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Studies on the Constituents of *Clematis* Species. V.[1] On the Saponins of the Root of *Clematis chinensis* Osbeck. (5)[2]

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Three triterpenoid prosapogenins tentatively named CP$_0$ (I), CP$_{2a}$ (VI) and CP$_{3a}$ (VIII) 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: I, hederagenin 23-*O*-α-L-arabinopyranoside; VI, hederagenin 23-*O*-β-D-glucopyranoside; VIII, olean-12-ene-28-oic acid-3β,24-diol (4-epihederagenin) 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside. I and VI are the first examples of 23-*O*-glycosides of oleanane-type triterpenes to be isolated from nature.

Six less polar genuine saponins were isolated and they appeared to be identical with prosapogenins CP$_4$, CP$_6$, CP$_7$, CP$_8$, CP$_9$ and CP$_{10}$,[1,4] on the basis of the results of high performance thin-layer chromatography (HPTLC).

Keywords——*Clematis chinensis*; Ranunculaceae; saponin; prosapogenin; hederagenin 23-*O*-glycoside; 4-epihederagenin 3-*O*-glycoside; ^{13}C-NMR

The chinese drug Wei Ling Xian (威霊仙) has been used as an analgesic, diuretic and antiinflammatory agent.[3] We have been working on saponin constituents of Formosan Wei Ling Xian (the root of *Clematis chinensis*) and have already reported the structural elucidation of sixteen prosapogenins, tentatively named CP$_1$—CP$_{10}$, CP$_{2b}$, CP$_{3b}$ and CP$_{7a}$—CP$_{10a}$, isolated from the alkaline hydrolysate of the crude saponin.[1,4] In a continuation of this work, other prosapogenins named CP$_0$, CP$_{2a}$ and CP$_{3a}$ were newly isolated by repeated column chromatography. This paper deals with the structural elucidation of these prosapogenins.[5] Among the prosapogenins mentioned above, six compounds, CP$_4$, CP$_6$, CP$_7$, CP$_8$, CP$_9$ and CP$_{10}$, appeared to exist in the crude drug as genuine saponins as determined by HPTLC, although their quantities were very small, as described in the experimental section.

CP$_0$ (I), colorless needles, mp 192—195°C (dec.), $[\alpha]_D$ +48.9°, was hydrolyzed with 2 N HCl–MeOH to give hederagenin (II) and methyl arabinoside. In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, I showed an anomeric proton signal at 4.90 ppm (d, J=5.2 Hz). ^{13}C signals observed in the carbon nuclear magnetic resonance (^{13}C-NMR) spectrum of I were assigned as shown in Table I, and the arabinose unit in I was suggested to be linked to the C$_{23}$ hydroxyl group of II by comparison of the ^{13}C-NMR spectral data of I with those of II. I was methylated according to Hakomori[6] to give the permethylate (III), whose anomeric proton signal was observed at 4.13 ppm (d, J=6.3 Hz). Methanolysis of III yielded methyl 2,3,4-tri-*O*-methyl-arabinopyranoside and an aglycone (IV) which, on acetylation, gave a monoacetate (V) exhibiting the acetoxymethylene proton signal at 3.96 ppm (2H, s). This observation indicated that V was the C$_{23}$-*O*-acetate and so IV was 3-*O*-methyl-hederagenin methyl ester. This identification was finally confirmed by comparing IV with a synthetic specimen from hederagenin. The mode of linkage of the L-arabinose unit was regarded as α on the basis of the coupling constants (J=5.2 (I), 6.3 Hz (III)) of the anomeric proton signals in the ^{1}H-NMR spectra of I and III.

Therefore, I was concluded to be hedragenin 23-*O*-α-L-arabinopyranoside.

CP$_{2a}$ (VI), colorless needles, mp 192—195°C (dec.), $[\alpha]_D$ +38.8°, was hydrolyzed with 2 N HCl–MeOH to give hederagenin and methyl glucoside. In the ^{1}H-NMR spectrum VI showed an anomeric proton signal at 4.87 ppm (d, J=7.0 Hz). The glucose unit in VI was suggested to be linked to the C$_{23}$ hydroxyl group of II from a comparison of the ^{13}C-NMR

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spectral data of VI with those of II. The permethylate (VII) of VI, which showed an anomeric proton signal at 4.11 ppm (d, $J=7.0$ Hz) in the ^{1}H-NMR spectrum, gave on methanolysis 3-*O*-methyl-hederagenin methyl ester (IV) and methyl 2,3,4,6-tetra-*O*-methyl-glucopyranoside. The mode of linkage of the D-glucose unit was regarded as β on the basis of the coupling constants ($J=7.0$ Hz) of the anomeric proton signals in the ^{1}H-NMR spectra of VI and VII.

Consequently, the structure of VI was established as hederagenin 23-*O*-β-D-glucopyranoside.

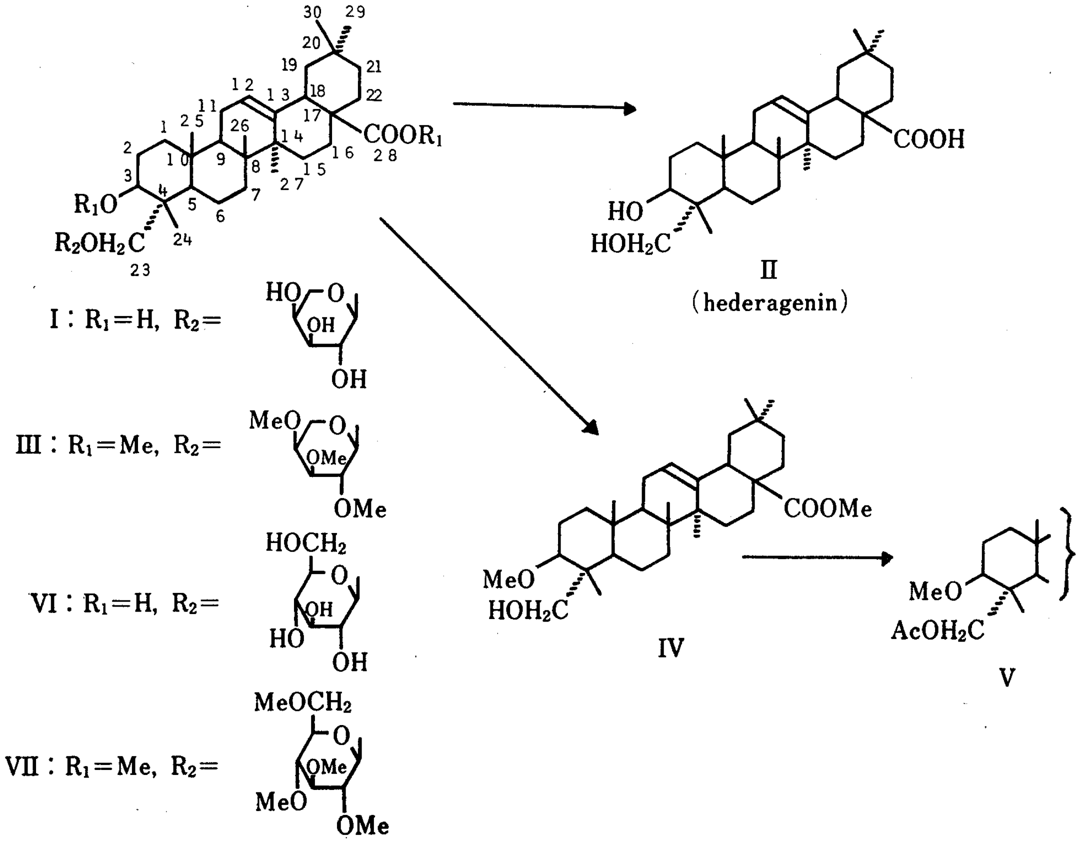

Chart 1

CP$_{3a}$ (VIII), colorless needles, mp 247—248°C (dec.), $[\alpha]_D$ $-4.4°$, was hydrolyzed with 2 N HCl–MeOH to give an aglycone (IX), methyl arabinoside and methyl rhamnoside. The infrared (IR) spectrum of IX (colorless needles, mp 305—307°C, $[\alpha]_D$ $+74.1°$) exhibited hydroxyl and carboxyl absorptions, and the methyl ester (X), colorless needles, mp 220—221°C, showed six tertiary methyl signals and one carbomethoxyl signal in the ^{1}H-NMR spectrum. The ^{1}H-NMR spectrum of the acetate (XI) of IX, colorless needles, mp 249—250°C, showed signals due to two acetoxyl groups at 2.06 (6H, s), one acetoxymethylene protons at 4.32 (2H, ABq, $J=12.0$ Hz, $\delta=26.4$ Hz) and one acetoxymethine proton at 4.67 ppm (1H, t-like). The mass spectra (MS) of both IX (M$^+$, *m/z* 472) and XI (M$^+$, *m/z* 556) exhibited relatively intense ion peaks at *m/z* 248 and 203, while X (M$^+$, *m/z* 486) and its acetate (XII) (M$^+$, *m/z* 570), colorless needles, mp 205—207°C, showed such peaks at *m/z* 262 and 203 in both cases. These are characteristic ion peaks due to the retro-Diels–Alder type fragmentation of olean-12-en- or urs-12-en-28-oic acid derivatives bearing no substituent on the C, D and E rings.[7] The MS data of the above compounds were very similar to those of the corresponding derivatives of hederagenin. Although IX failed to form an *O,O*-isopropylidene derivative in the conven-

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tional method, it readily gave one (XIII), colorless needles, mp 266—268°C, on reaction in dimethylformamide (DMF) with 2,2-dimethoxypropane and *p*-toluenesulfonic acid. The ^{1}H-NMR spectrum of XIII showed signals due to isopropylidene methyls at 1.37 and 1.44 (each 3H, s), methylene protons of $-CH_2-O-C(Me)_2-O-$ at 3.21 and 4.05 (each 1H, d, $J=11.5$ Hz) and the methine proton of $>CH-O-C(Me)_2-O-$ at 3.46 ppm (1H, m). These data suggested the presence of 3β,24-*O*-isopropylidene system in XIII.[8]

On the basis of all the above results and a comparison of the ^{13}C-NMR spectrum of IX with those of oleanolic acid and hederagenin, the structure of IX was established as olean-12-en-28-oic acid 3β,24-diol (*i.e.* the 4-epimer of hederagenin), which is a new triterpenoid.

The ^{1}H-NMR spectrum of VIII showed two anomeric proton signals at 5.12 (br s) and 5.66 ppm (br s), and the ^{13}C-NMR spectrum exhibited two anomeric carbon signals at 101.4 and 102.7 ppm. On mild acid hydrolysis, VIII gave a partial hydrolysis product (XIV) together with IX. XIV, colorless needles, mp 247—249°C (dec.), $[\alpha]_D$ +40.5°, yielded IX and methyl arabinoside on hydrolysis with 2 N HCl–MeOH. The ^{1}H-NMR spectrum of XIV showed an anomeric proton signal at 4.92 ppm (d, $J=6.6$ Hz), the coupling constant of which indicated that the mode of linkage of the arabinose unit was α. The permethylate (XV) of VIII, which showed two anomeric proton signals at 4.43 (d, $J=5.0$ Hz) and 5.12 ppm (d, $J=1.6$ Hz) in the ^{1}H-NMR spectrum, gave on methanolysis an aglycone (XVI), methyl 2,3,4-tri-*O*-methyl-rhamnopyranoside and methyl 3,4-di-*O*-methyl-arabinopyranoside. XVI, colorless needles, mp 190—192°C, showed a C_3–H signal at *ca.* 3.2 ppm (overlap) in the ^{1}H-NMR spectrum, while its acetate (XVII), colorless needles, mp 214—215°C, showed a signal at 4.55 ppm (1H, t-like). On the basis of the above data and a comparison of the ^{13}C-NMR spectrum of VIII with those of IX, the sugar moiety in VIII was indicated to be linked at the C_3 position. The configuration of the L-rhamnose unit was considered to be α on the basis of the ^{13}C-NMR spectral data[9] and the molecular rotation difference between XIV and VIII.

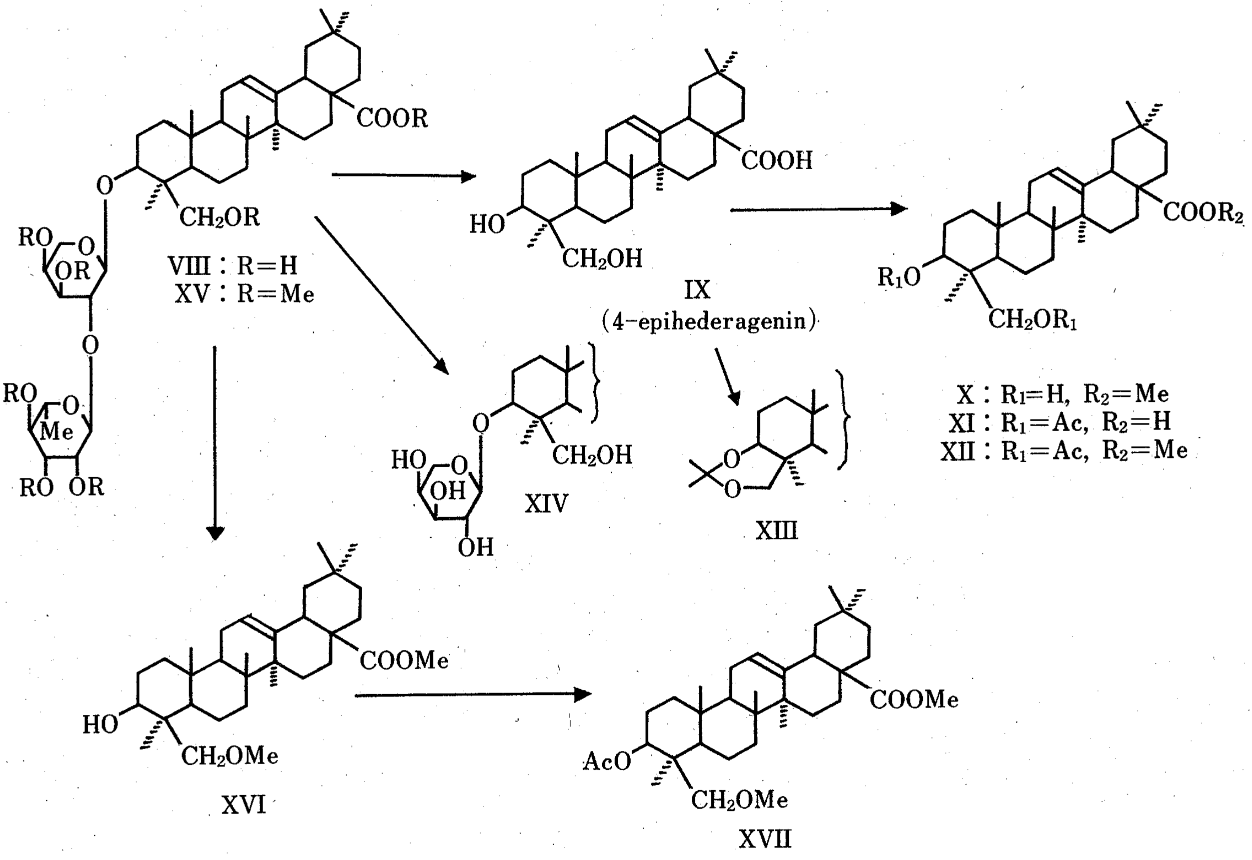

Chart 2

In addition, in the ^{13}C-NMR spectrum of VIII, the signal due to C_2 was observed at higher field by 2.5 ppm than in that of XIV, and the signals due to an L-arabinopyranosyl moiety appeared in unexpected positions compared with those of CP_2 (oleanolic acid 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside)[4a] and CP_{3b} (hederagenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside).[4c] Furthermore, an L-arabinopyranosyl anomeric proton signal was observed as a broad singlet in the ^{1}H-NMR spectrum. These findings are considered to reflect a conformational difference of the arabinose unit in VIII and in the other compounds mentioned above. That is, in VII the $^1C_4$ conformer is unexpectedly predominant.

TABLE I. ^{13}C-NMR Spectral Data in Pyridine-d_5[a]

	Ole.[b]	II	IX	CP_1[c]	I	VI	XIV	VIII	CP_{3b}[d]	CP_2[e]
C-1	39.0	38.9	38.8	38.8	38.6	38.5	38.6	38.4	39.0	39.0
2	28.1	27.6	28.5	26.2	27.4	27.4	28.4	25.9	26.2	26.5
3	78.2	73.7	80.3	82.0	72.3	72.5	89.2	91.1	81.2	88.9
4	39.4	42.9	43.3	43.5	42.6	42.9	44.4	43.9	43.6	39.5
5	55.9	48.8	56.5	47.7	49.5	48.8	56.4	56.3	47.8	56.0
6	18.8	18.7	19.1	18.2	18.8	18.6	18.9	19.2	18.2	18.6
7	33.3	33.0	33.6	33.0	33.0	33.1	33.5	33.5	32.9	33.3
8	39.8	39.8	39.8	39.8	39.8	39.7	39.8	39.8	39.8	39.8
9	48.2	48.2	48.3	48.2	48.2	48.1	48.0	48.0	48.2	48.1
10	37.5	37.3	37.3	37.0	37.3	37.3	36.8	36.9	36.9	37.0
11	23.8	23.8	24.1	23.8	23.8	23.8	24.1	24.0	23.8	23.8
12	122.7	122.7	122.7	122.7	122.7	122.7	122.7	122.5	122.7	122.7
13	145.0	145.0	145.1	145.0	145.2	145.2	145.0	144.8	145.0	145.0
14	42.2	42.2	42.2	42.2	42.3	42.2	42.2	42.2	42.2	42.2
15	28.4	28.4	28.5	28.4	28.3	28.3	28.4	28.3	28.4	28.1
16	23.8	23.8	23.8	23.8	23.8	23.8	23.8	23.8	23.8	23.8
17	46.7	46.7	46.8	46.7	46.8	46.7	46.8	46.7	46.7	46.7
18	42.1	42.0	42.1	42.0	42.1	42.0	42.1	42.0	42.0	42.0
19	46.6	46.5	46.6	46.5	46.5	46.6	46.5	46.5	46.5	46.6
20	31.0	31.0	31.0	31.0	31.0	31.0	31.0	31.0	31.0	31.0
21	34.3	34.3	34.3	34.3	34.3	34.3	34.3	34.3	34.3	34.4
22	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3
23	28.8	68.2	23.7	64.6	76.3	75.4	23.5	23.1	64.1	28.3
24	16.6	13.1	64.6	13.6	13.1	13.2	63.4	61.4[f]	14.0	17.0
25	15.6	16.0	16.0	16.1	15.9	16.0	15.4	15.6	16.1	15.5
26	17.4	17.5	17.3	17·5	17.5	17.5	17.3	17.3	17.4	17.4
27	26.2	26.2	26.2	26.2	26.3	26.3	26.1	26.1	26.2	26.2
28	180.3	180.4	180.4	180.4	180.4	180.5	180.4	180.1	180.4	180.4
29	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3	33.3
30	23.8	23.8	23.8	23.8	23.8	23.8	23.8	23.8	23.8	23.8
1′				106.7	104.6	105.5	106.6	102.7	104.4	104.9
2′				73.1	73.5	75.4	73.0	76.1	75.9	76.0
3′				74.8	74.0	78.8	74.6	66.0	74.7	73.8
4′				69.6	68.0	71.9	69.4	70.2	69.4	68.7
5′				67.0	64.9	78.6	66.6	63.4[f]	65.6	64.7
6′						63.0				
1′′								101.4	101.8	101.8
2′′								72.4	72.4	72.4
3′′								72.6	72.6	72.7
4′′								74.0	74.2	74.1
5′′								70.2	69.7	69.9
6′′								18.5	18.5	18.6

a) For details of the assignments of ^{13}C signals, refer to the previous paper.[1]
b) Ole.: oleanolic acid.
c) CP_1: hederagenin 3-*O*-α-L-arabinopyranoside.[4b]
d) CP_{3b}: hederagenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.[4c]
e) CP_2: oleanolic acid 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.[4a]
f) Assignment may be reversed.

On the other hand, in the cases of XV and the acetate of VIII, the $^4C_1$ conformer is considered to be predominant based on the coupling constants (both $J=5.0$ Hz) of the L-arabinopyranosyl anomeric proton signals in the ^{1}H-NMR spectra of these compounds.

On the basis of all the above results, the structure of VIII was established as 4-epihederagenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

Further work on 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, t: triplet, q: quartet, m: multiplet, br s: broad singlet). ^{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, and chemical shifts are given as δ (ppm). Measurement conditions were similar to those employed in the preceding paper[1] and the data are listed in Table I. MS were recorded on a JEOL JMS-D-100 mass spectrometer. 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 220°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) benzene–acetone (4: 1), d) benzene–acetone (15: 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)[4a] was chromatographed on silica gel (2 kg) with various solvent systems to give fractions 1—10 as described in the previous paper.[4c] Fr. 1 was rechromatographed on silica gel (50 g) with a gradient of $CHCl_3$–MeOH (MeOH 0—5%), and the product was recrystallized from MeOH to give CP_0 (20 mg). CP_{2a} and CP_{3a} were isolated from Fr. 2 and Fr. 3, respectively, as described in the previous paper.[4c]

CP_0 (I)——Colorless needles (from MeOH), mp 192—195°C (dec.), $[\alpha]_D$ +48.9° ($c=0.43$, MeOH). *Anal.* Calcd for $C_{35}H_{56}O_8 \cdot H_2O$: C, 67.49; H, 9.39. Found: C, 67.55; H, 9.32. IR ν_{max}^{KBr} cm^{-1}: 3400, 1690. ^{1}H-NMR (pyridine-d_5): 4.90 (1H, d, $J=5.2$ Hz, C_1–H of arabinose unit).

Hydrolysis of I——A solution of I (3 mg) in 2 N HCl–MeOH (2 ml) was heated under reflux on a water bath and was neutralized with Ag_2CO_3. The precipitate was filtered off and the filtrate was concentrated. The residue was crystallized from MeOH to give colorless prisms (II) (1.5 mg), mp 312—315°C. II was identified as hederagenin by direct comparison with an authentic sample (TLC (solv. a, b), IR). The mother liquor of crystallization was concentrated and analyzed by GLC-1 (as the trimethylsilylether derivative), which indicated the presence of methyl arabinoside (t_R 8′46″, 9′10″).

Permethylate (III) of I——I (10 mg) was methylated according to Hakomori.[6] The reaction mixture was diluted with ice-water and extracted with AcOEt. The AcOEt solution was washed with water and concentrated. The residue was subjected to column chromatography on silica gel (10 g) with a gradient of benzene–acetone (acetone 0—3%) to give the permethylate (III) (5 mg) as colorless needles (from MeOH), mp 177—178°C. IR ν_{max}^{KBr} cm^{-1}: no OH, 1725, 1100. ^{1}H-NMR ($CDCl_3$): 0.63 (3H), 0.72 (3H), 0.89 (3H), 0.92 (6H), 1.08 (3H) (each s, *tert*-Me × 6), 3.34, 3.46, 3.50, 3.61, 3.62 (each 3H, s, OMe × 5), 4.13 (1H, d, $J=6.3$ Hz, C_1–H of arabinose unit), 5.28 (1H, br s, C_{12}–H).

Methanolysis of III——A solution of III (4 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 IV (1.6 mg) and methyl 2,3,4-tri-*O*-methyl-arabinopyranoside (identified by TLC (solv. c), GLC-2 (t_R 8′07″)). IV, colorless needles (from MeOH), mp 197—198°C, was identified as 3-*O*-methyl-hederagenin methyl ester by direct comparison (TLC (solv. d), IR, ^{1}H-NMR). IV (1.2 mg) was acetylated with acetic anhydride (2 drops) and pyridine (3 drops), and the reaction mixture was treated by the usual procedure to give the acetate (V) (1 mg) as colorless needles (from MeOH), mp 144—145°C; this product was identified as 3-*O*-methyl-23-*O*-acetyl-hederagenin methyl ester by direct comparison with an authentic sample (TLC (solv. d), IR, ^{1}H-NMR).

3-O-Methyl-hederagenin Methyl Ester (IV)——Hederagenin (200 mg) in pyridine (2 ml) was tritylated with trityl chloride (400 mg) at room temperature for 15 h. The reaction mixture was concentrated and the residue was chromatographed on silica gel (50 g) with a gradient of benzene–AcOEt (AcOEt 0—10%) to give the tritylate (250 mg). The tritylate was methylated according to Hakomori and worked up as described for I. The product (250 mg) was treated with 0.1 N HCl–MeOH (5 ml) under reflux for 1 h, then the reaction mixture was worked up as described for I. The resulting methylate was chromatographed on silica gel (50 g) with a gradient of benzene–AcOEt (AcOEt 10—20%) to give IV (140 mg) as colorless needles (from MeOH),

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mp 197—198°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1}: 3460, 1725. ^{1}H-NMR ($CDCl_3$): 0.72, 0.83, 0.90, 0.92, 0.96, 1.12 (each 3H, s, *tert*-Me×6), 3.17 (1H, m, C_3–H), 3.36 (3H, s, C_3–OMe), 3.51 (2H, ABq, J=9.3 Hz, δ=23.9 Hz, –$C\underline{H}_2OH$), 3.62 (3H, s, COOMe), 5.28 (1H, br s, C_{12}–H). IV (50 mg) was acetylated with acetic anhydride (1.5 ml) and pyridine (2 ml) at room temperature for 20 h. The reaction mixture was concentrated and recrystallized from MeOH to give V (40 mg) as colorless needles, mp 144—145°C, IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1}: 1730, 1725, 1235, 1100, 1025. ^{1}H-NMR ($CDCl_3$): 0.73 (6H), 0.90 (3H), 0.94 (6H), 1.11 (3H) (each s, *tert*-Me×6), 2.08 (3H, s, –CH_2–OCO$\underline{\text{Me}}$), 3.31 (3H, s, C_3–OMe), 3.62 (3H, s, COOMe), 3.96 (2H, s, –$C\underline{H}_2$–OCOMe), 5.29 (1H, br s, C_{12}–H).

CP$_{2a}$ (VI)——Colorless needles (from MeOH), mp 192—195°C (dec.), $[\alpha]_D$ +38.8° (c=0.75, MeOH). *Anal.* Calcd for $C_{36}H_{58}O_9 \cdot H_2O$: C, 66.23; H, 9.26. Found: C, 66.41; H, 9.35. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1}: 3400, 1690. ^{1}H-NMR (pyridine-d_5): 4.87 (1H, d, J=7.0 Hz, C_1–H of glucose unit).

Hydrolysis of VI——VI (5 mg) was hydrolyzed and worked up as described for I to give hederagenin (2 mg) (identified by TLC, IR) and methyl glucoside (identified by GLC-1 (t_R 29′10″, 30′00″) as the trimethylsilylether derivative).

Permethylate (VII) of VI——VI (10 mg) was methylated and worked up as described for I. The product was chromatographed on silica gel (5 g) with a gradient of benzene–acetone (acetone 0—2%) to give the permethylate (VII) (8 mg) as a white powder (from dil. MeOH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1}: no OH, 1722, 1100. ^{1}H-NMR ($CDCl_3$): 0.63 (3H), 0.72 (3H), 0.91 (9H), 1.07 (3H) (each s, *tert*-Me×6), 3.31 (3H), 3.38 (3H), 3.51 (3H), 3.60 (9H) (each s, OMe×6), 4.11 (1H, d, J=7.0 Hz, C_1–H of glucose unit), 5.26 (1H, br s, C_{12}–H).

Methanolysis of VII——VII (5 mg) was methanolyzed and worked up as described for I to give 3-*O*-methyl-hederagenin methyl ester (IV) (2 mg) (identified by TLC (solv. d), IR, ^{1}H-NMR) and methyl 2,3,4,6-tetra-*O*-methyl-glucopyranoside (identified by TLC (solv. c), GLC-2 (t_R 8′02″, 8′50″)).

CP$_{3a}$ (VIII)——Colorless needles (from MeOH), mp 247—248°C (dec.), $[\alpha]_D$ −4.4° (c=0.34, MeOH). *Anal.* Calcd for $C_{41}H_{66}O_{12} \cdot 2H_2O$: C, 62.57; H, 8.97. Found: C, 62.70; H, 8.92. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1}: 3400, 1690. ^{1}H-NMR (pyridine-d_5): 0.83 (3H), 0.98 (6H), 1.02 (3H), 1.32 (3H), 1.43 (3H) (each s, *tert*-Me×6), 1.66 (3H, d, J=5.5 Hz, C_5–Me of rhamnose unit), 5.12 (1H, br s, C_1–H of arabinose unit), 5.44 (1H, br s, C_{12}–H), 5.66 (1H, br s, C_1–H of rhamnose unit). $\Delta[M]_D$: VIII−XIV, −277.6°, $[M]_D$ of methyl L-rhamnopyranoside: α, −111°; β, +170°.

Hydrolysis of VIII——VIII (30 mg) was hydrolyzed and worked up as described for I to give the aglycone (IX) (15 mg) and a sugar portion which was shown to contain methyl arabinoside (t_R 8′46″, 9′10″) and methyl rhamnoside (t_R 10′19″) by GLC-1 (as the trimethylsilylether derivative). IX, colorless needles (from MeOH), mp 305—307°C, $[\alpha]_D$ +74.1° (c=0.27, MeOH). *Anal.* Calcd for $C_{30}H_{48}O_4$: C, 76.23; H, 10.24. Found: C, 76.19; H, 10.22. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1}: 3400, 1690, 1030. ^{1}H-NMR (pyridine-d_5): 0.87, 0.96, 0.99, 1.02, 1.28, 1.56 (each 3H, s, *tert*-Me×6), 3.67, 4.50 (each 1H, d, J=10.7 Hz, C_4–$C\underline{H}_2OH$), 5.50 (1H, br s, C_{12}–H). MS *m/z*: 472 (M$^+$), 248, 203.

Acetate (XI) of IX——IX (13 mg) was acetylated in the usual way. The product was recrystallized from MeOH to give the acetate (XI) (13 mg) as colorless needles. mp 249—250°C, IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1}: 1730, 1260, 1240, 1045. ^{1}H-NMR ($CDCl_3$): 0.77 (3H), 0.96 (9H), 1.03 (3H), 1.16 (3H) (each s, *tert*-Me×6), 2.06 (6H, s, –OCOMe ×2), 2.88 (1H, m, C_{18}–H), 4.32 (2H, ABq, J=12.0 Hz, δ=26.4 Hz, C_4–$C\underline{H}_2$–OCOMe), 4.67 (1H, t-like, C_3–H), 5.36 (1H br s, C_{12}–H). MS *m/z*: 556 (M$^+$), 248, 203.

Methyl Ester (XII) of XI——MeI (2 ml) and Ag_2O (100 mg) were added to a solution of XI (12 mg) in MeOH (3 ml), and the reaction mixture was left standing for 1.5 h at room temperature, then filtered. The filtrate was concentrated and recrystallized from MeOH to give the methyl ester (XII) (12 mg) as colorless needles. mp 205—207°C, IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1}: 1750, 1730, 1260, 1235, 1045. ^{1}H-NMR ($CDCl_3$) : 0.74, 0.93, 0.95, 0.96, 1.04, 1.15 (each 3H, s, *tert*-Me×6), 2.06, 2.07 (each 3H, s, –OCOMe×2), 2.92 (1H, m, C_{18}–H), 3.68 (3H, s, COOMe), 4.32 (2H, ABq, J=12.0 Hz, δ=23.1 Hz, C_4–$C\underline{H}_2$–OCOMe), 4.66 (1H, t-like, C_3–H), 5.36 (1H, t-like, C_{12}–H). MS *m/z*: 570 (M$^+$), 262, 203.

Methyl Ester (X) of IX——A solution of XII (10 mg) in 2% HCl–MeOH (2 ml) was refluxed for 0.5 h, then neutralized with Ag_2CO_3. The precipitate was filtered off. The filtrate was concentrated and crystallized from MeOH to give X (6 mg) as colorless needles, mp 220—221°C, IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1}: 3270, 1730. ^{1}H-NMR ($CDCl_3$): 0.71, 0.87, 0.91, 0.94, 1.14, 1.25 (each 3H, s, *tert*-Me×6), 2.90 (1H, m, C_{18}–H), 3.39 and 4.26 (each 1H, d, J=11.5 Hz, C_4–$C\underline{H}_2OH$), 3.49 (1H, m, C_3–H), 3.69 (3H, s, COOMe), 5.36 (1H, br s, C_{12}–H). MS *m/z*: 486 (M$^+$), 262, 203.

Acetonide (XIII) of IX——A mixture of IX (4 mg), *p*-TsOH (5 mg), DMF (1 ml) and 2,2-dimethoxypropane (1 ml) was allowed to stand at room temperature for 15 h. The reaction mixture was neutralized with $NaHCO_3$ and concentrated. The residue was partitioned between AcOEt and water. The AcOEt-soluble part was concentrated and chromatographed on florisil (5 g) with benzene–AcOEt (1: 1) to give the acetonide (XIII) (2.5 mg) as colorless needles (from hexane), mp 266—268°C. ^{1}H-NMR ($CDCl_3$): 0.78, 0.91, 0.93, 1.11, 1.14, 1.22 (each 3H, s, *tert*-Me×6), 1.37 and 1.44 (each 3H, s, –O–C(Me)$_2$–O–), 3.21 and 0.45 (each 1H, d, J=11.5 Hz, C_4–CH_2–O–), 3.47 (1H, m, C_3–H), 5.30 (1H, br s, C_{12}–H).

Partial Hydrolysis of VIII——A solution of VIII (10 mg) in 0.2 N H_2SO_4–MeOH (2 ml) was refluxed for 1 h, then water was added. The mixture was concentrated to about half the initial volume, then extracted with AcOEt–*n*-BuOH (2: 1). The organic layer was washed with water and concentrated. The residue was chromatographed on silica gel (5 g) with a gradient of $CHCl_3$–MeOH (MeOH 0—6%) to give the aglycone

(IX) (4 mg) and a partial hydrolysis product (XIV) (1.8 mg). XIV, colorless needles, mp 247—249°C (dec.), $[\alpha]_D$ +40.5° (c=0.13, MeOH), IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3400, 1690. ^{1}H-NMR (pyridine-d_5): 0.79 (3H), 0.98 (6H), 1.02 (3H), 1.30 (3H), 1.55 (3H) (each s, *tert*-Me × 6), 4.92 (1H, d, J=6.6 Hz, C_1–H of arabinose unit), 5.49 (1H, br s, C_{12}–H). XIV (1 mg) was hydrolyzed with acid and worked up as described for I to give IX (trace) (identified by TLC (solv. a, b), IR) and methyl arabinoside (identified by GLC-1 as the trimethylsilylether derivative).

Permethylate (XV) of VIII——VIII (10 mg) was methylated and worked up as described for I. The product was recrystallized from MeOH to give the permethylate (XV) (4 mg) as colorless prisms, mp 203—204°C, IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: no OH, 1725. ^{1}H-NMR ($CDCl_3$): 0.71 (3H), 0.90 (3H), 0.92 (3H), 0.98 (3H), 1.11 (6H) (each s, *tert*-Me × 6), 3.24, 3.44, 3.46, 3.49, 3.50, 3.52, 3.62 (each 3H, s, OMe × 7), 4.43 (1H, d, J=5.0 Hz, C_1–H of arabinose unit), 5.12 (1H, d, J=1.6 Hz, C_1–H of rhamnose unit), 5.28 (1H, br s, C_{12}–H).

Peracetate of VIII——The peracetate of VIII was obtained as a white powder, as described in the previous paper.[4c] mp 140—142°C, *Anal.* Calcd for $C_{53}H_{78}O_{18}$: C, 64.62; H, 7.98. Found: C, 64.49; H, 8.12. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: no OH, 1750, 1240, 1050. ^{1}H-NMR ($CDCl_3$): 0.75 (3H), 0.93 (9H), 1.16 (6H) (each s, *tert*-Me × 6), 1.97—2.14 (18H, OCOMe × 6), 4.55 (1H, d, J=5.0 Hz, C_1–H of arabinose unit).

Methanolysis of XV——XV (2 mg) was methanolyzed and worked up as described for I to give the aglycone (XVI) (0.5 mg) and a methylated sugar portion which showed in TLC (solv. c) and GLC-2 the presence of methyl 2,3,4-tri-*O*-methyl-rhamnopyranoside (t_R 4′17″, 6′10″) and methyl 3,4-di-*O*-methyl-arabinopyranoside (t_R 16′02″, 32′05″). XVI, colorless needles (from MeOH), mp 190—192°C, IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3500, 1722. ^{1}H-NMR ($CDCl_3$): 0.69, 0.88, 0.89, 0.92, 1.12, 1.21 (each 3H, s, *tert*-Me × 6), 2.84 (1H, m, C_{18}–H), 3.19 and 3.88 (each 1H, d, J=9.3 Hz, C_4–CH_2–OMe), 3.31 (3H, s, C_{24}–OMe), 3.62 (3H, s, COOMe), 5.28 (1H, br s, C_{12}–H).

Acetate (XVII) of XVI——XVI (0.4 mg) was acetylated by the usual procedure. The product was crystallized from MeOH to give XVII (0.4 mg) as colorless needles, mp 214—215°C, IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1725, 1240. ^{1}H-NMR ($CDCl_3$): 0.72, 0.90, 0.92, 0.97, 0.99, 1.11 (eace 3H, s, *tert*-Me × 6), 2.05 (3H, s, OCOMe), 2.84 (1H, m, C_{18}–H), 3.28 (3H, s, C_{24}–OMe), 3.47 (2H, ABq, J=9.5 Hz, δ=26.7 Hz, C_4–CH_2–OMe), 3.62 (3H, s, COOMe), 4.55 (1H, t-like, C_3–H), 5.28 (1H, br s, C_{12}–H).

Isolation and Identification of Genuine Saponins——The fraction containing less polar saponins (described in Chart 1 in the previous paper[4a] as supernatant) was concentrated to give a brown syrupy residue, which was chromatographed on silica gel. Elution with $CHCl_3$–MeOH–H_2O (25: 6: 0.7) gave trace amounts of saponins A, B, C and D, and further elution with $CHCl_3$–MeOH–H_2O (25: 8: 1.2) gave trace amounts of E and F. They were examined by HPTLC using solvent systems $CHCl_3$–MeOH–H_2O (25: 11: 2), *sec*-BuOH–AcOEt–H_2O (8: 4: 1) and *n*-PrOH–$CHCl_3$–AcOH–H_2O (8: 2: 1: 1), and showed the same *Rf* values as prosapogenins CP_4, CP_6, CP_7, CP_8, CP_9 and CP_{10}, respectively.

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