[Chem. Pharm. Bull. 33(4)1400—1406(1985)]

Studies on the Constituents of *Hedera rhombea* BEAN. II.[1] On the Dammarane Triterpene Glycosides. (1)[2]

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(Received July 23, 1984)

Four triterpene glycosides, named Kizuta saponins K_4 (I), K_5 (II), K_7 (III) and K_{7C} (IV), were isolated from the stem and bark of *Hedera rhombea* BEAN (Araliaceae). On the basis of chemical and physicochemical evidence, they were characterized as follows: I, 3-*O*-acetyl-20(*S*)-dammar-24-ene-3β,6α,20,26-tetraol 26-*O*-β-D-glucopyranoside; II, 3-oxo-20(*S*)-dammar-24-ene-6α,20,26-triol 26-*O*-β-D-glucopyranoside; III, 20(*S*)-dammar-24-ene-3β,6α,20,26-tetraol 26-*O*-β-D-glucopyranoside; IV, 20(*S*)-dammar-24-ene-3β,20,26-triol 3,26-di-*O*-β-D-glucopyranoside.

Keywords——*Hedera rhombea*; Araliaceae; saponin; dammarane triterpene glycoside; Kizuta saponin K_4; Kizuta saponin K_5; Kizuta saponin K_7; Kizuta saponin K_{7C}

In the previous paper,[1] we reported the isolation and identification of four hederagenin glycosides, named Kizuta saponins K_3, K_6, K_{10} and K_{12}, from the stem and bark of *Hedera rhombea*. As a continuation of our work on the glycosidic constituents in this plant, eleven triterpene glycosides, named Kizuta saponins K_2, K_4 (I), K_5 (II), K_7 (III), K_{7A}, K_{7B}, K_{7C} (IV), K_8, K_9, K_{11} and K_{13}, were separated by repeated silica gel column chromatography of the crude saponin fraction as described in the experimental section. This paper deals with the structural elucidation of I—IV.

Saponin K_{7C} (IV), colorless needles (MeOH), mp 217—220 °C (dec.), $[\alpha]_D$ +2.8°, exhibited strong hydroxyl absorption bands in the infrared (IR) spectrum. Methanolysis of IV with 2 N HCl in MeOH afforded methyl glucoside from the sugar portion, and on enzymatic hydrolysis with cellulase, IV yielded a genuine aglycone (V) and a prosapogenin (VI). The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of V showed 30 carbon signals and the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of V exhibited signals ascribable to seven tertiary methyl groups and one trisubstituted olefin. Detailed examinations of the ^{1}H- and ^{13}C-NMR spectra of V suggested that V was a dammarane-type triterpene possessing three hydroxyl groups in its molecule. A comparison of the ^{13}C-NMR spectrum of V with that of dammarenediol-II (VII)[3] showed that most of the carbon signals of V, except those due to the terminal part of the side chain (C-23—C-27), were observed at almost the same positions as those of VII, while a comparison of V with that of "gypenoside XXI" (VIII), which was isolated from *Gynostemma pentaphyllum* by Takemoto *et al.*,[4] showed that the carbon resonances attributable to C-23—C-27 of V appeared at positions very similar to those of VIII. Based on the above findings, V was considered to be 20(*S*)-dammar-24-ene-3β,20,26-triol and this was confirmed by comparison of the thin-layer chromatographic (TLC) behavior, and IR, ^{1}H-NMR and ^{13}C-NMR spectra with those of an authentic sample.[5]

The prosapogenin (VI), colorless needles (MeOH), mp 209—213 °C (dec.), $[\alpha]_D$ +17.5°, exhibited an anomeric proton signal at δ 4.94 (d, J=7.1 Hz) in the ^{1}H-NMR spectrum and

showed an anomeric carbon signal at δ 107.0 in the ^{13}C-NMR spectrum. A comparison of the ^{13}C-NMR spectrum of VI with that of V showed that the signal assignable to C-3 of V was deshielded by 10.8 ppm and the signal due to C-2 of V was displaced to higher field by 1.6 ppm in the spectrum of VI, while signals attributable to C-20 and C-26 remained unshifted. Based on the above data, VI is considered to be the 3-*O*-β-D-glucopyranoside of V.

Compound IV exhibited two anomeric proton signals at δ 4.87 (d, J=7.3 Hz) and δ 4.94 (d, J=7.1 Hz) in the ^{1}H-NMR spectrum, and the ^{13}C-NMR spectrum of IV showed two anomeric carbon signals at δ 103.6 and 107.0. A comparison of the ^{13}C-NMR spectrum of IV with that of VI showed that the signals due to C-26 and C-25 of IV were observed at lower field by 7.0 ppm and at higher field by 3.9 ppm, respectively, than those of VI, while other carbon signals of IV except those due to the side chain were found at the same positions as those of VI. On the basis of the above results and the molecular rotation difference between IV and VI, IV was assigned as 20(*S*)-dammar-24-ene-3β,20,26-triol 3,26-di-*O*-β-D-glucopyranoside.

Saponin K_7 (III), a white powder (dil. MeOH), mp 128—132 °C (dec.), $[\alpha]_D$ +22.3°, showed strong hydroxyl absorption bands in the IR spectrum. On methanolysis it gave methyl glucoside and was hydrolyzed with cellulase to give a genuine aglycone (IX). IX, a white powder (dil. MeOH), mp 175—177 °C, $[\alpha]_D$ +48.7°, showed 30 carbon signals in the ^{13}C-NMR spectrum, and the ^{1}H-NMR spectrum of IX exhibited signals assignable to seven tertiary methyls, one hydroxymethyl and one trisubstituted olefin. On acetylation with acetic anhydride and pyridine at room temperature, IX gave a triacetate (X), which showed hydroxyl absorption in the IR spectrum. Detailed examinations of the ^{1}H- and ^{13}C-NMR spectra of IX suggested that IX was a dammarane triterpene having four hydroxyl groups in the molecule. A comparison of the ^{13}C-NMR spectrum of IX with those of V and 20(*S*)-protopanaxatriol (XI)[3b,6] showed that the signals due to carbons on the C and D rings and the side chain of IX were observed at similar positions to those of V, while signals due to carbons on the A and B rings of IX were found at similar positions to those of XI. The above data suggested that IX is 6α-hydroxy-V. Furthermore, the facts that a 4α-methyl signal was observed at very low field (δ 1.97) in the ^{1}H-NMR spectrum of IX measured in pyridine-d_5[6] and that the carbon signal due to C-28 of IX was found at lower field by 3.3 ppm than that of V, supported the presence of a 6α-hydroxyl group in IX. On the basis of these results, IX can be assigned as 20(*S*)-dammar-24-ene-3β,6α,20,26-tetraol.

Compound III exhibited one anomeric proton signal at δ 4.91 (d, J=7.1 Hz) in the ^{1}H-NMR spectrum, and the ^{13}C-NMR spectrum of III showed one anomeric carbon signal at δ 103.5. A comparison of the ^{13}C-NMR spectrum of III with that of IX showed that the carbon signal due to C-26 of III appeared at lower field by 7.0 ppm than that of IX. Based on the above data and the molecular rotation difference between III and IX, the structure of III was established as the 26-*O*-β-D-glucopyranoside of IX.

Saponin K_4 (I), a white powder (dil. MeOH), mp 116—120 °C (dec.), $[\alpha]_D$ +25.1°, showed ester group as well as hydroxyl group absorption bands in the IR spectrum. Compound I was methanolyzed to give methyl glucoside, and on enzymatic hydrolysis with crude hesperidinase I yielded a genuine aglycone (XII). XII, colorless needles (MeOH), mp 183 °C, $[\alpha]_D$ +56.7°, exhibited ester group absorption bands in the IR spectrum and showed a signal assignable to an acetyl group at δ 2.09 (3H, s) in the ^{1}H-NMR spectrum. The ^{13}C-NMR spectrum of XII showed 32 carbon signals, two of which were ascribable to an acetyl group. Alkaline hydrolysis of XII with 0.2 N KOH in 70% MeOH gave a desacetyl product, which was identified as IX by direct comparison (TLC, IR, ^{1}H-NMR). Thus, XII is a monoacetate of IX. A comparison of the ^{13}C-NMR spectrum of XII with that of IX showed that the signals due to C-3 and C-2 of XII appeared at lower field by 2.7 ppm and at higher field by 4.2 ppm, respectively, than those of IX. Based on the above findings, XII is considered to be the 3-*O*-

acetate of IX.

Compound I showed one anomeric proton signal at δ 4.91 (d, $J=7.3$ Hz) in the ^{1}H-NMR spectrum and showed one anomeric carbon signal in the ^{13}C-NMR spectrum. A comparison of the ^{13}C-NMR spectrum of I with those of XII and III revealed that the glucose unit in I is linked to the C-26 hydroxyl group of XII. Based on the above results and the molecular rotation difference between I and XII, I can be represented as 3-*O*-acetyl-20(*S*)-dammar-24-ene-3β,6α,20,26-tetraol 26-*O*-β-D-glucopyranoside.

Saponin K_5 (II), colorless needles (dil. MeOH), mp 133—135 °C (dec.), $[\alpha]_D$ +84.5 °, showed carbonyl and hydroxyl group absorptions in the IR spectrum. On methanolysis, II gave methyl glucoside, and on enzymatic hydrolysis with cellulase, it yielded a genuine aglycone (XIII). XIII, colorless needles (Et_2O), mp 161—163 °C, $[\alpha]_D$ +145.0 °, exhibited a carbonyl absorption band at 1690 cm^{-1} in the IR spectrum and showed the carbonyl carbon signal at δ 218.5 in the ^{13}C-NMR spectrum. The ^{1}H-NMR spectrum of XIII showed signals attributable to seven tertiary methyls, one hydroxymethyl and one trisubstituted olefin. On

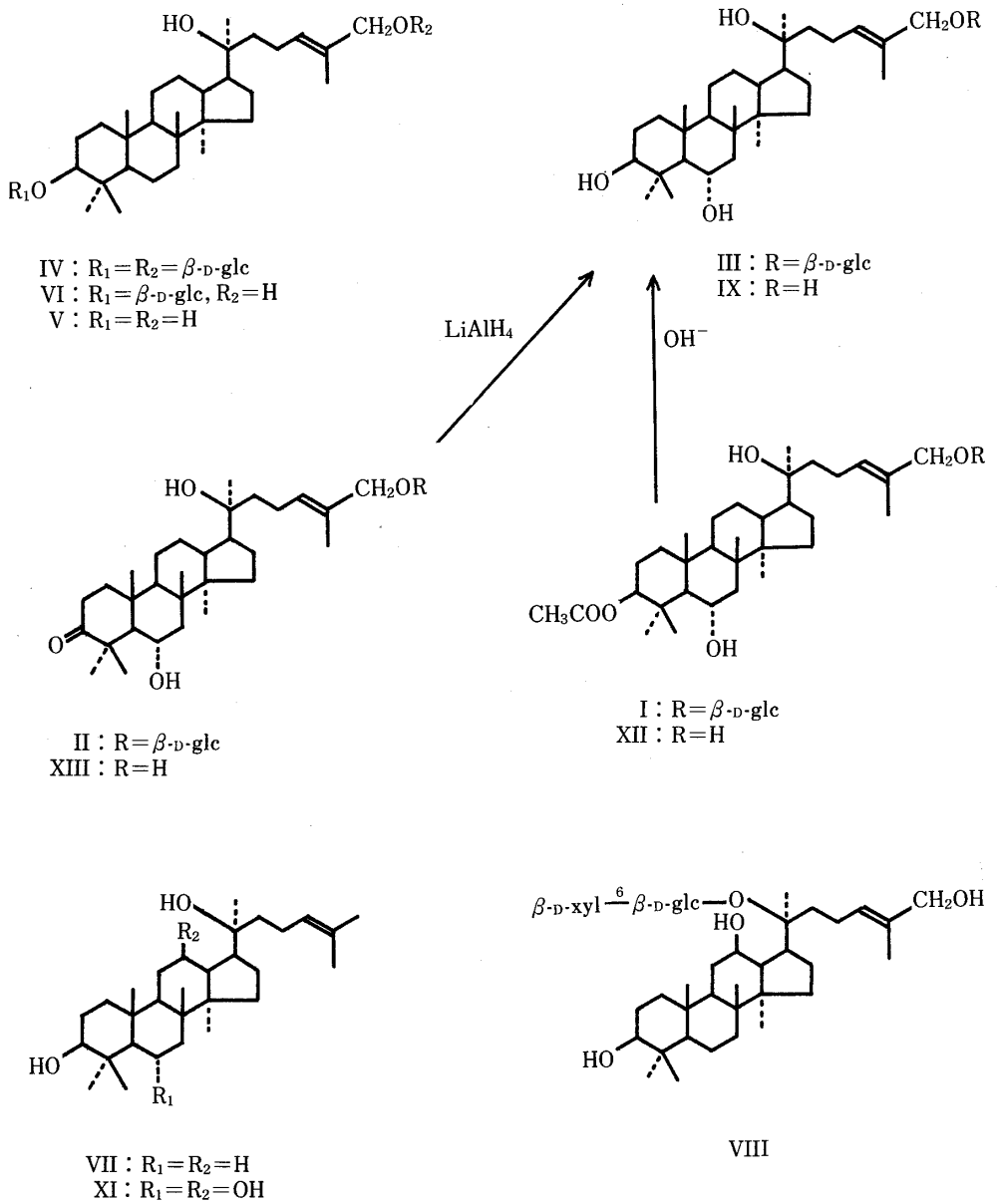

Chart 1

acetylation with acetic anhydride and pyridine at room temperature, XIII afforded a diacetate (XIV), which exhibited hydroxyl absorption bands in the IR spectrum. These data suggested that XIII is a dammarane triterpene possessing three hydroxyl groups and a keto group in the molecule. Compound XIII was treated with $LiAlH_4$ to give a tetraol, which was identifield as IX by direct comparison (TLC, IR, ^{1}H-NMR, ^{13}C-NMR). Thus, XIII was considered to be a keto derivative of IX. A comparison of the ^{13}C-NMR spectrum of XIII with that of IX

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

	VII	V	VIII[a]	XI[b]	IX	XII	XIII	I	II	III	VI	IV
C-1	39.6	39.6	39.7	39.3	39.5	38.8	40.2	38.8	40.2	39.5	39.3	39.4
C-2	28.4	28.4	28.3	28.0	28.1	23.9	33.4	23.9	33.4	28.1	26.8	26.8
C-3	78.2	78.2	78.4	78.3	78.6	81.3	218.5	81.3	218.6	78.6	89.0	89.0
C-4	39.6	39.6	39.7	40.2	40.4	38.9	47.8	38.9	47.8	40.4	39.7	39.7
C-5	56.5	56.5	56.6	61.7	62.0	61.6	59.6	61.6	59.6	61.9	56.5	56.5
C-6	18.8	18.8	18.9	67.6	67.9	67.4	66.9	67.4	66.9	67.8	18.5	18.5
C-7	35.8	35.8	35.4	47.4	48.1	47.9	45.9	47.9	45.9	48.1	35.7	35.7
C-8	40.8	40.8	40.3	41.1	41.9	41.7	41.4	41.7	41.3	41.8	40.7	40.7
C-9	51.2	51.2	50.5	50.1	50.9	50.6	49.7	50.6	49.6	50.7	51.1	51.1
C-10	37.5	37.5	37.5	39.3	39.5	39.3	38.4	39.2	38.4	39.5	37.0	37.1
C-11	22.0	22.0	31.0	31.9	22.0	21.9	22.9	21.9	22.9	21.9	21.9	21.9
C-12	25.4	25.4	70.4	70.9	25.4	25.4	25.3	25.3	25.3	25.4	25.3	25.3
C-13	42.6	42.7	49.7	48.1	42.2	42.1	42.4	42.1	42.4	42.2	42.6	42.6
C-14	50.7	50.7	51.6	51.6	50.7	50.7	50.6	50.7	50.6	50.7	50.7	50.7
C-15	31.7	31.7	30.7	31.3	31.7	31.6	31.6	31.6	31.6	31.6	31.7	31.7
C-16	28.2	28.2	26.8	26.8	28.2	28.0	28.1	28.0	28.1	28.1	28.1	28.2
C-17	50.5	50.5	51.9	54.6	50.5	50.5	50.4	50.4	50.4	50.4	50.4	50.4
C-18	16.6	16.6	16.4	17.5[c]	17.7	17.5	17.7	17.5	17.7	17.6	16.5	16.5
C-19	15.8	15.8	16.4	17.4[c]	17.4	17.3	16.1	17.3	16.1	17.3	15.7	15.7
C-20	74.2	74.2	83.7	72.9	74.2	74.1	74.3	74.1	74.3	74.1	74.2	74.1
C-21	26.2	26.1	22.5	26.9	26.1	26.1	26.1	26.1	26.0	26.0	26.0	26.0
C-22	41.9	41.8	36.2	35.7	41.8	41.7	41.7	41.5	41.5	41.5	41.8	41.6
C-23	23.3	22.9	23.0	22.9	22.9	22.9	22.9	22.9	23.0	22.9	22.9	22.9
C-24	126.3	125.7	125.8	126.2	125.7	125.7	125.8	129.4	129.4	129.4	125.7	129.4
C-25	130.9	136.2	136.2	130.6	136.3	136.2	136.2	132.2	132.3	132.2	136.1	132.2
C-26	25.8	68.2	68.3	25.8	68.2	68.2	68.3	75.2	75.2	75.2	68.2	75.2
C-27	17.7	14.0	14.2	17.7	14.0	14.0	13.9	14.3	14.2	14.2	14.0	14.3
C-28	28.7	28.7	28.8	31.9	32.0	31.3	32.2	31.3	32.2	32.0	28.1	28.2
C-29	16.3	16.3	16.4	16.4[c]	16.5	17.0	20.0	17.0	20.0	16.5	16.8	16.8
C-30	16.8	16.8	17.6	17.0	16.8	16.8	16.7	16.8	16.7	16.8	16.8	16.8
$\underline{C}H_3CO$						21.2		21.2				
$CH_3\underline{C}O$						170.9		170.9				
		20-*O*-Sugar										
	Glucose	C-1	98.2		26-*O*-Glucose		C-1	103.5	103.5	103.5		103.6
	moiety	C-2	75.0		moiety		C-2	75.2	75.2	75.2		75.2
		C-3	79.1				C-3	78.6[d]	78.6[d]	78.6[d]		78.7[d]
		C-4	71.8				C-4	71.8	72.0	71.8		71.8
		C-5	76.9				C-5	78.4[d]	78.2[d]	78.4[d]		78.5[d]
		C-6	70.0				C-6	62.9	63.1	62.9		62.9
	Xylose	C-1	105.5		3-*O*-Glucose		C-1				107.0	107.0
	moiety	C-2	74.8		moiety		C-2				75.7	75.8
		C-3	77.8				C-3				78.7[d]	78.8[d]
		C-4	71.1				C-4				71.9	71.9
		C-5	66.8				C-5				78.2[d]	78.3[d]
							C-6				63.1	63.1

a) Data are taken from ref. 4. *b*) Data are taken from ref. 3*b*. *c*, *d*) Assignments may be reversed in each column.

showed that a signal observed at δ 78.3 (C-3) in IX had disappeared and signals due to C-2 and C-4 were found at lower field by 5.3 and 7.4 ppm, respectively, than those of IX in the spectrum of XIII. Furthermore, the signals due to carbons on the B ring of XIII were found to be somewhat shifted and those on the C and D rings and the side chain of XIII were observed at similar positions to those of IX. On the basis of these results, the structure of XIII was established as 3-oxo-20(*S*)-dammar-24-ene-6α,20,26-triol.

Compound II exhibited one anomeric proton signal at δ 4.87 (d, J=7.3 Hz) in the ^{1}H-NMR spectrum and showed one anomeric carbon signal at δ 103.5 in the ^{13}C-NMR spectrum. A comparison of the ^{13}C-NMR spectrum of II with those of XIII and III revealed that the glucose unit in II was linked to C-26 of XIII. Based on the above facts and the molecular rotation difference between II and XIII, II was concluded to be the 26-*O*-β-D-glucopyranoside of XIII.

The sapogenins IX, XII and XIII are new dammarane-type triterpenes, and the glycosides I—IV are the first examples of dammarane triterpenes having the sugar unit at the C-26 position. The structures of other saponins will be reported in the next paper.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were taken at 100 MHz and at 25 MHz, respectively, with a JEOL JNM-FX-100 spectrometer and chemical shifts are given as δ (ppm) with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). 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 a glass column (2 m×4 mm i.d.) packed with 5% SE-30 on Chromosorb W (60—80 mesh); column temperature, 180 °C. TLC was performed on precoated Silica gel 60F_{254} plates and spots were detected by spraying dil. H_2SO_4 followed by heating.

Isolation——Fresh stems and barks of *Hedera rhombea* (28 kg) were extracted with hot MeOH three times. The MeOH solution was concentrated to give a brown residue, the warm water-soluble portion of which was extracted successively with Et_2O, AcOEt and BuOH. The AcOEt-soluble portion was concentrated and the residue was dissolved in AcOEt–MeOH. This solution was poured into petroleum benzin to give precipitates (crude saponin A, 180 g). The BuOH-soluble portion was concentrated and the residue was dissolved in MeOH; the solution was poured into AcOEt with stirring to give precipitates (crude saponin B, 300 g). The crude saponin A (110 g) was chromatographed on silica gel (2 kg) with AcOEt saturated with water to give Frs. 1—10. Fractions 1, 2 and 3 were recrystallized from MeOH to give saponin K_1 (trace), K_2 (yield from the starting material: 0.0001%) and K_3 (0.002%),[1)] respectively. Fraction 4 was chromatographed repeatedly on silica gel [solv.: AcOEt saturated with water; gradient of $CHCl_3$–MeOH (MeOH 0—5%)] to give saponin K_4 (I, 0.005%). Fraction 5 was chromatographed on silica gel with a gradient of $CHCl_3$–MeOH (MeOH 0—7%) to give saponin K_5 (II, 0.04%). Fraction 6 was chromatographed repeatedly on silica gel [solv.: AcOEt saturated with water; gradient of $CHCl_3$–MeOH (MeOH 0—10%)] to give saponin K_7 (III, 0.02%). Fraction 7 was recrystallized from MeOH to give saponin K_6 (0.006%).[1)] Fraction 8 was chromatographed repeatedly on silica gel [solv.: AcOEt saturated with water; gradient of $CHCl_3$–MeOH (MeOH 0—10%)] to give saponin K_{7A} (0.005%). The crude saponin B (100 g) was chromatographed on silica gel (2 kg) with $CHCl_3$–MeOH–H_2O (25:3:0.3→25:6:0.7→25:8:1.2→25:11:2) to give Frs. 11—17. Fraction 11 was chromatographed on silica gel with AcOEt–BuOH–H_2O (5:1:0.1) to give saponin K_7 (III, 0.02%) and K_{7A} (0.03%). Fraction 12 was chromatographed repeatedly on silica gel [solv.: AcOEt–BuOH–H_2O (5:1:0.1); gradient of $CHCl_3$–MeOH (MeOH 0—15%)] to give saponin K_{7B} (0.003%). Fraction 13 was recrystallized from MeOH to give saponin K_{7C} (IV, 0.002%). Fraction 14 was chromatographed repeatedly on silica gel [solv.: AcOEt–BuOH–H_2O (1:4:0.3); $CHCl_3$–MeOH–H_2O (25:8:1.2)] to give saponins K_8 (0.0002%) and K_9 (0.005%). Fraction 15 was chromatographed repeatedly on silica gel with BuOH saturated with water to give saponins K_{10} (0.1%)[1)] and K_{11} (0.0004%). Fraction 16 was chromatographed on silica gel with a gradient of $CHCl_3$–MeOH (MeOH 0—30%) to give saponin K_{13} (0.002%). Fraction 17 was chromatographed on silica gel with a gradient of $CHCl_3$–MeOH (MeOH 0—30%) to give saponin K_{12} (0.17%).[1)]

Saponin K_{7C} (IV)——Colorless needles (from MeOH), mp 217—220 °C (dec.), $[\alpha]_D^{20}$ +2.8° (c=1.00, MeOH). *Anal.* Calcd for $C_{42}H_{72}O_{13}\cdot 2H_2O$: C, 61.44; H, 9.33. Found: C, 61.25; H, 9.39. IR ν_{max}^{KBr} cm^{-1}: 3400, 1100—1000. ^{1}H-NMR (pyridine-d_5): 0.79 (3H), 0.95 (3H), 1.00 (6H), 1.31 (3H), (each s, *tert*-Me×5), 1.39 (3H, s, C_{20}-Me), 1.81 (3H, s, C_{25}-Me), 3.39 (1H, m, C_3-H), 4.87 (1H, d, J=7.3 Hz, C_1-H of 26-*O*-glucose unit), 4.94 (1H, d, J=7.1 Hz, C_1-H of 3-*O*-glucose unit), 5.73 (1H, t-like, C_{24}-H). ^{13}C-NMR: Table I. $\Delta[M]_D$: IV—VI=−86.9°. $[M]_D$ of methyl β-D-

glucopyranoside: $-66°$.

Methanolysis of IV——A solution of IV (10 mg) in 10% HCl–MeOH (2 ml) was heated under reflux on a water bath for 2 h and neutralized with Ag_2CO_3. The precipitates were filtered off and the filtrate was concentrated to give the residue, which was partitioned between a mixture of AcOEt–BuOH (2 : 1) and water. The aqueous layer was concentrated and analyzed by TLC [solv., $CHCl_3$–MeOH–H_2O (25 : 11 : 2)] and GLC (as the trimethylsilylether derivative), which revealed the presence of methyl glucoside.

Enzymatic Hydrolysis of IV——A suspension of IV (185 mg) in AcOH–AcONa buffer (pH 4.5, 135 ml) with cellulase (Tokyo Kasei Kogyo Co., 200 mg) was shaken at 37 °C for 30 d. The reaction mixture was then extracted with BuOH. The BuOH extract was washed with water and concentrated to give the residue, which was chromatographed on silica gel (15 g) with a gradient of $CHCl_3$–MeOH (MeOH 0—15%) to give an aglycone (V, 10 mg), a prosapogenin (VI, 60 mg) and unchanged IV (9 mg).

V, colorless needles (from MeOH), mp 196 °C. IR ν_{max}^{KBr} cm^{-1}: 3400, 1030—990. ^{1}H-NMR (pyridine-d_5): 0.88 (3H), 0.99 (6H), 1.06 (3H), 1.25 (3H) (each s, *tert*-Me × 5), 1.44 (3H, s, C_{20}-Me), 1.87 (3H, s, C_{25}-Me), 3.47 (1H, t-like, C_3-H), 4.33 (2H, s, C_{25}-CH_2OH), 5.84 (1H, t-like, C_{24}-H). ^{1}H-NMR ($CDCl_3$): 0.78, 0.85, 0.89, 0.97, 0.98, 1.16 (each 3H, s, *tert*-Me × 6), 1.69 (3H, s, C_{25}-Me), 3.20 (1H, m, C_3-H), 4.01 (2H, s, C_{25}-CH_2OH), 5.43 (1H, t-like, C_{24}-H). ^{13}C-NMR: Table I. V was identified as 20(*S*)-dammar-24-ene-3β,20-26-triol by direct comparison of TLC behavior and IR, ^{1}H-NMR and ^{13}C-NMR spectra with those of an authentic sample.

VI, colorless needles (from MeOH), mp 209—213 °C (dec.), $[\alpha]_D^{20}$ +17.5° (c=1.00, MeOH). *Anal.* Calcd for $C_{36}H_{62}O_8 \cdot H_2O$: C, 67.47; H, 10.07. Found: C, 67.19; H, 10.13. ^{1}H-NMR (pyridine-d_5): 0.80 (3H), 0.95 (3H), 1.01 (6H), 1.32 (3H) (each 3H, s, *tert*-Me × 5), 1.42 (3H, s, C_{20}-Me), 1.86 (3H, s, C_{25}-Me), 3.40 (1H, m, C_3-H), 4.31 (2H, s, C_{25}-CH_2OH), 4.94 (1H, d, J=7.1 Hz, C_1-H of glucose unit), 5.82 (1H, t-like, C_{24}-H). ^{13}C-NMR: Table I.

Saponin K_7 (III)——A white powder (from dil. MeOH), mp 128—132 °C (dec.), $[\alpha]_D^{20}$ +22.3° (c=0.97, MeOH). *Anal.* Calcd for $C_{36}H_{62}O_9 \cdot 2H_2O$: C, 64.07; H, 9.86. Found: C, 64.12; H, 9.91. IR ν_{max}^{KBr} cm^{-1}: 3400, 1100—1000. ^{1}H-NMR (pyridine-d_5): 0.99 (6H), 1.09 (3H) (each s, *tert*-Me × 3), 1.40 (3H, s, C_{20}-Me), 1.46 (3H, s, C_4-βMe), 1.82 (3H, s, C_{25}-Me), 1.99 (3H, s, C_4-αMe), 3.56 (1H, t-like, C_3-H), 4.91 (1H, d, J=7.1 Hz, C_1-H of glucose unit), 5.75 (1H, t-like, C_{24}-H). ^{13}C-NMR: Table I. $\Delta[M]_D$: III—IX = −89.5°. $[M]_D$ of methyl β-D-glucopyranoside: −66°. III (10 mg) was methanolyzed and worked up as described for IV to give methyl glucoside.

Enzymatic Hydrolysis of III——A suspension of III (200 mg) in AcOH–AcONa buffer (pH 5.0, 300 ml) with cellulase (400 mg) was shaken at 37 °C for 7 d. The reaction mixture was then extracted with Et_2O. The Et_2O extract was concentrated and the residue was chromatographed on silica gel (20 g) with a gradient of benzene–AcOEt (AcOEt 0—40%) to give an aglycone (IX, 70 mg). IX, colorless needles (from Et_2O), mp 175—177 °C, $[\alpha]_D^{20}$ +48.7° (c=1.10, MeOH). *Anal.* Calcd for $C_{30}H_{52}O_4$: C, 75.58; H, 10.99. Found: C, 75.48; H, 11.05. IR ν_{max}^{KBr} cm^{-1}: 3400, 1100—1000. ^{1}H-NMR (pyridine-d_5): 0.99 (6H), 1.08 (3H) (each s, *tert*-Me × 3), 1.41 (3H, s, C_{20}-Me), 1.44 (3H, s, C_4-βMe), 1.85 (3H, s, C_{25}-Me), 1.97 (3H, s, C_4-αMe), 3.53 (1H, t-like, C_3-H), 4.30 (2H, s, C_{25}-CH_2OH), 4.40 (1H, m, C_6-H), 5.81 (1H, t-like, C_{24}-H). ^{1}H-NMR ($CDCl_3$): 0.90 (6H), 0.98 (3H), 1.04 (3H), 1.15 (3H), 1.31 (3H) (each s, *tert*-Me × 6), 1.68 (3H, s, C_{25}-Me), 3.18 (1H, m, C_3-H), 3.99 (2H, s, C_{25}-CH_2OH), 4.14 (1H, m, C_6-H), 5.41 (1H, t-like, C_{24}-H). ^{13}C-NMR: Table I.

Acetylation of IX——IX (50 mg) in pyridine (1 ml) was treated with acetic anhydride (1 ml) at room temperature for 20 h. The reaction mixture was concentrated and the residue was chromatographed on silica gel (5 g) with a gradient of benzene–AcOEt (AcOEt 0—15%) to give the acetate (X, 40 mg). X, a white powder (from dil. MeOH), mp 60—61 °C. *Anal.* Calcd for $C_{36}H_{58}O_7$: C, 71.72; H, 9.70. Found: C, 71.41; H, 9.88. IR ν_{max}^{KBr} cm^{-1}: 3500, 1730, 1240, 1020. ^{1}H-NMR (pyridine-d_5): 0.89, 0.99, 1.03, 1.11, 1.20 (each 3H, s, *tert*-Me × 5), 1.40 (3H, s, C_{20}-Me), 1.72 (3H, s, C_{25}-Me), 2.03, 2.08, 2.10 (each 3H, s, MeCO × 3), 4.62 (2H, s, C_{25}-CH_2OCOMe), 4.68 (1H, t-like, C_3-H), 5.56 (1H, m, C_6-H), 5.68 (1H, t-like, C_{24}-H). ^{1}H-NMR ($CDCl_3$): 0.90, 0.91, 0.98, 1.03, 1.10, 1.14 (each 3H, s, *tert*-Me × 6), 1.66 (3H, s, C_{25}-Me), 2.04, 2.05, 2.07 (each 3H, s, MeCO × 3), *ca.* 4.45 (1H, m, C_3-H), 4.45 (2H, s, C_{25}-CH_2OCOMe), 5.38 (1H, m, C_6-H), 5.46 (1H, t-like, C_{24}-H).

Saponin K_4 (I)——A white powder (from Et_2O–$CHCl_3$), mp 116—120 °C (dec.), $[\alpha]_D^{20}$ +25.1° (c=1.50, MeOH). *Anal.* Calcd for $C_{38}H_{64}O_{10} \cdot 2H_2O$: C, 63.66; H, 9.56. Found: C, 63.83; H, 9.49. IR ν_{max}^{KBr} cm^{-1}: 3400, 1710, 1250, 1100—1000. ^{1}H-NMR (pyridine-d_5): 0.93, 0.99, 1.06, 1.32 (each 3H, s, *tert*-Me × 4), 1.40 (3H, s, C_{20}-Me), 1.65 (3H, s, C_4-αMe), 1.82 (3H, s, C_{25}-Me), 2.10 (3H, s, MeCO), 4.76 (1H, t-like, C_3-H), 4.91 (1H, d, J=7.3 Hz, C_1-H of glucose unit), 5.73 (1H, t-like, C_{24}-H). ^{13}C-NMR: Table I. $\Delta[M]_D$: I—XII = −123.0°. $[M]_D$ of methyl β-D-glucopyranoside = −66°. I (10 mg) was methanolyzed and worked up as described for IV to give methyl glucoside.

Enzymatic Hydrolysis of I——A suspension of I (750 mg) in AcOH–AcONa buffer (pH 5.0, 100 ml) with crude hesperidinase (Tanabe Pharm. Ind., Co., Ltd., 750 mg) was shaken at 37 °C for 4 d. The reaction mixture was extracted with AcOEt. The AcOEt extract was washed with water and concentrated. The residue (510 mg) was chromatographed on silica gel (50 g) with a gradient of benzene–AcOEt (AcOEt 0—30%) to give the aglycone (XII, 90 mg). XII, colorless needles (from MeOH), mp 183 °C, $[\alpha]_D^{20}$ +56.7° (c=1.50, MeOH). *Anal.* Calcd for $C_{32}H_{54}O_5$: C, 74.09; H, 10.49. Found: C, 74.03; H, 10.55. IR ν_{max}^{KBr} cm^{-1}: 3400, 1710, 1260, 1010. ^{1}H-NMR (pyridine-d_5): 0.93, 0.99, 1.05, 1.31 (each 3H, s, *tert*-Me × 4), 1.41 (3H, s, C_{20}-Me), 1.63 (3H, s, C_4-αMe), 1.85 (3H, s, C_{25}-Me), 2.09 (3H, s, MeCO), 4.30 (2H, s, C_{25}-CH_2OH), *ca.* 4.30 (1H, m, C_6-H), 4.76 (1H, t-like, C_3-H), 5.82 (1H, t-like, C_{24}-H). ^{1}H-NMR

($CDCl_3$): 0.90, 0.93, 1.04, 1.06, 1.15, 1.17 (each 3H, s, *tert*-Me × 6), 1.68 (3H, s, C_{25}-Me), 2.06 (3H, s, MeCO), 4.00 (2H, s, C_{25}-CH_2OH), 4.13 (1H, m, C_6-H), 4.45 (1H, t-like, C_3-H), 5.41 (1H, t-like, C_{24}-H). ^{13}C-NMR: Table I.

Alkaline Hydrolysis of XII——XII (10 mg) was treated with 0.2 N KOH in 70% MeOH at room temperature for 20 h. The reaction mixture was neutralized with 0.4 N H_2SO_4 and concentrated. The residue was partitioned between AcOEt and water. The AcOEt layer was concentrated and the residue was treated with dil. MeOH to give the deacetylated product (5 mg), which was identified as IX by TLC [solv., $CHCl_3$–MeOH (25 : 3), benzene–AcOEt (1 : 3)], IR and ^{1}H-NMR.

Saponin K_5 (II)——Colorless needles (dil. MeOH), mp 133—135 °C (dec.), $[\alpha]_D^{20}$ +84.5° (c = 1.04, MeOH). *Anal.* Calcd for $C_{36}H_{60}O_9 \cdot 4/3H_2O$: C, 65.43; H, 9.56. Found: C, 65.56; H, 9.40. IR ν_{max}^{KBr} cm^{-1}: 3400, 1690, 1100—1000. ^{1}H-NMR (pyridine-d_5): 0.78, 0.99, 1.01 (each 3H, s, *tert*-Me × 3), 1.39 (3H, s, C_{20}-Me), 1.66 (6H, s, *tert*-Me × 2), 1.82 (3H, s, C_{25}-Me), 4.87 (1H, d, J = 7.3 Hz, C_1-H of glucose unit), 5.73 (1H, t-like, C_{24}-H). ^{13}C-NMR: Table I. $\Delta[M]_D$: II—XIII = −149.9°. $[M]_D$ of methyl β-D-glucopyranoside: −66°. II (10 mg) was methanolyzed and worked up as described for IV to give methyl glucoside.

Enzymatic Hydrolysis of II——A suspension of II (7.6 g) in AcOH–AcONa buffer (pH 5.0, 760 ml) with cellulase (15 g) was shaken at 36—38 °C for 7 d. The reaction mixture was then extracted with AcOEt. The AcOEt extract was washed with water and concentrated. The residue was chromatographed on silica gel (500 g) with a gradient of hexane–AcOEt (AcOEt 0—50%) to give an aglycone (XIII, 4 g). XIII, colorless needles (from Et_2O), mp 161—163 °C, $[\alpha]_D^{20}$ +145° (c = 1.14, MeOH). *Anal.* Calcd for $C_{30}H_{50}O_4$: C, 75.90; H, 10.62. Found: C, 75.83; H, 10.67. IR ν_{max}^{KBr} cm^{-1}: 3400, 1690. ^{1}H-NMR (pyridine-d_5): 0.79, 1.00, 1.01 (each 3H, s, *tert*-Me × 3), 1.41 (3H, s, C_{20}-Me), 1.66, 1.67 (each 3H, s, *tert*-Me × 2), 1.85 (3H, s, C_{25}-Me), 4.13 (1H, m, C_6-H), 4.30 (2H, s, C_{25}-CH_2OH), 5.75 (1H, t-like, C_{24}-H). ^{1}H-NMR ($CDCl_3$): 0.74, 0.93, 1.02, 1.18, 1.31, 1.36 (each 3H, s, *tert*-Me × 6), 1.67 (3H, s, C_{25}-Me), 3.99 (2H, s, C_{25}-CH_2OH), *ca.* 3.99 (1H, m, C_6-H), 5.42 (1H, t-like, C_{24}-H). ^{13}C-NMR: Table I.

Acetylation of XIII——A solution of XIII (50 mg) in pyridine (0.5 ml) and acetic anhydride (0.3 ml) was allowed to stand at room temperature for 26 h. The reaction mixture was concentrated and the residue was treated with dil. MeOH to give the acetate (XIV) as a white powder (40 mg), mp 54—58 °C. IR ν_{max}^{KBr} cm^{-1}: 3475, 1710, 1235, 1020. ^{1}H-NMR (pyridine-d_5): 0.75, 0.96, 1.06, 1.25, 1.35, 1.38 (each 3H, s, *tert*-Me × 6), 1.72 (3H, s, C_{25}-Me), 2.02, 2.09 (each 3H, s, MeCO × 2), 4.60 (2H, s, C_{25}-CH_2OCOMe), 5.30 (1H, m, C_6-H), 5.67 (1H, t-like, C_{24}-H). ^{1}H-NMR ($CDCl_3$): 0.80 (3H), 0.91 (3H), 1.08 (6H), 1.15 (3H), 1.23 (3H), (each s, *tert*-Me × 6), 1.66 (3H, s, C_{25}-Me), 2.07 (6H, s, MeCO × 2), 4.45 (2H, s, C_{25}-CH_2OCOMe), 5.08 (1H, m, C_6-H), 5.46 (1H, t-like, C_{24}-H).

$LiAlH_4$ Reduction of XIII——$LiAlH_4$ (270 mg) was gradually added to a solution of XIII (268 mg) in tetrahydrofuran–Et_2O (1 : 1, 26 ml). Then AcOEt (50 ml) was added to the reaction mixture, followed by dil. AcOH (50 ml). The AcOEt-soluble portion was concentrated and the residue was chromatographed on silica gel (50 g) with a gradient of benzene–AcOEt (AcOEt 0—50%) to give the reduction product (120 mg) as a white powder (from dil. MeOH). This was identified as IX by direct comparison of TLC behavior [solv., Et_2O–AcOEt (1 : 1), $CHCl_3$–MeOH (25 : 3)] and IR, ^{1}H-NMR and ^{13}C-NMR spectra.

Acknowledgement We are grateful to Prof. O. Tanaka of Hiroshima University for his generous gifts of dammarenediol-II and related compounds, and to Prof. R. Stevenson of Brandeis University for an authentic sample of 20(*S*)-dammar-24-ene-3β,20,26-triol. Thanks are also due to Mrs. R. Igarashi of this University for micro analysis.

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