glucopyranoside by comparison with literature data (4). On comparison of the ^{13}C-nmr spectrum of **1** with that of **7**, significant glycosidation shifts at C-5 (−1.3 ppm) and C-6 (+6.6 ppm) were observed. Furthermore, methanolysis of the permethylether of **1** gave methyl 2,3,4-tri-*O*-methylarabinopyranoside and methyl 2,3,4-tri-*O*-methylglucopyranoside. Accordingly, the structure of **1** was determined to be laxogenin 3-*O*-α-L-arabinopyranosyl(1↦6)-β-D-glucopyranoside.

Compound **2**, mp 270–272°, showed an ir spectrum similar to that of **1**, suggesting it to be a spirostanol derivative. Compound **2** gave glucose, arabinose, and laxogenin



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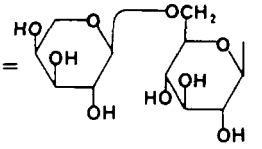

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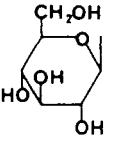

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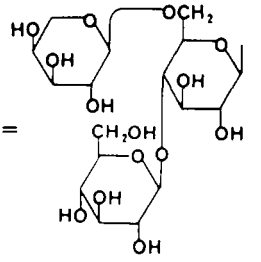

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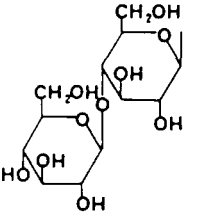
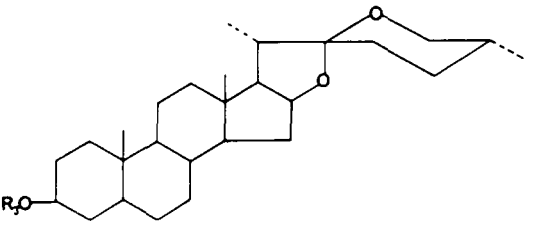


1 $R_1=S_1$, $R_2=H$
2 $R_1=S_2$, $R_2=H$
4 $R_1=S_2$, $R_2=OH$
5 $R_1=S_1$, $R_2=OH$
6 $R_1=R_2=H$

7 $R_1=S_3$, $R_2=H$
8 $R_1=S_4$, $R_2=H$
10 $R_1=H$, $R_2=OH$
11 $R_1=Ac$, $R_2=OAc$



3 $R_3=S_2$ **9** $R_3=H$

[**6**] on acid hydrolysis. The fabms of **2** showed a cationized molecular ion $[M + Na]^+$ at *m/z* 909 and a $[genin + H]^+$ ion at *m/z* 431, indicating that **2** was a triglycoside of laxogenin possessing two moles of glucose and one mole of arabinose. The ^{1}H-nmr spectrum of **2** showed the β configuration for glucose and the α configuration for arabinose. Partial hydrolysis of **2** afforded three prosapogenins and the aglycone, laxogenin. Two of the prosapogenins were identified by direct comparison with authentic samples of **7** and **1**. The third prosapogenin, **8**, yielded glucose and laxogenin on acid hydrolysis. On comparison of the ^{13}C-nmr spectrum of **8** with that of **7**, the C-4 signal of the glucopyranosyl moiety was more deshielded (+9.3 ppm) than that of **7**. Therefore, **8** was laxogenin 3-*O*-β-D-glucopyranosyl(1→4)-β-D-glucopyranoside. Methanolysis of the permethylether of **2** gave methyl 2,3,4-tri-*O*-methylarabinopyranoside, methyl 2,3,4,6-tetra-*O*-methylglucopyranoside, and methyl 2,3-di-*O*-methyl-

glucopyranoside. Consequently, **2** was laxogenin 3-*O*-β-D-glucopyranosyl(1↦4)-[α-L-arabinopyranosyl(1↦6)]-β-D-glucopyranoside.

Compound **3**, mp 279–280°, gave a positive Liebermann-Burchard test and showed 25 (*R*)-spiroketal absorption in its ir spectrum. Acid hydrolysis of **3** afforded glucose, arabinose, and an aglycone, which was identified as tigogenin [**9**] by direct comparison with an authentic sample (5,6). The fabms of **3** showed a pseudomolecular ion $[M + Na]^+$ at *m/z* 895 and a $[genin + H]^+$ ion at *m/z* 417, suggesting that **3** was a tigogenin trioside. The ^1H- and ^{13}C-nmr spectra of the 3-*O*-sugar moiety in **3** were identical with those of **2**. Thus, the structure of **3** was tigogenin 3-*O*-β-D-glucopyranosyl(1↦4)-[α-L-arabinopyranosyl(1↦6)]-β-D-glucopyranoside.

Compound **4**, mp 276–278°, was positive in the Liebermann-Burchard reaction. It showed strong absorption bands due to hydroxyl and carbonyl groups but no spiroketal side chain absorption in its ir spectrum. On acid hydrolysis, **4** gave glucose, arabinose, and an aglycone **10**. The eims of **10** exhibited a molecular ion at *m/z* 446 and a base peak at *m/z* 155, which suggested that **10** was a sapogenin possessing one hydroxyl substituent in ring F (7). The ^{13}C-nmr spectral data of **10** were very close to those of **6** except for chemical shifts of carbons on ring F, indicating the presence of 3β-hydroxyl and 6-keto functionalities. The ^1H-nmr spectrum of **10** showed three methyl signals at δ 0.78 (s), 0.78 (s), and 0.97 (d, $J = 6.2$ Hz), which were assigned to C-18, C-19, and C-21 methyl groups, respectively. However, the C-27 methyl doublet observed in the spectrum of **6** was absent in that of **10**. Acetylation of **10** gave diacetate **11**, which showed a molecular ion at *m/z* 530 and a base peak at *m/z* 197 in the eims. The ^1H-nmr spectrum of **11** showed an eight-line multiplet due to CH_2-27 at δ 3.88 together with a triplet due to H-26α at δ 3.51 ($J = 11.0$ Hz) and a doublet of doublets ascribable to H-26β at δ 3.65 ($J = 11.0$ and 3.2 Hz), suggesting the presence of an equatorial CH_2OH group at C-25 as observed in barbourgenin (8). In the ^1H-nmr spectrum of **10**, signals due to 25(*S*)-CH_2OH overlapped with C-3 and C-26 proton signals in the δ 3.41–3.79 region. Furthermore, comparison of the ^{13}C-nmr spectra of **6** and **10** showed the expected downfield shifts for the C-25 (+7.9 ppm) and C-27 (+47.1 ppm) signals, and upfield shifts for C-24 (−5.6 ppm) and C-26 (−2.1 ppm) due to the γ-gauche effect (9) exerted by the configuration of 25(*S*)-CH_2OH. From the above data, the structure of **10** was determined to be 25(*S*)-spirostan-6-one-3β,27-diol, not reported previously; it has been named sieboldogenin. The fabms of **4** showed a cationized molecular ion $[M + Na]^+$ at *m/z* 925, indicating that **4** was a sieboldogenin trioside. The ^{13}C-nmr signals of the sugar moiety of **4** were superimposable with those of **2** and **3**. Based upon the above data, the structure of **4** was established as sieboldogenin 3-*O*-β-D-glucopyranosyl(1↦4)-[α-L-arabinopyranosyl(1↦6)]-β-D-glucopyranoside.

Compound **5**, mp 266–267°, liberated glucose, arabinose, and sieboldogenin on acid hydrolysis. The fabms of **5** showed a pseudomolecular ion $[M + Na]^+$ at *m/z* 763, suggeting that **5** was a sieboldogenin disaccharide. The ^1H- and ^{13}C-nmr spectra of the 3-*O*-sugar moiety in **5** were identical with those of **1**. Thus, **5** was determined to be sieboldogenin 3-*O*-α-L-arabinopyranosyl(1↦6)-β-D-glucopyranoside.

Matsuura *et al.* (4) previously obtained **1** by chemical degradation of chinenoside 1, but isolation of **1** from a natural source has not been reported previously. The other glycosides, **2–5**, have also not been previously reported.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were taken on a Yanaco apparatus and are uncorrected. Optical rotations were measured on a Rudolph Autopol III automatic polarimeter. Ir spectra were obtained in KBr on a Mattson Polaris TM (Ft-ir) spectrophotometer. Elemental analysis was performed on a Perkin-Elmer 240C instrument. Nmr spectra were obtained on a Bruker AM-300 (300 MHz for ^1H nmr and 75.5 MHz for ^{13}C nmr) spectrometer using TMS as an internal standard and mea-

sured at room temperature. Chemical shifts are given as ppm. Eims and fabms were recorded on a Kratos MS 25 RFA mass spectrometer. Glc was run on a Shimadzu GC-9AM gas chromatograph. Tlc was carried out on precoated Si gel 60 F_{254} sheets (Merck), and detection was achieved by spraying 10% H_2SO_4 followed by heating. Sugars were run on precoated cellulose plates (Merck) and detected by aniline phthalate. Cc was conducted using Merck Si gel.

PLANT MATERIAL.—The subterranean parts of *S. sieboldii* were collected in Kyong Bug province of Korea in the summer of 1989, and authenticated by Prof. Chong Won Kim, Hyosung Women's University, Korea. A voucher specimen is deposited in the College of Pharmacy, Hyosung Women's University.

EXTRACTION AND FRACTIONATION.—The dried chopped subterranean plant parts (3.75 kg) were refluxed with hot MeOH (3 times, 12 h each) and evaporated in vacuo to give a residue (240 g), which was suspended in H_2O and extracted successively with Et_2O and *n*-BuOH. The *n*-BuOH solution was concentrated to give the *n*-BuOH-soluble fraction (90 g).

ISOLATION.—A portion of the *n*-BuOH-soluble fraction was subjected to cc over Si gel eluted with EtOAc saturated with H_2O/MeOH (gradient 0 to 10%) to give six fractions. The third fraction was rechromatographed over Si gel with $CHCl_3$-MeOH-H_2O (7:2:1) (lower layer) to give **1** (1 g) and **5** (0.1 g). The fifth fraction was subjected to repeated cc over Si gel using $CHCl_3$-MeOH-H_2O (7:2:1) (lower layer) to yield **2** (5 g) and **3** (0.5 g). The sixth fraction was rechromatographed over Si gel with $CHCl_3$-MeOH-H_2O (7:2.5:1) (lower layer) to give **4** (0.5 g).

Smilaxin A [**1**].—White needles from MeOH: mp 265–266°; $[\alpha]^{20}D$ −66.7° (c = 0.09, pyridine); ir ν max (KBr) 3438, 1706, 1059, 982, 921, 898, 866 cm^{-1}; fabms *m/z* (rel. int.) $[M+Na]^+$ 747 (13.3), $[genin+H]^+$ 431 (11.4); ^1H nmr see Table 1; ^{13}C nmr see Table 2. *Anal.* calcd for $C_{38}H_{60}O_{13}\cdot H_2O$, C 61.43, H 8.41; found C 61.61, H 8.27.

Smilaxin B [**2**].—White needles from MeOH: mp 270–272°; $[\alpha]^{21}D$ −20.0° (c = 0.13, MeOH); ir ν max (KBr) 3426, 1709, 1050, 984, 920, 899, 866 cm^{-1}; fabms *m/z* (rel. int.) $[M+Na]^+$ 909 (72.5), $[genin+H]^+$ 431 (76.0); ^1H nmr see Table 1; ^{13}C nmr see Table 2. *Anal.* calcd for $C_{44}H_{70}O_{18}\cdot 3H_2O$, C 56.15, H 8.14; found C 56.29, H 8.01.

TABLE 1. Partial ^1H-nmr Spectral Data for **1–5** and Related Compounds.[a]

Proton	Compound								
	1[b]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**6**[c]	**9**[c]	**10**[c]	**11**[c]
Me-18	0.65 s	0.64 s	0.65 s	0.64 s	0.62 s	0.78 s	0.76 s	0.78 s	0.78 s
Me-19	0.78 s	0.78 s	0.80 s	0.79 s	0.76 s	0.80 s	0.82 s	0.78 s	0.79 s
Me-21	1.13 d (7.0)	1.13 d (6.8)	1.12 d (6.9)	1.16 d (6.9)	1.14 d (7.0)	0.97 d (6.8)	0.96 d (6.8)	0.97 d (6.2)	0.98 d (6.8)
Me-27	0.70 d (5.7)	0.70 d (5.6)	0.67 d (5.5)			0.79 d (6.5)	0.79 d (6.2)		
H-3						3.56 m	3.58 m	3.41–3.79[d]	4.67 m
H-16						4.41 m	4.37 q (7.5)	4.43 m	4.42 m
H-26α						3.39 t (12.4)		3.41–3.79[d]	3.51 t (11.0)
H-26β						3.32–3.50 m		3.41–3.79[d]	3.65 dd (11.0, 3.2)
CH_2-27								3.41–3.79[d]	3.38[e]
Anomeric protons	4.78 d (10.7)	4.88 d (7.7)	4.91 d (7.8)	4.83 d (10.9)	4.81 d (10.6)				
	4.95 d (6.6)	5.04 d (7.3)	5.09 d (7.4)	5.08 d (7.9)	5.06 d (7.6)				
		5.42 d (7.5)	5.53 d (7.8)	5.48 d (7.9)					

[a]Data are δ (ppm), multiplicity, and J (in parentheses) in Hz.
[b]In pyridine-d_5.
[c]In $CDCl_3$.
[d]The signals of H-3, CH_2-26, and CH_2-27 were overlapped.
[e]Eight-line multiplet.

TABLE 2. ^{13}C-nmr Chemical Shifts of Compounds **1–10**.[a]

Carbon	Compound									
	1[b]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**6**[c]	**7**[b]	**8**[b]	**9**[c]	**10**[c]
C-1	36.8	36.7	37.1	36.7	36.7	36.7	36.9	36.7	37.0	36.7
C-2	29.6	29.4	29.9	29.4	29.5	30.7	29.5	29.4	31.4	30.7
C-3	77.0	77.0	77.5	76.9	77.0	70.6	77.0	76.9	71.3	70.4
C-4	27.0	26.9	34.8	26.9	27.0	30.1	27.0	27.0	38.2	30.1
C-5	56.5	56.5	44.6	56.4	56.4	56.8[d]	56.7	56.5[d]	44.8	56.8[d]
C-6	209.6	209.5	28.9	209.6	209.7	210.2	209.6	209.5	28.6	210.4
C-7	46.8	46.7	32.3	46.7	46.7	46.8	46.9	46.8	32.2	46.8
C-8	37.4	37.4	35.2	37.3	37.3	37.4	37.5	37.3	35.1	37.3
C-9	53.7	53.7	54.3	53.6	53.6	54.0	54.0	53.7	54.4	53.9
C-10	41.1	41.1	35.8	41.0	41.0	41.0	41.2	41.1	35.6	40.9
C-11	21.6	21.5	21.2	21.5	21.5	21.4	21.6	21.5	21.1	21.4
C-12	39.6	39.6	40.1	39.6	39.6	39.6	39.8	39.6	40.1	39.5
C-13	40.9	40.9	40.7	40.8	40.8	40.9	41.0	40.8	40.6	40.8
C-14	56.5	56.5	56.4	56.4	56.4	56.6[d]	56.7	56.4[d]	56.3	56.5[d]
C-15	31.9	31.8	32.1	31.8	31.8	31.6	31.9	31.8	31.8	31.6
C-16	80.9	80.8	81.0	80.8	80.9	80.5	80.9	80.8	80.8	80.5
C-17	62.9	62.8	62.5	62.8	62.8	62.2	62.9	62.8	62.2	62.1
C-18	16.5	16.5	16.6	16.4	16.4	16.4	16.6	16.5	16.5	16.4
C-19	13.1	13.1	12.3	13.0	13.1	13.2	13.3	13.1	12.3	13.2
C-20	42.0	42.0	41.9	42.0	42.0	41.7	42.0	41.9	41.6	41.7
C-21	15.0	15.0	15.0	14.9	15.0	14.4	14.4	15.0	14.5	14.4
C-22	109.3	109.3	109.2	109.6	109.7	109.3	109.3	109.2	109.2	109.5
C-23	31.8	31.8	31.8	31.5	31.5	31.4	31.4	31.7	31.5	31.6
C-24	29.2	29.2	29.2	24.0	24.0	28.8	28.8	29.2	28.8	23.2
C-25	30.6	30.6	30.6	39.1	39.1	30.3	30.3	30.6	30.3	38.2
C-26	66.9	67.2	67.3	64.4[d]	64.4[d]	66.9	66.9	66.9	66.8	64.8[e]
C-27	17.3	17.3	17.3	64.1[d]	64.1[d]	17.1	17.1	17.3	17.1	64.2[e]
Glc C-1	102.0	102.0	102.0	102.0	102.1		102.2	101.9		
C-2	75.2	74.7[d]	74.7[d]	74.7[e]	75.2		75.1	74.7		
C-3	78.5	76.5	76.5	76.5	78.5		78.3	76.4[e]		
C-4	71.9	81.1	81.1	81.0	71.8		71.9	81.1		
C-5	76.8	75.1	75.2	75.1	76.7		78.1	76.7[e]		
C-6	69.7	68.4	68.3	68.4	69.7		63.2	62.2[f]		
Glc C-1		104.9	104.9	104.9				104.9		
(↦^{4}Glc) C-2		74.8[d]	74.8[d]	74.8[e]				74.7		
C-3		78.4[e]	78.5[e]	78.4[f]				78.4		
C-4		71.8	71.8	71.8				71.4		
C-5		78.1[e]	78.2[e]	78.2[f]				78.1		
C-6		62.6	63.0	62.5				62.4[f]		
Ara C-1	105.4	105.6	105.7	105.6	105.5					
(↦^{6}Glc) C-2	72.3	72.5	72.6	72.5	72.3					
C-3	74.4	74.6	74.6	74.6	74.4					
C-4	69.1	69.7	69.8	69.7	69.1					
C-5	66.4	66.9	66.8	67.2	66.5					

[a]Chemical shifts are reported in ppm from TMS. Assignments were made by comparison with model compounds, literature data (4), and DEPT spectra.

[b]In pyridine-d_5.

[c]In $CDCl_3$.

[d,e,f]Assignments may be reversed in each column.

Smilaxin C [**3**].—White needles from MeOH: mp 279–280°; $[\alpha]^{21}D$ −100.0° (c = 0.1, pyridine); ir ν max (KBr) 3390, 1075, 980, 920, 900, 865 cm^{-1}; fabms *m/z* (rel. int.) $[M+Na]^+$ 895 (8.4), [genin + H]$^+$ 417 (9.9); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2. *Anal.* calcd for $C_{44}H_{70}O_{17}\cdot 2H_2O$, C 58.26, H 8.22; found C 58.30, H 8.37.

Sieboldiin A [**4**].—White plates from MeOH: mp 276–278°; $[\alpha]^{21}D$ −28.3° (c = 0.12, pyridine); ir ν max (KBr) 3407, 1711, 1075 cm^{-1}; fabms *m/z* (rel. int.) $[M+Na]^+$ 925 (1.7), $[genin+H]^+$ 447 (45.3); ^1H nmr see Table 1; ^{13}C nmr see Table 2. *Anal.* calcd for $C_{44}H_{70}O_{19}\cdot H_2O$, C 57.38, H 7.88; found C 57.49, H 7.78.

Sieboldiin B [**5**].—White plates from MeOH: mp 266–267°; $[\alpha]^{21}D$ −40.0° (c = 0.07, pyridine); ir ν max (KBr) 3447, 1710, 1073 cm^{-1}; fabms *m/z* (rel. int.) $[M+Na]^+$ 763 (20.1), $[genin+H]^+$ 447 (18.7); ^1H nmr see Table 1; ^{13}C nmr see Table 2. *Anal.* calcd for $C_{38}H_{60}O_{14}\cdot H_2O$, C 60.14, H 8.24; found C 61.30, H 8.23.

ACID HYDROLYSIS OF **1–5**.—Solutions of each glycoside (30 mg each) in 4 N HCl-dioxane (1:1) (9 ml) were refluxed for 30 min, and each reaction mixture was diluted with ice-H_2O. Precipitates were collected by filtration and purified by recrystallization from MeOH to afford the aglycones.

Laxogenin [**6**], the common aglycone from **1** and **2**, was crystallized from MeOH as white needles: mp 212–213°; $[\alpha]^{25}D$ −80.0° (c = 0.5, $CHCl_3$); ir ν max (KBr) 3480, 1706, 982, 920, 901, 868 cm^{-1}; eims *m/z* (rel. int.) $[M]^+$ 430 (4.1), $[M-H_2O]^+$ 412 (1.2), 358 (13.4), 316 (20.0), 287 (15.9), 139 (100.0), 115 (37.3); ^1H nmr see Table 1; ^{13}C nmr see Table 2. *Anal.* calcd for $C_{27}H_{42}O_4$, C 75.31, H 9.83; found C 75.46, H 9.97. Tigogenin [**9**], the aglycone from **3**, was crystallized from MeOH as white needles: mp 202–203°; ir ν max (KBr) 3480, 982, 920, 901, 868 cm^{-1}; eims *m/z* (rel. int.) $[M]^+$ 416 (44.1), $[M-H_2O]^+$ 398 (3.1), 344 (32.4), 302 (62.5), 287 (35.4), 273 (64.2), 239 (14.9), 139 (100.0); ^1H nmr see Table 1; ^{13}C nmr see Table 2. *Anal.* calcd for $C_{27}H_{44}O_3$, C 77.84, H 10.64; found C 77.76, H 10.61. Sieboldogenin [**10**], the common aglycone from **4** and **5**, was crystallized from MeOH as white plates: mp 209–210°; $[\alpha]^{17}D$ −62.0° (c = 0.1, $CHCl_3$); ir ν max (KBr) 3437, 1710 cm^{-1}; eims *m/z* (rel. int.) $[M]^+$ 446 (9.7), 361 (12.4), 357 (11.5), 316 (21.9), 287 (21.4), 155 (100.0); ^1H nmr see Table 1; ^{13}C nmr see Table 2. *Anal.* calcd for $C_{27}H_{42}O_5$, C 72.61, H 9.48; found C 72.52, H 9.40.

Each filtrate was neutralized with Ag_2CO_3 and filtered. The filtrates were concentrated and examined by tlc. In all hydrolysates, glucose and arabinose were detected as the common sugar components.

ACETYLATION OF SIEBOLDOGENIN [**10**].—Sieboldogenin was treated with Ac_2O in pyridine (1 ml each) at room temperature for 24 h. Workup in the usual manner gave **11**, which was recrystallized from MeOH as white needles: mp 213–214°; $[\alpha]^{17}D$ −77.0° (c = 0.1, $CHCl_3$); eims *m/z* (rel. int.) $[M]^+$ 530 (8.1), 400 (12.3), 358 (26.4), 343 (14.2), 329 (15.3), 298 (52.7), 197 (100.0), 173 (28.9), 128 (99.4); ^1H nmr see Table 1.

PERMETHYLATION OF **1** AND **2**.—Each compound (50 mg each) was permethylated with NaH (100 mg) and MeI (6 ml) by Hakomori's method (10). Each reaction product was chromatographed over Si gel with EtOAc to afford the hexa-*O*-methylether from **1** and the nona-*O*-methylether from **2**.

The hexa-*O*-methylether of **1**: white amorphous from MeOH; mp 82–83°; $[\alpha]^{21}D$ −55.7° (c = 0.07, $CHCl_3$); ^1H nmr ($CDCl_3$) δ 0.69 (3H, s, Me-18), 0.70 (3H, d, J = 4.2 Hz, Me-27), 0.72 (3H, s, Me-19), 0.90 (3H, d, J = 6.6 Hz, Me-21), 3.37, 3.42, 3.43, 3.49, 3.50, 3.52 (3H each, s, 6 Me), 4.03 (1H, d, J = 10.3 Hz, anomeric H), 4.23 (1H, d, J = 5.8 Hz, anomeric H).

The nona-*O*-methylether of **2**: white amorphous from MeOH; mp 81–83°; $[\alpha]^{21}D$ −37.5° (c = 0.08, $CHCl_3$); ^1H nmr ($CDCl_3$) δ 0.69 (3H, s, Me-18), 0.70 (3H, d, J = 4.8 Hz, Me-27), 0.73 (3H, s, Me-19), 0.91 (3H, d, J = 6.6 Hz, Me-21), 3.32, 3.37, 3.41, 3.45, 3.52, 3.53, 3.61 (3H each, s, 7Me), 3.47 (6H, s, 2Me), 4.01 (1H, d, J = 12.8 Hz, anomeric H), 4.23 (1H, d, J = 7.0 Hz, anomeric H), 4.24 (1H, d, J = 7.9 Hz, anomeric H).

METHANOLYSIS OF PERMETHYLETHERS OF **1** AND **2**.—Each permethylether (10 mg each) was refluxed with 2% methanolic HCl (10 ml) for 1 h. Each reaction mixture was concentrated to half volume and added to crushed ice. The resulting precipitates were filtered and recrystallized from MeOH to afford laxogenin [**6**] as the common aglycone. Each filtrate was neutralized with Ag_2CO_3, and the neutral solution was concentrated to dryness. The residues were examined by glc [column 5% DEGS + 1% H_3PO_4 on chromosorb W AW (100–200 mesh), 2.2 mm × 6 ft; column temperature 170°; carrier gas (N_2) 3.35 kg/cm^2], and methylated sugars were identified as methyl 2,3,4-tri-*O*-methylarabinopyranoside (Rt 1.98, 2.69), and methyl 2,3,4-tri-*O*-methylglucopyranoside (Rt 6.62, 9.09) from the hexa-*O*-methylether of **1**, and methyl 2,3,4-tri-*O*-methylarabinopyranoside (Rt 2.01, 2.63), methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (Rt 2.82, 3.70), and methyl 2,3-di-*O*-methylglucopyranoside (Rt 12.31, 14.83) from the nona-*O*-methylether of **2**.

PARTIAL HYDROLYSIS OF **1** AND **2**.—Compound **1** (60 mg) was refluxed with 1% methanolic H_2SO_4 (12 ml) for 10 min. After cooling, the reaction mixture was diluted with ice-H_2O. The precipitate was collected by filtration and dried. The residue (30 mg) was subjected to cc over Si gel with $CHCl_3$-MeOH-H_2O (7:2:0.1) to afford **6** (5 mg) and prosapogenin **7** (20 mg). Compound **7** was recrystallized from MeOH as white needles: mp 232–234°; $[\alpha]^{21}D$ −93.0° (c = 0.14, pyridine); ^1H nmr (pyridine-d_5) δ

0.63 (3H, s, Me-18), 0.72 (3H, d, $J = 5.9$ Hz, Me-27), 0.76 (3H, s, Me-19), 1.08 (3H, d, $J = 6.9$ Hz, Me-21), 4.82 (1H, d, $J = 7.7$ Hz, anomeric H); ^{13}C nmr see Table 2. Compound **2** (300 mg) was hydrolyzed in the same manner as described above. The precipitate was chromatographed to give **6** (5 mg) together with three prosapogenins, **7** (100 mg), **1** (20 mg), and **8** (40 mg) with $CHCl_3$-MeOH-H_2O (7:2:0.1). Compound **8** was recrystallized from MeOH as white needles: mp 254–256°; $[\alpha]^{21}D$ −49.1° ($c = 0.11$, pyridine); ^{1}H nmr (pyridine-d_5) δ 0.63 (3H, s, Me-18), 0.71 (3H, d, $J = 5.2$ Hz, Me-27), 0.79 (3H, s, Me-19), 1.13 (3H, d, $J = 6.8$ Hz, Me-21), 4.94 (1H, d, $J = 7.9$ Hz, anomeric H), 5.16 (1H, d, $J = 7.9$ Hz, anomeric H); ^{13}C nmr see Table 2.

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