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Studies on the Saponins of Ginseng. IV.[1] On the Structure and Enzymatic Hydrolysis of Ginsenoside-Ra_1[2]

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The presence of ginsenoside-Ra in Ginseng Radix has been demonstrated by thin-layer chromatography. Recently, ginsenoside-Ra was shown to be a mixture of more than two saponins. Among them, ginsenoside-Ra_1 was isolated and its structure was determined on the basis of spectral, chemical and enzymatic hydrolysis evidence as 20(*S*)-protopanaxadiol 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosido-20-*O*-*β*-D-xylopyranosyl(1→4)-*α*-L-arabinopyranosyl(1→6)-*β*-D-glucopyranoside (**1**). Besides compound K (**8**), three prosapogenins, 20(*S*)-protopanaxadiol 3-*O*-*β*-D-glucopyranosido-20-*O*-*β*-D-glucopyranoside (**7**), 20(*S*)-protopanaxadiol 3-*O*-*β*-D-glucopyranosido-20-*O*-*β*-D-xylopyranosyl(1→4)-*α*-L-arabinopyranosyl(1→6)-*β*-D-glucopyranoside (**9**), and 20(*S*)-protopanaxadiol 20-*O*-*β*-D-xylopyranosyl(1→4)-*α*-L-arabinopyranosyl(1→6)-*β*-D-glucopyranoside (**10**) were obtained in the course of enzymatic hydrolysis of ginsenoside-Ra_1.

Further, 20(*S*)-protopanaxadiol 3-*O*-*β*-D-glucopyranosido-20-*O*-*α*-L-arabinopyranosyl(1→6)-*β*-D-glucopyranoside (**5**), 20(*S*)-protopanaxadiol 20-*O*-*α*-L-arabinopyranosyl(1→6)-*β*-D-glucopyranoside (**6**), **7** and **8** were obtained by enzymatic hydrolysis of ginsenoside-Rb_2 (**4**). The course of enzymatic hydrolysis of ginsenosides with naringinase is also discussed.

Keywords——Ginseng root; Araliaceae; dammarane-type saponin; bisdesmoside; ginsenoside-Ra_1; 20(*S*)-protopanaxadiol; ^{13}C-NMR; naringinase

As we reported in the previous paper,[1,3] the structures of eleven saponins of Ginseng Radix (root of *Panax ginseng* C.A. MEYER; Araliaceae), ginsenoside-Ro, -Rb_1, -Rb_2, -Rb_3, -Rc, -Rd, -Re, -Rf, Rg_1[4] and -Rg_2, and 20-glucoginsenoside-Rf, have been established. The presence of ginsenoside-Ra in ginseng extract has been demonstrated by thin-layer chromatography (TLC),[5] but because of its low content and high polarity it was difficult to isolate in a pure state. The present paper deals with the structure determinations of ginsenoside-Ra_1 and new prosapogenins obtained by enzymatic hydrolysis of ginsenoside-Ra_1 and -Rb_2.

The crude saponin fraction from the methanolic extract of Ginseng Radix was worked up exactly according to the previous paper[3a] and the ginsenoside-Ra_1-containing fraction (Fr. 1 and Fr. 2 in the previous paper) was repeatedly purified by column chromatography on silica gel using chloroform–methanol–water and *n*-butanol saturated with water, followed by column chromatography on Sephadex LH-20 using methanol as a solvent to afford pure ginsenoside-Ra_1 (**1**) as a white powder (yield 0.01%).

The ^{13}C-nuclear magnetic resonance (NMR) spectrum of **1** showed 58 carbon signals. Among them, 30 signals were assigned to the aglycone, 20(*S*)-protopanaxadiol, by comparison with the ^{13}C-NMR spectra of ginsenoside-Rb_1, -Rb_2, -Rb_3 and -Rc, and the remaining 28 signals were attributable to the five sugar moieties, whose anomeric carbon signals appeared at δ 98.1, 104.9, 104.9, 105.9 and 106.7 ppm.

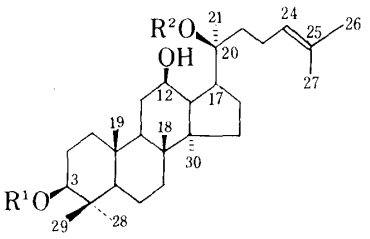
It is known that the 20-*O*-glycosyl moiety of ginsenoside is readily hydrolyzed by treatment with aqueous acetic acid. On partial hydrolysis with 50% acetic acid under heating for 4 h, **1** gave a prosapogenin (**2**) which was identified by direct comparison as protopanaxadiol 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranoside reported by Shibata *et al.*,[6] and an oligosaccharide (**3**), which gave glucose, arabinose and xylose on hydrolysis with 2 N hydrogen chloride. Based on the results described above, **1** was suggested to be a bisdesmoside of 20(*S*)-protopanaxadiol having two moles of glucose at the C-3 hydroxyl group in a sophorosyl type structure

an oligosaccharide consisting of one mol each of glucose, arabinose and xylose at the C-20 hydroxyl group.

The sugar sequence at the C-20 hydroxyl group of the aglycone was elucidated as follows.

The field desorption (FD) mass spectrum of **1** shows the molecular ion peak at *m/z* 1233 (M^++Na) and fragment peaks at *m/z* 1101 (M^+ +Na−pentose), 1071 (M^+ +Na−hexose), 969 (M^+ +Na−2 pentose), 909 (M^+ +Na−2 hexose), 807 (M^+ +Na−(2 pentose+hexose)), 789 (807−H_2O) and 628 (M^+ +Na−(2 pentose+2 hexose)) corresponding to the ions formed by stepwise elimination of pentosyl and hexosyl units from the molecular ion. The fragment pattern in the FD-mass spectrum of **1** suggests that the sequence of three monosaccharides of the triose at the C-20 hydroxyl group of the aglycone may be pentosyl→pentosyl→hexose or $\begin{matrix}\text{pentosyl}\searrow\\ \qquad\qquad\text{hexose.}\\ \text{pentosyl}\nearrow\end{matrix}$

To confirm the sugar sequence at the C-20 hydroxyl group, the enzymatic partial hydrolysis of ginsenoside-Rb_2 (**4**), which was assumed to be one of the prosapogenins of **1**, with commercial naringinase was examined as a model experiment. Kohda *et al.*[7] reported on the enzymatic hydrolysis of ginsenoside-Rb_1, -Rb_2 and -Rc with crude hesperidinase, naringinase, cellulase, amylase and emulsin. Among them, crude hesperidinase, naringinase and pectinase gave a minor genuine aglycone, 20(*S*)-protopanaxadiol, while the major product was a prosapogenin, compound K (**8**), which was obtained by Yoshioka *et al.*[8] by incubation of crude ginsenoside with soil bacteria. Our reinvestigation of the enzymatic hydrolysis of ginsenosides successfully yielded intermediate hydrolysis products, and this method was applied to the structure elucidation of **1**. On stepwise hydrolysis with commercial naringinase, **4** afforded four prosapogenins, namely 20(*S*)-protopanaxadiol 3-*O*-*β*-D-glucopyranosido-20-*O*-*α*-L-arabinopyranosyl(1→6)-*β*-D-glucopyranoside (**5**), 20(*S*)-protopanaxadiol 20-*O*-*α*-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranoside (**6**), 20(*S*)-protopanaxadiol 3-*O*-*β*-D-glucopyranosido-20-*O*-*β*-D-glucopyranoside(=ginsenoside-F_2,[9] **7**) and 20(*S*)-protopanaxadiol 20-*O*-*β*-D-glucopyranoside (=compound K, **8**). The formation of these prosapogenins from **4** revealed that the hydrolysis with naringinase began with attack on the terminal glucose of sophorose at the C-3 hydroxyl group and the reaction proceeded *via* attack on the C-3 glucose moiety or terminal arabinose moiety of an oligosaccharide at the C-20 hydroxyl group. Next, hydrolysis of **1** with commercial naringinase was carried out and the formation of prosapogenins **6**, **7** and **8** described above was detected on a TLC plate besides two new prosapogenins (**9** and **10**). The prosapogenins **9** and **10** were isolated and comparative studies on **9** and **10** with **1**, **4**, **5** and **6** by ^{13}C-NMR were carried out to establish the structure of the oligosaccharide moiety at C-20. According to reports on the assignment of carbon signals of ginseng sapogenins, saponins and other glycosides,[10] each carbon signal of **1**, **4**, **5**, **6**, **9**, and **10** could be assigned as shown in Table I. Based on the ^{13}C-NMR studies the structure of **1** was suggested to be 20(*S*)-protopanaxadiol 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosido-20-*O*-*β*-D-xylopyranosyl (1→4)-*α*-L-arabinopyranosyl(1→6)-*β*-D-glucopyranoside, and this was supported by



1: R^1=Glc(2→1β)Glc(1→, R^2=Xyl(1→4β)Ara(1→6α)Glc(1→β)

4: R^1=Glc(2→1β)Glc(1→, R^2=Ara(1→6α)Glc(1→β)

5: R^1=Glc(1→β), R^2=Ara(1→6α)Glc(1→β)

6: R^1=H- R^2=Ara(1→6α)Glc(1→β)

7: R^1=Glc(1→β), R^2=Glc(1→β)

8: R^1=H-, R^2=Glc(1→β)

9: R^1=Glc(1→β), R^2=Xyl(1→4β)Ara(1→6α)Glc(1→β)

10: R^1=H-, R^2=Xyl(1→4β)Ara(1→6α)Glc(1→β)

Chart 1

the fact that the ^{13}C-NMR spectrum of the sugar portion of **1** is superimposable with that of chikusetsusaponin-L_5 (**11**) isolated from leaves of *Panax japonicus* C.A. MEYER by Yahara *et al.*[10b]

In order to establish the structure of the sugar moiety at the C-20 hydroxyl group, **1** was methylated by Hakomori's method and the resulting methylate was treated with 50% acetic acid in methanol to afford an *O*-methylated prosapogenin and an *O*-methylated oligosaccharide. The latter was methanolyzed with 2 N hydrogen chloride in methanol, and methyl 2,3,4-tri-*O*-methyl-D-glucopyranoside, methyl 2,3-di-*O*-methyl-L-arabinopyranoside and methyl 2,3,4-tri-*O*-methyl-D-xylopyranoside were identified by TLC and gas liquid chromatography (GLC). Based on the $J_{C_1-H_1}$ values of the anomeric carbons and the coupling constants of anomeric protons, the configurations of the monosaccharides of **1** were assigned.

Consequently, the structure of **1** was established to be 20(*S*)-protopanaxadiol 3-*O*-β-D-glucopyranosyl (1→2)-β-D-glucopyranosido-20-*O*-β-D-xylopyranosyl (1→4)-α-L-arabinopyranosyl(1→6)-β-D-glucopyranoside as shown in Chart 1.

Recently, Besso *et al.*[11] reported the isolation of ginsenoside-Ra_1 and -Ra_2 from ginsenoside-Ra fraction and the structure of ginsenoside-Ra_1 independently proposed by his group is identical with that presented here.

Investigations on the biological activities of ginsenoside-Ra_1 and several prosapogenins are in progress.

TABLE I. ^{13}C-NMR Chemical Shifts of Ginsenoside-Ra_1 -Rb_2 and Related Compounds

	Compounds	**1**	**9**	**10**	**4**	**5**	**6**	**7**	**8**	Chikusetsu-saponin-L_5
Aglycone No.	1	39.3	39.6	39.4	39.1	39.2	39.5	39.2	39.4	
	2	26.8	26.6	28.1	26.6	26.7	28.1	26.7	28.2	
	3	89.1	88.8	78.1	89.0	88.8	78.1	88.8	78.1	
	4	39.7	39.6	39.4	39.5	39.7	39.5	39.7	39.5	
	5	56.5	56.4	56.3	56.3	56.4	56.3	56.4	56.4	
	6	18.5	18.5	18.7	18.3	18.6	18.7	18.5	18.8	
	7	35.3	35.1	35.1	35.0	35.2	35.2	35.1	35.2	
	8	40.2	40.0	40.0	39.9	40.1	40.0	40.0	40.1	
	9	50.3	50.2	50.2	50.0	50.2	50.3	50.2	50.3	
	10	37.0	36.9	37.3	36.7	37.0	37.4	36.9	37.4	
	11	30.8	30.7	30.7	30.4	30.8	30.7	30.8	30.8	
	12	70.2	70.1	70.1	70.1	70.2	70.2	70.3	70.2	
	13	49.6	49.4	49.4	49.3	49.5	49.4	49.4	49.5	
	14	51.5	51.3	51.3	51.3	51.4	51.4	51.5	51.4	
	15	30.8	30.7	30.7	30.4	30.7	30.7	30.8	30.8	
	16	26.8	26.6	26.6	26.6	26.7	26.6	26.7	26.7	
	17	51.5	51.3	51.3	51.5	51.7	51.6	51.7	51.7	
	18	16.3	16.2	16.3	16.1	16.3	16.3	16.3	16.3	
	19	16.1	16.0	16.0	15.9	16.0	16.0	16.0	16.0	
	20	83.5	83.4	83.5	83.4	83.5	83.5	83.3	83.3	
	21	22.3	22.2	22.1	22.1	22.4	22.2	22.5	22.4	
	22	36.3	36.1	36.2	36.0	36.2	36.2	36.1	36.1	
	23	23.2	23.1	23.0	23.0	23.2	23.1	23.3	23.3	
	24	126.0	125.8	125.8	125.7	125.9	125.8	125.9	125.9	
	25	131.1	131.0	131.0	131.0	131.1	131.0	130.9	130.9	
	26	25.8	25.7	25.7	25.7	25.8	25.8	25.8	25.8	
	27	17.9	17.8	17.8	17.7	17.9	17.9	17.8	17.8	
	28	28.2	28.1	28.6	27.9	28.2	28.7	28.2	28.7	
	29	16.6	16.7	16.3	16.4	16.8	16.3	16.8	16.3	
	30	17.5	17.4	17.4	17.3	17.4	17.4	17.4	17.4	
C-20 Sugars										
Glucose	1	98.1	97.8	97.9	97.9	98.1	98.0	98.2	98.2	97.9
	2	74.9	74.7	74.7	74.7	74.9	74.9	75.1	75.1	74.6

	Compounds	1	9	10	4	5	6	7	8	Chikusetsu-saponin-L_5
	3	79.1	79.0	79.0	78.9	79.1	79.1	79.1	79.2	79.1
	4	71.8	71.6	71.6	71.4	71.8	71.7	71.5	71.6	71.7
	5	77.0	76.6	76.7	76.4	76.7	76.6	78.3	78.2	76.6
	6	69.9	69.6	69.6	70.1	69.2	69.1	62.8	62.8	69.7
Arabinose	1	104.9	104.8	104.8	104.4	104.6	104.5			104.8
	2	72.9	72.6	72.6	71.9	72.1	72.1			72.7
	3	73.8	73.7	73.7	73.8	74.1	74.0			73.7
	4	78.3	78.5	78.5	68.4	68.6	68.5			78.4
	5	65.6	65.6	65.5	65.4	65.5	65.5			65.5
Xylose	1	106.7	106.6	106.7						106.7
	2	75.3	75.2	75.2						75.2
	3	78.0	78.1	78.1						78.4
	4	71.0	70.8	70.8						70.8
	5	67.2	67.1	67.1						67.2
C-3 Sugars										
Glucose	1	104.9	106.6		104.9	106.9		106.9		
	2	83.5	75.6		82.8	75.8		75.7		
	3	78.0	78.1		77.7	78.3		78.3		
	4	71.8	71.8		71.9	71.9		71.9		
	5	78.0	78.5		78.0	78.7		78.7		
	6	62.9	62.9		62.5	63.1		63.1		
Glucose	1	105.9			105.5					
	2	76.7			76.7					
	3	78.3			78.0					
	4	71.8			71.4					
	5	78.0			77.7					
	6	62.9			62.5					

Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus (hot-stage type) and are uncorrected. The IR spectra were recorded with a JASCO IRA-2 unit and the ^{1}H-NMR (100 MHz) and the ^{13}C-NMR spectra were recorded with a JEOL model FX-100 spectrometer with tetramethylsilane as an internal standard. The optical rotations were measured with a Yanagimoto model OR-50 automatic polarimeter and FD mass spectra were recorded with a JEOL JMS-01SG-2 machine. GLC was run on a Shimadzu GC-6A unit with a flame ionization detector. TLC was performed on Kieselgel G-60 F_{254} (Merck) using solvent A, $CHCl_3$–MeOH–H_2O (7: 3: 0.4 v/v); solvent B, $CHCl_3$–MeOH–AcOEt–H_2O (2: 2: 4: 1 v/v, lower phase); or solvent C, BuOH saturated with H_2O. Detection was achieved by spraying 10% H_2SO_4 followed by heating.

Isolation of 1——According to the previous paper,[3a] a crude saponin fraction was obtained as a white powder from the butanol-soluble fraction of the methanolic extract of Ginseng Radix (root of *Panax ginseng* C.A. Meyer, Araliaceae). The crude saponin fraction was subjected to column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (65: 35: 10 v/v, lower phase), to obtain five fractions (Fr. 1—Fr. 5). Each fraction was examined by TLC and Fr. 2 (ginsenoside-Rb_1-rich fraction) was repeatedly subjected to column chromatography on silica gel with solvent A. The ginsenoside-Ra_1-rich fraction obtained was purified by repeated column chromatography on silica gel using solvent C. On the other hand, Fr. 1 (ginsenoside-Ro-rich fraction) was methylated with CH_2N_2 and the reaction mixture was repeatedly purified in the same way as in the case of Fr. 2. Crude ginsenoside-Ra_1 was purified by column chromatography on Sephadex LH-20 using MeOH, and finally **1** was obtained as a white powder from BuOH saturated with water (yield 0.01%).

Properties of 1——A white powder from BuOH saturated with H_2O, (mp 202—206°C), $[\alpha]_D^{25}$ +14.0° (c=1.00, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3360 (OH), FD MS (emitter current, 16—18 mA; accelerating voltage, 5 kV; cathode voltage, −7 kV; sample temp., 150—180°C) m/z: 1233 (M^++Na, $C_{58}H_{98}O_{26}$=1210), 1101 (M^++Na−pentose), 1071 (M^++Na−hexose), 969 (M^++Na−2 pentose), 909 (M^++Na−2 hexose), 807 (M^++Na−2 pentose−hexose), 789 (m/z 807−H_2O), 628 (m/z 807−hexose), 467 (2 pentose+hexose+Na). $J_{C_1-H_1}$ values of anomeric carbon signals in the ^{13}C-NMR (in pyridine) δ: 98.1 (J=159.3 Hz, glucose), 104.9 (J=158.1 Hz, arabinose), 104.9 (J=158.1 Hz, glucose), 105.9 (J=157.7 Hz, glucose), 106.7 (J=163.0 Hz, xylose).

Acetylation of 1——A solution of **1** (25 mg) in pyridine (1.5 ml) and Ac_2O (1.5 ml) was heated at 75°C for 3 h and the reaction mixture was poured into ice-water. The precipitate was collected by filtration,

washed with water and dried. The product was purified by column chromatography on silica gel with benzene–acetone (5: 1 v/v) to afford an acetate of **1** (9 mg) as a colorless crystalline powder from EtOH, mp 138—139°C. *Anal.* Calcd for $C_{90}H_{130}O_{42}$: C, 57.37; H, 6.96. Found: C, 57.35; H, 7.12.

Partial Hydrolysis of 1 with 50% AcOH——A solution of **1** (50 mg) in 50% AcOH (5 ml) was heated for 4 h on a water bath. The precipitate was filtered off, washed with water and dried. The product was crystallized from MeOH to give a prosapogenin (**2**) as colorless needles (25 mg), mp 260—264°C (lit.[6] 262—264°C). This product was shown to be identical with an authentic sample by comparing TLC behavior (*Rf* 0.44 (solvent A); *Rf* 0.46 (solvent C)) and by mixed fusion.

The filtrate was concentrated under reduced pressure and the residue was subjected to column chromatography on Sephadex LH-20 with MeOH to afford a prosapogenin (**2**) and an oligosaccharide (**3**, 15 mg), which was crystallized from MeOH to give colorless prisms, mp 196—198°C.

Hydrolysis of 3 with 2 N HCl——A solution of **3** (5 mg) in 2 N HCl–60% dioxane (1 ml) was refluxed for 3 h and the reaction mixture was diluted with water. The aqueous solution was neutralized with Amberlite IR-45 and evaporated to dryness *in vacuo*. The residue was examined by TLC and GLC. TLC (plate, DC-Fertigplatten Cellulose F; solvent, BuOH–AcOH–H_2O (4: 1: 5 v/v, upper phase); detection, aniline hydrogen phthalate): *Rf* 0.25 (glucose), 0.29 (arabinose), 0.32 (xylose). GLC (column, 5% SE-52 3 mm × 2 m; column temp. 155°C; injection temp. 200°C; carrier gas, N_2 1 kg/cm²; samples, TMS derivatives): t_R(min) 21.5, 33.3 (glucose), 6.3, 7.2, 8.0 (arabinose), 9.4, 11.9 (xylose).

Methylation of 1 by Hakomori's Method——According to the previous papers, **1** (50 mg) was methylated by Hakomori's method with NaH (125 mg), dimethylsulfoxide (DMSO, 5 ml) and CH_3I (1 ml). The reaction mixture was diluted with water and extracted with $CHCl_3$. The $CHCl_3$ solution was dried on Na_2SO_4 and filtered. The filtrate was evaporated to dryness and the residue was subjected to column chromatography on Sephadex LH-20 using MeOH followed by silica gel column chromatography using hexane–acetone (5: 1 v/v) to afford per-*O*-methylginsenoside-Ra_1 (20 mg) as a syrup. ^{1}H NMR (in $CDCl_3$) δ: 0.80 (3H, s, CH_3), 0.84 (6H, s, 2 × CH_3), 0.92 (3H, s, CH_3), 1.00 (3H, s, CH_3), 1.20 (3H, s, CH_3), 1.59 (3H, s, CH_3), 1.67 (3H, s, CH_3), 3.29 (3H, s, OCH_3), 3.35 (3H, s, OCH_3), 3.41 (9H, s, 3 × OCH_3), 3.46 (15H, s, 5 × OCH_3), 3.50 (3H, s, OCH_3), 3.53 (3H, s, OCH_3), 3.56 (6H, s, 2 × OCH_3), 3.58 (6H, s, 2 × OCH_3), 4.22 (2H, d, J = 7 Hz), 4.36 (2H, d, J = 7 Hz), 4.60 (1H, d, J = 7 Hz).

Methanolysis of Per-*O*-methylate of 1 with 50% AcOH in MeOH——A solution of per-*O*-methylate of **1** (20 mg) in methanolic 50% AcOH (2 ml was heated on a water bath for 4 h. The reaction mixture was concentrated to dryness and the residue was subjected to column chromatography on silica gel using benzene–acetone (3: 1 v/v) followed by Sephadex LH-20 column chromatography with MeOH to afford an *O*-methylated prosapogenin (10 mg) as a syrup and a per-*O*-methyloligosaccharide (5 mg) as a syrup. The anomeric protons of both products were examined by ^{1}H-NMR. Prosapogenin: NMR (in $CDCl_3$) δ: 4.20 (1H, d, J = 7 Hz), 4.61 (1H, d, J = 7 Hz). Per-*O*-methyloligosaccharide: NMR (in $CDCl_3$) δ: 4.23 (1H, d, J = 7 Hz), 4.35 (1H, d, J = 7 Hz), 4.50 (1/2H, d, J = 7 Hz), 5.25 (1/2H, d, J = 3 Hz).

Methanolysis of Per-*O*-methyloligosaccharide with Methanolic 2 N HCl——A solution of the above-described per-*O*-methyloligosaccharide (2 mg) in methanolic 2 N HCl (0.5 ml) was refluxed at 70°C for 2 h. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated to dryness under reduced pressure and the residue was examined by TLC (solvent: hexane–acetone (3: 1 v/v)) and GLC (column, 5% NPGS 3 mm × 2 m; column temp., 160°C; injection temp., 230°C; carrier gas, N_2 1 kg/cm²). *Rf* 0.22, t_R(min) 6.8, 9.1 (methyl 2,3,4-tri-*O*-methyl-D-glucopyranoside), *Rf* 0.28, t_R(min) 4.1, 5.2 (methyl 2,3-di-*O*-methyl-L-arabinopyranoside), *Rf* 0.49, t_R(min) 1.5, 1.9 (methyl 2,3,4-tri-*O*-methyl-D-xylopyranoside).

Enzymatic Hydrolyses of 4 and 1 with Commercial Naringinase——(a) Hydrolysis of **4**: Naringinase (200 mg, Sigma Co. Ltd., Lot No. 705140) was added to a solution of **4** (500 mg) in 10% EtOH (40 ml), and the mixture was incubated at 37°C for 24 h. The reaction mixture was extracted with BuOH saturated with water and the BuOH extract was evaporated to dryness *in vacuo*. The residue was subjected to column chromatography on silica gel using $CHCl_3$–MeOH (87: 13—80: 20 v/v) followed by rechromatography on silica gel using solvent B to afford four prosapogenins, 20(*S*)-protopanaxadiol 3-*O*-*β*-D-glucopyranosido-20-*O*-*α*-L-arabinopyranosyl(1→6)-*β*-D-glucopyranoside (**5**, 95 mg), 20(*S*)-protopanaxadiol 20-*O*-*α*-L-arabinopyranosyl(1→6)-*β*-D-glucopyranoside (**6**, 35 mg), 20(*S*)-protopanaxadiol 3-*O*-*β*-D-glucopyranosido-20-*O*-*β*-D-glucopyranoside (**7**, 115 mg) and 20(*S*)-protopanaxadiol 20-*O*-*β*-D-glucopyranoside (**8**, 42 mg).

(b) Hydrolysis of **1**: Commercial naringinase (100 mg) was added to a solution of **1** (200 mg) in 10% EtOH (30 ml). The reaction mixture was incubated and treated by the method described above to afford five prosapogenins, **6** (trace), **7** (2.6 mg), **8** (4.5 mg), 20(*S*)-protopanaxadiol 3-*O*-*β*-D-glucopyranosido-20-*O*-*β*-D-xylopyranosyl(1→4)-*α*-L-arabinopyranosyl(1→6)-*β*-D-glucopyranoside (**9**, 20 mg) and 20(*S*)-protopanaxadiol 20-*O*-*β*-D-xylopyranosyl(1→4)-*α*-L-arabinopyranosyl(1→6)-*β*-D-glucopyranoside (**10**, 82 mg). **5**: A colorless crystalline powder from BuOH, mp 184—186°C, $[\alpha]_D^{20}$ +25.0° (c = 1.00, MeOH), ^{13}C-NMR (Table I), *Anal.* Calcd for $C_{47}H_{80}O_{17}\cdot 3/2H_2O$: C, 59.78; H, 8.86. Found: C, 59.71; H, 8.93. **6**: A colorless crystalline powder from AcOEt–MeOH, mp 159—162°C, $[\alpha]_D^{17}$ +21.9° (c = 0.99, MeOH), ^{13}C-NMR (Table I), TLC: *Rf* 0.52 (solv. A); 0.48 (solv. C). *Anal.* Calcd for $C_{41}H_{70}O_{12}\cdot 2H_2O$: C, 62.25; H, 9.43. Found: C, 62.39; H, 9.39. Prosapogenin **6** (52.5 mg) was heated with pyridine (2 ml) and Ac_2O (2 ml) at 75°C for 3 h, then the reaction mixture was poured into ice-water. The precipitate was collected by filtration, washed with water

and dried. The product was purified by column chromatography on silica gel with benzene–acetone (10: 1 v/v) to afford an octaacetate (30 mg), which was crystallized from EtOH to afford colorless needles, mp 213—214°C, $[\alpha]_D^{20}$ +3.7° (c=0.94, $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: OH (nil), 1750, 1742, 1730 (OAc). ^1H NMR (in $CDCl_3$) δ: 0.85 (9H, s, 3×CH_3), 0.91 (3H, s, CH_3), 0.96 (3H, s, CH_3), 1.17 (3H, s, CH_3), 1.59, 1.66 (3H each, s, CH_3), 1.98 (6H, s, 2×OAc), 2.03 (15H, s, 5×OAc), 2.07 (3H, s, OAc). **7**: A colorless crystalline powder from BuOH, mp 185—189°C, $[\alpha]_D^{20}$ +29.8° (c=1.04, MeOH), ^{13}C-NMR (Table I), TLC: *Rf* 0.45 (solv. A); 0.52 (solv. C), *Anal.* Calcd for $C_{42}H_{72}O_{13}\cdot 4H_2O$: C, 58.85; H, 9.41. Found: C, 59.16; H, 9.41. This compound appeared to be identical with ginsenoside F_2.[9] **8**: A white powder after being dried, TLC: *Rf* 0.70 (solv. A); 0.76 (solv. C). Prosapogenin **8** (66 mg) was heated with pyridine (2 ml) and Ac_2O (2 ml) at 75°C for 3 h, and the reaction mixture was treated by the same procedure as described above. A hexaacetate of **8** was obtained as colorless n edles from EtOH, mp 176.5—178°C (lit.[8] mp 177—178°C), $[\alpha]_D^{20}$ +9.3° (c= 0.86, $CHCl_3$) (lit.[8] $[\alpha]_D$ +9.6° (c=0.94, $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: OH (nil), 1760, 1735 (OAc), ^1H NMR (in $CDCl_3$) δ: 0.85 (9H, s, 3×CH_3), 0.93 (3H, s, CH_3), 0.96 (3H, s, CH_3), 1.17 (3H, s, CH_3), 1.57, 1.64 (3H each, s, CH_3), 1.98 (6H, s, 2×OAc), 2.02 (12H, s, 4×OAc), which appeared to be identical with compound K.[8] **9**: A colorless crystalline powder from aqueous EtOH, (mp 187—189°C), $[\alpha]_D^{17}$ 17.5° (c=0.84, MeOH), ^{13}C NMR (Table I), *Anal.* Calcd for $C_{52}H_{88}O_{21}\cdot H_2O$: C, 58.52; H, 8.50. Found: C, 58.31; H, 8.51. **10**: Colorless needles from aqueous EtOH, mp 178—180°C, $[\alpha]_D^{20}$ +12.1° (c=0.99, MeOH), *Anal.* Calcd for $C_{46}H_{78}O_{16}\cdot H_2O$: C, 61.04; H, 8.91. Found: C, 61.33; H, 9.24.

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