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Studies on the Constituents of *Clematis* Species. II.[1] On the Saponins of the Root of *Clematis chinensis* Osbeck. (2)[2]

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Four triterpenoid prosapogenins tentatively named CP_1, CP_3, CP_4 and CP_5 were isolated from the alkaline hydrolysate of the crude saponin obtained from the root of *Clematis chinensis* Osbeck. On the basis of chemical and physicochemical evidence, they were characterized as follows: CP_1 (I), hederagenin 3-O-α-L-arabinopyranoside; CP_3 (II), oleanolic acid 3-O-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside; CP_4 (VI), oleanolic acid 3-O-β-D-ribopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside; CP_5 (VIII), hederagenin 3-O-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

Keywords——*Clematis chinensis* Osbeck; Ranunculaceae; prosapogenin; oleanolic acid glycoside; hederagenin glycoside

In the preceding paper,[1] we reported the isolation and structural elucidation of four prosapogenins tentatively named CP_2, CP_6, CP_7 and CP_8, which were isolated from the alkaline hydrolysate of the crude saponin obtained from the root of *Clematis chinensis* Osbeck (Chinese drug: Wei Ling Xian (威靈仙)). In a continuation of this work, four prosapogenins tentatively named CP_1, CP_3, CP_4 and CP_5 were isolated as described in the experimental section. This paper deals with their isolation and structural elucidation.

CP_1 (I), colorless needles, mp 231—233° (dec.), $[\alpha]_D$ +51.2°, was hydrolyzed with acid to give hederagenin and arabinose, and was identified as hederagenin 3-O-α-L-arabinopyranoside by comparison of its *Rf* value on thin-layer chromatography (TLC) and of its infrared (IR) and proton magnetic resonance (^{1}H-NMR) spectra with those of an authentic sample.[1]

CP_3 (II), a white powder, mp 241—243° (dec.), $[\alpha]_D$ −4.5°, is composed of oleanolic acid, arabinose, rhamnose and xylose. It was partially hydrolyzed with 0.5 N H_2SO_4 in 75% EtOH for 1 hr to give oleanolic acid 3-O-α-L-arabinopyranoside (III)[1] and oleanolic acid 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (IV, $=CP_2$),[1] together with oleanolic acid and unchanged II. II was methylated according to Hakomori[4] to give the permethylate (V). The ^{1}H-NMR spectrum of V showed three anomeric proton signals at 4.39 (1H, doublet, J=5.5 Hz), 4.48 (1H, doublet, J=6.5 Hz) and 5.06 ppm (1H, singlet), which were assigned to the anomeric protons of the arabinose, xylose and rhamnose units, respectively, by comparison with the ^{1}H-NMR spectrum of the permethylate of IV. V was subjected to methanolysis to give methyl oleanolate and methyl pyranosides of 2,3 4-tri-O-methyl-D-xylose, 2,4-di-O-methyl-L-rhamnose and 3,4-di-O-methyl-L-arabinose. The mode of linkage of the terminal D-xylose unit was regarded as β on the basis of the coupling constant of its anomeric proton signal in the ^{1}H-NMR spectrum of V and the molecular rotation difference between II and IV.

Consequently, the structure of CP_3 was established as oleanolic acid 3-O-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

1) Part I: H. Kizu and T. Tomimori, *Chem. Pharm. Bull.*, **27**, 2388 (1979).
2) Presented in part at the 26th Annual Meeting of the Japanese Society of Pharmacognosy, Tokyo, Nov. 1979.
3) Location: *3 Ho, Kanagawa-machi, Kanazawa.*
4) S. Hakomori, *J. Biochem.*, **55**, 205 (1964).

CP_4 (VI), colorless needles, mp 236—238° (dec.), $[\alpha]_D$ −24.3°, consisting of oleanolic acid, arabinose, rhamnose and ribose, was partially hydrolyzed with 0.5 N H_2SO_4 in 75% EtOH for 1 hr to give III and IV, together with oleanolic acid and unchanged VI. The ^{1}H-NMR spectrum of the permethylate (VII) of VI showed three anomeric proton signals at 4.30 (1H, doublet, J=5.5 Hz, arabinose unit), 4.98 (1H, doublet, J=4.8 Hz, ribose unit) and 5.14 ppm (1H, singlet, rhamnose unit). On methanolysis, VII liberated methyl oleanolate and methyl pyranosides of 2,3,4-tri-O-methyl-D-ribose, 2,4-di-O-methyl-L-rhamnose and 3,4-di-O-methyl-L-arabinose. The β-linkage of the terminal D-ribose unit was suggested by the molecular rotation difference between IV and VI.

On the basis of these results, VI was established as oleanolic acid 3-O-β-D-ribopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

CP_5 (VIII), a white powder, mp 229—232° (dec.), $[\alpha]_D$ +6.0°, is composed of hederagenin, arabinose, rhamnose and xylose. It was partially hydrolyzed with 0.5 N H_2SO_4 in 75% EtOH for 1 hr to give I and hederagenin 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (IX),[1] together with hederagenin and unchanged VIII. The ^{1}H-NMR spectrum of the permethylate (X) of VIII exhibited three anomeric proton signals at 4.30 (1H, doublet, J=6.0 Hz), 4.53 (1H, doublet, J=5.8 Hz) and 5.14 ppm (1H, singlet), which were assigned to the anomeric protons of the arabinose, xylose, and rhamnose units, respectively, by comparison with the ^{1}H-NMR spectrum of the permethylate of IX. X was methanolyzed to yield 23-O-methyl-hederagenin methylester and the same methylated sugars as in the case of V. The molecular rotation difference between IX and VIII and the coupling constant of the anomeric proton signal in the ^{1}H-NMR spectrum of X indicated that the mode of linkage of the terminal D-xylose unit in VIII was β.

Thus, the structure of VIII was established as hederagenin 3-O-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside; this compound has already been isolated from *Akebia quinata*.[5]

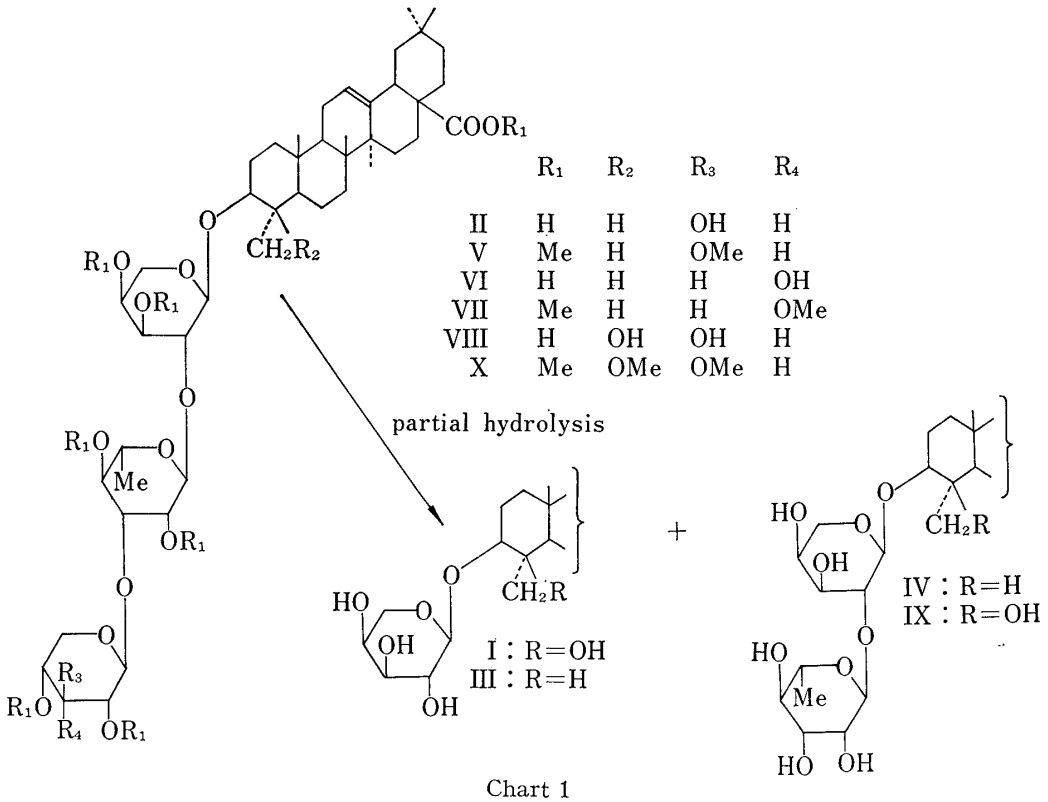

Chart 1

5) R. Higuchi and T. Kawasaki, *Chem. Pharm. Bull.*, **24**, 1021 (1976).

Work on other prosapogenins and genuine saponins in this plant is in progress.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus, and are uncorrected. ^{1}H-NMR spectra were taken at 100 MHz with a JEOL-JNM-MH-100 spectrometer in $CDCl_3$ solution unless otherwise stated, and chemical shifts are given as δ (ppm) with tetramethylsilane as an internal standard. IR spectra were obtained with a JASCO-IR-A-2 spectrometer. Optical rotations were measured with a JASCO-DIP-4 digital polarimeter. Gas-liquid chromatography (GLC) was run on a Shimadzu GC-6AM unit with a flame ionization detector, using glass columns (2 m×4 mmϕ) packed with 5% SE-30 on Chromosorb W (60—80 mesh) (GLC-1) or with 15% 1,4-butanediol succinate on Chromosorb W (100—120 mesh) (GLC-2); column temperature 150° (GLC-1) or 198° (GLC-2). TLC was performed on Kieselgel G (Merck) using the following solvent systems: a) $CHCl_3$–MeOH–HCOOH (15: 1: trace), b) toluene–HCOOH–HCOOEt (5: 1: 4), c) $CHCl_3$–MeOH–H_2O (25: 8: 1.2), d) benzene–acetone (5: 2), and spots were detected by spraying 10% H_2SO_4 followed by heating.

Isolation——A prosapogenin mixture (100 g) obtained by alkaline hydrolysis of the crude saponin (340 g)[1] was chromatographed on silica gel (2 kg), eluting with $CHCl_3$–MeOH–H_2O (25: 3: 0.3), to give crude CP_1 (1.7 g), a mixture of CP_3 and CP_4 (1.9 g), and a mixture of CP_5 and CP_6 (7.2 g). Crude CP_1 (1.7 g) was rechromatographed on silica gel (170 g), eluting with a gradient of $CHCl_3$–MeOH (MeOH 0—6%), to give pure CP_1 (50 mg). A mixture of CP_3 and CP_4 (950 mg) was acetylated with acetic anhydride (10 ml) and pyridine (10 ml) at room temperature for 20 hr. The reaction mixture was treated by the usual procedure to give the acetates, which were subjected to silica gel (100 g) column chromatography, eluting with a gradient of benzene–HCOOEt (HCOOEt 0—20%), to give the peracetates of CP_3 and CP_4. Each acetate thus obtained was deacetylated with 0.5 N KOH at room temperature for 20 hr, neutralized with dil. H_2SO_4, then extracted with *n*-BuOH. After removal of the solvent, the residue was passed through a silica gel (30 g) column, eluting with $CHCl_3$–MeOH (85: 15), to give CP_3 (160 mg) and CP_4 (470 mg). A mixture of CP_5 and CP_6 (1 g) was acetylated and worked up as described above to give CP_5 (140 mg) and CP_6 (430 mg).

CP_1 (I)——Colorless needles (MeOH), mp 231—233° (dec.), $[\alpha]_D$ +51.2° (c=0.97, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400, 1690. ^{1}H-NMR (in pyridine-d_5): 0.89 (3H, singlet), 0.93 (6H, singlet), 1.00 (6H, singlet), 1.23 (3H, singlet), 4.87 (1H, doublet, J=7.0 Hz, anomeric H), 5.40 (1H, broad singlet, C_{12}–H). *Anal.* Calcd for $C_{35}H_{56}O_8 \cdot H_2O$: C, 67.49; H, 9.39. Found: C, 67.22; H, 9.42. I (10 mg) in 2 N HCl–MeOH (2 ml) was heated under reflux for 2 hr. The reaction mixture was neutralized with Ag_2CO_3 and the precipitate was filtered off. The filtrate was concentrated and the residue was crystallized from MeOH to give colorless needles (4 mg), mp>300°, which were identified as hederagenin by direct comparison with an authentic sample (TLC (solv. a, b), IR). The filtrate after crystallization was examined by TLC (solv. c) and GLC-1 (as the trimethylsilylether derivative), revealing the presence of methyl arabinoside (retention time (t_R) 8.7 min). I was identified as hederagenin 3-O-α-L-arabinopyranoside[1] by direct comparison (TLC, IR, ^{1}H-NMR, mixed fusion).

CP_3 (II)——A white powder (dil. MeOH), mp 241—243° (dec.), $[\alpha]_D$ −4.5° (c=1.00, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400, 1690. *Anal.* Calcd for $C_{46}H_{74}O_{15} \cdot H_2O$: C, 62.42; H, 8.66. Found: C, 62.18; H, 8.70. $\Delta[M]_D$: II—IV, −68.3°, $[M]_D$ of methyl D-xylopyranoside: α, +253°; β, −108°.

Hydrolysis of II——i) II (10 mg) was hydrolyzed with 2 N HCl–MeOH (2 ml) under reflux for 2 hr and worked up in the same way as I to give the aglycone (4 mg) and a mixture of methyl glycosides. The aglycone was crystallized from MeOH to give colorless needles, mp 307—308°, which were identified as oleanolic acid by TLC (solv. a,b) and IR. The sugar portion was treated with 2 N HCl aq. on a boiling water bath for 2 hr and after neutralization with Ag_2CO_3, the precipitate was filtered off. The filtrate was concentrated and analyzed by GLC-1 (as the trimethylsilylether derivative), revealing the presence of arabinose (t_R 12.8, 14.7 min), rhamnose (t_R 13.6, 18.6 min) and xylose (t_R 19.7, 26.4 min).

ii) II (100 mg) was partially hydrolyzed with 0.5 N H_2SO_4 in 75% EtOH (5 ml) under reflux for 1 hr. After neutralization with 0.5 N KOH, the reaction mixture was concentrated and partitioned with water and *n*-BuOH. The *n*-BuOH extract was concentrated and chromatographed on silica gel (10 g), eluting with $CHCl_3$–MeOH–H_2O (25: 3: 0.3), to give oleanolic acid 3-O-α-L-arabinopyranoside (III) (34 mg) and oleanolic acid 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (IV, =CP_2)[1] (11 mg). These were identical with the corresponding authentic samples as determined by direct comparison (TLC (solv. c), IR, ^{1}H-NMR, mixed fusion).

Permethylate (V) of II——II (40 mg) was methylated according to Hakomori.[4] The reaction mixture was diluted with ice-water and extracted with AcOEt. The AcOEt extract was concentrated and the residue (42 mg) was passed through a silica gel (10 g) column, eluting with benzene–acetone (91: 9), to give the permethylate (V) as a white powder (24 mg) (dil. MeOH), mp 121—122°. IR (KBr): no OH. ^{1}H-NMR: 4.39 (1H, doublet, J=5.5 Hz, C_1–H of arabinose unit), 4.48 (1H, doublet, J=6.5 Hz, C_1–H of xylose unit), 5.06 (1H, singlet, C_1–H of rhamnose unit). *Anal.* Calcd for $C_{54}H_{90}O_{15}$: C, 66.23; H, 9.26. Found: C, 66.31; H, 9.42.

Methanolysis of V——V (15 mg) was methanolyzed with 2 N HCl–MeOH (2 ml) under reflux for 2 hr and treated in the same way as I to give the aglycone (5 mg) as colorless needles, mp 197—198°; this material was identified as methyl oleanolate by direct comparison (TLC, IR, mixed fusion) with an authentic sample. The mother liquor of crystallization was examined by TLC (solv. d) and GLC-2, revealing the presence of methyl pyranosides of 2,3,4-tri-O-methyl-D-xylose (t_R 4.7, 5.7 min), 2,4-di-O-methyl-L-rhamnose (t_R 10.4 min) and 3,4-di-O-methyl-L-arabinose (t_R 16.8, 33.7 min).

CP_4 (VI)——Colorless needles (MeOH), mp 236—238° (dec.), $[\alpha]_D$ −24.3° (c=2.75, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400, 1690. *Anal.* Calcd for $C_{46}H_{74}O_{15}\cdot H_2O$: C, 62.42; H, 8.66. Found: C, 62.32; H, 8.69. $\Delta[M]_D$: VI—IV, −239.7°, $[M]_D$ of methyl D-ribopyranoside: α, +170°; β, −186°.

Hydrolysis of VI——i) VI (10 mg) was hydrolyzed with 2 N HCl–MeOH (2 ml) under reflux for 2 hr and treated in the same way as I to give the aglycone (3 mg) as colorless needles; this compound was identified as oleanolic acid by TLC (solv. a,b) and IR. The sugar portion was treated with 2 N HCl aq., worked up in the same way as II, and analyzed by GLC-1, revealing the presence of arabinose (t_R 12.8, 14.7 min), rhamnose (t_R 13.6, 18.6 min) and ribose (t_R 15.4, 16.2 min).

ii) VI (200 mg) was partially hydrolyzed with 0.5 N H_2SO_4 in 75% EtOH (10 ml) under reflux for 1 hr and treated in the same way as II. The resulting hydrolysate was chromatographed on silica gel (20 g), eluting with $CHCl_3$–MeOH–H_2O (25: 3: 0.3), to give III (40 mg) and IV (33 mg), which were identical with the corresponding authentic samples on direct comparison (TLC (solv. c), IR, ^{1}H-NMR, mixed fusion).

Permethylate (VII) of VI——VI (100 mg) was methylated and treated in the same way as II. The product was passed through a silica gel (10 g) column, eluting with benzene–acetone (9: 1), to give the permethylate (VII) (68 mg) as a white powder, mp 123—125°. IR (KBr): no OH. ^{1}H-NMR: 4.35 (1H, doublet, J=5.5 Hz, C_1–H of arabinose unit), 4.98 (1H, doublet, J=4.8 Hz, C_1–H of ribose unit), 5.11 (1H, singlet, C_1–H of rhamnose unit). *Anal.* Calcd for $C_{54}H_{90}O_{15}$: C, 66.23; H, 9.26. Found: C, 66.28; H, 9.35.

Methanolysis of VII——VII (20 mg) was methanolyzed and worked up in the same way as I to give the aglycone (7 mg) as colorless needles; this compound was identified as methyl oleanolate by direct comparison (TLC, IR, mixed fusion). Methylated sugars in the mother liquor were identified as methyl pyranosides of 2,3,4-tri-O-methyl-D-ribose (t_R 8.0, 11.9 min), 2,4-di-O-methyl-L-rhamnose (t_R 10.4 min) and 3,4-di-O-methyl-L-arabinose (t_R 16.8, 33.7 min) by TLC (solv. d) and GLC-2.

CP_5 (VIII)——A white powder (dil. MeOH), mp 229—232° (dec.), $[\alpha]_D$ +6.0° (c=1.00, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400, 1690. *Anal.* Calcd for $C_{46}H_{74}O_{16}\cdot H_2O$: C, 61.31; H, 8.50. Found: C, 61.71; H, 8.72. $\Delta[M]_D$: VIII—IX, −88.1°, $[M]_D$ of methyl D-xylopyranoside: α, +253°; β, −108°.

Hydrolysis of VIII——i) VIII (10 mg) was hydrolyzed with 2 N HCl–MeOH (2 ml) under reflux for 2 hr and worked up in the same way as II to give the aglycone (3 mg) as colorless needles, together with arabinose, rhamnose and xylose. The aglycone was identified as hederagenin by direct comparison (TLC, IR) with an authentic sample.

ii) VIII (100 mg) was partially hydrolyzed with 0.5 N H_2SO_4 in 75% EtOH (5 ml) under reflux for 1 hr and worked up in the same way as II. The resulting hydrolysate was chromatographed on silica gel (10 g), eluting with $CHCl_3$–MeOH–H_2O (25: 3: 0.3), to give hederagenin 3-O-α-L-arabinopyranoside (I) (26 mg) and hederagenin 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (IX) (9 mg). These were identical with the corresponding authentic samples[1] as judged by direct comparison (TLC, IR, mixed fusion).

Permethylate (X) of VIII——VIII (30 mg) was methylated and treated in the same way as II. The resulting product was passed through a silica gel (8 g) column, eluting with benzene–acetone (91: 9), to give the permethylate (X) as a white powder (dil. MeOH) (22 mg), mp 117—122°. IR (KBr): no OH. ^{1}H-NMR: 4.30 (1H, doublet, J=6.0 Hz, C_1–H of arabinose unit), 4.53 (1H, doublet, J=5.8 Hz, C_1–H of xylose unit), 5.14 (1H, singlet, C_1–H of rhamnose unit). *Anal.* Calcd for $C_{55}H_{99}O_{16}$: C, 65.45; H, 9.19. Found: C, 65.52; H, 9.28.

Methanolysis of X——X (15 mg) was methanolyzed and worked up as described for I to give the aglycone (6 mg) as colorless needles and the same methylated sugars as VI. The aglycone was identified as 23-O-methyl-hederagenin methylester by direct comparison (TLC, IR, mixed fusion).

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