2-(1-Aminoethyl)-5-chloro-3-phenylindole (XII)——A solution of XI (32 g) in dry ether (300 ml) was added dropwise to a suspension of $LiAlH_4$ (10.6 g) in dry ether (500 ml) below 10°. The mixture was heated under reflux for 9 hr. After cooling, H_2O (100 ml) was added cautiously, followed by conc. HCl (150 ml). The precipitate was collected by filtration and washed succesively with ether, 5% HCl and H_2O to give 19.0 g (55.2%) of the hydrochloride of XII, mp 171.5—175° (decomp.). The hydrochloride (0.3 g) was suspended in CH_2Cl_2 and made basic with 28% NH_4OH. After stirring, the CH_2Cl_2 layer was separated and evaporated. The residue was recrystallized from isopropyl ether to give the free base of XII (0.18 g) as colorless prisms, mp 119.5—120.5°. IR ν_{max}^{Nujol} cm^{-1}: 3340, 3140, 3000, 1600. NMR ($CDCl_3$) δ: 1.42 (3H, d, J=6 Hz, CH_3), 1.77 (2H, s, D_2O exchangeable, NH_2), 4.60 (1H, q, J=6 Hz, CH), 7.60—7.11 (8H, m, aromatic H), 9.20 (1H, s, D_2O exchangeable, indole NH). *Anal.* Calcd. for $C_{16}H_{15}N_2Cl$: C, 70.98; H, 5.58; N, 10.35; Cl, 13.09. Found: C, 70.86; H, 5.60; N, 10.55; Cl, 13.09.

7-Chloro-1,3-dihydro-3-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one (XIII)——A solution of chromium trioxide (2.9 g) in H_2O (3 ml) was added dropwise to a mixture of the hydrochloride of XII (0.5 g) in acetic acid (15 ml) with stirring. The mixture was stirred at room temperature for 24 hr. The reaction mixture was neutralized with 28% NH_4OH (15 ml) and extracted with benzene. After evaporation of the solvent, the residue was crystallized from petroleum ether to give 0.35 g (75.4%) of XIII, mp 199.5—201.5°. Recrystallization from EtOH gave colorless prisms, mp 222—223° (lit[6] mp 220—221°). IR ν_{max}^{Nujol} cm^{-1}: 3180, 3100, 3020, 1680. NMR ($CDCl_3$) δ 1.75 (3H, d, J=6.5 Hz, CH_3), 3.75 (1H, q, J=6.5 Hz, CH), 7.75—7.11 (8H, m, aromatic H), 10.01 (1H, s, D_2O exchangeable, NH). *Anal.* Calcd. for $C_{16}H_{13}ON_2Cl$: C, 67.49; H, 4.60; N, 9.84; Cl, 12.45. Found: C, 67.17; H, 4.73; N, 9.99; Cl, 13.18.

Acknowledgement The authors wish to thank Mr. Isao Katsuki and Mr. Kazuo Mori for skillful technical assistance.

6) S.C. Bell, T.S. Sulkowski, C. Gochman, and S.J. Childress, *J. Org. Chem.* **27**, 562 (1962).

Chem. Pharm. Bull. 23(12)3282—3285(1975)

UDC 547.918.02.04 : 581.192

Studies on the Constituents of Himalayan Ginseng, *Panax pseudoginseng*. II.[1] The Structures of the Saponins (2)

Noriko Kondo and Junzo Shoji

School of Pharmaceutical Sciences, Showa University[2]

(Received April 15, 1975)

The chemical structures of saponin B (I) (=chikusetsusaponin IV), $C_{47}H_{74}O_{18}$, $[\alpha]_D^{18}$ −9.10° (pyridine) and saponin C (III) (=desarabinofuranosylchikusetsusaponin IV), $C_{42}H_{66}O_{14}$, $[\alpha]_D^{18}$ +15.8° (MeOH), which were isolated from *Panax pseudoginseng* subsp. *himalaicus* var. *angustifolius* (Araliaceae), were established on the basis of physical data and chemical investigations.

In our previous paper,[1] we reported the structural elucidation of saponin A(=chikusetsusaponin V[3]) and saponin D(=ginsenoside Rb_1[1,4]), which were isolated from the rhizoma of *Panax pseudoginseng* subsp. *himalaicus* var. *angustifolius*. The present paper deals with the structure determination of saponin B(I) and C(III).

Saponin B(I), $C_{47}H_{74}O_{18}\cdot 4H_2O$, $[\alpha]_D^{18}$ −9.10° (in pyridine) forms a white powder reprecipitated from methanol–ethyl acetate. The infrared (IR) spectrum of I shows the presence

1) Part I: N. Kondo, J. Shoji, and O. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **21**, 2705 (1973).
2) Location: *Hatanodai, Shinagawa-ku, Tokyo.*
3) N. Kondo, Y. Marumoto, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **19**, 1103 (1971).
4) S. Sanada, N. Kondo, J. Shoji, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **22**, 421 (1974).

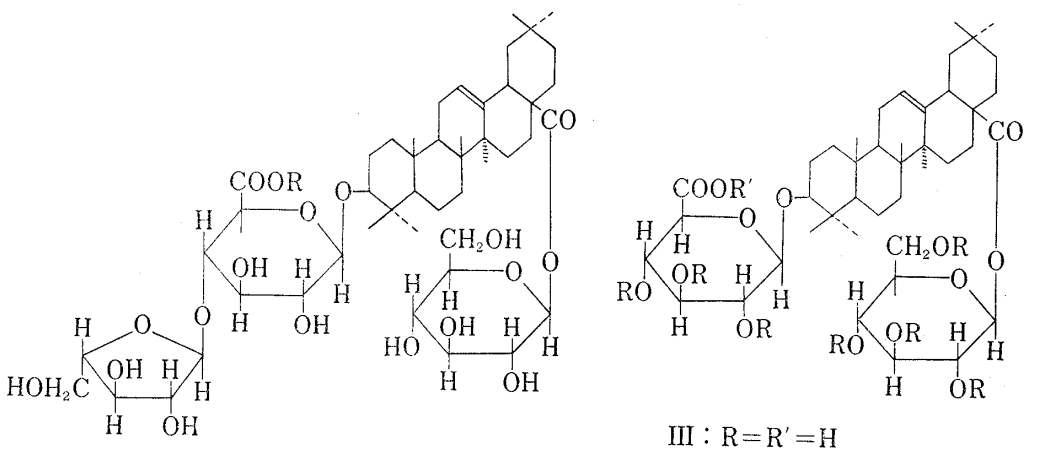

Chart 1

of hydroxyl group (3400 cm^{-1}), ester group (1750 cm^{-1}, shoulder) and carboxyl group (1720 cm^{-1}).

On methylation with diazomethane, I gave a monomethyl ester (II), $C_{48}H_{76}O_{18}\cdot H_2O$, $[\alpha]_D^{17}$ −21.5° (in methanol), which shows the ester band at 1740 cm^{-1} in the IR spectrum and an O-methyl signal at 3.69 ppm (3H(s)) in the nuclear magnetic resonance (NMR) spectrum. Hydrolysis of I with diluted hydrogen chloride gave oleanolic acid, arabinose, glucose and glucuronic acid.

The properties of saponin B and its methyl ester mentioned above strongly suggest that saponin B must be identical with chikusetsusaponin-IV (=Araloside A), the saponin of *P. Japonicum* C.A. MEYER[5] and *Aralia manschurica*,[6] the structure of which has been established as I. The identity of saponin B with chikusetsusaponin-IV was finally proved by the direct comparison.

Saponin C(III) forms a white powder, $C_{42}H_{66}O_{14}\cdot 3H_2O$, $[\alpha]_D^{18}$ +15.8° (in methanol) by repeated precipitation from *n*-butanol saturated with water. The IR spectrum of III shows the presence of hydroxyl group (3400 cm^{-1}), ester group (1750 cm^{-1} shoulder) and carboxyl group (1730 cm^{-1}). Hydrolysis of III with diluted hydrogen chloride gave oleanolic acid, glucose and glucuronic acid. On methylation with diazomethane, III gave a monomethyl ester(IV), $C_{43}H_{68}O_{14}\cdot 2H_2O$, $[\alpha]_D^{18}$ +12.3° (in methanol), which shows the ester band at 1740 cm^{-1} in the IR spectrum and an O-methyl signal at 3.68 ppm (3H(s)) in the NMR spectrum. Per-O-methylsaponin C(V), $C_{50}H_{82}O_{14}$, $[\alpha]_D^{20}$ +14.5° (in chloroform), prepared from III by repeated methylation by the Kuhn's method,[7] gave, on reduction with lithium aluminium hydride, 2,3,4,6-tetra-O-methyl-D-glucose and 2,3,4,6-tetra-O-methyl-D-sorbitol.

The results of the foregoing experiments suggested that glucose residue of saponin C attached to a carboxyl group of either oleanolic acid or glucuronic acid in ester form. To confirm the position of glucosyl ester, β-D-glucopyranosyl oleanolate-(3)-β-D-glucuronopyranoside (III) was prepared from chikusetsusaponin IV (I) as follows.

Hydrolysis of chikusetsusaponin IV (I) with 50% acetic acid under reflux for 2 hr gave a prosapogenin (III), $C_{42}H_{66}O_{14}\cdot 3H_2O$, $[\alpha]_D^{16}$ +15.8° (in methanol). The IR spectrum of this prosapogenin shows the presence of hydroxyl group (3400 cm^{-1}), ester group (1750 cm^{-1} shoulder) and carboxyl group (1730 cm^{-1}). On methylation with diazomethane, the pro-

5) N. Kondo, J. Shoji, N. Nagumo, and N. Komatsu, *Yakugaku Zasshi*, **89**, 846 (1969).
6) N.K. Kochetkov, A.J. Khorlin, and V.E. Vaskovsky, *Tetrahedron Letters*, **1962**, 713; *idem*, *Izv. Akad. Nauk SSSR Ser Khim*, **1963**, 1398, 1409.
7) R. Kuhn, *Angew. Chem.*, **67**, 32 (1955).

sapogenin gave a monomethyl ester, $C_{43}H_{68}O_{14}\cdot 2H_2O$, $[\alpha]_D^{18}$ +11.5° (in methanol) which shows the ester band at 1740 cm^{-1} in the IR spectrum and an O-methyl signal at 3.68 ppm (3H(s) $COOCH_3$) in the NMR spectrum. On repeated methylation by the Kuhn's method, the prosapogenin gave octa-O-methylprosapogenin (V), $C_{50}H_{82}O_{14}$, colorless needles, mp 149°. All of the properties of β-D-glucopyranosyl oleanolate-(3)-β-D-glucuronopyranoside and its derivatives completely agreed with those of saponin C and its derivatives. The identity of both compounds finally proved by the direct comparison.

As we pointed out in the previous paper, the morphological similarity of *Panax pseudoginseng* subsp. *himalaicus* var. *angustifolius* to *P. japonicum* C.A. MEYER, which grows in Japan and has been investigated by us, has urged us to study the constituents of both plants, comparatively. The chemotaxonomical observation on both plants will be discussed in the next paper.

Experimental

All melting points were taken on a Yanagimoto micro melting points apparatus and uncorrected. IR absorption spectra were obtained with a Hitachi Model 215. NMR spectra were measured with a Hitachi Model R-20 High Resolution NMR spectrometer and a Hitachi Model R-22 High Resolution NMR spectrometer with tetramethylsilane as an internal standard. The chemical shifts are reported in δ and the solvents used are indicated. Gas chromatography was run on a Hitachi Model K-53 with hydrogen flame ionization detector.

Isolation of Saponin B and C——As we reported in our previous paper,[1] the total glycoside fraction obtained from 300 g of dried rhizoma was submitted to column chromatography over silica gel using ethyl acetate saturated with water and the same solvent containing 5—25% MeOH. The crude saponin B and C were purified by chromatography on silicic acid using $CHCl_3$–MeOH–H_2O (65 : 35 : 10 the lower phase) and chromatographycally pure saponin B (0.6 g) and saponin C (1.2 g) were obtained.

Saponin B (I)——The pure saponin B (I) was obtained as a white powder by repeated precipitation from MeOH–AcOEt, (mp 235° (decomp.)), $[\alpha]_D^{18}$ −9.10° (c=0.66 pyridine). *Anal.* Calcd. for $C_{47}H_{74}O_{18}\cdot 4H_2O$: C, 56.50; H, 8.27. Found: 56.57; H, 7.82. IR ν_{max}^{Nujol} cm^{-1}: 3200—3500 (OH), 1750 (shoulder, COOR), 1720 (COOH).

Hydrolysis of I——I (50 mg) was hydrolyzed with 5 ml of 2N HCl in dioxane–H_2O (1 : 7 v/v) under reflux on a water bath for 4 hr. The reaction mixture was treated as described in the previous paper[8] and oleanolic acid, glucose and glucuronic acid were identified by thin-layer chromatography (TLC) and gas-liquid chromatography (GLC). TLC (plate, silica gel H; solvent, $CHCl_3$–MeOH–H_2O=65 : 35 : 10 the lower phase) *Rf* 0.28 (glucose), 0.37 (arabinose), 0.55 (glucuronolactone). GLC (column: 5% SE-52 on Chromosorb w, 3 mm×2 m; column temperature: 180°; injection temperature: 230°; carrier gas: N_2 flow 1.0 kg/cm^2; samples: TMS derivatives) t_R (min) 3.4, 3.7 (arabinose), 7.6 (glucuronolactone), 9.8, 14.4 (glucose).

Methylation of I with CH_2N_2 (Formation of Chikusetsusaponin IV Monomethyl Ester (II))——I (100 mg) was dissolved in MeOH and excess CH_2N_2 in ether was added and allowed to stand for 4 hr. The reaction mixture was treated as described in the previous paper to afford a monomethyl ester, a white powder from aqueous EtOH, $[\alpha]_D^{17}$ −21.5° (c=0.19, MeOH). *Anal.* Calcd. for $C_{48}H_{76}O_{18}\cdot H_2O$: C, 57.94; H, 8.25. Found: C, 57.67; H, 8.35. IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH, broad), 1740 (COOR). NMR (in C_5D_5N): 3.69 (3H(s) $COOC\underline{H}_3$). Saponin B monomethyl ester (II) was identified with chikusetsusaponin IV monomethyl ester by comparing TLC (plate, silica gel H; solvent, $CHCl_3$–MeOH–H_2O (7 : 3 : 1 the lower phase) *Rf* 0.29), IR spectra and NMR spectra.

Saponin C (III)——The pure saponin C (III) forms a white powder by repeated precipitation from *n*-BuOH saturated with water–ethyl acetate, (mp 216° (decomp.)), $[\alpha]_D^{18}$ +15.8° (c=0.62 MeOH). *Anal.* Calcd. for $C_{42}H_{66}O_{14}\cdot 3H_2O$: C, 60.10; H, 8.65. Found: C, 60.43; H, 8.18. IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH), 1730 (broad, COOH), 1750 (COOR shoulder), 1630 (>C=C<).

Hydrolysis of III——III (50 mg) was hydrolyzed with 5 ml of 2N HCl in dioxane–H_2O (1 : 7 v/v) under reflux on a water bath for 4 hr. After cooling the precipitate was filtered and dried. The product was identified as oleanolic acid by mixed fusion and by comparing IR spectra and TLC (plate, silica gel H; solvent, benzene–acetone=3 : 1 *Rf* 0.61). The filtrate was neutralized with Ag_2CO_3 and evaporated *in vacuo*. The residue was identified as glucose and glucuronic acid by comparing with authentic samples by TLC and GLC. TLC (plate, silica gel H; solvent, $CHCl_3$–MeOH–H_2O=65 : 35 : 10 the lower phase) *Rf* 0.25 (glucose), 0.55 (glucuronolactone). GLC (column: 5% SE-52 on Chromosorb w; column temperature: 160°; injection

8) N. Kondo and J. Shoji, *Yakugaku Zasshi*, **88**, 325 (1968).

temperature: 210°; N_2 flow 1 kg/cm²; samples: TMS derivatives) t_R (min) 10.8 (glucuronolactone), 14.2, 20.8 (glucose).

Methylation of III with CH_2N_2——III (100 mg) was methylated with CH_2N_2 and the reaction mixture was treated as usual. The product was recrystallized from MeOH to afford colorless needles (IV), mp 210°, $[\alpha]_D^{18}$ +12.3° (c=0.16 MeOH). *Anal.* Calcd. for $C_{43}H_{68}O_{14}\cdot 2H_2O$: C, 61.11; H, 8.59. Found: C, 60.90; H, 8.58. IR ν_{max}^{Nujol} cm⁻¹: 3400 (OH), 1740 (COOR). NMR (in C_5D_5N): 3.68 (3H (s) $COOC\underline{H}_3$), 4.92 (1H (d) J=7 Hz anomeric H), 5.42 (1H, broad, >C=C<$\underline{H}$), 6.20 (1H (d) J=7 Hz anomeric H).

Per-O-methylation of III——According to the previous paper,[1] III (500 mg) was methylated by the Kuhn's method. The product was submitted to chromatography over silica gel using hexane–acetone (3: 1) followed by recrystallization from MeOH, affording per-O-methylsaponin C (V) as colorless needles, mp 149°, $[\alpha]_D^{20}$ +14.5° (c=0.86 $CHCl_3$). *Anal.* Calcd. for $C_{50}H_{82}O_{14}$: C, 66.19; H, 9.01. Found: C, 66.13; H, 8.75. IR ν_{max}^{Nujol} cm⁻¹: OH (nil.), 1750 (COOR). NMR (in $CDCl_3$): 0.78—1.12 (3H (s)×7 $C\underline{H}_3$), 3.34—3.80 (3H (s)×8 $OC\underline{H}_3$), 4.33 (1H (d) J=7 Hz anomeric H), 5.35 (2H, >C=C<$\underline{H}$ and anomeric H).

Reductive Cleavage of V with $LiAlH_4$——V (30 mg) in absolute ether was reduced with $LiAlH_4$ (10 mg) under reflux for 2 hr and the reaction mixture was treated by the same method as described in the previous papers. The chloroform soluble fraction was examined by TLC (solvent: benzene–acetone=2: 1) to reveal the presence of two kinds of methylated monosaccharides, which were identified with authentic samples of 2,3,4,6-tetra-O-methylglucose and 2,3,4,6-tetra-O-methylsorbitol by TLC and GLC. TLC (plate: silica gel H; solvent: benzene–acetone=2: 1) *Rf* 0.08 (2,3,4,6-tetra-O-methylsorbitol), 0.22 (2,3,4,6-tetra-O-methylglucose). GLC (column: 3% SE-30 on Chromosorb w 3 mm×1 m; column temperature: 150° N_2 flow 1 kg/cm²; samples: TMS derivatives). t_R (min) 2.9 (2,3,4,6-tetra-O-methylglucose), 6.3 (2,3,4,6-tetra-O-methylsorbitol).

Partial Hydrolysis of Chikusetsusaponin IV (I) with 50% Acetic Acid (Formation of III)——Chikusetsusaponin IV (700 mg) was heated with 50% AcOH for 2 hr on a water bath. The reaction mixture was extracted with *n*-BuOH saturated with water and the BuOH solution was evaporated *in vacuo*. The residue was purified by chromatography over silica gel using $CHCl_3$–MeOH–H_2O=7: 3: 1 (the lower phase) to afford a prosapogenin (160 mg), a white powder from *n*-BuOH saturated with water–ethyl acetate, (mp 218° (decomp.)), $[\alpha]_D^{16}$ +21.8° (c=0.28 MeOH). IR ν_{max}^{Nujol} cm⁻¹: 3400 (OH), 1730 (broad, COOH), 1750 (COOR sohulder), 1630 (>C=C<).

Methylation of Prosapogenin with CH_2N_2——The prosapogenin was methylated with CH_2N_2 by the method described above. The product was recrystallized from MeOH to afford colorless needles, mp 211°, $[\alpha]_D^{17}$ +11.5° (c=0.43 MeOH). IR ν_{max}^{Nujol} cm⁻¹: 3400 (OH), 1740 (COOR). *Anal.* Calcd. for $C_{43}H_{68}O_{14}\cdot 2H_2O$: C, 61.11; H, 8.59. Found: C, 61.55; H, 8.51. NMR (in C_5D_5N): 3.69 (3H (s) $COOC\underline{H}_3$), 4.92 (1H (d) J=7 Hz anomeric H), 5.40 (1H (broad) >C=C<$\underline{H}$), 6.20 (1H (d) J=7 Hz anomeric H). Prosapogenin monomethyl ester was identified with IV by a mixed fusion, and comparing IR and NMR spectra.

Per-O-methylation of Prosapogenin——The prosapogenin was methylated by the Kuhn's method as described above to afford per-O-methylprosapogenin, colorless needles from MeOH, mp 150°, $[\alpha]_D^{21}$ +9.86° (c=0.50 $CHCl_3$). *Anal.* Calcd. for $C_{50}H_{82}O_{14}$: C, 66.19; H, 9.01. Found: C, 66.47; H, 9.06. IR ν_{max}^{Nujol} cm⁻¹: OH (nil.), 1750 (COOR). NMR (in $CDCl_3$): 0.75—1.22 (3H (s)×7 $C\underline{H}_3$), 3.31—3.75 (3H (s)×8 $OC\underline{H}_3$), 4.29 (1H (d) J=7 Hz anomeric H), 5.34 (2H >C=C<$\underline{H}$ and anomeric H). TLC (plate: silica gel H; solvent: benzene–acetone=3: 1) *Rf* 0.67. Per-O-methylprosapogenin was identified with V by a mixed fusion and by comparing TLC, IR spectra and NMR spectra.

Acknowledgement The authors express their gratitude to Prof. H. Hara, Faculty of Sciences, University of Tokyo, Prof. O. Tanaka, Institute of Pharmaceutical Sciences, School of Medicine, Hiroshima University and other members of the expedition for their encouragement throughout the course of this work. Thanks are also due to the members of Analytical Laboratory of the School of Pharmaceutical Sciences, Showa University for elemental analyses and NMR spectral measurement.