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

HARUHISA KIZU, MICHIYO KOSHIJIMA and TSUYOSHI TOMIMORI*

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

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On the basis of chemical and physicochemical evidence, the structures of four dammarane triterpene glycosides, named Kizuta saponins K_{7A} (I), K_{7B} (VII), K_9 (X) and K_{13} (XII), which were isolated from the stem and bark of *Hedera rhombea* BEAN (Araliaceae), were established as follows: I, 3-oxo-20(*S*)-dammar-24-ene-6α,20,21,26-tetraol 26-*O*-β-D-glucopyranoside; VII, 20(*S*)-dammar-24-ene-3β,6α,20,21,26-pentaol 26-*O*-β-D-glucopyranoside; X, 20(*S*)-dammar-24-ene-3β,6α,20,26-tetraol 3,26-di-*O*-β-D-glucopyranoside; XII, 20(*S*)-dammar-24-ene-3β,6α,20,26-tetraol 3-*O*-β-sophoroside-26-*O*-β-D-glucopyranoside.

Keywords——*Hedera rhombea*; Araliaceae; saponin; dammarane triterpene glycoside; Kizuta saponin K_{7A}; Kizuta saponin K_{7B}; Kizuta saponin K_9; Kizuta saponin K_{13}

In the previous papers,[1,3] we reported the isolation of fifteen glycosidic constituents from the stem and bark of *Hedera rhombea* BEAN (Araliaceae). The structures of eight glycosides, named Kizuta saponins K_3, K_4, K_5, K_6, K_7, K_{7C}, K_{10} and K_{12}, were also reported.[1,3] This paper describes the structural elucidation of four (Kizuta saponins K_{7A}, K_{7B}, K_9, K_{13}) of the remaining seven glycosides.

Saponin K_{7A} (I), a white powder (dil. MeOH), mp 181—185 °C (dec.), $[\alpha]_D$ +79.5°, exhibited absorption bands of carbonyl and hydroxyl groups in the infrared (IR) spectrum and was methanolyzed with 2 N HCl in MeOH to give methyl glucoside from the sugar portion. On enzymatic hydrolysis with cellulase, I yielded a genuine aglycone (II). Compound II, colorless needles (Et_2O–hexane), mp 143—145 °C, $[\alpha]_D$ +132.0°, showed signals due to five quaternary carbons, five methines, eleven methylenes, six methyls, one ketone and two olefinic carbons in the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of II showed signals ascribable to six tertiary methyls, two hydroxymethyls, one methine on carbon bearing a hydroxyl group and one trisubstituted olefin. Detailed examinations of these data suggested that II was a dammarane triterpene possessing four hydroxyl groups and one keto group as well as one trisubstituted double bond in the molecule. A comparison of the ^{13}C-NMR spectrum of II with that of 3-oxo-20(*S*)-dammar-24-ene-6α,20,26-triol (III, the aglycone of Kizuta saponin K_5[1]) showed that most of the signals of II except those due to C-12, -13, -17, -20, -21 and -22, were observed at positions similar to those of III, while the C-20-methyl signal found at δ 26.1 in the spectrum of III was absent, but instead a signal attributable to hydroxymethyl carbon appeared at δ 66.8 in the spectrum of II. These findings suggested that II is 21-hydroxy-III. This was also supported by the ^{1}H-NMR spectrum of II, in which the C-20-methyl signal observed at δ 1.41 in the spectrum of III was not found, but instead a signal assignable to C-20-hydroxymethyl appeared at δ 4.05. However, these data do not define the configuration at C-20. Finally, the structure of II was confirmed by the following facts. Catalytic hydrogenation of II resulted in IV, which was assigned as 3-oxo-dammarane-6α,20,21-triol based on the ^{1}H- and ^{13}C-NMR spectral data. Compound IV was partially

tosylated with *p*-toluenesulfonylchloride (*p*-TsCl) in pyridine at room temperature for 2 h to give the 21-*O*-tosylate (V) as a major product. Compound V was treated with $LiAlH_4$ to give a triol derivative (VI), which was identical with the compound derived from III on catalytic hydrogenation followed by $LiAlH_4$ reduction (Chart 1). Consequently, the structure of II was concluded to be 3-oxo-20(*S*)-dammar-24-ene-6α,20,21,26-tetraol.

Compound I exhibited one anomeric proton signal at δ 4.86 (d, J=7.1 Hz) in the ^{1}H-NMR spectrum and one anomeric carbon signal in the ^{13}C-NMR spectrum. A comparison of the ^{13}C-NMR spectrum of I with those of II and Kizuta saponin K_5[1] revealed that the glucose unit in I was linked to C-26 of II. Based on these findings and the molecular rotation difference between I and II, I was concluded to be the 26-*O*-β-D-glucopyranoside of II.

Saponin K_{7B} (VII), a white powder (dil. MeOH), mp 131—134 °C (dec.), $[\alpha]_D$ +23.0°, exhibited strong hydroxyl absorption bands in the IR spectrum. Compound VII was

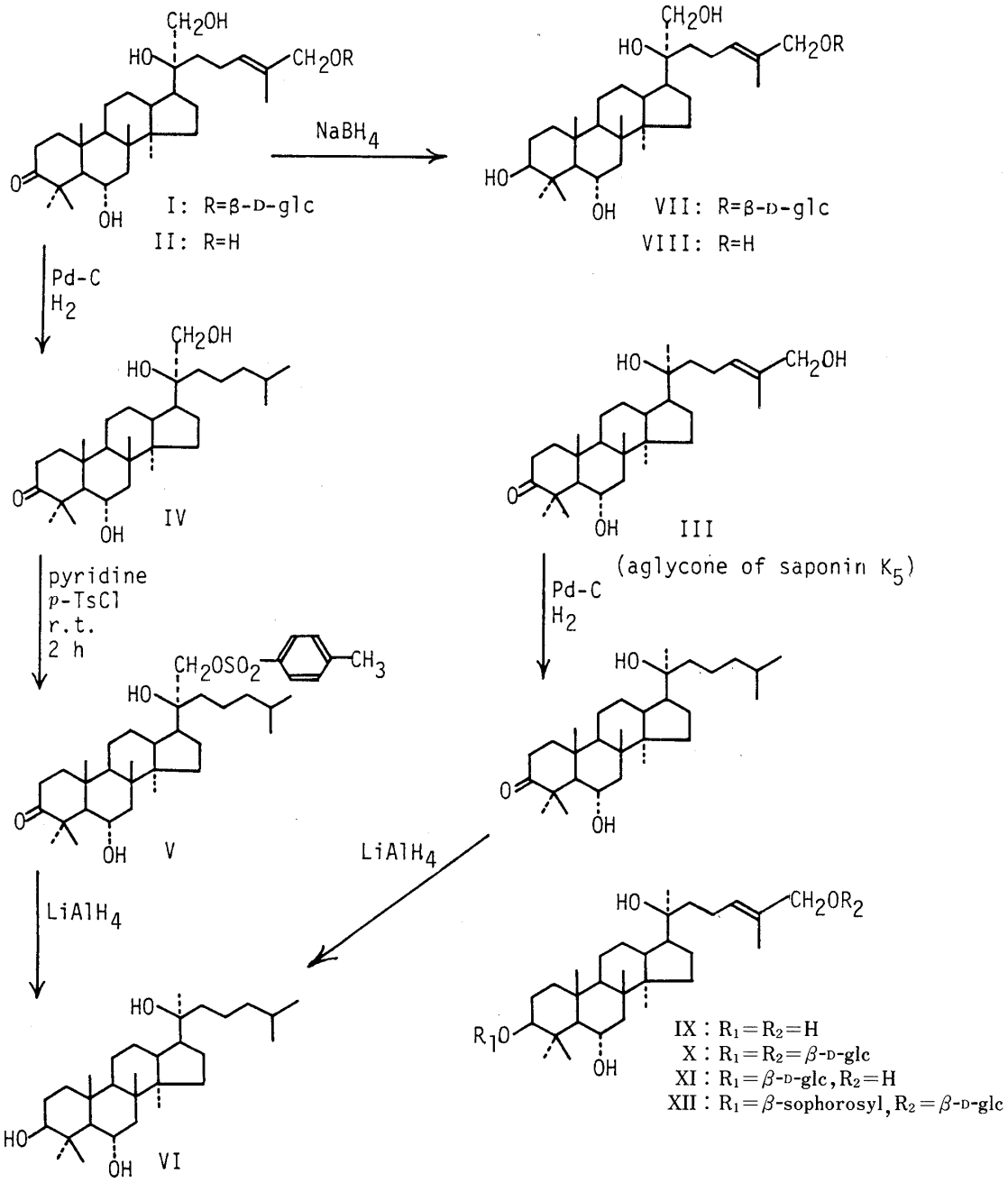

Chart 1

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

	III[1)]	II	VIII	IX[1)]	I	VII	XI	X	XII
C-1	40.2	40.1	39.4	39.5	40.1	39.5	39.3	39.3	39.3
C-2	33.4	33.4	28.0	28.1	33.4	28.1	26.7	26.7	26.8
C-3	218.5	219.1	78.6	78.6	219.1	78.6	89.7	89.6	89.6
C-4	47.8	47.7	40.3	40.4	47.7	40.4	39.0	39.0	39.0
C-5	59.6	59.2	61.8	62.0	59.2	61.9	62.0	61.9	62.0
C-6	66.9	66.8	67.8	67.9	66.8	67.8	67.6	67.6	67.6
C-7	45.9	45.9	47.9	48.1	45.8	48.1	48.0	48.0	48.1
C-8	41.4	41.2	41.8	41.9	41.2	41.9	41.8	41.7	41.8
C-9	49.7	49.6	50.8	50.9	49.6	50.9	50.7	50.7	50.7
C-10	38.4	38.2	39.4	39.5	38.2	39.5	40.6	40.6	40.7
C-11	22.9	22.8	21.9	22.0	22.8	21.9	21.9	21.9	21.9
C-12	25.3	24.7	24.7	25.4	24.7	24.8	25.4	25.3	25.4
C-13	42.4	41.5	41.4	42.2	41.5	41.5	42.2	42.1	42.2
C-14	50.6	50.4	50.5	50.7	50.4	50.6	50.7	50.7	50.7
C-15	31.6	31.7	31.6	31.7	31.7	31.8	31.6	31.6	31.6
C-16	28.1	28.1	28.0	28.2	28.0	28.2	28.1	28.1	28.1
C-17	50.4	46.2	46.2	50.5	46.1	46.3	50.4	50.4	50.4
C-18	17.7	17.8	17.5	17.7	17.8	17.6	17.5	17.5	17.5
C-19	16.1	16.0	17.3	17.4	16.0	17.4	17.3	17.3	17.3
C-20	74.3	76.7	76.7	74.2	76.6	76.6	74.2	74.1	74.1
C-21	26.1	66.8	66.8	26.1	66.8	66.8	26.1	26.0	26.0
C-22	41.7	36.4	36.2	41.8	36.2	36.2	41.6	41.5	41.6
C-23	22.9	22.8	22.8	22.9	22.9	22.9	22.9	22.9	22.9
C-24	125.8	125.8	125.9	125.7	129.5	129.5	125.7	129.4	129.4
C-25	136.2	136.2	136.0	136.3	132.2	132.2	136.2	132.2	132.3
C-26	68.3	68.2	68.2	68.2	75.2	75.2	68.2	75.2	75.2
C-27	13.9	14.0	14.0	14.0	14.3	14.3	14.0	14.2	14.2
C-28	32.2	32.2	31.9	32.0	32.2	32.0	31.5	31.4	31.4
C-29	20.0	19.9	16.4	16.5	19.9	16.5	17.0	17.0	16.8
C-30	16.7	16.5	16.7	16.8	16.6	16.8	16.8	16.8	16.8
				C-1	103.5	103.6		103.5	103.6
				C-2	75.2	75.2		75.2	75.2
		26-*O*-Glucose moiety		C-3	78.6[a)]	78.6[a)]		78.6[a)]	78.7[a)]
				C-4	72.0	71.8		71.7	71.9
				C-5	78.4[a)]	78.4[a)]		78.4[a)]	78.5[a)]
				C-6	62.8	62.9		62.8	62.9
				C-1			107.3	107.3	105.5
				C-2			76.0	75.9	83.5
		3-*O*-Sugar moiety		C-3			78.8[a)]	78.8[a)]	78.1[a)]
		(inner glucose)		C-4			72.0	71.9	71.9
				C-5			78.3[a)]	78.3[a)]	78.5[a)]
				C-6			63.2	63.1	62.9
				C-1					106.1
				C-2					77.1
		3-*O*-Sugar moiety		C-3					78.1[a)]
		(terminal glucose)		C-4					71.9
				C-5					78.1[a)]
				C-6					62.9

a) Assignments may be reversed in each column.

methanolyzed to give methyl glucoside and was hydrolyzed with cellulase to yield a genuine aglycone (VIII). Compound VIII, a white powder (Et_2O), mp 110—114 °C, $[\alpha]_D$ +51.9 °, showed signals due to five quaternary carbons, six methines, eleven methylenes, six methyls

and two olefinic carbons in the ^{13}C-NMR spectrum. The ^{1}H-NMR spectrum of VIII exhibited signals ascribable to six tertiary methyls, two hydroxymethyls, two methines on carbon bearing a hydroxyl group and one trisubstituted olefin. A comparison of the ^{13}C-NMR spectrum of VIII with those of 20(*S*)-dammar-24-ene-3β,6α,20,26-tetraol (IX, the aglycone of Kizuta saponin K_7[1]) and II showed that the signals due to carbons on the A and B rings of VIII were observed at almost the same positions as those of IX and the signals due to carbons on the C and D rings and the side chain of VIII were found at almost the same positions as those of II. These data suggested that VIII is 20(*S*)-dammar-24-ene-3β,6α,20,21,26-pentaol. This was confirmed by the fact that VIII was identical with the $NaBH_4$ reduction product of II.

Compound VII showed one anomeric proton signal at δ 4.90 (d, J=7.3 Hz) in the ^{1}H-NMR spectrum and one anomeric carbon signal in the ^{13}C-NMR spectrum. A comparison of the ^{13}C-NMR spectrum of VII with those of VIII and I revealed that the glucose unit in VII was linked to C-26 of VIII. Furthermore, VII was identified as the $NaBH_4$ reduction product of I. Based on these results, the structure of VII was established as 20(*S*)-dammar-24-ene-3β,6α,20,21,26-pentaol 26-*O*-β-D-glucopyranoside.

Saponin K_9 (X), a white powder (MeOH–AcOEt), mp 144—147 °C (dec.), $[\alpha]_D$ +10.8°, exhibited strong hydroxyl absorption bands in the IR spectrum and was methanolyzed to yield methyl glucoside. On enzymatic hydrolysis with cellulase, X afforded a genuine aglycone and a prosapogenin (XI). The aglycone was identified as IX by direct comparison of thin-layer chromatographic (TLC) behavior and IR, ^{1}H-NMR and ^{13}C-NMR spectra. Compound XI was considered to be the 3-*O*-β-D-glucopyranoside of IX on the basis of a comparison of the ^{13}C-NMR spectral data with that of IX. Compound X showed two anomeric proton signals at δ 4.87 (d, J=7.1 Hz) and at δ 4.98 (d, J=7.1 Hz) in the ^{1}H-NMR spectrum. These findings and a comparison of the ^{13}C-NMR spectrum of X with those of IX, XI and Kizuta saponin K_7[1] revealed that X is the 3,26-di-*O*-β-D-glucopyranoside of IX.

Saponin K_{13} (XII), a white powder (MeOH–AcOEt), mp 175—179 °C (dec.), $[\alpha]_D$ +3.0°, was methanolyzed to give methyl glucoside from the sugar portion. On enzymatic hydrolysis with cellulase, XII yielded a genuine aglycone and a prosapogenin, which were identified as IX and XI, respectively, by direct comparison of the TLC behavior and IR, ^{1}H-NMR and ^{13}C-NMR spectra, with those of authentic samples. Compound XII showed three anomeric carbon signals in the ^{13}C-NMR spectrum. A comparison of the ^{13}C-NMR spectrum of XII with that of X showed that the signals attributable to the aglycone moiety and the 26-*O*-glucose moiety of XII were observed at almost the same positions as those of X. The carbon signals ascribable to the 3-*O*-sugar unit of XII were found at almost the same positions as those of some ginseng saponins[4] which have the β-sophorose moiety at the C-3 position. Based on these results, the structure of XII was concluded to be 20(*S*)-dammar-24-ene-3β,6α,20,26-tetraol 3-*O*-β-sophoroside-26-*O*-β-D-glucopyranoside.

Experimental[5]

Isolation——The procedures for isolation of the saponins were described in the previous paper.[1]

Saponin K_{7A} (I)——A white powder (from dil. MeOH), mp 181—185 °C (dec.), $[\alpha]_D^{20}$ +79.5° (c=1.00, MeOH). *Anal.* Calcd for $C_{36}H_{60}O_{10}\cdot 2H_2O$: C, 62.77; H, 9.36. Found: C, 62.82; H, 9.30. IR ν_{max}^{KBr} cm^{-1}: 3400, 1690, 1100—1000. ^{1}H-NMR (pyridine-d_5): 0.77 (3H), 1.00 (3H), 1.02 (3H), 1.66 (6H) (each s, *tert*-Me×5), 1.81 (3H, s, C_{25}-Me), 4.86 (1H, d, J=7.1 Hz, C_1-H of glucose unit), 5.73 (1H, br s, C_{24}-H). ^{13}C-NMR: Table I. $\Delta[M]_D$: I—II = −128.5°. $[M]_D$ of methyl β-D-glucopyranoside: −66°.

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

Enzymatic Hydrolysis of I——A solution of I (4 g) in AcOH–AcONa buffer (pH 5.0, 2.3 l) with cellulase (Tokyo Kasei Co., 4 g) was shaken at 37 °C for 4 d. The reaction mixture was extracted with AcOEt. The AcOEt-soluble portion was washed with water and then concentrated. The residue (2.4 g) was chromatographed on silica gel (300 g) with a gradient of $CHCl_3$–MeOH (MeOH 0—7%) to give an aglycone (II, 830 mg). II, colorless needles (from Et_2O–hexane), mp 143—145 °C, $[\alpha]_D^{20}$ +132.0° ($c=0.5$, MeOH). *Anal.* Calcd for $C_{30}H_{50}O_5$: C, 73.43; H, 10.27. Found: C, 73.48; H, 10.32. IR ν_{max}^{KBr} cm^{-1}: 3400, 1690. ^{1}H-NMR (pyridine-d_5): 0.78, 1.01, 1.03, 1.68, 1.69 (each 3H, s, *tert*-Me × 5), 1.86 (3H, s, C_{25}-Me), 4.05 (2H, s, C_{20}-CH_2OH), *ca.* 4.2 (1H, m, C_6-H), 4.31 (2H, s, C_{25}-CH_2OH), 5.85 (1H, t-like, C_{24}-H). ^{13}C-NMR: Table I.

Catalytic Hydrogenation of II——A solution of II (354 mg) in EtOH (30 ml) was stirred with Pd–C (5%, 200 mg) under hydrogen at room temperature. The reaction mixture was filtered and the filtrate was concentrated to give the crude product, which was chromatographed on silica gel (50 g) with $CHCl_3$–MeOH–H_2O (25 : 1.5 : 0.06) to give the pure reduction product (IV, 100 mg). IV, colorless plates (from acetone), mp 141—145 °C. IR ν_{max}^{KBr} cm^{-1}: 3400, 1690, 1030. ^{1}H-NMR (pyridine-d_5): 0.78 (3H, s, *tert*-Me), 0.86 (6H, d, $J=6.3$ Hz, C_{25}-Me × 2), 1.00 (3H), 1.05 (3H), 1.65 (6H) (each s, *tert*-Me × 4), 3.98 (2H, s, C_{20}-CH_2OH), *ca.* 4.1 (1H, m, C_6-H). ^{13}C-NMR (pyridine-d_5): 40.1 (C-1), 33.4 (2), 218.8 (3), 47.7 (4), 59.2 (5), 66.8 (6), 45.9 (7), 41.2 (8), 49.7 (9), 38.2 (10), 22.9 (11), 24.7 (12), 41.5 (13), 50.4 (14), 31.8 (15), 28.1 (16), 46.2 (17), 17.8 (18), 16.0 (19), 76.7 (20), 66.8 (21), 36.8 (22), 22.1 (23), 40.4 (24), 28.3 (25), 22.8 (26, 27), 32.1 (28), 19.9 (29), 16.6 (30).

Partial Tosylation of IV——A solution of IV (170 mg) in pyridine (2.5 ml) with *p*-TsCl (500 mg) was allowed to stand for 2 h at room temperature. The reaction mixture was concentrated and the residue was chromatographed on silica gel (40 g) with a gradient of benzene–AcOEt (AcOEt 0—12%) to give a monotosylate (V, 146 mg) as a major product. ^{1}H-NMR ($CDCl_3$): 0.75 (3H, s, *tert*-Me), 0.83 (6H, d, $J=6.3$ Hz, C_{25}-Me × 2), 0.88, 0.98, 1.31, 1.33 (each 3H, s, *tert*-Me × 4), 2.45 (3H, s, $C_{4'}$-Me), 3.92 (2H, s, C_{20}-CH_2OTs), 4.03 (1H, m, C_6-H), 7.35 (2H, d, $J=8.3$ Hz, $C_{2'}$, $C_{5'}$-H), 7.80 (2H, d, $J=8.3$ Hz, $C_{3'}$, $C_{5'}$-H). ^{13}C-NMR ($CDCl_3$): 39.9 (C-1), 33.1 (2), 219.5 (3), 47.3 (4), 59.2 (5), 67.8 (6), 45.5 (7), 41.1 (8), 49.1 (9), 38.2 (10), 22.2 (11), 24.1 (12), 41.1 (13), 49.9 (14), 31.1 (15), 27.2 (16), 45.7 (17), 17.6 (18), 15.8 (19), 75.5 (20), 74.1 (21), 35.3 (22), 21.1 (23), 39.6 (24), 27.9 (25), 22.5, 22.6 (26, 27), 32.0 (28), 19.6 (29), 16.1 (30), 145.2 (1'), 128.1 (2', 6'), 130.1 (3', 5'), 133.0 (4'), 21.7 (4'-Me).

$LiAlH_4$ Reduction of V——$LiAlH_4$ (15 mg) was gradually added to a solution of V (100 mg) in Et_2O (5 ml), then AcOEt (30 ml) and dil. AcOH (30 ml) were added to the reaction mixture. The AcOEt-soluble portion was concentrated and the residue was chromatographed on silica gel (20 g) with a gradient of benzene–AcOEt (AcOEt 0—20%) to give a reduction product (VI, 37 mg) as a major product. VI was identified as 20(*S*)-dammarane-3β,6α,20-triol by direct comparison of the TLC behavior and IR, ^{1}H-NMR and ^{13}C-NMR spectra, with those of an authentic sample.

Catalytic Hydrogenation of III——A solution of III (600 mg) in EtOH (40 ml) was stirred with Pd–C (5%, 200 mg) under hydrogen at room temperature. The reaction mixture was treated in the same way as described for II. The crude product was chromatographed on silica gel (50 g) with a gradient of benzene–AcOEt (AcOEt 0—20%) to give 3-oxo-20(*S*)-dammarane-6α,20-diol (110 mg) as a major product. A white powder (from dil. MeOH), mp 151—155 °C. IR ν_{max}^{KBr} cm^{-1}: 3450, 1690. ^{1}H-NMR (pyridine-d_5): 0.80 (3H, s, *tert*-Me), 0.89 (6H, d, $J=6.2$ Hz, C_{25}-Me × 2), 1.01, 1.04, 1.41, 1.67, 1.69 (each 3H, s, *tert*-Me × 5), 4.16 (1H, m, C_6-H). ^{1}H-NMR ($CDCl_3$): 0.76 (3H, s, *tert*-Me), 0.88 (6H, d, $J=6.3$ Hz, C_{25}-Me × 2), 0.92, 1.02, 1.14, 1.32, 1.34 (each 3H, s, *tert*-Me × 5), 3.98 (1H, m, C_6-H). ^{13}C-NMR (pyridine-d_5): 40.1 (C-1), 33.4 (2), 219.0 (3), 47.7 (4), 59.2 (5), 66.8 (6), 45.8 (7), 41.2 (8), 49.5 (9), 38.3 (10), 22.9 (11), 25.3 (12), 42.2 (13), 50.6 (14), 31.6 (15), 28.1 (16), 50.2 (17), 17.8 (18), 15.9 (19), 74.2 (20), 26.3 (21), 42.2 (22), 22.2 (23), 40.2 (24), 28.4 (25), 22.8 (26, 27), 32.1 (28), 19.9 (29), 16.7 (30).

$LiAlH_4$ Reduction of 3-Oxo-20(*S*)-dammarane-6α,20-diol——$LiAlH_4$ (10 mg) was gradually added to a solution of 3-oxo-20(*S*)-dammarane-6α,20-diol (50 mg) in Et_2O (5 ml), and the reaction mixture was treated in the same way as described for V. The product was passed through a silica gel (10 g) column [solv., gradient of benzene–AcOEt (AcOEt 0—20%)] to give 20(*S*)-dammarane-3β,6α,20-triol (28 mg) as a major product. Colorless needles (from MeOH), mp 102—105 °C. IR ν_{max}^{KBr} cm^{-1}: 3400, 1040. ^{1}H-NMR (pyridine-d_5): 0.89 (6H, d, $J=6.1$ Hz, C_{25}-Me × 2), 1.01 (6H), 1.12 (3H) (each s, *tert*-Me × 3), 1.41 (3H, s, C_{20}-Me), 1.47 (3H, s, C_4-βMe), 2.00 (3H, s, C_4-αMe), 3.56 (1H, t-like, C_3-H), 4.41 (1H, m, C_6-H). ^{1}H-NMR ($CDCl_3$): 0.87 (6H, d, $J=6.1$ Hz, C_{25}-Me × 2), 0.90 (6H), 0.98 (3H), 1.04 (3H), 1.12 (3H), 1.32 (3H) (each s, *tert*-Me × 6), 3.17 (1H, m, C_3-H), 4.07 (1H, m, C_6-H). ^{13}C-NMR (pyridine-d_5): 39.6 (C-1), 28.2 (2), 78.6 (3), 40.4 (4), 61.9 (5), 67.8 (6), 48.1 (7), 41.9 (8), 50.8 (9), 39.6 (10), 21.9 (11), 25.4 (12), 42.2 (13), 50.7 (14), 31.7 (15), 28.2 (16), 50.4 (17), 17.6 (18), 17.4 (19), 74.2 (20), 26.3 (21), 42.2 (22), 22.2 (23), 40.3 (24), 28.4 (25), 22.8, 22.9 (26, 27), 32.0 (28), 16.5 (29), 16.8 (30).

Saponin K_{7B} (VII)——A white powder (from dil. MeOH), mp 131—134 °C (dec.), $[\alpha]_D^{23}$ +23.0° ($c=0.66$, MeOH). *Anal.* Calcd for $C_{36}H_{62}O_{10}\cdot 2H_2O$: C, 62.58; H, 9.63. Found: C, 62.47; H, 9.66. IR ν_{max}^{KBr} cm^{-1}: 3400, 1100—1000. ^{1}H-NMR (pyridine-d_5): 0.98, 1.01, 1.11 (each 3H, s, *tert*-Me × 3), 1.46 (3H, s, C_4-βMe), 1.82 (3H, s, C_{25}-Me), 2.00 (3H, s, C_4-αMe), 3.55 (1H, t-like, C_3-H), 4.03 (2H, s, C_{20}-CH_2OH), 4.90 (1H, d, $J=7.3$ Hz, C_1-H of glucose unit), 5.77 (1H, t-like, C_{24}-H). ^{13}C-NMR: Table I. $\Delta[M]_D$: VII—VIII = −104.9°. $[M]_D$ of methyl β-D-glucopyranoside: −66°.

Enzymatic Hydrolysis of VII——VII (100 mg) was hydrolyzed with cellulase (200 mg) in the same way as

described for I to give an aglycone (VIII, 55 mg). VIII, a white powder (from Et_2O), mp 110—114 °C, $[\alpha]_D^{23}$ +51.9° ($c=0.54$, MeOH). *Anal.* Calcd for $C_{30}H_{52}O_5$: C, 73.13; H, 10.64. Found: C, 73.26; H, 10.66. IR ν_{max}^{KBr} cm^{-1}: 3380, 1035. ^{1}H-NMR (pyridine-d_5): 0.97, 1.00, 1.10 (each 3H, s, *tert*-Me × 3), 1.43 (3H, s, C_4-βMe), 1.84 (3H, s, C_{25}-Me), 1.96 (3H, s, C_4-αMe), 3.53 (1H, m, C_3-H), 4.01 (2H, s, C_{20}-CH_2OH), 4.28 (2H, s, C_{25}-CH_2OH), *ca.* 4.4 (1H, m, C_6-H), 5.82 (1H, t-like, C_{24}-H). ^{13}C-NMR: Table I.

$NaBH_4$ Reduction of I——$NaBH_4$ (10 mg) was added to a solution of I (55 mg) in MeOH (5 ml), then the reaction mixture was neutralized with 1% HCl–MeOH and filtered. The filtrate was concentrated and the residue was chromatographed on silica gel (10 g) with $CHCl_3$–MeOH–H_2O (25 : 3.8 : 0.3) to give a reduction product (14 mg), the TLC behavior and IR, ^{1}H-NMR and ^{13}C-NMR spectra of which coincided with those of VII.

$NaBH_4$ Reduction of II——II (170 mg) in MeOH (5 ml) was reduced with $NaBH_4$ (20 mg) and then the reaction mixture was treated in the same way as described for I. The product was chromatographed on silica gel (16 g) with a gradient of $CHCl_3$–MeOH (MeOH 0—8%) to give a reduction product (94 mg), the TLC behavior and IR, ^{1}H-NMR and ^{13}C-NMR spectra of which coincided with those of VIII.

Saponin K_9 (X)——A white powder (from MeOH–AcOEt), mp 144—147 °C (dec.), $[\alpha]_D^{20}$ +10.8° ($c=1.05$, MeOH). *Anal.* Calcd for $C_{42}H_{72}O_{14}\cdot 3H_2O$: C, 59.00; H, 9.19. Found: C, 59.15; H, 9.22. IR ν_{max}^{KBr} cm^{-1}: 3400, 1100—1000. ^{1}H-NMR (pyridine-d_5): 0.90, 1.01, 1.04 (each 3H, s, *tert*-Me × 3), 1.38 (3H, s, C_4-βMe), 1.40 (3H, s, C_{20}-Me), 1.81 (3H, s, C_{25}-Me), 2.05 (3H, s, C_4-αMe), 3.45 (1H, m, C_3-H), 4.87 (1H, d, $J=7.3$ Hz, C_1-H of C_3-*O*-glucose unit), 4.98 (1H, d, $J=7.1$ Hz, C_1-H of C_{26}-*O*-glucose unit), 5.73 (1H, t-like, C_{24}-H). ^{13}C-NMR: Table I. X (10 mg) was methanolyzed and worked up in the same way as described for I to give methyl glucoside.

Enzymatic Hydrolysis of X——A solution of X (50 mg) in AcOH–AcONa buffer (pH 4.5, 50 ml) with cellulase (200 mg) was shaken at 37 °C for 6 d. The reaction mixture was extracted with BuOH. The BuOH layer was washed with water and then concentrated. The residue was chromatographed on silica gel (10 g) with a gradient of $CHCl_3$–MeOH (MeOH 0—8%) to give an aglycone (1.5 mg), and then with $CHCl_3$–MeOH–H_2O (25 : 3 : 0.3) to give a prosapogenin (XI, 13 mg). The aglycone was identified as IX by direct comparison of the TLC behavior and IR, ^{1}H-NMR and ^{13}C-NMR spectra with those of an authentic sample. XI, a white powder (from dil. MeOH), mp 138—142 °C (dec.), $[\alpha]_D^{23}$ +32.8° ($c=0.80$, MeOH). *Anal.* Calcd for $C_{36}H_{62}O_9\cdot H_2O$: C, 65.82; H, 9.82. Found: C, 65.72; H, 9.88. IR ν_{max}^{KBr} cm^{-1}: 3400, 1100—1000. ^{1}H-NMR (pyridine-d_5): 0.91, 1.03, 1.05 (each 3H, s, *tert*-Me × 3), 1.43 (6H, s, C_4-βMe, C_{20}-Me), 1.87 (3H, s, C_{25}-Me), 2.08 (3H, s, C_4-αMe), 3.49 (1H, m, C_3-H), 4.32 (2H, s, C_{25}-CH_2OH), 5.13 (1H, d, $J=7.1$ Hz, C_1-H of glucose unit), 5.83 (1H, t-like, C_{24}-H). ^{13}C-NMR: Table I.

Saponin K_{13} (XII)——A white powder (MeOH–AcOEt), mp 175—179 °C (dec.), $[\alpha]_D^{20}$ +3.0° ($c=0.76$, MeOH). *Anal.* Calcd for $C_{48}H_{82}O_{19}\cdot 4H_2O$: C, 55.69; H, 8.76. Found: C, 55.46; H, 8.81. IR ν_{max}^{KBr} cm^{-1}: 3400, 1100—1000. ^{1}H-NMR (pyridine-d_5): 0.90, 1.02, 1.06 (each 3H, s, *tert*-Me × 3), 1.40 (3H, s, C_{20}-Me), 1.53 (3H, s, C_4-βMe), 1.83 (3H, s, C_{25}-Me), 2.02 (3H, s, C_4-αMe), 3.36 (1H, m, C_3-H), 4.92 (2H, d, $J=7.1$ Hz, anomeric H × 2), 5.43 (1H, d, $J=6.8$ Hz, anomeric H), 5.73 (1H, br s, C_{24}-H). ^{13}C-NMR: Table I. XII (10 mg) was methanolyzed and worked up in the same way as described for I to give methyl glucoside.

Enzymatic Hydrolysis of XII——A solution of XII (65 mg) in AcOH–AcONa buffer (pH 5.0, 65 ml) with cellulase (277 mg) was shaken at 37 °C for 6 d. The reaction mixture was treated in the same way as described for X. The product was chromatographed on silica gel (5 g) with a gradient of $CHCl_3$–MeOH (MeOH 0—8%), then with $CHCl_3$–MeOH–H_2O (25 : 3 : 0.3→25 : 4 : 0.4→25 : 5 : 0.5→25 : 6 : 0.6) to give an aglycone (4.5 mg) and a prosapogenin (8.4 mg), which were identified as IX and XI, respectively, by direct comparison of the TLC behavior and IR, ^{1}H-NMR and ^{13}C-NMR spectra with those of authentic samples.

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References and Notes

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5) The instruments used to obtain the physical data were the same as those described in the previous paper.[1)]