[Chem. Pharm. Bull.
30(3) 859—865 (1982)]

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

Haruhisa Kizu and Tsuyoshi Tomimori*

School of Pharmacy, Hokuriku University, 3 Ho, Kanagawa-machi, Kanazawa, 920-11, Japan

(Received July 31, 1981)

Four triterpenoid prosapogenins named CP_{7a}, CP_{8a}, CP_{9a} and CP_{10a} have been 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_{7a} (I), oleanolic acid 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside; CP_{8a} (VI), hederagenin 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside; CP_{9a} (XI), oleanolic acid 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside; CP_{10a} (XIII), hederagenin 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

Keywords——*Clematis chinensis*; Ranunculaceae; prosapogenin; oleanolic acid glycoside; hederagenin glycoside; ^{13}C-NMR

In the previous papers,[1,3] we reported the isolation and structural elucidation of twelve prosapogenins tentatively named CP_1–CP_{10}, CP_{2b} and CP_{3b}, which had been isolated from the alkaline hydrolysate of the crude saponin obtained from the root of *Clematis chinensis* Osbeck (Chinese drug: Wei Ling Xian (威霊仙)). In a continuation of the study, four new prosapogenins tentatively named CP_{7a}, CP_{8a}, CP_{9a} and CP_{10a} were isolated by repeated silica gel column chromatography as described in the experimental section. The present paper deals with the structural elucidation of these compounds.[4]

CP_{7a} (I), a white powder, $[\alpha]_D$ −18.1°, was hydrolyzed with acid to give oleanolic acid, arabinose, glucose, rhamnose and xylose. The carbon nuclear magnetic resonance (^{13}C-NMR) spectrum of I showed four anomeric carbon signals at 101.6, 103.7, 104.6 and 106.7 ppm. I was partially hydrolyzed with 0.5 N H_2SO_4 in 75% EtOH to give three partial hydrolysis products, II, III and IV, together with oleanolic acid and unchanged I. Enzymatic hydrolysis of I with cellulase gave IV and unchanged I. II, III and IV were identified as 3-*O*-α-L-arabinopyranoside, 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (*i.e.* CP_2), and 3-*O*-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (*i.e.* CP_3) of oleanolic acid, respectively, as described in detail in the experimental section.[3] These results suggest that I has one glucose unit attached to the sugar chain of IV. I was methylated according to Hakomori[5] to give the permethylate (V). In the proton nuclear magnetic resonance (^1H-NMR) spectrum, V showed four anomeric proton signals at 4.28 (1H, d, J=7.0 Hz), 4.41 (1H, d, J=5.2 Hz), 4.52 (1H, d, J=7.0 Hz) and 5.04 ppm (1H, s), which were assigned as those of the glucose, arabinose, xylose and rhamnose units, respectively, by comparison with the ^1H-NMR spectrum of the permethylate of IV.[3b] V was methanolyzed to give methyl oleanolate, methyl 2,3-di-*O*-methyl-xylosides and methyl pyranosides of 2,3,4,6-tetra-*O*-methyl-glucose, 2,4-di-*O*-methyl-rhamnose and 3,4-di-*O*-methyl-arabinose. The mode of linkage of the terminal D-glucose unit was regarded as β based on the coupling constant (J=7.0 Hz) of its anomeric proton signal in the ^1H-NMR spectrum of V and the molecular rotation difference between IV and I.

On the basis of all the above results, the structure of I was established as oleanolic acid 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

CP_{8a} (VI), a white powder, $[\alpha]_D$ −8.1°, is composed of hederagenin, arabinose, glucose, rhamnose and xylose. In the ^{13}C-NMR spectrum, the carbon signals due to the sugar moiety of VI are observed to be almost superimposable on those of I, suggesting that VI possesses the same sugar moiety as I. On partial hydrolysis with mild acid, VI yielded three partial hydrolysis products, VII, VIII and IX, together with hederagenin and unchanged VI. Enzymatic hydrolysis of VI with emulsin gave IX and unchanged VI. VII, VIII and IX were identified as 3-*O*-α-L-arabinopyranoside (*i.e.* CP_1), 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (*i.e.* CP_{3b}) and 3-*O*-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (*i.e.* CP_5) of hederagenin, respectively.[1,3b] VI is, therefore, suggested to be the compound having a glucose unit combined with IX. The ^1H-NMR spectrum of the permethylate (X) of VI showed four anomeric proton signals at 4.27 (2H, d, J=7.0 Hz), 4.53 (1H, d, J=7.0 Hz) and 5.11 ppm (1H, s), which were assigned as those of the arabinose and glucose, xylose and rhamnose units, respectively, by comparison with ^1H-NMR spectrum of the permethylate of IX.[3b] X was methanolyzed to yield 23-*O*-methyl-hederagenin methyl ester and the same methylated sugars as in the case of V. The mode of linkage of the terminal D-glucose unit was regarded as β based on the coupling constant (J=7.0 Hz) of its anomeric

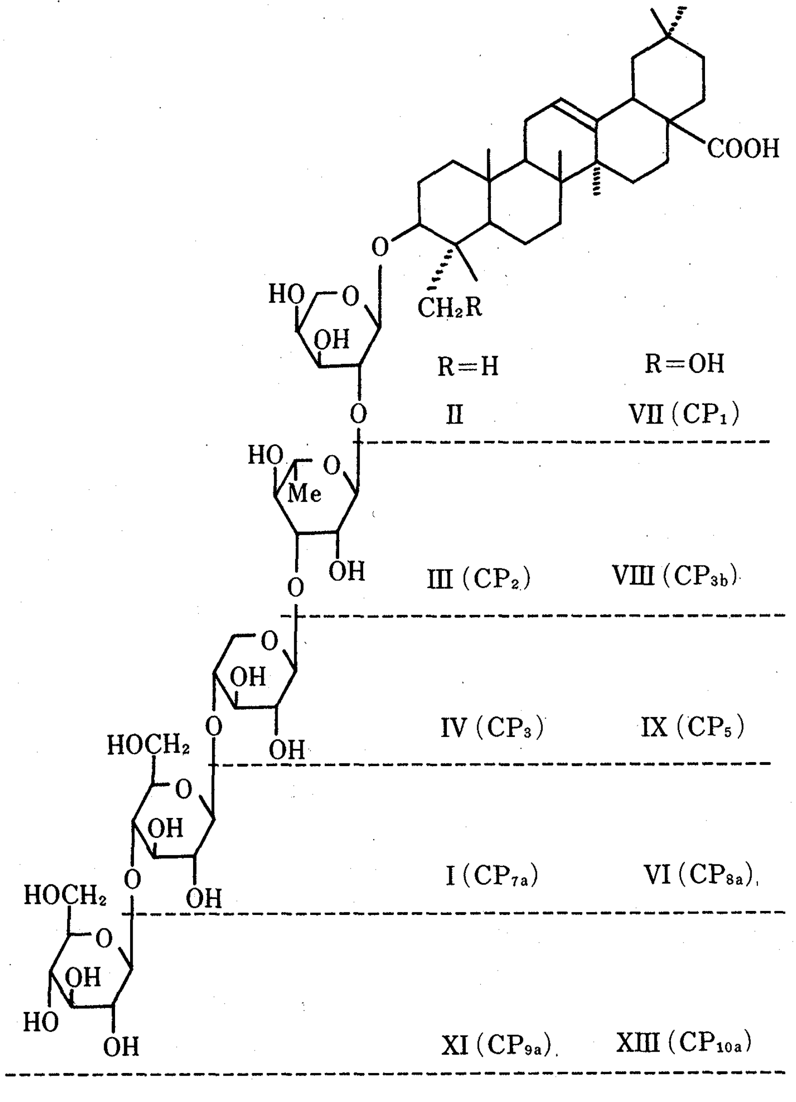

Chart 1

proton signal in the ^{1}H-NMR spectrum of X and the molecular rotation difference between IX and VI.

From these results, the structure of VI was established as hederagenin 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

CP$_{9a}$ (XI), a white powder, $[\alpha]_D$ −16.8°, consisting of oleanolic acid, arabinose, glucose, rhamnose and xylose, was partially hydrolyzed with mild acid to give four partial hydrolysis products, II, III, IV and I, together with oleanolic acid and unchanged XI. On enzymatic hydrolysis with cellulase, XI gave IV and I together with unchanged XI. The ^{1}H-NMR spectrum of the permethylate (XII) of XI exhibited five anomeric proton signals at 4.31 (2H, d, J=7.0 Hz), 4.41 (1H, d, J=5.5 Hz), 4.53 (1H, d, J=7.0 Hz) and 5.08 ppm (1H, s) ,which were assigned as those of the glucose (×2), arabinose, xylose and rhamnose units, respectively, by comparison with the ^{1}H-NMR spectrum of V. XII was methanolyzed to yield methyl oleanolate, methyl 2,3,6-tri-*O*-methyl-glucopyranoside and the same methylated sugars as in the case of V. The mode of linkage of the terminal D-glucose unit was regarded as β on the basis of the coupling constant (J=7.0 Hz) of its anomeric proton signal in the ^{1}H-NMR spectrum of XII and the molecular rotation difference between I and XI.

TABLE I. ^{13}C-NMR Chemical Shifts in Pyridine-$d_5$$^{a)}$

	Aglycone moieties$^{b)}$					Sugar moieties$^{b)}$			
	IV	IX	I	VI		IV	IX	I	VI
C-1	38.8	39.1	39.1	39.2	Arabinose				
2	26.5	26.2	26.6	26.2	C-1	105.1	104.6	104.6	104.2
3	88.8	81.2	89.1	81.4	2	75.4	75.5	76.1	75.9
4	39.5	43.6	39.6	43.7	3	74.3	75.0	73.4	74.3
5	56.0	47.7	56.2	47.9	4	69.0	69.6	68.4	69.0
6	18.8	18.1	18.7	18.3	5	65.4	66.2	64.4	65.3
7	33.3	32.9	33.3	33.0					
8	39.6	39.7	39.9	39.9	Rhamnose				
9	48.0	48.2	48.2	48.3	C-1	101.4	101.4	101.6	101.5
10	37.0	36.9	37.2	37.0	2	71.8	71.9	71.9	71.9
11	23.8	23.8	23.9	23.9	3	82.8	83.1	83.1	83.3
12	122.7	122.7	122.7	122.6	4	72.9	73.0	72.8	72.5
13	144.9	144.9	145.1	145.1	5	69.6	69.6	69.8	69.7
14	42.1	42.1	42.3	42.3	6	18.3	18.4	18.3	18.3
15	28.1	28.3	28.4	28.5					
16	23.8	23.8	23.9	23.9	Xylose				
17	46.6	46.6	46.8	46.8	C-1	107.2	107.5	106.7	106.9
18	41.9	42.0	42.1	42.1	2	75.4	75.2	74.3	74.3
19	46.6	46.6	46.8	46.8	3	78.2	78.3	76.2	76.2
20	30.9	31.0	31.0	31.0	4	70.9	71.0	77.9$^{c)}$	78.0$^{c)}$
21	34.3	34.2	34.5	34.4	5	67.2	67.3	64.8	64.9
22	33.3	33.3	33.3	33.3					
23	28.1	64.1	28.4	64.5	Glucose				
24	17.0	14.1	17.0	13.9	C-1			103.7	103.7
25	15.5	16.1	15.6	16.1	2			75.2	75.2
26	17.2	17.4	17.4	17.5	3			78.6$^{c)}$	78.6$^{c)}$
27	26.1	26.2	26.2	26.2	4			71.9	71.9
28	180.3	180.3	180.3	180.3	5			78.2$^{c)}$	78.2$^{c)}$
29	33.3	33.3	33.3	33.3	6			62.8	62.8
30	23.8	23.8	23.9	23.9					

a) Measurement conditions: spectral width, 5000 Hz; pulse width, 8 μsec (pulse flipping angle, *ca.* 55°); concentration, *ca.* 0.2—0.3 M; temperature, 23°C; acquisition time, 0.8192 sec; number of data points, 8192; pulse repetition, 1.8—2.0 sec; number of pulses, 2000—50000.

b) For details of assignments of ^{13}C signals, refer to the following paper: S. Seo, Y. Tomita, K. Tori and Y. Yoshimura, *J. Am. Chem. Soc.*, **100**, 3331 (1978).

c) These assignments may be reversed in each column.

On the basis of all the above results, the structure of XI was established as oleanolic acid 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

CP_{10a} (XIII), a white powder, $[\alpha]_D$ −10.1°, is composed of hederagenin, arabinose, glucose, rhamnose and xylose. XIII was partially hydrolyzed with mild acid to give four partial hydrolysis products, VII, VIII, IX and VI, together with hederagenin and unchanged XIII. On enzymatic hydrolysis with emulsin, XIII gave IX and VI together with unchanged XIII. The ^{1}H-NMR spectrum of the permethylate (XIV) of XIII showed five anomeric proton signals at 4.18—4.38 (3H, m), 4.54 (1H, d, J=7.0 Hz) and 5.12 ppm (1H, s). XIV was methanolyzed to yield 23-O-methyl-hederagenin methyl ester and the same methylated sugars as in the case of XII. The mode of linkage of the terminal D-glucose unit was regarded as β based on the molecular rotation difference between VI and XIII.

From these results, the structure of XIII was established as hederagenin 3-O-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

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 or a JEOL JNM-FX-100 spectrometer and chemical shifts are given as δ (ppm) with tetramethylsilane (TMS) as an internal standard (s: singlet, d: doublet, m: multiplet). ^{13}C-NMR spectra were taken at 25 MHz with a JEOL JNM-FX-100 spectrometer in pyridine-d_5 using TMS as an internal standard. Chemical shifts are given as δ (ppm). The ^{13}C chemical shift data are listed in Table I. 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, programmed from 150°C (20 min hold) to 240°C at 5°C/min (GLC-1) or 198°C (GLC-2). TLC was performed on Kieselgel G (Merck) or HPTLC plates (Silica gel $60F_{254}$, Merck) using the following solvent systems: a) $CHCl_3$–MeOH–HCOOH (100: 8: 1), b) toluene–HCOOH–HCOOEt (5: 1: 4), c) $CHCl_3$–MeOH–H_2O (25: 8: 1.2), d) *sec*-BuOH–AcOEt–H_2O (8: 4: 1), e) *n*-PrOH–$CHCl_3$–H_2O–AcOH (8: 2: 1: 1), f) benzene–acetone (5: 2), g) benzene–acetone (1: 1), h) $CHCl_3$–MeOH (50: 1). Spots were detected by spraying dil. H_2SO_4 followed by heating.

Isolation——A prosapogenin mixture (100 g) obtained by alkaline hydrolysis of the crude saponin (340 g)[3a] was chromatographed on silica gel to give "Fr. 1—Fr. 10" as described in the preceding paper.[1] "Fr. 7" was chromatographed on silica gel with *sec*-BuOH–AcOEt–H_2O (8: 4: 1) repeatedly to give CP_{7a} (800 mg) and CP_7 (2.3 g). "Fr. 8" was chromatographed under the same conditions as "Fr. 7" to give CP_{8a} (1.2 g) and CP_8 (3.2 g). CP_{9a} and CP_{10a} were isolated as described in the preceding paper.[1]

CP_{7a} (I)——A white powder (from MeOH), $[\alpha]_D$−18.1° (c=0.81, MeOH). *Anal.* Calcd for $C_{52}H_{84}O_{20}\cdot 2H_2O$: C, 58.63; H, 8.33. Found: C, 58.46; H, 8.43. IR ν_{max}^{KBr} cm^{-1}: 3400, 1690. $\Delta[M]_D$: I—IV, −147.3°, $[M]_D$ of methyl D-glucopyranoside: α, +309°; β, −66°.

Hydrolysis of I——A solution of I (20 mg) in 2 N HCl–MeOH (2 ml) was heated under reflux for 2 h. 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 (5 mg), mp 306—308°C, which were identified as oleanolic acid by direct comparison (TLC (solv. a, b), IR, ^{1}H-NMR) with an authentic sample. The mother liquor of crystallization was concentrated and treated with 2 N HCl aq. on a boiling water bath for 3 h. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was concentrated and analyzed by GLC-1 (as the trimethylsilylether derivative), which indicated the presence of arabinose (t_R 13′07″, 15′10″), rhamnose (t_R 14′00″, 19′14″), xylose (t_R 20′07″, 24′26″) and glucose (t_R 29′58″, 32′46″).

Partial Hydrolysis of I——I (200 mg) was hydrolyzed with 0.5 N H_2SO_4 in 75% EtOH (30 ml) under reflux for 45 min. The reaction mixture was neutralized with 0.5 N KOH and concentrated. The residue was partitioned between water and *n*-BuOH. The *n*-BuOH extract was concentrated and chromatographed on silica gel (40 g) with a gradient of $CHCl_3$–MeOH (MeOH 0—10%) to give oleanolic acid (25 mg), II (40 mg) and III (12 mg), then with $CHCl_3$–MeOH–H_2O (25: 4: 0.3) to give IV (trace), and finally with $CHCl_3$–MeOH–H_2O (25: 6: 0.5) to recover I (35 mg). II and III were identified as oleanolic acid 3-*O*-α-L-arabinopyranoside and CP_2, respectively, by direct comparison (TLC (solv. c, d, e), IR, ^{1}H-NMR).[3a] IV was examined by TLC (solv. c, d, e) and its mobilities were in good agreement with those of CP_3.

Enzymatic Hydrolysis of I——I (200 mg) in AcOH-AcONa buffer solution (pH 5.5, 100 ml) containing cellulase (Tokyo Kasei Kogyo Co., 200 mg) was shaken at 37°C for 5 d. The reaction mixture was extracted with *n*-BuOH. After removal of the solvent, the residue was subjected to column chromatography on silica gel (30 g) with $CHCl_3$–MeOH–H_2O (25: 4: 0.3→25: 6: 0.5) to give IV (80 mg) and unchanged I (60 mg). IV (10 mg) was hydrolyzed with acid and worked up in the same way as I to give oleanolic acid, arabinose, rhamnose and xylose. IV (20 mg) was partially hydrolyzed with 0.5 N H_2SO_4 in 75% EtOH (5 ml) under reflux for 1 h. The reaction mixture was neutralized with 0.5 N KOH and concentrated. The residue was partitioned with water and *n*-BuOH. The *n*-BuOH extract was washed with water and concentrated. The residue was examined by TLC (solv. b, c, d), which showed four spots. The *Rf* values were identical with those of oleanolic acid, oleanolic acid 3-*O*-α-L-arabinopyranoside, CP_2 and CP_3, respectively. IV was also identified as CP_3 by direct comparison (TLC (solv. c, d, e), IR, ^{1}H-NMR, ^{13}C-NMR).

Permethylate (V) of I——I (100 mg) was methylated according to Hakomori.[5] The reaction mixture was diluted with ice-water and extracted with AcOEt. The AcOEt extract was concentrated and the residue was chromatographed on silica gel (10 g) to give the permethylate (V) (68 mg) as a white powder (from dil. MeOH). *Anal.* Calcd for $C_{63}H_{106}O_{20}$: C, 63.94; H, 9.03. Found: C, 63.79; H, 9.12. IR (KBr): no OH. ^{1}H-NMR ($CDCl_3$): 4.28 (1H, d, $J=7.0$ Hz, C_1–H of glucose unit), 4.41 (1H, d, $J=5.2$ Hz, C_1–H of arabinose unit), 4.52 (1H, d, $J=7.0$ Hz, C_1–H of xylose unit), 5.04 (1H, s, C_1–H of rhamnose unit).

Methanolysis of V——V (30 mg) was methanolyzed with 2 N HCl–MeOH (2 ml) under reflux for 2 h and worked up in the same way as I to give the aglycone (8 mg) as colorless needles, mp 196—197°C; this product was identified as methyl oleanolate by direct comparison (TLC (solv. f, h), IR, ^{1}H-NMR). The mother liquor of crystallization was examined by TLC (solv. f, g) and GLC-2, which indicated the presence of methyl 2,3-di-*O*-methyl-xyloside (t_R 11′22″ 12′05″, 13′10″) and methyl pyranosides of 2,3,4,6-tetra-*O*-methyl-glucose (t_R 8′02″, 10′58″), 2,4-di-*O*-methyl-rhamnose (t_R 9′26″) and 3,4-di-*O*-methyl-arabinose (t_R 15′05″, 29′58″).

CP_{8a} (VI)——A white powder (from dil. MeOH), $[\alpha]_D -8.1°$ ($c=1.20$, MeOH). *Anal.* Calcd for $C_{52}H_{84}O_{21} \cdot 2H_2O$: C, 57.76; H, 8.20. Found: C, 57.61; H, 8.38. IR ν_{max}^{KBr} cm^{-1}: 3400, 1690. $\Delta[M]_D$: VI—VII, −137.3°, $[M]_D$ of methyl D-glucopyranoside: α, +309°; β, −66°.

Hydrolysis of VI——A solution of VI (20 mg) in 2 N HCl–MeOH (2 ml) was heated under reflux for 2 h and worked up in the same way as I to give the aglycone (6 mg), arabinose, glucose, rhamnose and xylose. The aglycone, colorless prisms, mp 310—312°C, was identified as hederagenin by direct comparison (TLC (solv. a, b), IR, ^{1}H-NMR).

Partial Hydrolysis of VI——VI (300 mg) was hydrolyzed with 0.5 N H_2SO_4 in 75% EtOH (50 ml) under reflux for 1 h and worked up in the same way as I. The product was chromatographed on silica gel (50 g) with a gradient of $CHCl_3$–MeOH (MeOH 0—10%) to give hederagenin (30 mg), VII (65 mg) and VIII (15 mg), then with $CHCl_3$–MeOH–H_2O (25: 5: 0.4) to give IX (5 mg), and finally with $CHCl_3$–MeOH–H_2O (25: 7: 1) to recover unchanged VI (60 mg). VII and VIII were identified as CP_1 and CP_{3b}, respectively, by direct comparison (TLC (solv. c, d, e), IR, ^{1}H-NMR).[1,3b)] IX was examined by TLC (solv. c, d, e) and its mobilities were in good agreement with those of CP_5.

Enzymatic Hydrolysis of VI——VI (100 mg) in citric acid-Na_2HPO_4 buffer solution (pH 5.0, 50 ml) was treated with almond emulsin (P-L Biochem. Inc., 20 mg) at 37°C for 5 d. The reaction mixture was extracted with *n*-BuOH. The *n*-BuOH extract was washed with water and concentrated. The residue was chromatographed on silica gel (20 g) with $CHCl_3$–MeOH–H_2O (25: 4: 0.3→25: 6: 0.5) to give IX (55 mg) and unchanged VI (25 mg). IX (10 mg) was hydrolyzed with 2 N HCl–MeOH and worked up in the same way as I to give hederagenin, arabinose, rhamnose and xylose. IX (20 mg) was partially hydrolyzed with 0.5 N H_2SO_4 in 75% EtOH (5 ml) for 1 h and worked up in the same way as I. The product was examined by TLC (solv. b, c, d, e), which showed four spots. The *Rf* values were identical with those of hederagenin, CP_1, CP_{3b} and CP_5. IX was identified as CP_5 by direct comparison (TLC, IR, ^{1}H-NMR, ^{13}C-NMR).[3b)]

Permethylate (X) of VI——VI (150 mg) was methylated and worked up in the same way as I. The product was chromatographed on silica gel (15 g) with benzene–acetone (17: 3) to give the permethylate (X) (100 mg) as a white powder (from dil. MeOH). *Anal.* Calcd for $C_{64}H_{108}O_{21}$: C, 63.34; H, 8.97. Found: C, 63.41; H, 9.04. IR (KBr): no OH. ^{1}H-NMR ($CDCl_3$): 4.27 (1H, d, $J=7.0$ Hz, C_1–H of glucose and arabinose units), 4.53 (1H, d, $J=7.0$ Hz, C_1–H of xylose unit), 5.11 (1H, s, C_1–H of rhamnose unit).

Methanolysis of X——X (30 mg) was methanolyzed and worked up in the same way as V to give the aglycone (11 mg) and the same methylated sugars as in the case of V. The aglycone, colorless needles, mp 190—191°C, was identified as 23-*O*-methyl-hederagenin methyl ester by direct comparison (TLC (solv. f, h), IR, ^{1}H-NMR).

CP_{9a} (XI)——A white powder (from MeOH), $[\alpha]_D -16.8°$ ($c=0.92$, MeOH). *Anal.* Calcd for $C_{58}H_{94}O_{25} \cdot 4H_2O$: C, 55.14; H, 8.14. Found: C, 55.02; H, 8.27. IR ν_{max}^{KBr} cm^{-1}: 3400, 1690. $\Delta[M]_D$: XI—I, −13.6°, $[M]_D$ of methyl D-glucopyranoside: α, +309°; β, −66°.

Hydrolysis of XI——A solution of XI (30 mg) in 2 N HCl–MeOH (3 ml) was heated under reflux for 2 h and worked up in the same way as I to give oleanolic acid together with arabinose, glucose, rhamnose and xylose.

Partial Hydrolysis of XI——XI (300 mg) was hydrolyzed with 0.5 N H_2SO_4 in 75% EtOH (30 ml) under reflux for 1 h and worked up in the same way as I. The product was chromatographed on silica gel (50 g) with a gradient of $CHCl_3$–MeOH (MeOH 0—10%) to give oleanolic acid (18 mg), II (35 mg) and III (10 mg), then with $CHCl_3$–MeOH–H_2O (25: 5: 0.4) to give IV (trace), and finally with $CHCl_3$–MeOH–H_2O (25: 8: 1.2) to give I (trace) and unchanged XI (45 mg). II and III were identified as oleanolic acid 3-*O*-α-L-arabinopyranoside and CP_2, respectively, by direct comparison (TLC (solv. b, c, d), IR, ^{1}H-NMR). IV and I were examined by TLC (solv. c, d, e) and their mobilities were in good agreement with those of CP_3 and CP_{7a}, respectively.

Enzymatic Hydrolysis of XI——XI (200 mg) in AcOH–AcONa buffer solution (pH 5.5, 100 ml) was treated with cellulase (200 mg) at 37°C for 5 d. The reaction mixture was worked up in the same way as I. The product was chromatographed on silica gel (30 g) with $CHCl_3$–MeOH–H_2O (25: 4: 0.3→25: 6: 0.6→25: 8: 1.2) to give IV (35 mg), I (45 mg) and unchanged XI (60 mg). IV was identified as CP_3 by direct comparison (TLC (solv. c, d, e), IR, ^{1}H-NMR, ^{13}C-NMR).[4b)] I (10 mg) was acid-hydrolyzed to give oleanolic acid, arabinose, glucose, rhamnose and xylose. I (20 mg) was partially hydrolyzed with 0.5 N H_2SO_4 in 75% EtOH for 1 h and worked up in the same way as I to give five compounds. Their *Rf* values on TLC (solv. b, c, d, e) were identical with those of oleanolic acid, oleanolic acid 3-*O*-α-L-arabinopyranoside, CP_2, CP_3 and CP_{7a}. I was identified as CP_{7a} by direct comparison (TLC, IR, ^{1}H-NMR, ^{13}C-NMR).

Permethylate (XII) of XI——XI (200 mg) was methylated and worked up in the same way as I. The reaction product was chromatographed on silica gel (20 g) with a gradient of benzene–acetone (acetone 0—20%) to give the permethylate (XII) (145 mg) as a white powder (dil. MeOH). *Anal.* Calcd for $C_{72}H_{122}O_{25}$: C, 62.32; H, 8.86. Found: C, 62.23; H, 8.96. IR (KBr): no OH. ^{1}H-NMR ($CDCl_3$): 4.31 (2H, d, J=7.0 Hz, C_1–H of glucose unit×2), 4.41 (1H, d, J=5.5 Hz, C_1–H of arabinose unit), 4.53 (1H, d, J=7.0 Hz, C_1–H of xylose unit), 5.08 (1H, s, C_1–H of rhamnose unit).

Methanolysis of XII——XII (65 mg) was methanolyzed and worked up in the same way as V to give the aglycone (10 mg) as colorless needles; this product was identified as methyl oleanolate by direct comparison (TLC (solv. f, h), IR, ^{1}H-NMR). The methylated sugar portion was analyzed by TLC (solv. f, g) and GLC-2, which indicated the presence of methyl 2,3,6-tri-*O*-methyl-glucopyranoside (t_R 24′26″, 31′34″) and the same methylated sugars as in the case of V.

CP_{10a} (XIII)——A white powder (from dil. MeOH), $[\alpha]_D$ −10.1° (c=1.00, MeOH). *Anal.* Calcd for $C_{58}H_{94}O_{26}\cdot 4H_2O$: C, 54.45; H, 8.04. Found: C, 54.35; H, 7.88. IR ν_{max}^{KBr} cm^{-1}: 3400, 1690. $\Delta[M]_D$: XIII—VI, −37.4°, $[M]_D$ of methyl D-glucopyranoside: α, +309°; β, −66°.

Hydrolysis of XIII——XIII (20 mg) was hydrolyzed with 2 N HCl–MeOH and worked up in the same way as I to give the aglycone (3 mg) as colorless prisms together with arabinose, glucose, rhamnose and xylose. The aglycone was identified as hederagenin by direct comparison (TLC (solv. a, b), IR).

Partial Hydrolysis of XIII——XIII (400 mg) was hydrolyzed with 0.5 N H_2SO_4 in 75% EtOH (50 ml) under reflux for 1 h and worked up in the same way as I. The product was chromatographed on silica gel (50 g) with a gradient of $CHCl_3$–MeOH (MeOH 0—12%) to give hederagenin (20 mg), VII (50 mg) and VIII (12 mg), then with $CHCl_3$–MeOH–H_2O (25: 6: 0.5) to give IX (trace), and finally with $CHCl_3$–MeOH–H_2O (25: 8: 1.2) to give VI (trace) and XIII (50 mg). VII and VIII were identified as CP_1 and CP_{3b}, respectively, by direct comparison (TLC (solv. b, c, d), IR, ^{1}H-NMR). IX and VI were examined by TLC (solv. c, d, e) and their mobilities were in good agreement with those of CP_5 and CP_{8a}, respectively.

Enzymatic Hydrolysis of XIII——XIII (200 mg) in citric acid-Na_2HPO_4 buffer solution (100 ml) was treated with emulsin (30 ml) at 37°C for 5 d. The reaction mixture was worked up in the same way as I. The product was chromatographed on silica gel (20 g) with $CHCl_3$–MeOH–H_2O (25: 4: 0.3→25: 6: 0.6→25: 8: 1.2) to give IX (50 mg), VI (40 mg) and unchanged XIII (50 mg). IX was identified as CP_5 by direct comparison (TLC (solv. c, d, e), IR, ^{1}H-NMR, ^{13}C-NMR). VI was acid-hydrolyzed to give hederagenin, arabinose, glucose, rhamnose and xylose. On partial hydrolysis with 0.5 N H_2SO_4 in 75% EtOH (5 ml) for 1 h, VI (20 mg) gave five compounds. Their *Rf* values on TLC (solv. b, c, d, e) were identical with those of hederagenin, CP_1, CP_{3b}, CP_5 and CP_{8a}. VI was identified as CP_{8a} by direct comparison (TLC, IR, ^{1}H-NMR, ^{13}C-NMR).

Permethylate (XIV) of XIII——XIII (150 mg) was methylated and worked up in the same way as I. The product was chromatographed on silica gel (20 g) with benzene–acetone (17: 3) to give the permethylate (XIV) (110 mg) as a white powder (from dil. MeOH). *Anal.* Calcd for $C_{73}H_{124}O_{26}$: C, 61.84; H, 8.82. Found: C, 61.90; H, 8.97. IR (KBr): no OH. ^{1}H-NMR ($CDCl_3$): 4.18—4.38 (3H, m, anomeric H×3), 4.54 (1H, d, J=7.0 Hz, C_1–H of xylose unit), 5.12 (1H, s, C_1–H of rhamnose unit).

Methanolysis of XIV——XIV (50 mg) was methanolyzed and worked up in the same way as V to give the aglycone (9 mg) and the same methylated sugars as in the case of XII. The aglycone, colorless needles (from MeOH), mp 190—191°C, was identified as 23-*O*-methyl-hederagenin methyl ester by direct comparison (TLC (solv. f, h), IR, ^{1}H-NMR).

Acknowledgement We are grateful to Prof. T. Kawasaki of Kyushu University for his encouragement and valuable advice. Thanks are also due to Mrs. R. Igarashi of this university for microanalysis.

References and Notes

1) Part III: H. Kizu and T. Tomimori, *Chem. Pharm. Bull.*, **28**, 3555 (1980).
2) H. Kizu and T. Tomimori, presented at the 27th Annual Meeting of the Japanese Society of Pharmacognosy, Nagoya, Sep. 1980, Abstract Papers, p. 19.
3) *a*) H. Kizu and T. Tomimori, *Chem. Pharm. Bull.*, **27**, 2388 (1979); *b*) *Idem*, *ibid.*, **28**, 2827 (1980).
4) It is assumed in this paper that the monosaccharide components of prosapogenins (except for arabinose), *i.e.*, glucose (D), rhamnose (L) and xylose (D) have the absolute configurations shown in parentheses, as in the cases of other naturally occurring saponins hitherto reported.
5) S. Hakomori, *J. Biochem.*, **55**, 205 (1964).