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

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Four triterpenoid saponins, which were tentatively named prosapogenins CP_2, CP_6, CP_7 and CP_8, were isolated from the alkaline hydrolysate of crude saponin obtained from *Clematis chinensis* Osbeck. On the basis of chemical and physicochemical evidence, they were characterized as follows: CP_2 (I), oleanolic acid 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside; CP_6 (III), hederagenin 3-O-β-D-ribopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside; CP_7 (VII), oleanolic acid 3-O-β-D-glucopyranosyl-(1→4)-β-D-ribopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside; CP_8 (IX), hederagenin 3-O-β-D-glucopyranosyl-(1→4)-β-D-ribopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

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

The root of *Clematis chinensis* Osbeck (Ranunculaceae) is a source of the Chinese drug "Wei Ling Xian (威霊仙)" which has been used as an analgesic, diuretic and antiinflammatory agent.[3] The literature contains no reports on the constituents of this plant, but it may contain saponins, since several kinds of saponins, for example, clematosides,[4] songarosides[5] and vitalbosides,[6] have been isolated from *Clematis mandschurica*, *C. songarica* and *C. vitalba*, respectively. The present paper deals with the triterpenoid saponins of Formosan "Wei Ling Xian," which was confirmed to have been obtained from the root of *C. chinensis* Osbeck.[7]

The methanol extract of the material was fractionated as shown in Chart 1. The crude saponin obtained from the *n*-butanol-soluble fraction as precipitates on treatment with methanol and acetone contained more than ten coponents as determined by thin-layer chromatography (TLC) (Fig. 1). It was hydrolyzed, without further purification, with 0.5N KOH to give a mixture of at least ten prosapogenins, which were tentatively named prosapogenins CP_1–CP_{10} in order of increasing polarity (Fig. 1). Among them, CP_2, CP_6, CP_7 and CP_8 were isolated in pure states by repeated column chromatography, monitored by TLC.

We report here the identification of CP_2 and the structural elucidations of CP_6, CP_7 and CP_8.

1) Presented in part at the 25th Annual Meeting of the Japanese Society of Pharmacognosy, Fukuoka, Dec. 1978.
2) Location: *3 Ho, Kanagawa-machi, Kanazawa.*
3) "Zhong Yao Zhi (中葯誌)," Vol. 1, ed. by the Pharmaceutical Institute, Chinese Academy of Medical Science, Peking, 1961, p. 328.
4) V. Ya. Chirva, V.P. Konyukhov, *Izv. Akad. Nauk Mold. SSR, Ser. Biol. Khim. Nauki*, **1969**, 43 [*C.A.*, **71**, 70851 (1969)].
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6) V. Ya. Chirva, P.K. Kintya, V.N. Mel'nikov, Ref. Dokl. Soobshch. Mendeleevsk, S'ezd Obshch. Prikl. Khim., 11th 1975, 6, 109 (Russ) ed. by V.N. Rodinskaya "Nauka": Moscow, USSR [*C.A.*, **88**, 148971 (1978)].
7) The material was kindly identified by Prof. T. Namba, Research Institute for Wakan-Yaku (Traditional Crude Drugs), Medical and Pharmaceutical University of Toyama.

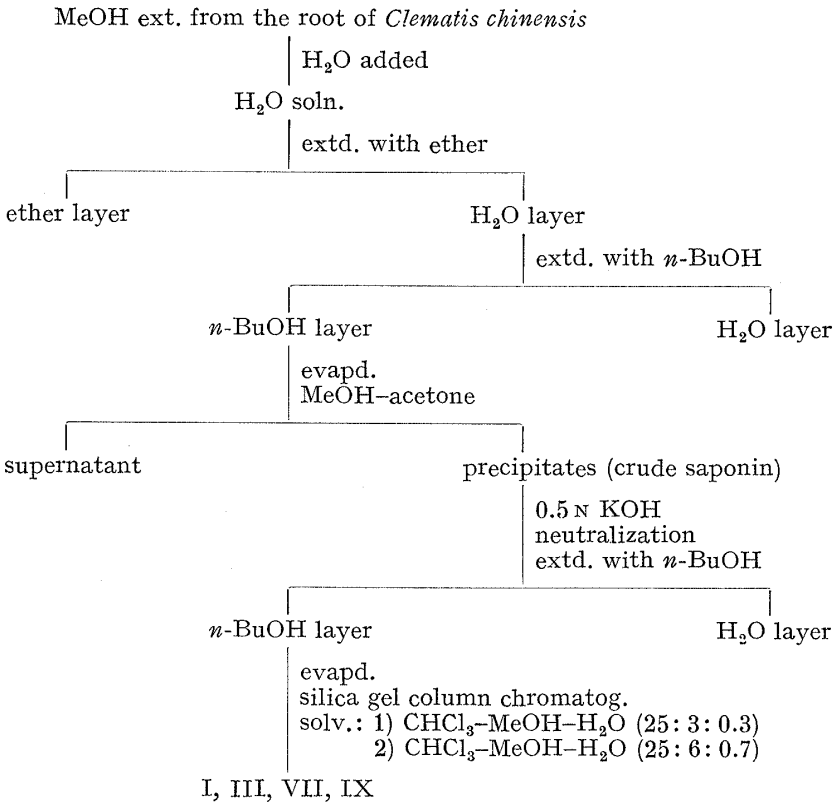

Chart 1

CP_2 (I), colorless needles, mp 220—224° (dec.), $[\alpha]_D$ +10°, was hydrolyzed with 2 N H_2SO_4 in 50% EtOH under reflux for 2 hr to give oleanolic acid, arabinose and rhamnose. I was methylated according to Hakomori[8] to give the permethylate (II). The proton magnetic resonance (PMR) spectrum of II showed two anomeric proton signals at 4.40 (1H, doublet, J=5.0 Hz) and 5.14 ppm (1H, singlet). On methanolysis, II gave methyl oleanolate, methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside and methyl 3,4-di-O-methyl-L-arabinopyranoside. On the basis of these data, I was considered to be oleanolic acid 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (this has already been isolated from *Akebia quinata*[9]) and was identical with authentic sample[10] on direct comparison.

CP_6 (III), a white powder, mp 242—246° (dec.), $[\alpha]_D$ −11.2°, is composed of hederagenin, arabinose, rhamnose and ribose. It was partially hydrolyzed with 2 N H_2SO_4 in 50% EtOH for 10 min to give hederagenin 3-O-α-L-arabinopyranoside[11] (IV) and hederagenin 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside[11] (V) together with hederagenin and unchanged III. The permethylate (VI) of III prepared according to Hakomori showed three one-proton signals at 4.33 (doublet, J=6.5 Hz), 5.12 (doublet, J=5.0 Hz) and 5.24 ppm (singlet), which were ascribable to the anomeric protons of the arabinose, ribose and rhamnose units, respectively, by comparison with the PMR spectrum of the permethylate of V. VI was methanolyzed to give 23-O-methyl hederagenin methylester and methylated sugars which were identified as 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 on the basis of their *Rf* values on TLC and retention times (t_R) on gas–liquid chromatography (GLC) in comparison with those of authentic samples.

8) S. Hakomori, *J. Biochem.*, **55**, 205 (1964).
9) R. Higuchi and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **24**, 1021 (1976).
10) The authentic sample was kindly provided by Prof. T. Kawasaki of Kyushu University.
11) M. Shimizu, M. Arisawa, N. Morita, H. Kizu, and T. Tomimori, *Chem. Pharm. Bull.* (Tokyo), **26**, 655 (1978).

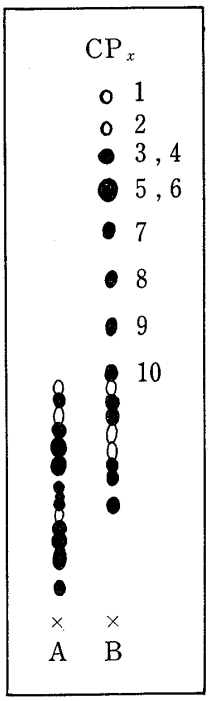

Fig. 1. TLC Patterns of Crude Saponin (A) and Its Alkaline Hydrolysate (B)

Plate, Kieselgel G;
solv., $CHCl_3$–MeOH–H_2O (25: 14: 3).

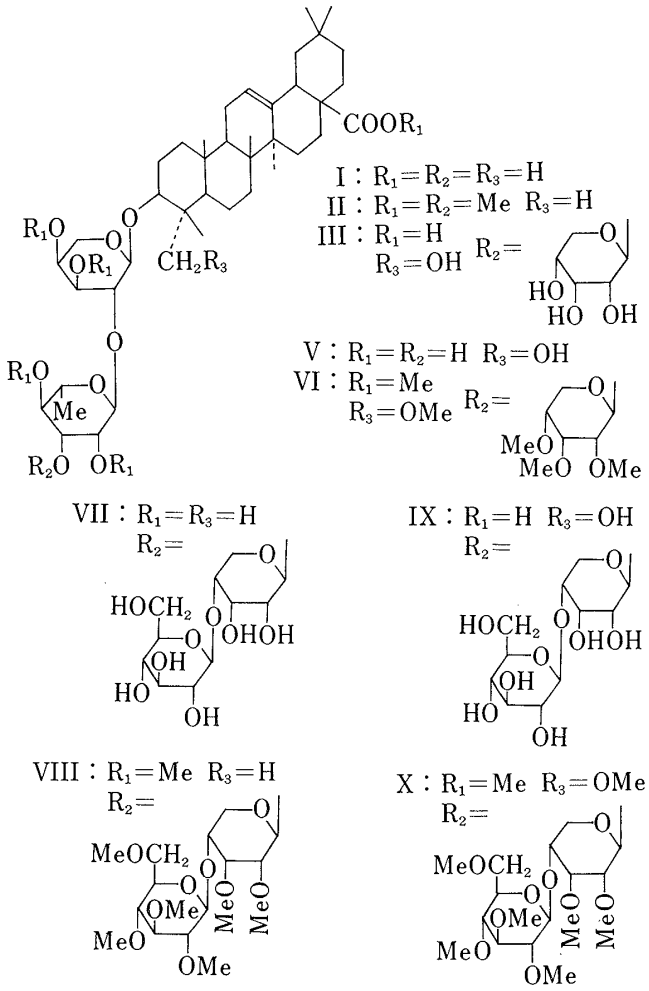

Chart 2

The mode of linkage of the D-ribose unit was regarded as β on the basis of the molecular rotation difference between III and V.

Consequently III was identified as hederagenin 3-O-β-D-ribopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

CP_7 (VII), a white powder, mp 252—254° (dec.), $[\alpha]_D$ −31.5°, consisting of oleanolic acid, arabinose, glucose, rhamnose and ribose, was partially hydrolyzed with acid to give oleanolic acid 3-O-α-L-arabinopyranoside[12] and I together with oleanolic acid and unchanged VII. The PMR spectrum of the permethylate (VIII) of VII showed four one-proton signals at 4.28 (doublet, J=7.0 Hz), 4.41 (doublet, J=4.8 Hz), 4.89 (doublet, J=7.0 Hz) and 5.05 ppm (singlet), which were assigned to the anomeric protons of the glucose, arabinose, ribose and rhamnose units, respectively. VIII was subjected to methanolysis to give methyl oleanolate, methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside, methyl 2,3-di-O-methyl-D-ribopyranoside and the corresponding furanoside, methyl 2,4-di-O-methyl-L-rhamnopyranoside and methyl 3,4-di-O-methyl-L-arabinopyranoside. The mode of linkage of the terminal D-glucose unit was regarded as β based on the coupling constant of its anomeric proton signal in the PMR spectrum of VIII. The β-configuration of the D-ribose unit was suggested by the molecular rotation difference between VII and I. Although both methyl 2,3-di-O-methyl-D-ribopyranoside and the corresponding furanoside were formed, the ring form of the β-D-ribose unit in VII was regarded as pyranose type based on the following data. As shown in Table

12) The authentic sample was isolated from *Fatsia japonica*. T. Aoki and T. Suga, *Phytochemistry*, **17**, 771 (1978).

Table I. Coupling Constants of Anomeric Protons in Methyl β-D-Riboside Derivatives[a]

Derivatives (in $CDCl_3$)	Pyranose type	Furanose type
2,3,4(5)[b]-Tri-O-methyl	5.8 Hz	≅0
2,3,4(5)[b]-Tri-O-acetyl	3.5 Hz	≅0
2,3-Di-O-methyl	1.8 Hz	≅0
2,3-Di-O-methyl-4(5)[b]-O-acetyl	3.8 Hz	≅0
Methyl β-D-riboside[c]	5.0 Hz	≅0

a) All the derivatives were synthesized in our laboratory.
b) Arabic numerals in parentheses indicate the position in the furanose type compounds.
c) In D_2O.

I, the anomeric proton signals of methyl β-D-ribopyranoside derivatives are observed as a doublet, while those of methyl β-D-ribofuranoside derivatives appear almost as a singlet. These data rule out the possibility that the ring form of the D-ribose unit in VII is a furanose type.

On the basis of these results, VII can be formulated as oleanolic acid 3-O-β-D-glucopyranosyl-(1→4)-β-D-ribopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

CP_8 (IX), a white powder, mp 237—241° (dec.), $[\alpha]_D$ −20.3°, is composed of hederagenin, arabinose, glucose, rhamnose and ribose. It was partially hydrolyzed with acid to give IV and V together with hederagenin and unchanged IX. The PMR spectrum of the permethylate (X) of IX exhibited four anomeric proton signals at 4.33 (2H, doublet, J=6.0 Hz, arabinose and glucose units), 4.97 (1H, doublet, J=7.0 Hz, ribose unit) and 5.18 ppm (1H, singlet, rhamnose unit). Methanolysis of X yielded 23-O-methyl hederagenin methylester and the same methylated sugars as VIII. The β-linkage of the D-glucose unit was suggested by the coupling constant of its anomeric proton signal in the PMR spectrum of X. The mode of linkage of the D-ribose unit was regarded as β, based on the molecular rotation difference between IX and V. The ring form of the β-D-ribose unit was considered to be pyranose type, since its anomeric proton signal was observed as a doublet (J=7.0 Hz) in the PMR spectrum of X.

Thus, the structure of IX was established as hederagenin 3-O-β-D-glucopyranosyl-(1→4)-β-D-ribopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

I, III, VII and IX were obtained as prosapogenins from the alkaline hydrolysate of the crude saponin; this is the first time that III, VII and IX have been isolated from nature.

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. PMR spectra were taken at 100 MHz with a JEOL-JNM-MH-100 spectrometer in $CDCl_3$ solution, and chemical shifts are given as δ (ppm) with tetramethylsilane as an internal standard. Infrared spectra (IR) were obtained with a JASCO-IR-A-2 spectrometer. Optical rotations were measured with a JASCO-DIP-4 digital polarimeter. 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, 120°, 160° (GLC-1), or 198° (GLC-2). TLC was performed on Kieselgel G (Merck) using the following solvent systems: a) $CHCl_3$–MeOH–H_2O (25:6:0.7), b) *n*-BuOH–AcOH–H_2O (4:1:2), c) benzene–acetone–H_2O (4:11:2, upper layer), d) benzene–acetone (5:2), e) $CHCl_3$–MeOH–HCOOH (15:1:trace), f) toluene–HCOOH–HCOOEt (5:1:4), and spots were detected by spraying 10% H_2SO_4 followed by heating.

Isolation of Saponins——The root of *Clematis chinensis* imported from Formosa was extracted with hot MeOH. The MeOH extract was evaporated down and treated as shown in Chart 1. The crude saponin obtained from the *n*-BuOH-soluble fraction was hydrolyzed with 0.5 N KOH on a boiling water bath for 0.5 hr, neutralized with dil. H_2SO_4 then extracted with *n*-BuOH. The *n*-BuOH solution was washed with

water and evaporated down to give a prosapogenin mixture, which was chromatographed over silica gel, eluting with $CHCl_3$–MeOH–H_2O (25 : 3 : 0.3) to give CP_2 and CP_6, and then with $CHCl_3$–MeOH–H_2O (25 : 6 : 0.7) to give CP_7 and CP_8.

CP_2 (I)——Colorless needles (MeOH), mp 220—224° (dec.), $[\alpha]_D$ +10° (c=1.23, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400, 1690. *Anal.* Calcd. for $C_{41}H_{66}O_{11}\cdot H_2O$: C, 65.40; H, 9.10. Found: C, 65.12; H, 9.47.

Hydrolysis of I——I was hydrolyzed with 2 N H_2SO_4 in 50% EtOH for 2 hr on a boiling water bath. After cooling, the reaction mixture was concentrated to half volume. The precipitates were collected by filtration and crystallized from MeOH to give colorless needles, mp 299—301°, which were identified as oleanolic acid by direct comparison (TLC (solv. e, f), IR, mixed melting point). The filtrate of the hydrolysate was neutralized with saturated aq. $Ba(OH)_2$ and the white precipitate ($BaSO_4$) was removed by centrifugation (3000 rpm, 0.5 hr). The supernatant was concentrated under reduced pressure and the residue was examined by TLC (solv. b) and GLC-1 (as the trimethylsilylether derivative), revealing the presence of arabinose and rhamnose.

Permethylate (II) of I——I was methylated according to Hakomori.[8] The reaction mixture was diluted with ice-water and extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated down and the residue was passed through a silica gel column (eluent, benzene–acetone (97 : 3)) to give a white powder (II), mp 207—211°, IR: no OH. PMR: 4.40 (1H, doublet, J=5.0 Hz, C_1–H of arabinose residue), 5.14 (1H, singlet, C_1–H of rhamnose residue). *Anal.* Calcd. for $C_{47}H_{78}O_{11}$: C, 68.92; H, 9.60. Found: C, 69.09; H, 9.72.

Methanolysis of II——II was boiled with 2 N HCl in MeOH for 2 hr. The reaction mixture was neutralized with Ag_2CO_3 and the precipitate was filtered off. The filtrate was evaporated down and the residue was crystallized from MeOH to give colorless needles, mp 197—198°, which were identified as methyl oleanolate (TLC, IR, mixed melting point). The mother liquor of crystallization was examined by TLC (solv. d) and GLC-2, revealing the presence of methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside (t_R 4.28 min) and methyl 3,4-di-O-methyl-L-arabinopyranoside (t_R 16.24, 32.40 min).

CP_6 (III)——A white powder (dil. MeOH), mp 242—246° (dec.), $[\alpha]_D$ −11.2° (c=0.92, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400, 1690. *Anal.* Calcd. for $C_{46}H_{74}O_{16}\cdot 2H_2O$: C, 60.11; H, 8.55. Found: C, 60.45; H, 8.71. $\Delta[M]_D$: III—V, −240°, $[M]_D$ of methyl D-ribopyranoside: α, +170°; β, −186°.

Hydrolysis of III——i) III was hydrolyzed with 2 N H_2SO_4 in 50% EtOH for 2 hr and worked up in the same way as for I to give the aglycone together with arabinose, rhamnose and ribose. The aglycone was crystallized from MeOH to give colorless prisms, mp>300°, which were identified as hederagenin by direct comparison with an authentic sample (TLC (solv. e, f), IR).

ii) III was partially hydrolyzed with 2 N H_2SO_4 in 50% EtOH under reflux for 10 min. After neutralization with 0.5 N KOH, the reaction mixture was concentrated to about half volume and extracted with *n*-BuOH, then the *n*-BuOH extract was evaporated down and chromatographed over silica gel, eluting with $CHCl_3$–MeOH–H_2O (25 : 3 : 0.3) to give hederagenin 3-O-α-L-arabinopyranoside (IV) as colorless needles, mp 229—231° (dec.), and hederagenin 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (V), colorless needles, mp 249—251° (dec.). These were identical with the corresponding authentic samples as determined by direct comparison (TLC (solv. a, b, c), IR).

Permethylate (VI) of III——III was methylated in the same way as I to give the permethylate (VI) as a white powder (dil. MeOH), mp 130—132°, IR: no OH. PMR: 4.33 (1H, doublet, J=6.0 Hz, C_1–H of arabinose residue), 5.12 (1H, doublet, J=5.0 Hz, C_1–H of ribose residue), 5.24 (1H, singlet, C_1–H of rhamnose residue). *Anal.* Calcd. for $C_{55}H_{92}O_{16}$: C, 65.45; H, 9.19. Found: C, 65.48; H, 9.41.

Methanolysis of VI——VI was methanolyzed and treated in the same way as II to afford 23-O-methyl hederagenin methylester and a mixture of methylated sugars which were identified as the methyl pyranosides of 2,3,4-tri-O-methyl-D-ribose (t_R 7.60, 11.36 min), 2,4-di-O-methyl-L-rhamnose (t_R 10.00 min) and 3,4-di-O-methyl-L-arabinose (t_R 16.24, 32.40 min) by TLC (solv. d) and GLC-2.

CP_7 (VII)——A white powder (dil. MeOH), mp 252—254° (dec.), $[\alpha]_D$ −31.5° (c=1.85, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400, 1690. *Anal.* Calcd. for $C_{52}H_{84}O_{20}\cdot 2H_2O$: C, 58.63; H, 8.33. Found: C, 58.49; H, 8.52. $\Delta[M]_D$: VII-I, −383°, $[M]_D$ of methyl D-ribopyranoside: α, +170°; β, −186°; $[M]_D$ of methyl β-D-glucopyranoside: −66°.

Hydrolysis of VII——VII was hydrolyzed with acid in the same manner as I to give oleanolic acid, arabinose, glucose, rhamnose and ribose, and partially hydrolyzed in the same way as III to give two products, one of which was identified as oleanolic acid 3-O-α-L-arabinopyranoside, and the other as I by direct comparison (TLC (solv. a, b, c), IR).

Permethylate (VIII) of VII——VII was methylated and worked up in the same way as I to give a syrup, which was passed through a silica gel column, eluting with benzene–acetone (9 : 1) to give the permethylate (VIII) as a white powder (dil. MeOH), mp 129—131°, IR: no OH. PMR: 4.28 (1H, doublet, J=7.0 Hz, C_1–H of glucose residue), 4.41 (1H, doublet, J=4.8 Hz, C_1–H of arabinose residue), 4.89 (1H, doublet, J=7.0 Hz, C_1–H of ribose residue), 5.05 (1H, singlet, C_1–H of rhamnose residue). *Anal.* Calcd. for $C_{63}H_{106}O_{20}$: C, 63.94; H, 9.03. Found: C, 64.02; H, 9.15.

Methanolysis of VIII——VIII was methanolyzed in the same manner as II to afford methyl oleanolate and methylated sugars which were identified by TLC (sov. d) and GLC-2 as methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside (t_R 8.64, 11.64 min), methyl 2,3-di-O-methyl-D-ribopyranoside (t_R 12.20, 15.36 min) and the

corresponding furanoside (t_R 15.60, 29.56 min), methyl 2,4-di-O-methyl-L-rhamnopyranoside (t_R 10.00 min) and methyl 3,4-di-O-methyl-L-arabinopyranoside (t_R 16.24, 32.40 min). The acetates of the methylated sugars prepared in the usual manner were identical with those of authentic samples on GLC-2.

CP_S (IX)——A white powder (dil. MeOH), mp 237—241° (dec.), $[\alpha]_D$ −20.3° (c=1.71, MeOH, IR ν_{max}^{KBr} cm^{-1}: 3400, 1690. *Anal.* Calcd. for $C_{52}H_{84}O_{21}\cdot 2H_2O$: C, 57.76; H, 8.20. Found: C, 57.53; H, 8.46. $\Delta[M]_D$: IX—V, −353°, $[M]_D$ of methyl D-ribopyranoside: α, +170°; β, −186°; $[M]_D$ of β-D-glucopyranoside: −66°.

Hydrolysis of IX——IX was acid-hydrolyzed in the same way as I to give hederagenin, arabinose, glucose, rhamnose and ribose, and partially hydrolyzed with acid and worked up as described for III to give IV and V.

Permethylate (X) of IX——IX was methylated and worked up in the same way as I to give the permethylate (X) as a white powder (dil. MeOH), mp 128—129°, IR: no OH. PMR: 4.33 (2H, doublet, J=6.0 Hz, C_1–H of arabinose and glucose residues), 4.97 (1H, doublet, J=7.0 Hz, C_1–H of ribose residue), 5.18 (1H, singlet, C_1–H of rhamnose residue). *Anal.* Calcd. for $C_{64}H_{102}O_{21}$: C, 63.34; H, 8.97. Found: C, 63.38; H, 9.02.

Methanolysis of X——X was methanolyzed and worked up as described for II to give 23-O-methyl hederagenin methylester and the same methylated sugars as VIII.

Syntheses of Reference Compounds[13]——i) Permethylate of D-Ribose: A solution of D-ribose (5 g) in anhydrous 0.1 N HCl–MeOH (200 ml) was heated under reflux for 2 hr. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated down under reduced pressure to give a mixture of methyl D-ribosides, which were acetylated with acetic anhydride (50 ml) and pyridine (50 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 column chromatography, eluting benzene–AcOEt (10: 1) to give methyl 2,3,5-tri-O-acetyl-β-D-ribofuranoside, methyl 2,3,4-tri-O-acetyl-β-D-ribopyranoside and a mixture of methyl 2,3,4-tri-O-acetyl-α-D-ribopyranoside and methyl 2,3,5-tri-O-acetyl-α-D-ribofuranoside. The acetates thus obtained were deacetylated with 22% NH_4OH in 25% MeOH and methylated according to Kuhn[14] to give methyl 2,3,5-tri-O-methyl-β-D-ribofuranoside and methyl 2,3,4-tri-O-methyl-β-D-ribopyranoside.

ii) Methyl 2,3-Di-O-methyl-D-ribosides: A mixture of methyl D-ribosides (5 g) in pyridine (15 ml) was tritylated with trityl chloride (5 g) at room temperature for 70 hr. The reaction mixture was evaporated down and the residue was partitioned with water and $CHCl_3$. The $CHCl_3$ solution, which contained methyl 5-O-trityl α- and β-D-ribofuranosides, was evaporated down and methylated according to Kuhn. The reaction mixture was evaporated down, treated with 2 N HCl–MeOH under reflux for 2 hr and neutralized with Ag_2CO_3. The precipitate was filtered off and filtrate was evaporated down to give a syrup, which was chromatographed on silica gel, eluting with a gradient of benzene–acetone (acetone 0—25%) to give methyl 2,3-di-O-methyl-β-D-ribopyranoside, methyl 2,3-di-O-methyl-β-D-ribofuranoside, methyl 2,3-di-O-methyl-α-D-ribopyranoside and methyl 2,3-di-O-methyl-α-D-ribofuranoside. These four compounds were acetylated by the usual procedure to give the corresponding mono acetates.

iii) Methyl 2,4-Di-O-methyl-α-L-rhamnopyranoside: Methyl 4-O-methyl-α-L-rhamnopyranoside prepared according to Butler *et al.*[15] was partially methylated according to Purdie[16] and the products were separated by silica gel column chromatography (eluent, benzene–acetone (95: 5)) to give methyl 2,4-di-O-methyl-α-L-rhamnopyranoside. This was acetylated by the usual procedure to give methyl 2,4-di-O-methyl-3-O-acetyl-α-L-rhamnopyranoside.

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13) The structures of the reference compounds were supported by their chemical and physicochemical data ($[\alpha]_D$, PMR, GLC, TLC).
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