[Chem. Pharm. Bull. 31(7)2281—2287(1983)]

Further Studies on Dammarane-Saponins of Sanchi-Ginseng

HIROMICHI MATSUURA,[a] RYOJI KASAI,[b] OSAMU TANAKA,*[,b] YUH-ICHIRO SARUWATARI,[a] TOHRU FUWA,[a] and JUN ZHOU[c]

Central Research Laboratories, Wakunaga Pharmaceutical Co., Ltd.,[a] Shimokotachi 1624, Koda-cho, Takata-gun, Hiroshima 729-64, Japan, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine,[b] Kasumi, Minami-ku, Hiroshima 734, Japan and Kunming Institute of Botany, Academia Sinica,[c] Kunming, Yunnan, China

(Received December 16, 1982)

Three new saponins, named notoginsenosides-R3 (**11**), -R4 (**12**), and -R6 (**13**) were isolated from Sanchi-Ginseng, roots of *Panax notoginseng*. The structures of these saponins were established as 20(*S*)-protopanaxatriol 6-*O*-*β*-D-glucopyranosyl-20-*O*-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranoside (**11**), 20(*S*)-protopanaxadiol 3-[*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl]-20-*O*-*β*-D-xylopyranosyl(1→6)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranoside (**12**), and 20(*S*)-protopanaxatriol 6-*O*-*β*-D-glucopyranosyl-20-*O*-*α*-D-glucopyranosyl(1→6)-*β*-D-glucopyranoside (**13**), respectively. Besides these saponins, two known saponins, 20-*O*-glucoginsenoside-Rf (**9**) and gypenoside XVII (**10**), were also isolated and identified.

From corms of this plant, ginsenosides-Rb_1 (**1**), -Rb_2 (**26**), -Rd (**2**), -Re (**3**), -Rg_1 (**4**), and notoginsenoside-R1 (**5**) were isolated in relatively high yields.

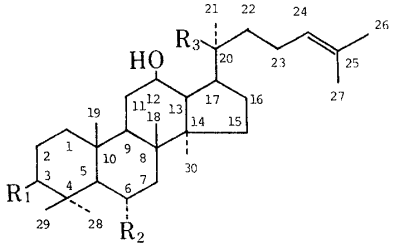
Keywords——Sanchi-Ginseng; *Panax notoginseng*; dammarane-saponin; notoginsenosides-R3, -R4, -R6; 20-*O*-gluco-ginsenoside-Rf; gypenoside XVII; ^{13}C NMR of oligoglycoside; FD-MS; *α*-1,6-glucobioside; corm saponin

Several known Ginseng saponins, ginsenosides-Rb_1 (**1**), -Rd (**2**), -Re (**3**), and -Rg_1 (**4**), have been isolated[1] from a well-known Chinese traditional crude drug, Sanchi-Ginseng (=Tienchi-Ginseng), roots of *Panax notoginseng* (BURK.) F. H. CHEN (Araliaceae, cultivated in Yunnan, China). Recently, we reported the isolation and structure determination of two new dammarane-saponins, notoginsenosides-R1 (**5**) and -R2 (**6**), from this crude drug as well as the identification of two additional known Ginseng saponins, ginsenosides-Rg_2 (**7**) and -Rh_1 (**8**).[2] Saponins of flower-buds,[3] leaves, and seeds[4] of this plant were also investigated. As a continuation of our chemical studies on this drug, the present paper reports the isolation and structure elucidation of a further three new minor saponins and the identification of two known minor saponins. The saponin composition of corms of this plant is also described.

A crude saponin fraction of the methanolic extract of this drug was subjected to chromatography on silica gel, highly porous polymer and octadecyldimethylsilylated (ODS) silica gel columns and finally to preparative high performance liquid chromatography (HPLC) on an ODS silica gel column, affording hitherto unidentified saponins, **9**—**13**, along with saponins reported already. Saponin **9** (yield: 0.005%) was proved to be identical with 20-*O*-gluco-ginsenoside-Rf, a minor saponin of Ginseng roots,[5] and saponin **10** (yield: 0.036%) was identified as gypenoside XVII, which has been isolated from *Gynostemma pentaphyllum* MAKINO by Takemoto *et al.*[6]

New saponins **11** (yield: 0.007%) and **13** (yield: 0.002%) were named notoginsenosides-R3 and -R6. Assignments of ^{13}C nuclear magnetic resonance (NMR) signals of dammarane-sapogenins[7] and -saponins,[2,4,8,9] as well as the glycosylation shifts of a variety of aliphatic

glycosides,[10] have been elaborated. In the ^{13}C NMR spectra of both saponins, carbon signals due to the aglycone moiety appeared at almost the same positions as those of **4**, indicating that both **11** and **13** must be saponins of 20(*S*)-protopanaxatriol (**14**) having sugar units at both the 6- and 20-hydroxyl groups. On mineral acid hydrolysis, **11** and **13** afforded glucose and the anomeric carbon signals of both saponins indicated the presence of three glucosyl units. This conclusion was supported by a cluster ion at *m/z* 985 $(M+Na)^+$ observed in the field desorption mass spectra (FD-MS) of **11** and **13**. It has been reported that the glycosyl linkage at the C-20 hydroxyl group of dammarane-saponins is rather unstable, being readily hydrolyzed even under mildly acidic conditions[11] and in the electron impact mass spectra (EI-MS) of the acetates or trimethylsilyl (TMSi) ethers, M^+ or fragment ions having an *O*-glycosyl group at the C-20 position could not be observed.[12,13] The EI-MS of the acetates of **11** and **13** exhibited a fragment ion at *m/z* 331 (glucosyl $(Ac)_4^+$) and a pair of ions at *m/z* 812 and 813 which appeared in the spectrum of the octaacetate of **4** and were assigned as **15** and **16**, respectively[12] (see Chart 2). Further, the EI-MS of the TMSi ethers of **11** and **13** showed a fragment ion at *m/z* 583 (**17**, Chart 2) which is characteristic of a 1,6-glucobiose unit.[13]



	R_1	R_2	R_3
1:	O-glc$^{2\ 1}$glc	H	O-glc$^{6\ 1}$glc
2:	O-glc$^{2\ 1}$glc	H	O-glc
3:	OH	O-glc$^{2\ 1}$rha	O-glc
4:	OH	O-glc	O-glc
5:	OH	O-glc$^{2\ 1}$xyl	O-glc
6:	OH	O-glc$^{2\ 1}$xyl	OH
7:	OH	O-glc$^{2\ 1}$rha	OH
8:	OH	O-glc	OH
9:	OH	O-glc$^{2\ 1}$glc	O-glc
10:	O-glc	H	O-glc$^{6\ 1}$glc
11:	OH	O-glc	O-glc$^{6\ 1}$glc
12:	O-glc$^{2\ 1}$glc	H	O-glc$^{6\ 1}$glc$^{6\ 1}$xyl
13:	OH	O-glc	O-glc$^{6\ 1}$glc′
14:	OH	OH	OH
19:	OH	H	OH
26:	O-glc$^{2\ 1}$glc	H	O-glc$^{6\ 1}$ara (p)

glc, β-D-glucopyranosyl; glc′, α-D-glucopyranosyl; xyl, β-D-xylopyranosyl; rha, α-L-rhamnopyranosyl; ara (p), α-L-arabinopyranosyl.

Chart 1

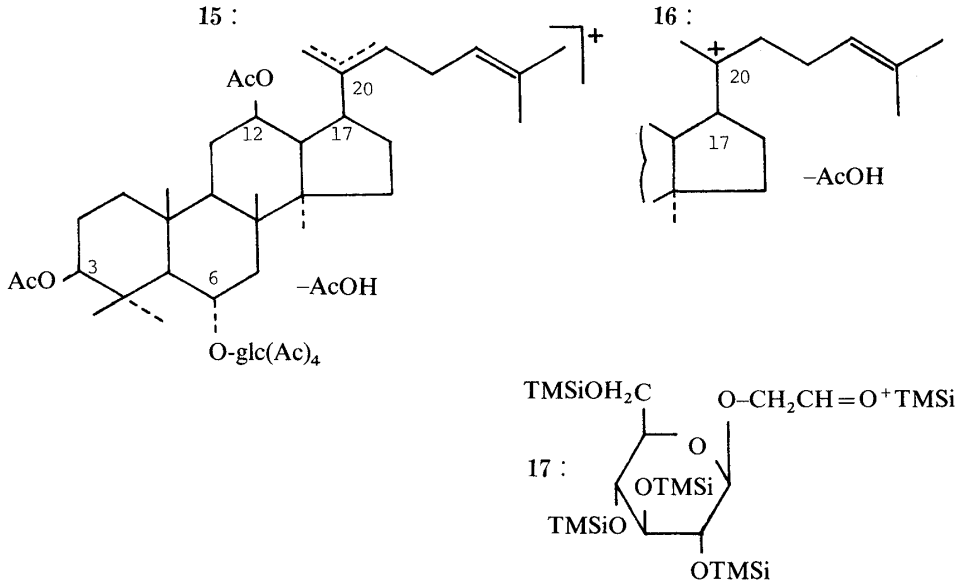


Chart 2

TABLE I. ^{13}C NMR Chemical Shifts: Aglycone Moiety (in C_5D_5N)

	14	**4**	**11**	**13**	**19**	**1**	**12**
C- 1	39.2	39.5	39.5	39.6	39.5	39.1	39.0
C- 2	28.0	27.6	27.7	27.7	28.2	26.6	26.6
C- 3	78.3	78.6	78.0	78.0	77.9	89.3	89.2
C- 4	40.2	40.1	40.1	40.3	39.5	39.6	39.5
C- 5	61.7	61.3	61.2	61.4	56.3	56.3	56.3
C- 6	67.6	77.8	78.9	79.4	18.7	18.6	18.4
C- 7	47.4	44.9	44.9	45.1	35.2	35.1	35.1
C- 8	41.1	41.0	40.9	41.1	40.0	39.9	39.9
C- 9	50.1	49.9	49.7	49.9	50.4	50.1	50.0
C-10	39.3	39.5	39.5	39.6	37.3	36.8	36.7
C-11	31.9	30.8[b]	30.5[b]	30.7	32.0	30.8	30.6
C-12	70.9	70.3	70.0	70.4	70.9	70.1	70.0
C-13	48.1	48.9	48.9	48.9	48.5	49.3	49.2
C-14	51.6	51.3	51.1	51.3	51.6	51.3	51.3
C-15	31.3	30.6[b]	30.9[b]	30.7	31.8	30.8	30.6
C-16	26.8	26.4	26.5	26.6	26.8	26.6	26.6
C-17	54.6	51.6	51.1	51.3	54.7	51.6	51.3
C-18	17.5[a]	17.4[a]	17.6[a]	17.5[a]	16.2[a]	16.2[a]	16.1[a]
C-19	17.4[a]	17.4[a]	17.6[a]	17.5[a]	15.8[a]	15.9[a]	15.9[a]
C-20	72.9	83.3	83.2	83.4	72.9	83.5	83.5
C-21	26.9	22.3	22.2	22.5	26.9	22.6	22.3
C-22	35.7	35.9	35.9	36.0	35.8	36.1	36.1
C-23	22.9	23.2	23.0	23.2	22.9	23.1	22.8
C-24	126.2	125.8	125.7	126.0	126.2	125.8	125.7
C-25	130.6	130.9	130.7	130.9	130.6	131.0	130.9
C-26	25.8	25.7	25.7	25.7	25.8	25.8	25.7
C-27	17.7[a]	17.7[a]	17.6[a]	17.9[a]	17.6[a]	17.9[a]	17.8[a]
C-28	31.9	31.6	31.5	31.7	28.6	28.0	27.9
C-29	16.4[a]	16.2[a]	16.1[a]	16.3[a]	16.4[a]	16.5[a]	16.5[a]
C-30	17.0[a]	17.0[a]	17.4[a]	17.2[a]	17.0[a]	17.3[a]	17.3[a]

a, b) Assignments in any column may be reversed, though those given here are preferred.

TABLE II. ^{13}C NMR Chemical Shifts: Sugar Moiety

	4	11	27[c]	13	1	12
3- (or 6-) glc 1	105.7	105.7		105.8	105.0	105.0
(inner) glc 2	75.3	75.1		75.4[b]	82.9	82.6
glc 3	80.0[a]	79.7[a]		79.9[a]	77.2[a]	77.7[a]
glc 4	71.6[b]	71.5		71.8	71.5	71.4[b]
glc 5	79.3[a]	79.3[a]		79.1[a]	78.0[a]	77.7[a]
glc 6	62.9	62.6		62.7	62.6	62.6
3-glc 1					105.6	105.2
(terminal) glc 2					76.7	76.5
glc 3					78.8[a]	78.6[a]
glc 4					71.5	71.4[b]
glc 5					78.0[a]	77.7[a]
glc 6					62.6	62.6
20-glc 1	98.1	97.9		97.9	97.9	97.8
(inner) glc 2	74.9	74.6		75.2[b]	74.9	74.7
glc 3	78.8[a]	78.0[a]		78.7[a]	78.0[a]	77.7[a]
glc 4	71.3[b]	71.5		71.8	71.5	71.4[b]
glc 5	77.8[a]	76.7		76.1	76.7	76.5
glc 6	62.6	71.5		68.0	71.5	71.2[b]
20-glc 1		105.0	101.3	100.3	105.0	105.0
glc 2		74.6	74.0[a]	73.9	74.9	74.7
glc 3		78.0[a]	75.3	75.2[b]	78.0[a]	77.7[a]
glc 4		71.5	72.1	71.8	71.5	71.4[b]
glc 5		78.0[a]	73.8[a]	73.9	78.0[a]	76.5
glc 6		62.6	62.8	62.7	62.6	69.6
20-xyl 1						105.2
xyl 2						74.7
xyl 3						77.7[a]
xyl 4						71.0
xyl 5						66.7

a, b) Assignments in any column may be reversed, though those given here are preferred.
c) Methyl α-D-glucopyranoside.[10]
glc, β- or α-D-glucopyranosyl; xyl, β-D-xylopyranosyl.

On partial hydrolysis with hot 50% acetic acid, **11** afforded gentiobiose. The carbon signals due to the sugar moiety of **11** were revealed to consist of signals assignable to the 6-*O*-β-D-glucopyranosyl unit of **4** and to the 20-*O*-β-gentiobiosyl unit of **1** (see Table II). It follows that **11** should be formulated as the 6-*O*-β-D-glucopyranosyl-20-*O*-β-gentiobioside of **14**.

On mild hydrolysis with hot 50% acetic acid, **13** yielded a glucobiose which was identified as isomaltose (**18**) by gas chromatography-mass spectrometry (GC-MS) of its TMSi ether in comparison with that of an authentic sample. In the ^{13}C NMR spectrum of **13**, a set of signals assignable to a β-isomaltoside of a tertiary alcohol and signals due to the 6-*O*-β-D-glucopyranosyl unit of **4** were observed at the expected positions. The α-glucopyranosyl linkage was further substantiated by the $J_{C_1-H_1}$ (164 Hz) value of an anomeric carbon signal at δ 100.3, which is characteristic of the 6-α-glucosyl linkage of **18** (Table II). These results led to the formulation of **13** as 20(*S*)-protopanaxatriol 6-*O*-β-D-glucopyranosyl-20-*O*-α-D-glucopyranosyl(1→6)-β-D-glucopyranoside. It is noteworthy that **13** is the first example of the saponin having an α-glucosyl linkage.

The carbon signals of **12** due to the aglycone moiety appeared at almost the same positions as those of **1**, indicating that **12** must be a glycoside of 20 (*S*)-protopanaxadiol (**19**) having glycosyl linkages at both the 3- and 20-hydroxyl groups. Inspection of the anomeric

TABLE III. Comparison of Yields of Saponins from Various Parts of *Panax notoginseng*

	Main roots	Corms	Leaves[4)]	Flower-buds[3)]	Seeds[4)] (%)
20(*S*)-Protopanaxadiol type					
Ginsenoside-Rb_1	1.8	5.2	0.03	0.4	0.01
Ginsenoside-Rb_2	—	0.12	—	0.1	—
Ginsenoside-Rb_3	—		0.71	—	1.20
Ginsenoside-Rc	—		0.39	1.0	0.42
Ginsenoside-Rd	0.20	1.0	—	0.1	0.067
Ginsenoside-F_2	—		—	0.1	—
Gypenoside IX	—		0.03	—	0.014
Gypenoside XVII	0.036		—	—	—
Notoginsenoside-Fa	—		0.01	—	0.087
Notoginsenoside-Fc	—		0.05	—	0.15
Notoginsenoside-Fe	—		0.005	—	—
Notoginsenoside-R4	0.028		—	—	—
20(*S*)-Protopanaxatriol type					
Ginsenoside-Re	0.15	0.63	—	—	—
Ginsenoside-Rg_1	1.9	5.7	—	—	—
Ginsenoside-Rg_2	0.03		—	—	—
Ginsenoside-Rh_1	0.01		—	—	—
Notoginsenoside-R1	0.16	1.1	—	—	—
Notoginsenoside-R2	0.04		—	—	—
Notoginsenoside-R3	0.007		—	—	—
Notoginsenoside-R6	0.002		—	—	—

carbon signals revealed the presence of five monosaccharide units in this saponin. On mineral acid hydrolysis, **12** yielded glucose and xylose and the FD-MS spectrum of **12** exhibited a cluster ion at *m/z* 1263 $(M+Na)^+$ followed by ions due to the stepwise elimination of monosaccharide units, indicating the presence of one xylosyl and four glucosyl units. The EI-MS spectrum of the acetate exhibited fragment ions at *m/z* 259 (xylosyl $(Ac)_3^+$), 547 ((xylosyl-glucosyl) $(Ac)_6^+$), 835 ((xylosyl-glucosyl-glucosyl) $(Ac)_9^+$), 331 (glucosyl $(Ac)_4^+$), and 619 ((glucosyl-glucosyl) $(Ac)_7^+$). The mild hydrolysis of **12** with hot 50% acetic acid gave an oligosaccharide (**20**) and a prosapogenin (**21**), of which the latter afforded methyl 2,3,4,6-tetra-*O*-methyl-glucopyranoside (**22**) and methyl 3,4,6-tri-*O*-methyl-glucopyranoside (**23**) on methylation followed by methanolysis, and showed the same *Rf* value on thin-layer chromatogram (TLC) as the prosapogenin (β-sophoroside of 20-epimeric mixture of protopanaxadiol) previously obtained from **1** by the same treatment.[11)] Permethylation of **20** followed by methanolysis afforded methyl 2,3,4-tri-*O*-methyl-xylopyranoside (**24**) and methyl 2,3,4-tri-*O*-methyl-glucopyranoside (**25**). A comparison of the ^{13}C NMR spectrum of **12** with that of **1** showed that a set of signals assignable to a terminal β-xylopyranosyl unit appeared in the spectrum of **12** and each of the 5- and 6-C signals of four β-D-glucopyranosyl units of **1** were displaced to the positions expected for the glycosylation shifts induced by 6-*O*-β-D-xylosylation, while all other carbon signals remained almost unshifted. On the basis of these results, the structure of **12** can be assigned as 20(*S*)-protopanaxadiol 3-[*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]-20-*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside.

Previously, Otsuka *et al.* reported the presence of saponin in the corm (rhizome part at the head of the root) of *Panax ginseng* C. A. MEYER in relatively large amounts.[14)] The same part of Sanchi-Ginseng collected in Yunnan, China, was extracted with methanol and the

methanolic extract was subjected to repeated chromatography on ODS silica gel, silica gel, and highly porous polymer columns, affording **1**, ginsenoside-Rb_2 (**26**), **2**—**5** in relatively higher yields than from the main roots, as shown in Table III. It is noteworthy that **26** has not been detected in the main roots (Sanchi-Ginseng), and the corms seem to be a promising source of biologically active dammarane-saponins, especially **1** (yield: 5.2%) and **4** (yield: 5.7%).

Experimental

The ^{13}C NMR spectra were taken on a JEOL PFT-100 spectrometer (25.15 MHz) in pyridine-d_5 and chemical shifts are given in the δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. The EI-MS spectra were recorded on JEOL 01-SG-2 and JEOL JMS-DX300 mass spectrometers at 75 and 70 eV, respectively. The FD-MS spectra were taken on a JEOL JMX-DX300 machine with an emitter heating current of 22—28 mA.

Identification of the known saponins was carried out as described in previous papers.[2,9]

Isolation of Saponins——Powdered Sanchi-Ginseng (0.7 kg) was extracted five times with hot MeOH (1 l) for 2 h each. The MeOH extract suspended in H_2O was extracted with 1-BuOH (saturated with H_2O). The combined BuOH layer was concentrated to dryness *in vacuo* and the residue (67 g) was subjected to silica gel column chromatography (gradient elution with $CHCl_3$–MeOH–H_2O (50 : 10 : 1 (homogeneous)→7 : 3 : 0.5 (homogeneous)→13 : 7 : 2 (lower phase)) to give fractions I—IX.

Fr. V (2.8 g) was applied to a column of silanized silica gel (Merck) and eluted with 40% aqueous MeOH and then MeOH. The fraction eluted with 40% aqueous MeOH was subjected to repeated column chromatography, first on silica gel (solvent: $CHCl_3$–MeOH–H_2O = 7 : 3 : 0.5), and then on reversed-phase highly porous polymer (MCI CHP20P, Mitsubishi Chemical Ind. Ltd.) (solvent: 55% aqueous MeOH), affording **9** (yield: 0.005%), **11** (yield: 0.007%), and **13** (yield: 0.002%). The MeOH-eluted fraction was subjected to repeated column chromatography, first on highly porous polymer (solvent: 80% aqueous MeOH), and then on silica gel (solvent: $CHCl_3$–MeOH–H_2O = 7 : 3 : 0.5), giving a mixture of **2** and **10**. This saponin mixture was further purified by preparative HPLC, affording **10** (yield: 0.036%). HPLC conditions: Waters' radial compression separative system; column, Radial Pak A (ODS silica gel, 8 mm × 10 cm); solvent, 80% aqueous MeOH; flow rate, 1.3 ml/min; monitored with a differential refractometer (Waters R-401).

Fr. IX (5.4 g) was chromatographed on a column of highly porous polymer (solvent: 70% aqueous MeOH) to give a saponin mixture. This mixture was subjected to repeated silica gel column chromatography (solvent: $CHCl_3$–MeOH–H_2O = 13 : 7 : 2, lower phase), giving **12** (yield: 0.028%).

Properties of 11—13——Notoginsenoside-R3 (**11**): white powder (reprecipitated from EtOH–EtOAc), $[\alpha]_D^{16}$ +23.7° (c = 0.97, MeOH). *Anal.* Calcd for $C_{48}H_{82}O_{19} \cdot 3\frac{1}{2}H_2O$: C, 56.18; H, 8.74. Found: C, 56.20; H, 8.66. FD-MS (m/z): 985 $(M+Na)^+$, 823 $(M+Na-glucosyl)^+$.

Notoginsenoside-R4 (**12**): White powder (reprecipitated from EtOH–EtOAc), $[\alpha]_D^{16}$ +8.9° (c = 1.0, MeOH). *Anal.* Calcd for $C_{59}H_{100}O_{27} \cdot 2H_2O$: C, 55.47; H, 8.21. Found: C, 55.27; H, 8.00. FD-MS (m/z): 1279 $(M+K)^+$, 1263 $(M+Na)^+$, 1131 $(M+Na-xylosyl)^+$, 1101 $(M+Na-glucosyl)^+$, 939 $(M+Na-glucosyl\text{-}glucosyl)^+$, 807 $(M+Na-glucosyl\text{-}glucosyl\text{-}xylosyl)^+$.

Notoginsenoside-R6 (**13**): White powder (reprecipitated from EtOH–EtOAc), $[\alpha]_D^{17}$ +44.3° (c = 0.50, MeOH). *Anal.* Calcd for $C_{48}H_{82}O_{19} \cdot 4H_2O$: C, 55.69; H, 8.76. Found: C, 55.53; H, 8.47. FD-MS (m/z): 985 $(M+Na)^+$, 823 $(M+Na-glucosyl)^+$. $J_{C_1-H_1}$ values of anomeric carbon signals in the ^{13}C NMR spectrum, δ 97.9 (J = 156.2 Hz), 100.3 (J = 164.1 Hz), 105.8 (J = 152.3 Hz).

Partial Hydrolysis of 11—13——A saponin in 50% aqueous AcOH was heated at 70 °C for 4 h. After dilution with H_2O, the reaction mixture was extracted with 1-BuOH (saturated with H_2O). The BuOH layer was concentrated to dryness *in vacuo* to give a prosapogenin, while the aqueous layer was deionized on Amberlite IR-45 (OH form), giving an oligosaccharide after freeze-drying. The prosapogenin from **12** showed the same *Rf* value on TLC on silica gel (solvent: $CHCl_3$–EtOAc–MeOH–H_2O = 2 : 4 : 2 : 1, lower phase) as the prosapogenin of **1** (ginsenoside-Rg_3).

GC-MS Analysis of TMSi–Sugars——The sugars obtained from **11** and **13** by partial hydrolysis, as well as authentic gentiobiose (Nakarai Chemical Ltd.) and isomaltose (Tokyo Chemical Industry Co. Ltd.) were trimethylsilylated according to the previous paper[2] and the resulting TMSi–sugars were subjected to GC-MS. GC-MS conditions: 2% OV-1 on Chromosorb W (80—100 mesh); glass column, 2 mm × 1 m; column temperature, 240 °C; injection temperature, 260 °C; carrier gas, He (40 ml/min); accelerating voltage, 3 kV. Gentiobiose–TMSi: t_R (min), 17.8; m/z 583 (glucosyl $(TMSi)_4$–O–CH_2–CH=O$^+$TMSi), 569 (glucosyl $(TMSi)_4$–O$^+$=CH–O–TMSi), 451 (glucosyl $(TMSi)_4^+$). Isomaltose–TMSi; t_R (min), 11.5, 14.5, 17.6; giving the mass spectrum as same as gentiobiose–TMSi.

Permethylation followed by Methanolysis of 20 and 21——According to Hakomori's method,[15] **20** and **21** were methylated with NaH and DMSO, and CH_3I, respectively. Each reaction mixture was poured into H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O and dried on Na_2SO_4. The $CHCl_3$ solution was concentrated to

dryness *in vacuo* and the residue was subjected to column chromatography on silica gel (solvent: $CHCl_3$–MeOH = 70 : 1). A solution of the resulting permethylated oligosaccharide (**20**) or prosapogenin (**21**) in 5% methanolic HCl was refluxed for 4 h. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was concentrated to dryness, and the resulting hydrolysate was subjected to GC on a glass column (4 mm × 2 m) packed with 5% NPGS on Chromosorb WAW, column temperature, 170 °C; carrier gas, N_2 (1.0 kg/cm^2). t_R (min): **22** (5.6, 7.8), **23** (14.6, 16.8), **24** (2.6, 3.0), **25** (13.2, 16.8).

Isolation of Saponins from Corms——The corms (48 g) were extracted with hot MeOH. The MeOH extract (19.0 g) was applied to a column of silanized silica gel and eluted with 10% aqueous MeOH and then MeOH. The fraction eluted with MeOH was chromatographed on silica gel (solvent: $CHCl_3$–MeOH–H_2O = 7 : 3 : 0.5) to give six fractions (Fr.), I—VI.

Fr. II was purified by chromatography on highly porous polymer (solvent: 70% aqueous MeOH), affording **4** (yield: 5.7%).

Fr. III was applied to a column of silanized silica gel and eluted with 40% aqueous MeOH and then MeOH. The fraction eluted with 40% aqueous MeOH was chromatographed on highly porous polymer (solvent: 55% aqueous MeOH) to give **3** (yiled: 0.63%) and **5** (yield: 1.1%). The fraction eluted with MeOH was further purified by reversed-phase chromatography on ODS silica gel (solvent: 70% aqueous MeOH), affording **2** (yield: 1.0%).

Fr. IV was subjected to silica gel column chromatography (solvent: 1-BuOH–EtOAc–H_2O = 4 : 1 : 2, upper phase), giving **26** (yield: 0.12%).

Fr. V was further purified by chromatography on highly porous polymer (solvent: 80% aqueous MeOH) to give **1** (yield: 5.2%).

Acknowledgement The authors are grateful to Prof. Cheng-yih Wu, Director of Kunming Institute of Botany, Academia Sinica, for his encouragement and valuable advice.

References

1) S. Sanada and J. Shoji, *Jpn. J. Pharmacol.*, **32**, 96 (1978); M.-Z. Wu, *Acta Botanica Yunnanica*, **1**, 119 (1979); J. X. Wei, J. F. Wang, L. Y. Chang, and Y. C. Du, *Acta Pharmaceutica Sinica*, **15**, 359 (1980).
2) J. Zhou, M.-Z. Wu, S. Taniyasu, H. Besso, O. Tanaka, Y. Saruwatari, and T. Fuwa, *Chem. Pharm. Bull.*, **29**, 2844 (1981).
3) S. Taniyasu, O. Tanaka, T.-R. Yang, and J. Zohu, *Planta Medica*, **44**, 124 (1982).
4) T.-R. Yang, R. Kasai, J. Zhou, and O. Tanaka, *Phytochemistry*, "in press."
5) S. Sanada and J. Shoji, *Chem. Pharm. Bull.*, **26**, 1694 (1978).
6) T. Takemoto, S. Arihara, K. Yoshikawa, T. Nakajima, and M. Okuhira, The 24th Symposium on the Chemistry of Natural Products, Osaka, Oct. 1981.
7) J. Asakawa, R. Kasai, K. Yamasaki, and O. Tanaka, *Tetrahedron*, **33**, 1935 (1977).
8) S. Yahara, O. Tanaka, and I. Nishioka, *Chem. Pharm. Bull.*, **26**, 3010 (1978); O. Tanaka and S. Yahara, *Phytochemistry*, **17**, 1353 (1978).
9) H. Besso, R. Kasai, Y. Saruwatari, T. Fuwa, and O. Tanaka, *Chem. Pharm. Bull.*, **30**, 2380 (1982); H. Besso, R. Kasai, J. X. Wei, J. F. Wang, Y. Saruwatari, T. Fuwa, and O. Tanaka, *ibid.*, **30**, 4534 (1982); R. Kasai, H. Besso, O. Tanaka, Y. Saruwatari, and T. Fuwa, *ibid.*, **31**, 2120 (1983).
10) R. Kasai, M. Suzuo, J. Asakawa, and O. Tanaka, *Tetrahedron Lett.*, **1977**, 175; S. Seo, Y. Tomita, K. Tori, and Y. Yoshimura, *J. Am. Chem. Soc.*, **100**, 3331 (1978); R. Kasai, M. Okihara, J. Asakawa, K. Mizutani, and O. Tanaka, *Tetrahedron*, **35**, 1427 (1979); K. Mizutani, R. Kasai, and O. Tanaka, *Carbohydr. Res.*, **87**, 19 (1980).
11) S. Shibata, T. Ando, and O. Tanaka, *Chem. Pharm. Bull.*, **14**, 1157 (1966).
12) T. Komori, O. Tanaka, and Y. Nagai, *Organic Mass Spectrometry*, **9**, 744 (1974).
13) R. Kasai, K. Matsuura, O. Tanaka, S. Sanada, and J. Shoji, *Chem. Pharm. Bull.*, **25**, 3277 (1977).
14) H. Otsuka, Y. Morita, Y. Ogiwara, and S. Shibata, *Planta Medica*, **32**, 9 (1977).
15) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).