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Studies on the Saponins of Ginseng. III.[1] Structures of Ginsenoside-Rb_3 and 20-Glucoginsenoside-Rf

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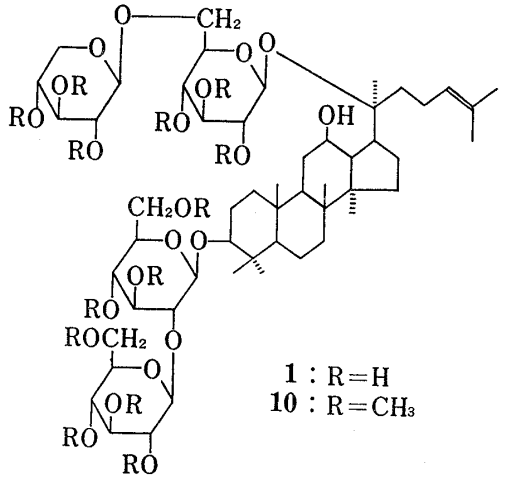
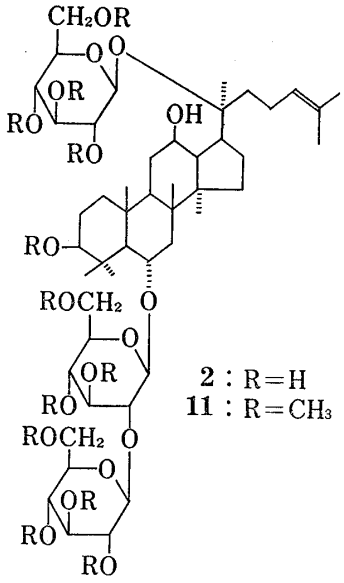
The chemical structures of ginsenoside-Rb_3 and 20-glucoginsenoside-Rf, which were isolated from Ginseng (the root of *Panax ginseng* C.A. Meyer; Araliaceae), were established to be 20*S*-protopanaxadiol-3-[*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside]-20-[*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranoside] (**1**) and 20*S*-protopanaxatriol-6-[*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside]-20-*O*-β-D-glucopyranoside (**2**), respectively.

Keywords——ginsenoside-Rb_3; 20-glucoginsenoside-Rf; dammarane-type saponins; bisdesmosides; ginseng; *Panax ginseng* C.A. Meyer; Araliaceae; 20*S*-protopanaxadiol; 20*S*-protopanaxatriol

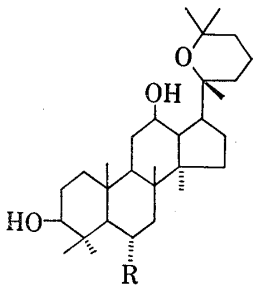
As we reported in the previous papers,[1,3] the structures of nine saponins of Ginseng (root of *Panax ginseng* C.A. Meyer; Araliaceae), ginsenoside-Ro, Rb_1, Rb_2, -Rc, -Rd, -Re, -Rf, -Rg_1,[4]



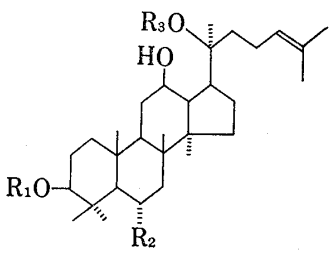
1 : R=H
10 : R=CH_3



2 : R=H
11 : R=CH_3



3 : R=H
4 : R=OH



5: $R_1=R_3=H$, $R_2=OH$
6: $R_1=R_2=H$, $R_3=\overset{\beta}{\text{——}}$D-glucose
7: $R_1=R_2=R_3=H$
8: $R_1=\overset{\beta}{\text{——}}$D-glucose$\overset{2\beta\,1}{\text{——}}$D-glucose, $R_2=R_3=H$
9: $R_1=R_3=H$, $R_2=-O\overset{\beta}{\text{——}}$D-glucose$\overset{2\beta\,1}{\text{——}}$D-glucose

Chart 1

1) Part II: S. Sanada, N. Kondo, J. Shoji, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **22**, 2407 (1974).
2) Location: *1-5-8 Hatanodai, Shinagawa-ku, Tokyo, 142, Japan.*
3) S. Sanada, N. Kondo, J. Shoji, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **22**, 421 (1974).
4) Y. Nagai, O. Tanaka, and S. Shibata, *Tetrahedron*, **27**, 881 (1971).

and $-Rg_2$, have been established. The present paper deals with the structure determination of two minor saponins named ginsenoside-Rb_3(**1**) and 20-glucoginsenoside-Rf(**2**).

1, $C_{53}H_{90}O_{22}$, and **2**, $C_{48}H_{82}O_{19}$ were isolated from the fraction 3 reported in the previous paper[3] by repeated column chromatography on silica gel. The yield of each saponin was 0.005% on the dried crude drug basis.

Infrared (IR) spectra of **1** and **2** show the presence of hydroxyl groups (3420 cm^{-1}) and a double bond (1620 cm^{-1}). On hydrolysis with 2N hydrogen chloride, **1** gave panaxadiol (**3**),[5] D-glucose and D-xylose, while **2** gave panaxatriol (**4**)[6] and D-glucose.

According to Tanaka and Kohda,[7] the enzymatic hydrolysis of ginsenoside-Rg_1[4] afforded its genuine sapogenin, 20*S*-protopanaxatriol(**5**),[6] while a mixture of ginsenoside-Rb_1,[3] $-Rb_2$,[3] and $-Rc$[3] gave compound K(**6**)[8] (=20-*O*-β-D-glucopyranosyl 20*S*-protopanaxadiol) as a main product. In our study, the enzymatic hydrolysis with pectinase(Sigma)[9] followed by de Mayo's oxidative degradation[10] of **1** gave 20*S*-protopanaxadiol(**7**)[11] as a genuine aglycone, while **2** afforded **5** only by enzymatic hydrolysis.

It has been known that the 20-*O*-glycosyl moiety of ginsenoside is readily hydrolyzed by the treatment with aqueous acetic acid. On the partial hydrolysis with 50% acetic acid under reflux for 4 hr, **1** gave 20*S*-protopanaxadiol 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside(**8**) and a biose which consists of glucose and xylose, while **2** gave ginsenoside-Rf(**9**) and glucose.

Further, on methylation by the Hakomori's method,[12] **1** afforded a trideca-*O*-methylate(**10**), colorless needles from methanol, while **2** gave a dodeca-*O*-methylate(**11**), a white powder from aqueous methanol. The IR spectra of **10** and **11** show the hydroxyl absorption band at 3438 cm^{-1}. Based on the previous study on the IR spectra of *O*-methylated ginsenosides, the position of the hydroxyl group which resists to the Hakomori's permethylation is assigned to C-12 of each aglycone. The methanolysis of **10** with 5% methanolic hydrogen chloride gave methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside, methyl 3,4,6-tri-*O*-methyl-D-glucopyranoside, methyl 2,3,4-tri-*O*-methyl-D-glucopyranoside, and methyl 2,3,4-tri-*O*-methyl-D-xylopyranoside, while **11** afforded methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside and methyl 3,4,6-tri-*O*-methyl-D-glucopyranoside by the same methanolysis.

The configurations of each monosaccharide of **1** and **2** were revealed to be β by the coupling constant (J=7 Hz) of each anomeric proton signal of **10** and **11** in nuclear magnetic resonance(NMR) spectra.

Consequently, the structures of ginsenoside-Rb_3 and 20-glucoginsenoside-Rf were established to be 20*S*-protopanaxadiol-3-[*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside]-20-[*O*-β-D-xylopyranosyl(1→6)-β-D-glucopyranoside] and 20*S*-protopanaxatriol-6-[*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside]-20-*O*-β-D-glucopyranoside, respectively.

Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus and uncorrected. Optical rotations were measured with a Yanagimoto OR-50 automatic polarimeter. IR spectra were obtained with a Hitachi Model EPI-2 and NMR spectra were taken at 90 MHz with a Hitachi Model R-22 High Resolution NMR spectrometer and chemical shifts are given in δ (ppm) scale with tetramethylsilane as the internal standard. Gas–liquid chromatography (GLC) was run on a Hitachi Model K-53 with flame ionization detector. Thin-layer chromatography (TLC) was performed on Kieselgel H (Merck) by using the following

5) M. Nagai, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **19**, 2349 (1971).
6) S. Shibata, O. Tanaka, K. Soma, and Y. Iida, *Tetrahedron Lett.*, **1976**, 391.
7) H. Kohda and O. Tanaka, *Yakugaku Zasshi*, **95**, 246 (1975).
8) I. Yoshioka, T. Sugawara, K. Imai, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 2418 (1972).
9) T.D. Lin, N. Kondo, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **24**, 253 (1976).
10) J.J. Dugan and P. de Mayo, *Canad. J. Chem.*, **43**, 2033 (1965).
11) M. Nagai, T. Ando, N. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **20**, 1212 (1972).
12) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).

solvent system: a) hexane–acetone=2:1, b) hexane–acetone=7:2, c) benzene–acetone=3:1, d) BuOH–AcOH–H_2O=4:1:5 (upper phase), e) $CHCl_3$–MeOH–H_2O=7:3:1 (lower phase), f) $CHCl_3$–MeOH–AcOEt–H_2O=2:2:4:1 (lower phase). Detection was made by spraying 10% H_2SO_4 followed by heating.

Isolation of 1 and 2——Fraction 3 reported in the previous paper[3] was submitted to column chromatography on silica gel with BuOH–AcOEt–H_2O (4:1:2 upper phase) to separate into four fractions (Fr. 3-I, -II, -III, and -IV). Fr. 3-I and Fr. 3-III were purified by column chromatography on silica gel eluted with $CHCl_3$–MeOH–H_2O (65:35:10 lower phase) to afford **2** from the former and **1** from the latter, respectively. Ginsenoside-Rb_3 (**1**): a white powder from isopropanol, (mp 193—195°), $[\alpha]_D^{28}$+19.39° (c=0.98 MeOH), *Anal.* Calcd. for $C_{53}H_{90}O_{22}\cdot 4H_2O$: C, 55.26; H, 8.57. Found: C, 54.87; H, 8.10. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1620 (>C=C<). 20-Glucoginsenoside-Rf (**2**): a white powder from isopropanol, (mp 182—184°), $[\alpha]_D^{28}$ +21.00° (c=1.01 MeOH), *Anal.* Calcd. for $C_{48}H_{82}O_{19}\cdot 4H_2O$: C, 55.69; H, 8.76. Found: C, 55.43; H, 8.49. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1620 (>C=C<).

Hydrolyses of 1 and 2 with 2 N HCl——**1** and **2** were hydrolyzed with 2 N HCl (4 N HCl–50% dioxane=1:1 v/v) on a boiling water bath for 4 hr. The reaction mixture was diluted with water and extracted with $CHCl_3$. The $CHCl_3$ extractive was washed with water, dried over anhyd. Na_2SO_4 and concentrated to dryness. The aqueous layer was neutralized with Amberlite IR-4B and evaporated *in vacuo* to dryness. The $CHCl_3$ soluble fraction (genin) and water soluble fraction (sugars) were identified by TLC and GLC by comparing with authentic samples. Genin: TLC (solvent c) *Rf* 0.54 (**3**), 0.32 (**4**). Sugars: TLC (solvent d) *Rf* 0.10 (glucose), 0.30 (xylose); GLC (column: 5% SE-52 on Chromosorb W 3 mm×2 m; column temp.: 180°; carrier gas: N_2 1 kg/cm^2; samples: TMS derivatives) t_R (min) 11.7, 16.8 (glucose), 2.1, 3.0 (xylose).

The residue of the aqueous layer originated from **1** was chromatographed on cellulose powder using $CHCl_3$–MeOH–H_2O=7:3:1 (lower phase) to afford glucose and xylose. Glucose: $[\alpha]_D^{28}$+49.0° (c=0.66 H_2O) (D-glucose lit.[13] $[\alpha]_D$+52.7°); xylose: $[\alpha]_D^{28}$+14.7° (c=0.30 H_2O) (D-xylose lit.[13] $[\alpha]_D$ +18.8°).

Identification of Genuine Aglycone of 1 and 2——Each ginsenoside was dissolved in McIlivaine buffer solution (80 ml, pH 4.3) with addition of pectinase (40 mg, Sigma) and a drop of toluene. Each mixture was stirred at 37° for 48 hr. The reaction mixture of **2** was extracted with $CHCl_3$. The extractive was evaporated *in vacuo* and the residue was purified by column chromatography on silica gel using hexane-acetone=2:1 to afford **5**, which was identified by TLC and GLC. On the other hand, the reaction mixture of **1** was extracted with butanol saturated with water. The extractive was evaporated *in vacuo* and the residue was treated with $NaIO_4$ by the de Mayo's method described in the previous paper.[14] The product was purified by column chromatography on silica gel using hexane–acetone=2:1 to afford **7**, which was identified by TLC and GLC. TLC (solvent a): *Rf* 0.30 (**7**), 0.23 (**5**). GLC (column: glass column 1.5% SE-30 DMCS on Chromosorb W, 3 mm×2 m; carrier gas: N_2 flow 1.5 kg/cm^2; samples: TMS derivatives): i) column temp. 265°: t_R (min) 8.3 (**7**); cf. 9.3 (20*R*-protopanaxadiol). ii) column temp. 270°: t_R (min); 7.4 (**5**); cf. 8.3 (20*R*-proropanaxatriol).

Partial Hydrolyses of 1 and 2 with 50% AcOH——**1** and **2** were heated with 50% AcOH for 4 hr at 70°, respectively. The reaction mixture was extracted with BuOH saturated with water. Each BuOH solution was evaporated *in vacuo* and the residue was purified by chromatography on silica gel eluted with $CHCl_3$–MeOH–AcOEt–H_2O=2:2:4:1 (lower phase). **1** gave a prosapogenin, colorless needles from MeOH, mp 260—264°, which were identified as **8** by comparing TLC (solvent e, *Rf* 0.50) and IR spectra and by mixed fusion, while **2** gave a white powder from acetone, (mp 197—198°), which was identified as **9** by comparing TLC (solvent f, *Rf* 0.22) and IR spectra.

The aqueous layer of **1** was evaporated *in vacuo* to dryness. The residue was hydrolyzed with 2 N HCl and the reaction mixture was treated by the same method described above to afford glucose and xylose which were identified by TLC and GLC. On the other hand, the aqueous layer of **2** was evaporated *in vacuo* to dryness. The presence of glucose in the residue was proved by TLC and GLC.

Methylation of 1 and 2 by the Hakomori's Method——**1** and **2** were methylated by the Hakomori's method described in the previous paper and the products were purified by column chromatography on silica gel eluted with hexane–acetone=3:1 to afford **10** and **11**, respectively. **10**: colorless needles from MeOH, mp 195°, $[\alpha]_D^{28}$±0.00° (c=0.91 $CHCl_3$), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3438 (OH). NMR (in $CDCl_3$) δ: 0.84 (3H, s, CH_3), 0.88 (3H×2, s, CH_3), 0.92 (3H, s, CH_3), 1.02 (3H, s, CH_3), 1.32 (3H, s, CH_3), 1.62 (3H, s, CH_3), 1.70 (3H, s, CH_3), 3.33—3.60 (3H×13, s, OCH_3), 4.25 (1H, d, J=7 Hz, anomeric H), 4.29 (1H, d, J=7 Hz, anomeric H), 4.45 (1H, d, J=7 Hz, anomeric H), 4.66 (1H, d, J=7 Hz, anomeric H). **11**: a white powder from aq. MeOH, (mp 92—93°), $[\alpha]_D^{28}$+14.10° (c=0.99, $CHCl_3$), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3438 (OH). NMR (in $CDCl_3$) δ: 0.88 (3H, s, CH_3), 0.92 (3H, s, CH_3), 0.95 (3H, s, CH_3), 1.01 (3H, s, CH_3), 1.26 (3H, s, CH_3), 1.33 (3H, s, CH_3), 1.59 (3H, s, CH_3), 1.67 (3H, s, CH_3), 3.33—3.63 (3H×12, s, OCH_3), 4.34 (1H, d, J=7 Hz, anomeric H), 4.48 (1H, d, J=7 Hz, anomeric H), 4.65 (1H, d, J=7 Hz, anomeric H).

Methanolyses of 10 and 11——**10** and **11** were methanolyzed with methanolic 5% HCl under reflux for 2 hr, respectively. The reaction mixture was neutralized with Ag_2CO_3 and evaporated to dryness. The

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14) N. Kondo, J. Shoji, and O. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **21**, 2702 (1973).

residue was examined by TLC and GLC by comparing with authentic samples. TLC (solvent b): *Rf* 0.40, 0.58 (methyl 2,3,4,6-tetra-*O*-methylglucopyranoside), 0.19 (methyl 3,4,6-tri-*O*-methylglucopyranoside), 0.15 (methyl 2,3,4-tri-*O*-methylglucopyranoside), 0.49 (methyl 2,3,4-tri-*O*-methylxylopyranoside). GLC (column: 5% NPGS on Chromosorb W, 3 mm × 2 m; column temp.: 175°; carrier gas: N_2 1 kg/cm^2): t_R 4.7, 6.6 (methyl 2,3,4,6-tetra-*O*-methylglucopyranoside), 12.2 (methyl 3,4,6-tri-*O*-methylglucopyranoside), 10.0 (methyl 2,3,4-tri-*O*-methylglucopyranoside), 3.7, 5.0 (methyl 2,3,4-tri-*O*-methylxylopyranoside).

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