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Studies on the Constituents of *Clematis* Species. III.[1] On the Saponins of the Root of *Clematis chinensis* Osbeck. (3)[2]

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Four triterpenoid prosapogenins named CP_{2b}, CP_{3b}, CP_9 and CP_{10} 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_{2b} (I), oleanolic acid 3-O-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranoside; CP_{3b} (IV), hederagenin 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside; CP_9 (V), oleanolic acid 3-O-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-ribopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside; CP_{10} (X), hederagenin 3-O-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-ribopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

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

In the previous papers,[1,4] we reported the isolation and structural elucidation of eight prosapogenins tentatively named CP_1—CP_8, 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 prosapogenins tentatively named CP_{2b}, CP_{3b}, CP_9 and CP_{10} were isolated by column chromatography as described in the experimental section. This paper deals with the identification of CP_{3b} and the structural elucidation of CP_{2b}, CP_9 and CP_{10}.[5]

CP_{2b} (I), colorless needles, mp 255—256° (dec.), $[\alpha]_D$ +37.4°, was hydrolyzed with acid to give oleanolic acid, arabinose and xylose. I was partially hydrolyzed with 0.5 N H_2SO_4 in 75% EtOH for 1 hr to yield only one prosapogenin (II), consisting of oleanolic acid and arabinose, together with oleanolic acid and unchanged I. II was identified as oleanolic acid 3-O-α-L-arabinopyranoside by direct comparison with an authentic sample.[4] I was methylated according to Hakomori[6] to give the permethylate (III), which showed a molecular ion peak at *m/z* 804 in the mass spectrum (MS). The proton magnetic resonance (^{1}H-NMR) spectrum of III showed two anomeric proton signals that overlapped at 4.53 ppm (2H, d, J=6.0 Hz), which were assigned as those of the L-arabinose unit (α-configulation) and the D-xylose unit (β-configulation). III was methanolyzed with 2N HCl–MeOH to give methyl oleanolate, methyl 2,3,4-tri-O-methyl-D-xylopyranoside and methyl 3,4-di-O-methyl-L-arabinopyranoside.

From these results, the structure of I was established as oleanolic acid 3-O-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranoside.

1) Part II: H. Kizu and T. Tomimori, *Chem. Pharm. Bull.*, **28**, 2827 (1980).
2) H. Kizu and T. Tomimori, Abstract of Paper, the 100th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, Apr. 1980, p. 184.
3) Location: *3 Ho, Kanagawa-machi, Kanazawa.*
4) H. Kizu and T. Tomimori, *Chem. Pharm. Bull.*, **27**, 2388 (1979).
5) It is assumed in this paper that the monosaccharide ingredients of these prosapogenins (except for arabinose), *i.e.*, glucose (D), rhamnose (L), ribose (D) and xylose (D) have the absolute configurations shown in parentheses, as in the case of other naturally occurring saponins hitherto reported.
6) S. Hakomori, *J. Biochem.*, **55**, 205 (1964).

CP_{3b} (IV), colorless needles, mp 248—250° (dec.), $[\alpha]_D$ +18.8°, consisting of hederagenin, arabinose and rhamnose, was proved to be identical with hederagenin 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside by comparing its *Rf* value on thin-layer chromatography (TLC), and its infrared (IR) and ^{1}H-NMR spectra with those of an authentic sample.[7]

CP_9 (V), a white powder, $[\alpha]_D$ −27.5°, is composed of oleanolic acid, arabinose, glucose, rhamnose and ribose. V was partially hydrolyzed with 0.5N H_2SO_4 in 75% EtOH for 1 hr to give four partial hydrolysis products, II, VI, VII (trace) and VIII (trace), together with oleanolic acid and unchanged V. Enzymatic hydrolysis of V with cellulase gave VIII and unchanged V. VI was identified as oleanolic acid 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (=CP_2), based on the general properties and spectral data.[4] VII was examined by TLC with several solvent systems and its mobilities coincided well with those of β-D-ribopyranoside of VI, *i.e.* CP_4.[1] VIII was acid-hydrolyzed to yield oleanolic acid, arabinose, glucose, rhamnose and ribose, and on partial hydrolysis gave II, VI and VII (trace), together with oleanolic acid and unchanged VIII. The ^{1}H-NMR spectrum of the permethylate of VIII showed four anomeric proton signals at 4.28 (1H, d, J=7.0 Hz), 4.41 (1H, d, J=4.8 Hz), 4.89 (1H, d, J=7.0 Hz) and 5.05 ppm (1H, s), which were assigned as those of the glucose, arabinose, ribose and rhamnose units, respectively, by comparison with ^{1}H-NMR spectra of the permethylates of VI and VII.[1,4] The permethylate of VIII gave on methanolysis methyl oleanolate, methyl 2,3-di-O-methyl-D-ribofuranoside and methyl pyranosides of 2,3,4,6-tetra-O-methyl-D-glucose, 2,3-di-O-methyl-D-ribose, 2,4-di-O-methyl-L-rhamnose and 3,4-di-O-methyl-L-arabinose. On the basis of the above results, VIII was identified as β-D-glucopyranoside of VII, *i.e.* CP_7.[4] The ^{1}H-NMR spectrum of the permethylate (IX) of V showed five anomeric proton signals at 4.32 (2H, d, J=7.0 Hz), 4.40 (1H, d, J=5.0 Hz), 4.89 (1H, d, J=6.5 Hz) and 5.08 ppm (1H, s), which were assigned as those of the glucose (×2), arabinose, ribose and rhamnose units, respectively, by comparison with the ^{1}H-NMR spectra of the permethylates of VI, VII and VIII.[1,4] IX was methanolyzed to give methyl oleanolate, methyl 2,3-di-O-methyl-D-ribofuranoside and methyl pyranosides of 2,3,4,6-tetra-O-methyl-D-glucose, 2,3-di-O-methyl-D-ribose, 2,4-di-O-methyl-L-rhamnose and 3,4-di-O-methyl-L-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 ^{1}H-NMR spectrum of IX.

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

CP_{10} (X), a white powder, $[\alpha]_D$ −19.3°, is composed of hederagenin, arabinose, glucose, rhamnose and ribose. On partial hydrolysis with 0.5N H_2SO_4 in 75% EtOH, X yielded four partial hydrolysis products, XI, IV, XII (trace) and XIII (trace), together with hederagenin and unchanged X. Enzymatic hydrolysis with emulsin afforded XIII and unchanged X. XI and IV were identified as 3-O-α-L-arabinopyranoside and 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside of hederagenin (*i.e.* CP_1[1] and CP_{3b}), respectively, by direct comparison. XII was examined by TLC with several solvent systems, and its mobilities coincided well with those of β-D-ribopyranoside of IV, *i.e.* CP_6.[4] XIII was acid-hydrolyzed to give hederagenin, arabinose, glucose, rhamnose and ribose, and partially hydrolyzed with acid to yield XI, IV and XII (trace), together with hederagenin and unchanged XIII. The ^{1}H-NMR spectrum of the permethylate of XIII showed four anomeric proton signals at 4.33 (2H, d, J=6.0 Hz, arabinose and glucose units), 4.97 (1H, d, J=7.0 Hz, ribose unit) and 5.18 ppm (1H, s, rhamnose unit). Methanolysis of the permethylate of XIII yielded 23-O-methylhederagenin methyl ester and the same methylated sugars as in the case of the permethylate of VIII. On the basis of the above results, XIII was identified as β-D-glucopyranoside of XII, *i.e.* CP_8.[4] The ^{1}H-NMR spectrum of the permethylate (XIV) of X showed five anomeric

7) M. Shimizu, M. Arisawa, N. Morita, H. Kizu, and T. Tomimori, *Chem. Pharm. Bull.*, **26**, 655 (1978).

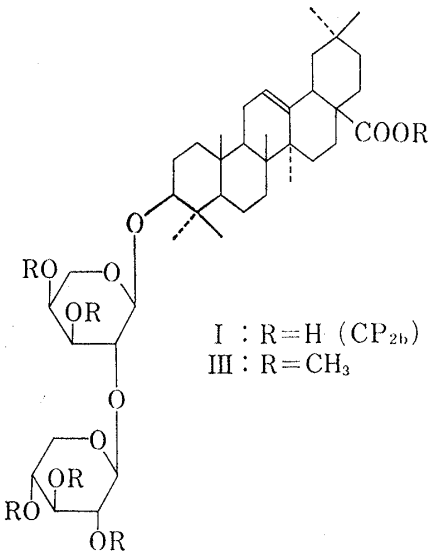

Chart 1

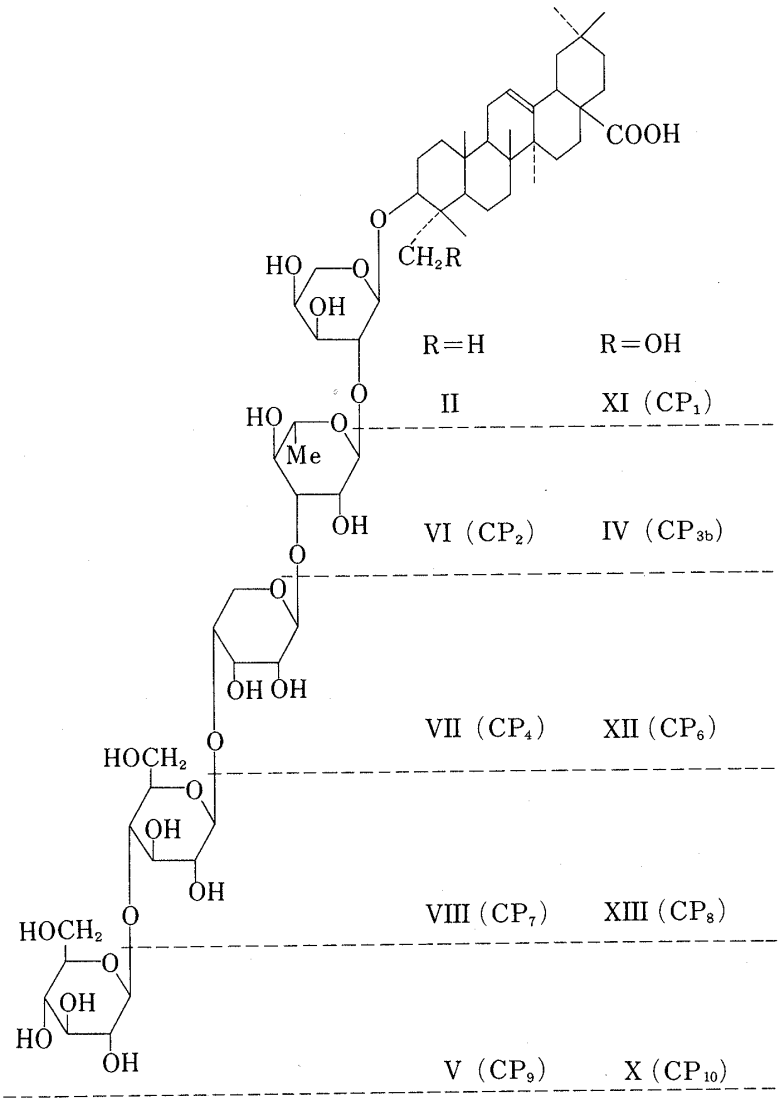

Chart 2

proton signals at 4.20—4.36 (3H, m), 4.88 (1H, d, J=7.0 Hz) and 5.11 ppm (1H, s). XIV gave on methanolysis 23-O-methyl-hederagenin methyl ester and the same methylated sugars as in the case of IX. The mode of linkage of the terminal D-glucose unit was regarded as β on the basis of the molecular rotation difference between XIII and X.

On the basis of all the above results, the structure of X was established as hederagenin 3-O-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-ribopyranosyl-(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 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. MS were recorded on a JEOL JMS-D-100 mass spectrometer. 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° (20 min hold) to 240° at 5°/min (GLC-1) or 198° (GLC-2). TLC was performed on Kieselgel G (Merck) with 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) $CHCl_3$–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)[4] was chromatographed on silica gel (2 kg) with the following solvent systems to give fractions 1 to 10.

Eluent	Fr. No.	Composition	Yield (g)
$CHCl_3$–MeOH–H_2O (25: 3: 0.3)	1	Crude CP_1	1.7
	2	CP_{2a},[8] CP_2, CP_{2b}	1.4
$CHCl_3$–MeOH–H_2O (25: 6: 0.7)	3	CP_{3a},[8] CP_{3b}	0.9
	4	CP_3, CP_4	1.9
	5	Crude CP_6	5.0
	6	CP_5, CP_6	7.2
$CHCl_3$–MeOH–H_2O (25: 7: 1)	7	CP_{7a},[8] CP_7	13.9
	8	CP_{8a},[8] CP_8	9.8
$CHCl_3$–MeOH–H_2O (25: 7.5: 1.1)	9	CP_{9a},[8] CP_9	6.1
	10	CP_{10a},[8] CP_{10}	7.8

Fr. 2 was rechromatographed on silica gel (140 g) with *n*-BuOH–AcOEt–H_2O (13: 90: 6) to give CP_{2a}[8] (25 mg), CP_2[4] (250 mg) and CP_{2b} (38 mg). Fr. 3 was acetylated with acetic anhydride (9 ml) and pyridine (9 ml) at room temperature for 40 hr. The reaction mixture was treated by the usual procedure to give the acetates, which were chromatographed on silica gel (100 g) with a gradient of benzene–AcOEt (AcOEt 0—20%) to give the peracetates of CP_{3a} and CP_{3b}. Each acetate thus obtained was deacetylated with 0.5 N KOH at room temperature for 20 hr, neutralized with dil. H_2SO_4 and extracted with *n*-BuOH. Each *n*-BuOH extract was concentrated and the residue was recrystallized from MeOH to give CP_{3a}[8] (35 mg) and CP_{3b} (50 mg), respectively. Fr. 9 (6 g) was subjected to repeated silica gel column chromatography with *n*-PrOH–$CHCl_3$–H_2O–AcOH (8: 2: 1: 1) to give CP_{9a}[8] (150 mg) and CP_9 (600 mg). Fr. 10 (7 g) was chromatographed under the same conditions as Fr. 9 to give CP_{10a}[8] (195 mg) and CP_{10} (680 mg).

CP_{2b} (I)——Colorless needles (MeOH), mp 255—256° (dec.), $[\alpha]_D$ +37.4° (c=0.45, MeOH), *Anal.* Calcd for $C_{40}H_{64}O_{11}\cdot H_2O$: C, 65.02; H, 9.00. Found: C, 64.86; H, 9.08. IR ν_{max}^{KBr} cm^{-1}: 3400, 1690.

Hydrolysis of I——I (8 mg) was hydrolyzed with 2 N HCl–MeOH (2 ml) 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 (3 mg), mp 306—308°, which were identified as oleanolic acid by direct comparison (TLC (solv. a, b), IR, mixed fusion) 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 hr. The reaction mixture was neutralized with Ag_2CO_3 and the precipitate was filtered off. The filtrate was concentrated and analyzed by GLC-1 (as the trimethylsilyl ether derivative), which indicated the presence of arabinose (t_R 13′07″, 15′10″) and xylose (t_R 20′07″, 24′26″).

Partial Hydrolysis of I——I (50 mg) was hydrolyzed with 0.5 N H_2SO_4 in 75% EtOH (2 ml) under reflux for 1 hr. The reaction mixture was neutralized with 0.5 N KOH and extracted with *n*-BuOH. After removal of the solvent, the residue was subjected to column chromatography on silica gel (8 g) with a gradient of $CHCl_3$–MeOH (MeOH 0—10%) to give oleanolic acid (16 mg), II (8 mg) and unchanged I (13 mg). II (6 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 oleanolic acid (2 mg) and arabinose. II was identical with oleanolic acid 3-O-α-L-arabinopyranoside upon direct comparison (TLC (solv. a, b, c), IR, ^{1}H-NMR) with an authentic sample.[4]

Permethylate (III) of I——I (20 mg) was methylated according to Hakomori. The reaction mixture was diluted with ice-water and extracted with AcOEt. The AcOEt extract was concentrated and the residue was passed through a silica gel (10 g) column. Elution with hexane–acetone (4: 1) gave the permethylate (III) (17 mg) as colorless needles (dil. MeOH), mp 179—180°. *Anal.* Calcd for $C_{46}H_{76}O_{11}$: C, 68.63; H, 9.52. Found: C, 68.66; H, 9.50. IR (KBr): no OH. ^{1}H-NMR: 4.53 (2H, d, J=6.0 Hz, anomeric H×2). MS m/z (%): 804 (1), 453 (91), 335 (5), 262 (67), 203 (59), 175 (100).

Methanolysis of III——III (12 mg) was methanolyzed 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 (5 mg) as colorless needles, mp 196—198°; this material was identified as methyl oleanolate by direct comparison (TLC (solv. f, g), IR, mixed fusion). The mother liquor of crystallization was examined by TLC (solv. f) and GLC-2, which indicated the presence of methyl pyranosides of 2,3,4-tri-O-methyl-D-xylose (t_R 4′14″, 5′07″) and 3,4-di-O-methyl-L-arabinose (t_R 15′12″, 30′24″).

CP_{3b} (IV)——Colorless needles (MeOH), mp 248~250° (dec.), $[\alpha]_D$ +18.8° (c=0.67, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 1690. ^{1}H-NMR (in pyridine-d_5): 1.62 (3H, d, J=6.0 Hz, C_5-CH_3 of rhamnose unit), 5.10 (1H, d, J=5.5 Hz, C_1–H of arabinose unit), 5.48 (1H, br. s, C_{12}–H), 6.10 (1H, s, C_1–H of rhamnose unit). IV (10 mg) was hydrolyzed with 2 N HCl–MeOH (2 ml) for 2 hr and worked up in the same way as I to give the aglycone (4 mg) as colorless plates together with arabinose and rhamnose. The aglycone was identified as hederagenin

8) Work on this compound is in progress.

by direct comparison (TLC (solv. a, b), IR). IV was identified as hederagenin 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside[7] by direct comparison (TLC (solv. c, d), IR, ^{1}H-NMR).

CP_9 (V)——A white powder (MeOH), $[\alpha]_D$ −27.5° (c=0.79, MeOH). *Anal.* Calcd for $C_{58}H_{94}O_{25}\cdot 4H_2O$: C, 55.14; H, 8.14. Found: C, 54.98; H, 8.03. IR ν_{max}^{KBr} cm^{-1}: 3400, 1690.

Hydrolysis of V——A solution of V (20 mg) in 2 N HCl–MeOH (2 ml) was heated under reflux for 2 hr and worked up in the same way as I to give the aglycone (5 mg) as colorless needles; this material was identified as oleanolic acid by direct comparison (TLC, IR). The sugar portion was examined by GLC-1 (as the trimethylsilyl ether derivative), which indicated the presence of arabinose (t_R 13′07″, 16′10″), rhamnose (t_R 14′00″, 19′14″), ribose (t_R 15′41″, 16′43″) and glucose (t_R 29′58″, 32′46″).

Partial Hydrolysis of V——V (300 mg) was hydrolyzed with 0.5 N H_2SO_4 in 75% EtOH (30 ml) under reflux for 1 hr and worked up in the same way as I. The resulting hydrolysate was subjected to column chromatography on silica gel (30 g). Elution was carried out first with a gradient of $CHCl_3$–MeOH (MeOH 0—10%) to give oleanolic acid (26 mg), II (29 mg) and VI (35 mg), then with $CHCl_3$–MeOH–H_2O (25: 6: 0.7) to give VII (trace) and VIII (trace), and finally with $CHCl_3$–MeOH–H_2O (25: 8: 1.2) to recover unchanged V (55 mg). II, colorless needles (MeOH), mp 245—250° (dec.), was identified as oleanolic acid 3-O-α-L-arabinopyranoside[4] by direct comparison (TLC, IR, ^{1}H-NMR). VI, colorless needles (MeOH), mp 227—229° (dec.), was identified as CP_2[4] by direct comparison (TLC, IR, ^{1}H-NMR). VII was examined by TLC with solvent systems c, d, and e and its mobilities were in good agreement with those of CP_4.[1]

Enzymatic Hydrolysis of V——A solution of V (100 mg) in AcOH–AcONa buffer solution (pH 5.5, 60 ml) containing cellulase (Tokyo Kasei Kogyo Co., 100 mg) was shaken at 37° for 5 days. 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: 7: 1→25: 8: 1.2) to give VIII (30 mg) and unchanged V (50 mg). VIII (10 mg) was hydrolyzed with 2 N HCl–MeOH under reflux for 2 hr and worked up in the same way as I to give oleanolic acid (3 mg, identified by TLC and IR), arabinose, glucose, rhamnose and ribose. VIII (10 mg) was partially hydrolyzed with 0.5 N H_2SO_4 in 75% EtOH (2 ml) under reflux for 1 hr and worked up in the same way as I. The resulting hydrolysate was examined by TLC with solvent systems c, d and e, which indicated the presence of oleanolic acid, II, VI, VII (trace) and unchanged VIII. VIII (30 mg) was methylated and worked up in the same way as I. The product was chromatographed on silica gel (8 g) with benzene–acetone (9: 1) to give the permethylate (16 mg) as a white powder (dil. MeOH). IR (KBr): no OH. ^{1}H-NMR: 4.28 (1H, d, J=7.0 Hz, C_1–H of glucose unit), 4.41 (1H, d, J=4.8 Hz, C_1–H of arabinose unit), 4.89 (1H, d, J=7.0 Hz, C_1–H of ribose unit), 5.05 (1H, s, C_1–H of rhamnose unit). The permethylate (15 mg) was methanolyzed 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 (3 mg), which was identified as methyl oleanolate by direct comparison (TLC, IR). The methylated sugar portion was analyzed by TLC (solv. f) and GLC-2, which indicated the presence of methyl 2,3-di-O-methyl-D-ribofuranoside (t_R 15′05″ (overlap), 28′19″) and methyl pyranosides of 2,3,4,6-tetra-O-methyl-D-glucose (t_R 8′02″, 10′58″ (overlap)), 2,4-di-O-methyl-L-rhamnose (t_R 9′26″), 2,3-di-O-methyl-D-ribose (t_R 10′58″ (overlap), 15′05″ (overlap)) and 3,4-di-O-methyl-L-arabinose (t_R 15′05″ (overlap), 29′58″). The methylated sugar portion was acetylated in the usual manner and analyzed by GLC-2, which indicated the presence of methyl 2,3-di-O-methyl-5-O-acetyl-D-ribofuranoside (t_R 16′36″, 28′07″) and methyl pyranosides of 2,3,4,6-tetra-O-methyl-D-glucose (t_R 8′02″, 10′58″), 2,4-di-O-methyl-3-O-acetyl-L-rhamnose (t_R 10′36″), 2,3-di-O-methyl-4-O-acetyl-D-ribose (t_R 13′00″, 19′00″ (overlap)) and 3,4-di-O-methyl-2-O-acetyl-L-arabinose (t_R 19′00″ (overlap), 22′58″).

Permethylate (IX) of V——V (100 mg) was methylated and worked up in the same way as I. The product was chromatographed on silica gel (10 g) with benzene–acetone (85: 15) to give permethylate (IX) (75 mg) as a white powder (dil. MeOH). *Anal.* Calcd for $C_{72}H_{122}O_{25}$: C, 62.32; H, 8.86. Found: C, 62.16; H, 8.90. IR (KBr): no OH. ^{1}H-NMR: 4.32 (2H, d, J=7.0 Hz, C_1–H of glucose unit×2), 4.40 (1H, d, J=5.0 Hz, C_1–H of arabinose unit), 4.89 (1H, d, J=6.5 Hz, C_1–H of ribose unit), 5.08 (1H, s, C_1–H of rhamnose unit).

Methanolysis of IX——IX (50 mg) was methanolyzed 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 (11 mg) as colorless needles, mp 197—198°; this product was identified as methyl oleanolate by direct comparison (TLC (solv. f, g), IR, ^{1}H-NMR). The methylated sugar portion was analyzed by TLC (solv. f) and GLC-2, which indicated the presence of methyl 2,3-di-O-methyl-D-ribofuranoside (t_R 15′05″ (overlap), 28′19″) and methyl pyranosides of 2,3,4,6-tetra-O-methyl-D-glucose (t_R 8′04″, 10′58″ (overlap)), 2,4-di-O-methyl-L-rhamnose (t_R 9′26″), 2,3-di-O-methyl-D-ribose (t_R 10′58″ (overlap), 15′05″ (overlap)), 3,4-di-O-methyl-L-arabinose (t_R 15′05″ (overlap), 29′58″) and 2,3,6-tri-O-methyl-D-glucose (t_R 24′26″, 31′34″). The methylated sugar portion was acetylated in the usual manner and analyzed by GLC-2, which indicated the presence of methyl 2,3-di-O-methyl-5-O-acetyl-D-ribofuranoside (t_R 16′36″, 28′07″) and methyl pyranosides of 2,3,4,6-tetra-O-methyl-D-glucose (t_R 8′02″, 10′58″), 2,4-di-O-methyl-3-O-acetyl-L-rhamnose (t_R 10′36″), 2,3-di-O-methyl-4-O-acetyl-D-ribose (t_R 13′00″, 19′00″ (overlap)), 3,4-di-O-methyl-2-O-acetyl-L-arabinose (t_R 19′00″ (overlap), 22′58″) and 2,3,6-tri-O-methyl-4-O-acetyl-D-glucose (t_R 24′00″, 32′17″).

CP_{10} (X)——A white powder (dil. MeOH), $[\alpha]_D$ −19.3° (c=1.00, MeOH). *Anal.* Calcd for $C_{58}H_{94}O_{26}\cdot 4H_2O$: C, 54.45; H, 8.04. Found: C, 54.28; H, 7.97. IR ν_{max}^{KBr} cm^{-1}: 3400, 1690. $\Delta[M]_D$: X—XIII, −20.9°, $[M]_D$ of methyl D-glucopyranoside: α, +309°; β, −66°.

Hydrolysis of X——X (25 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 (6 mg) as colorless plates together with arabinose, glucose, rhamnose and ribose. The aglycone was identified as hederagenin by direct comparison (TLC (solv. a, b), IR).

Partial Hydrolysis of X——X (350 mg) was hydrolyzed with 0.5 N H_2SO_4 in 75% EtOH (20 ml) under reflux for 1 hr and worked up in the same way as I. The hydrolysate was chromatographed on silica gel (50 g) with $CHCl_3$–MeOH–H_2O (25: 3: 0.3→25: 6: 0.7→25: 8: 1.2) to give hederagenin (29 mg), XI (32 mg), IV (30 mg), XII (trace), XIII (trace) and unchanged X (62 mg). XI, colorless needles, mp 226—229° (dec.), was identified as CP_1 by direct comparison (TLC, IR, ^{1}H-NMR). IV, colorless needles, mp 245—248° (dec.), was identified as CP_{3b} by direct comparison (TLC, IR, ^{1}H-NMR). XII was examined by TLC (solv. c, d, e) and its mobilities were in good agreement with those of CP_6.[4]

Enzymatic Hydrolysis of X——A solution of X (100 mg) in citric acid–Na_2HPO_4 buffer solution (pH 5.0, 60 ml) was treated with almond emulsin (P–L Biochem. Inc., 20 mg) at 37° for 5 days. The reaction mixture was extracted with *n*-BuOH. The extract was washed with water and concentrated. The residue was chromatographed on silica gel (30 g) with $CHCl_3$–MeOH–H_2O (25: 7: 1→25: 8: 1.2) to give XIII (48 mg) and unchanged X (34 mg). XIII (15 mg) was hydrolyzed with 2 N HCl–MeOH (2 ml) and worked up in the same way as I to give hederagenin (4 mg) (identified by TLC and IR) together with arabinose, glucose, rhamnose and ribose. XIII (20 mg) was partially hydrolyzed with 0.5 N H_2SO_4 in 75% EtOH (2 ml) under reflux for 1 hr and worked up in the same way as I. The resulting hydrolysate was examined by TLC (solv. a, b, c, d), which indicated the presence of hederagenin, XI, IV, XII (trace) and unchanged XIII. XIII (40 mg) was methylated and worked up in the same way as I. The product was chromatographed on silica gel (8 g) with benzene–acetone (9: 1) to give the permethylate (25 mg) as a white powder (dil. MeOH). IR (KBr): no OH. ^{1}H-NMR: 4.33 (2H, d, J=6.0 Hz, C_1–H of arabinose and glucose units), 4.97 (1H, d, J=7.0 Hz, C_1–H of ribose unit), 5.18 (1H, s, C_1–H of rhamnose unit). The permethylate (20 mg) was methanolyzed 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), which was identified as 23-O-methyl-hederagenin methyl ester by direct comparison (TLC, IR). The methylated sugar portion was analyzed by TLC (solv. f) and GLC-2, which indicated the presence of the same methylated sugars as in the case of the permethylate of VIII.

Permethylate (XIV) of X——X (100 mg) was methylated and treated in the same manner as I. The product was chromatographed on silica gel (20 g) with benzene–acetone (85: 15) to give the permethylate (XIV) (81 mg) as a white powder (dil. MeOH). *Anal.* Calcd for $C_{73}H_{124}O_{26}$: C, 61.84; H, 8.82. Found: C, 61.70; H, 8.86. IR (KBr): no OH. ^{1}H-NMR: 4.20—4.26 (3H, m, anomeric H×3), 4.88 (1H, d, J= 7.0 Hz, C_1–H of ribose unit), 5.11 (1H, s, C_1–H of rhamnose unit).

Methanolysis of XIV——XIV (60 mg) was methanolyzed 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 (12 mg) as colorless needles, mp 186—187°; this material was identified as 23-O-methyl-hederagenin methyl ester by direct comparison (TLC, IR, ^{1}H-NMR). The methylated sugar portion and its acetate were analyzed by GLC-2, which indicated the presence of the same methylated sugars and their acetates as in the case of IX.

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