[Chem. Pharm. Bull. 19(6)1103—1107(1971)]

UDC 547.597.02 : 581.192 : 582.892

Studies on the Constituents of Panacis Japonici Rhizoma. IV.[1] The Structure of Chikusetsusaponin V

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(Received October 5, 1970)

The chemical structure of chikusetsusaponin V, $C_{48}H_{76}O_{19}$ $2H_2O$, mp 240—241°, $[\alpha]_D^{22}$ +2.85° (MeOH), a main saponin isolated from the rhizome of *Panax japonicum* was established to be β-D-glucopyranosyl oleanate-(3)-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranoside as represented by formula (**1**).

In our previous papers[1,3,4], it has been reported that three kinds of saponin were isolated from a crude drug, "Chikusetsu-Ninjin" (rhizome of *Panax japonicum* C.A. Meyer; Araliaceae) and the structure of chikusetsusaponin IV agreed with a tonic saponin, araroside A[5] and chikusetsusaponin III was determined to be 20(*S*)-protopanaxadiol-3-[β-D-glucopyranosyl-(1→2)]-[β-D-xylopyranosyl(1→6)]-β-D-glucopyranoside.[1]

In the present paper, the structure elucidation of chikusetsusaponin V which leads to the assignment of the structure **1** is described.

Chikusetsusaponin V (**1**), $C_{48}H_{76}O_{19}\cdot 2H_2O$, white powder, mp 240—241°, $[\alpha]_D^{22}$ +2.85° (MeOH), has been isolated from methanol extract of the crude drug by repeated column chromatography and precipitation from methanol–ethyl acetate. The infrared (IR) spectrum of **1** indicates the presence of hydroxyl group (3300 cm^{-1}), ester group (1742 cm^{-1}) and carboxyl group (1725 cm^{-1}) as the functional groups.

On methylation with diazomethane in methanol, **1** gave monomethylester (**2**), $C_{49}H_{78}O_{19}\cdot 4H_2O$, IR ν_{max}^{KBr}: 1747 cm^{-1} (ester), NMR $\delta_{TMS}^{CDCl_3}$: 3.52 ppm (3H(s)×1, $COOCH_3$). As we reported in previous paper,[3] **1**, on acid hydrolysis, afforded oleanolic acid as an aglycone and D-glucose and D-glucuronic acid as the sugar components.

Per-O-methylchikusetsusaponin V(**3**), $C_{59}H_{98}O_{19}$, $[\alpha]_D^{26}$ −74.05° ($CHCl_3$), prepared by repeated methylation by the Kuhn method,[6] gave, on reduction with $LiAlH_4$ in absolute ether, erythrodiol bioside (**4**), $C_{48}H_{82}O_{12}$, $[\alpha]_D^{27}$ +36.27° ($CHCl_3$), 2,3,4,6-tetra-O-methyl-D-glucose and 2,3,4,6-tetra-O-methyl-D-sorbitol.

Methanolysis of erythrodiol bioside with 2N HCl in methanol gave erythrodiol (**5**), mp 234—236°, methyl 2,3,4,6-tetra-O-methyl-D-glucoside (**6**) and methyl 3,4-di-O-methyl-D-glucoside (**7**). Theformation of 2,3,4,6-tetra-O-methyl-D-glucose and 2,3,4,6-tetra-O-methyl-D-sorbitol by reductive degradation of per-O-methylchikusetsusaponin V with $LiAlH_4$ suggested that one glucose residue attached to the carboxyl group of either oleanolic acid or glucuronic acid in ester form.

To confirm the position of the ester bonding with glucose, a microbial method for splitting the glycosidic linkage was applied. The cultural condition employed accorded to the general

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2) Location: *Hatanodai, Shinagawa-ku, Tokyo.*
3) N. Kondo and J. Shoji, *Yakugaku Zasshi*, **88**, 325 (1968).
4) N. Kondo, J. Shoji, N. Nagumo, and N. Komatsu, *Yakugaku Zasshi*, **89**, 846 (1969).
5) N.K. Kochetkov, A.J. Khorlin, and V.E. Vaskovsky, *Tetrahedron Letters*, **1962**, 713; N.K. Kochetkov, A. J. Khorlin, and V.E. Vaskovsky, *Izv. Akad. Nauk SSSR Ser Khim.*, **1963**, 1398, 1409.
6) R. Kuhn, *Angew. Chem.*, **67**, 32 (1955).

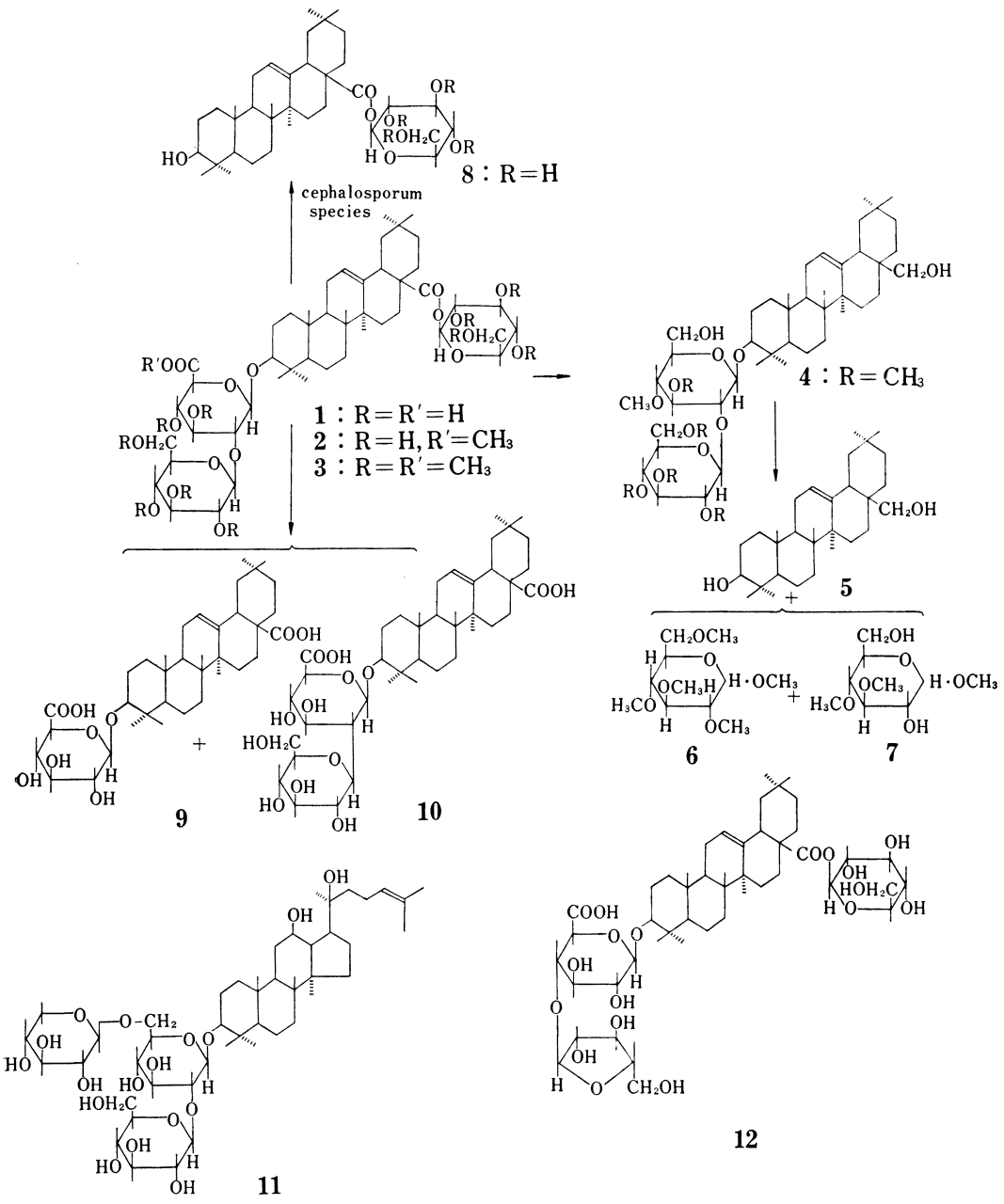

Chart 1

procedure reported by Yoshioka & Kitagawa[7] using *cephalosporium* species which was selected stam by us.[4] From the $CHCl_3$ extract of cultural medium a solid residue was obtained and recrystallized from acetone giving a colorless needles. The product and its acetate were

7) I. Yoshioka, M. Fujio, M. Osamura, and I. Kitagawa, *Tetrahedron Letters*, **1966**, 6306.

identified to be compound O (**8**) and its acetate[4,7] by thin-layer chrometography (TLC), mixed fusion and IR spectra with the authentic samples.

The above results suggested the structure of **1** to be represented β-D-glucopyranosyl oleanate-3-D-glucopyranosyl (1→2)-D-glucuronopyranoside.

Partial hydrolysis of **1** with 2% H_2SO_4 gave prosapogenin 1(**9**), $C_{36}H_{56}O_9$, $[\alpha]_D^{22}+15.26°$ (MeOH) and prosapogenin 2(**10**), $C_{42}H_{66}O_{14}\cdot H_2O$, $[\alpha]_D^{22}+4.69°$(MeOH) which were isolated from the hydrolysate by repeated column chromatography.

On hydrolysis with 2N HCl in dioxane–water prosapogenin **1** gave oleanolic acid and glucuronic acid but prosapogenin 2 gave oleanolic acid, glucuronic acid and glucose respectively.

For the determination of the configuration of the glycoside linkage, Klyne's rule[8] was applied to the prosapogenins and furthermore the chemical shifts and the coupling constants of the anomeric proton signals of per-O-methylchikusetsusaponin V (**3**) and partial O-methylated erythrodiol bioside (**4**) were examined. The results were summerised in Table I.

TABLE I

Glucose→glucuronic acid	NMR anomer H δ 4.65 $J=7$ cps	β
	$[M]_D$·prosapogenin-2−$[M]_D$·prosapogenin-1 = −59.15°	β
Glucuronic acid→genin	NMR anomer H δ 4.35 $J=7$ cps	β
	$[M]_D$·prosapogenin-1−$[M]_D$·oleanolic acid = −218.68°	β
Glucose→genin (Compound O)	NMR anomer H δ 5.55 $J=9$ cps[7]	β
	$[M]_D$·Chikusetsusaponin V−$[M]_D$·prosapogenin-2 = −10.09°	β
	Methyl-α-D-glucopyranoside $[M]_D$ +276.02°	
	Methyl-β-D-glucopyranoside $[M]_D$ −61.56°	
	Methyl-α-D-glucuronopyranoside $[M]_D$ +95.68°	
	Methyl-β-D-glucuronopyranoside $[M]_D$ −205.92°	

The configuration of the three sugars of **1** was assigned to be all β-form. The total structure of chikusetsusaponin V was suggested to be β-D-glucopyranosyl oleanate-(3)-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranoside.

As we reported in previous papers, Panacis Japonici rhizoma is one of the famous crude drug in Japan and has been used as the substitute drug of "Ginseng." The chemical structures of three main saponins contained in this crude drug are shown by formula (**1**), (**11**),[1] and (**12**).[4,5] The investigation of the pharmacological action of these saponins and the comparative study on the saponin of other Araliaceous plant are now in progress.

Experimental

All melting points were determined on Yanagimoto Micro Melting Point apparatus and uncorrected. Infrared absorption spectra were measured with Hitachi Model EPI-2. Nuclear magnetic resonance (NMR) spectra were measured with Japan Electron Co, JNM. 4H-100 spectrometer and Hitachi Model R-20 High Resolution NMR Spectrometer with tetramethylsilane as a internal standard. The chemical shifts are reported in δ and the solvents used are indicated. Gas chromatograph used was Hitachi Model K-53 with hydrogen flame injection detecter.

Chikusetsusaponin V (1)——As we reported in previous paper, **1** was obtained from the crude saponin fraction of the rhizome of *Panax japonicum* by repeated column chromatography on silica gel[9] with 10—25% MeOH–AcOEt saturated with water and finally on silicic acid[9] with $CHCl_3$–MeOH–H_2O (65:35:10 lower phase). The pure chikusetsusaponin V was obtained in white powder by repeated precipitation from

8) W. Klyne, *Biochem. J.*, **47**, xli (1950).
9) The approximate PH values of 10% aqueous slurry of silica gel and silicic acid are 7 to the former and 4 to the latter.

MeOH–AcOEt, mp 240—241°, $[\alpha]_D^{25}$+2.85° (c=2.01, MeOH). *Anal.* Calcd. for $C_{48}H_{76}O_{19}\cdot 2H_2O$: C, 57.06; H, 8.02. Found: C, 57.36; H, 8.26. IR ν_{max}^{Nujol} cm^{-1}: 3300 (OH), 1742 (COOR), 1725 (COOH).

Hydrolysis of Chikusetsusaponin V (1)——**1** was hydrolysed with 2N HCl–50% dioxane–H_2O (1:1) by refluxing on water-bath for 4 hr. The reaction mixture was treated as described in previous paper and oleanolic acid, D-glucose and D-glucuronic acid were detected.

Methylation of 1 with CH_2N_2 (Formation of Chikusetsusaponin V Monomethylester (2))——**1** (125 mg) was dissolved in MeOH and excess CH_2N_2 in ether was added and allowed to stand for few minutes. The reaction mixture was concentrated and the residue was purified by column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (65:35:10 lower phase). The product was purified by reprecipitation from EtOH–AcOEt to give white powder, mp 245° (decomp.). *Anal.* Calcd. for $C_{49}H_{78}O_{19}\cdot 4H_2O$: C, 56.42; H, 8.31. Found: C, 56.56; H, 8.55. IR ν_{max}^{KBr} cm^{-1}: 1747 (ester). NMR $\delta_{TMS}^{CDCl_3}$: 3.52 (3H (s), $COOCH_3$).

Per-O-methylation of 1 (Formation of Per-O-methylchikusetsusaponin V (3))——According to the Kuhn method, chikusetsusaponin V (1 g) in dimethylformamide (5 ml) was methylated with Ag_2O (6 g) and methyl iodide (6 ml) at room temperature for 90 hr under stirring. The reaction mixture was filtered and the filtrate was methylated again in the same way with additional Ag_2O (4 g) and methyl iodide (4 ml). After 90hr the reaction mixture was filtered and the filtrate was diluted with water and then KCN (solid) was added to dissolve the silver salt. The reaction mixture was extracted with $CHCl_3$ several time, washed with water, dried over Na_2SO_4 and evaporated. The residue was submitted to chromatography on silica gel using benzene–acetone (4:1) as a solvent, followed by reprecipitation from dilute EtOH, affording the per-O-methylate as white powder, mp 118°, $[\alpha]_D^{26}$−74.05° (c=0.47, $CHCl_3$). *Anal.* Calcd. for $C_{59}H_{98}O_{19}$: C, 63.74; H, 8.88. Found: C, 63.51; H, 8.94. IR ν_{max}^{Nujol} cm^{-1}: OH (nil), 1753 (ester). NMR $\delta_{TMS}^{CDCl_3}$: 0.73—1.1 (3H(s)×7, CH_3), 3.3—3.7 (3H(s)×11, OCH_3), 4.35 (1H(d, J=7 cps), Anomer H), 4.6 (1H(d, J=7 cps), Anomer H), 5.27—5.30 (2H, >C=C<H and Anomer H).

Reductive Cleavage of Per-O-methylchikusetsusaponin V (3) with $LiAlH_4$——Per-O-methylchikusetsusaponin V (3) (500 mg) in absolute $(C_2H_5)_2O$ was reduced with $LiAlH_4$ (170 mg) under refluxing for 2 hr. The reaction mixture was treated with water under ice cooling to decompose the excess $LiAlH_4$ and then acidified with 2% H_2SO_4 to dissolve the precipitate. The solution was extracted with ether, washed with water, dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel using benzene–acetone (2:1) as a solvent, followed by reprecipitation from diluted MeOH, affording the hexa-O-methyl-erythrodiol bioside (**4**) as white powder, mp 135°, $[\alpha]_D^{27}$+36.27° (c=0.74, $CHCl_3$). *Anal.* Calcd. for $C_{48}H_{82}O_{12}$: C, 67.76; H, 9.76. Found: C, 67.23; H, 9.81. NMR $\delta_{TMS}^{CDCl_3}$ (ppm): 0.83—1.15 (3H(s)×7, CH_3), 3.34—3.62 (3H(s)×6, OCH_3), 4.35 (1H (d, J=7 cps), Anomer H), 4.65 (1H (d, J=7 cps), Anomer H), 5.16 (1H (m) >C=C<H).

The aqueous layer was extracted with $CHCl_3$, washed with water, dried over Na_2SO_4 and evaporated. The residue was submitted to chromatography on silica gel using benzene–acetone (2:1) as a solvent. Two products were separated and identified to be 2,3,4,6-tetra-O-methyl-D-glucose and 2,3,4,6-tetra-O-methyl-D-sorbitol by comparing with the authentic samples.

TLC: plate, Kieselgel H; solvent, benzene–acetone (2:1); *Rf* 2,3,4,6-tetra-O-methyl-D-glucose 0.33, 2,3,4,6-tetra-O-methyl-D-sorbitol 0.20.

GLC: (a) Column, 5% NPGS on Chromosorb W, 3 mm×2 m; column temperature 150°; N_2 flow 0.6 kg/cm^2; t_R (min) 2,3,4,6-tetra-O-methyl-D-glucose TMS ether, 8.7, 9.3; 2,3,4,6-tetra-O-methyl-D-sorbitol TMS ether, 9.7. (b) Column, 3% SE-30 on Chromosorb W, 3 mm×1 m; column temperature: 150°; N_2 flow: 0.6 kg/cm^2; t_R (min) 2,3,4,6-tetra-O-methyl-D-glucose TMS ether, 3.1; 2,3,4,6-tetra-O-methyl-D-sorbitol TMS ether, 5.7.

Acidic Hydrolysis of Hexa-O-methyl-erythrodiol Bioside (4)——The above hexa-O-methyl-erythrodiol bioside (130 mg) was refluxed with 13 ml of 2 N HCl in MeOH for 2 hr, the solution was neutralized with Ag_2CO_3 and evaporated to dryness. The residue was chromatographed on silica gel with benzene–acetone (4:1) and three products were isolated and identified to be erythrodiol, methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside and methyl 3,4-di-O-methyl-D-glucopyranoside by comparing with the authentic samples. Besides these methylated sugars, a partially methylated biose was isolated and characterised by further methanolysis with 2.5 N HCl in MeOH affording methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside and methyl 3,4-di-O-methyl-D-glucopyranoside.

Erythrodiol——Colorless needles (from $(CH_3)_2CO$), mp 234—236°. The identification was carried out by comparing with the authentic sample by mixed mp, TLC (plate, Kieselgel H; solvent, benzene–acetone (4:1), *Rf* 0.60) and IR spectra.

Methyl 2,3,4,6-Tetra-O-methyl-D-glucopyranoside (6) and Methyl 3,4-Di-O-methyl-D-glucopyranoside (7)——TLC: plate, Kieselgel H; solvent, benzene–acetone (4:1); *Rf*, (**6**) 0.34, 0.46; (**7**) 0.10 (0.21 trace).

GLC: (a) Column, 5% NPGS on Chromosorb W, 3 mm×2 m; column temperature 110°; N_2 flow, 1.0 kg/cm^2; t_R (min), (**6**) 3.7, 4.8; (**7**) 3.2. (b) Column, 3% SE-30 on Chromosorb W, 3 mm×1 m; column temperature: 110°; N_2 flow: 1.0 kg/cm^2; t_R (min), (6) 4.0, 4.8; (7) 12.5.

Microbiological Cleavage of Chikusetsusaponin V (Formation of Compound O (8))——According to the cleavage method of saponin by Yoshioka & Kitagawa, chikusetsusaponin V (900 mg) was cultivated at

27° for 10 days under shaking with *cephalosporium* species, which was used for the hydrolysis of chikusetsusaponin IV. The culture medium was extracted with $CHCl_3$, washed with water, dried over Na_2SO_4 and evaporated. The residue was recrystallized from acetone, affording colorless needles, mp 245—246°, and identified with the authentic compound O which was obtained from chikusetsusaponin IV by comparing mixed mp, TLC (plate; Kieselgel H; solvent, $CHCl_3$–MeOH, (6:1), *Rf* 0.48) and IR spectra. The product was derivated to the acetate by usual manner and identified to be acetyl compound O by comparing with the authentic sample by mixed mp (mp 116—118°), TLC (above condition *Rf* 0.80) and IR spectra.

Partially Hydrolysis of Chikusetsusaponin V——Chikusetsusaponin V (3.4 g) was heated with 100 ml of 2% H_2SO_4 for 4 hr. The precipitate was collected by filtration, washed with water and then dried. The mixture of prosapogenin was roughly separated by chromatography on silica gel with *n*-BuOH saturated with water and then isolated on silicic acid with $CHCl_3$–MeOH–H_2O (65:35:10, lower phase) to give prosapogenin **1** and prosapogenin **2**.

Prosapogenin 1 (**9**)——White powder from acetone–hexane, mp 210° (decomp.), $[\alpha]_D^{22}$+15.26° (*c*=2.40, MeOH). *Anal.* Calcd. for $C_{36}H_{56}O_9$: C, 68.32; H, 8.92. Found: C, 68.63; H, 8.92. IR ν_{max}^{Nujol} cm^{-1}: 1720 (COOH), 1688 (COOH).

Prosapogenin 2 (**10**)——Colorless needles from EtOH, mp 235° (decomp.), $[\alpha]_D^{22}$+4.69° (*c*=1.07, MeOH). *Anal.* Calcd. for $C_{42}H_{66}O_4 \cdot H_2O$: C, 67.06; H, 8.36. Found: C, 62.17; H, 8.23. IR ν_{max}^{Nujol} cm^{-1}: 1723 (COOH), 1692 (COOH). Prosapogenin **1** and **2** were hydrolysed with 2 N HCl–50% Dioxane–H_2O (1:3) by refluxing on water bath for 3 hr. Each reaction mixture was treated as usual, and glucuronic acid from the former and glucose and glucuronic acid from the latter were identified.

Acknowledgement We are deeply grateful to Prof. N. Komatsu and Mr. N. Nagumo for their guidance and useful discussion throughout this work. Thanks are also due to the members of Analytical Laboratory of this school for elemental analysis and for the measurement of infrared and nuclear magnetic resonance spectra.