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Studies on Nepalese Crude Drugs. III.[1] On the Saponins of *Hedera nepalensis* K. KOCH.[2]

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Twelve saponins, tentatively named HN-saponins A (I), B (II), D_1 (III), D_2 (IV), E (V), F (VI), H (VII), I (VIII), K (X), M (XII), N (XIII) and P (XIV), were isolated from the stem and bark of *Hedera nepalensis* K. KOCH. (Araliaceae). Compounds II, V, XII, XIII and XIV were identified as Kizuta saponins K_3, K_6, K_{10}, K_{11} and K_{12}, respectively, which have been isolated from *Hedera rhombea* BEAN. On the basis of chemical and physicochemical evidence, other saponins were identified as follows: I, a mixture of the β-D-glucopyranosides of campesterol (trace), stigmasterol and β-sitosterol; III, hederagenin 3-*O*-β-D-glucopyranoside; IV, oleanolic acid 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside; VI, 3-*O*-α-L-arabinopyranosyl-hederagenin 28-*O*-β-D-glucopyranosyl ester; VII, hederagenin 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester; VIII, oleanolic acid 3-*O*-β-D-glucuronopyranoside; X, hederagenin 3-*O*-β-D-glucuronopyranoside.

Compounds VI and VII were isolated from nature for the first time, though they had previously been derived from Akebia seed saponin D and Kizuta saponin K_{12}, respectively, by partial hydrolysis.

Keywords——Nepalese crude drug; *Hedera nepalensis*; Araliaceae; hederagenin monodesmoside; hederagenin monodesmosyl ester; hederagenin bisdesmoside; oleanolic acid monodesmoside

Hedera nepalensis K. KOCH. (Nepalese name: "Dudela") is an evergreen woody climber of the family Araliaceae, which is distributed in Nepal, China and India. In Nepal, the stem, leaves and berries of this plant have been used as folk medicines (rubefacients, diaphoretics and cathartics).[3] No work has been done on the constituents of this plant, however. As a part of our studies on Nepalese crude drugs, the saponin constituents of this plant have now been examined. We also wished to compare the constituents with those of *Hedera rhombea* which have already been reported by us.[4]

The stem and bark of *H. nepalensis*, which were collected in Central Nepal, were extracted with MeOH. The water-soluble portion of the MeOH ext. was successively extracted with Et_2O, AcOEt and BuOH. The AcOEt- and BuOH-soluble fractions were each subjected to repeated silica gel column chromatography, to give twelve saponins, tentatively named HN-saponins A (I), B (II), D_1 (III), D_2 (IV), E (V), F (VI), H (VII), I (VIII), K (X), M (XII), N (XIII) and P (XIV), as described in the experimental section. This paper deals with their structural identification.

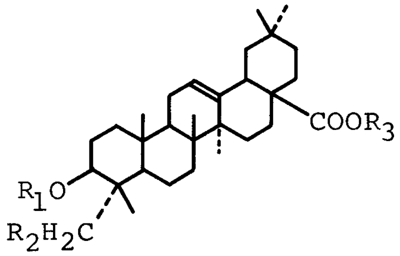
Compounds II, V, XII, XIII and XIV, were identified as Kizuta saponins K_3, K_6, K_{10}, K_{11} and K_{12}, respectively, which were isolated from the stem and bark of *H. rhombea*, by direct comparison of the infrared (IR), proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra with those of corresponding authentic samples.[4a,d] In the same manner, compound IV was identified as prosapogenin CP_2,[5] which was isolated from the alkaline hydrolysate of the crude saponin of *Clematis*

chinensis.

HN-saponin A (I) showed a single spot on thin-layer chromatography (TLC). However, the aglycone fraction obtained on methanolysis was shown to contain three compounds by gas-liquid chromatography (GLC). The retention times (t_R) of these compounds coincided with those of campesterol, stigmasterol and β-sitosterol, respectively. The sugar fraction of the methanolysate contained only methyl glucoside. The ^{13}C-NMR spectrum of I showed signals assignable to 1 mol of β-D-glucopyranose moiety[6] as well as those due to the mixed aglycone moieties. Based on these findings, compound I is considered to be a mixture of the β-D-glucopyranosides of campesterol, stigmasterol and β-sitosterol.

HN-saponin D_1 (III), colorless needles (MeOH), mp 231—233 °C (dec.), was methanolyzed to give hederagenin and methyl glucoside. The ^{1}H-NMR spectrum of III showed one anomeric proton signal at 5.14 (d, J=7.1 Hz). Based on these results and a comparison of the ^{13}C-NMR spectrum of I with that of hederagenin,[6,7] the structure of III was concluded to be hederagenin 3-*O*-β-D-glucopyranoside.

HN-saponin F (VI), colorless needles (dil. MeOH), mp 202—205 °C (dec.), $[\alpha]_D$ +36.0 °, was methanolyzed to yield hederagenin, methyl arabinoside and methyl glucoside. On alkaline hydrolysis with 0.5 N KOH, VI afforded II. The ^{1}H-NMR spectrum of VI exhibited two anomeric proton signals at 4.98 (d, J=7.0 Hz) and 6.30 ppm (d, J=7.1 Hz), and the ^{13}C-NMR spectrum of VI showed two anomeric carbon signals at 95.8 and 106.7 ppm. Based on these findings, VI was considered to be 3-*O*-α-L-arabinopyranosyl-hederagenin 28-*O*-β-D-glucopyranosyl ester, and this was confirmed by direct comparison of the IR, ^{1}H-NMR and ^{13}C-NMR spectra with those of an authentic sample[4d] which was obtained by partial hydrolysis of Kizuta saponin K_{12} (XIV).



	R_1	R_2	R_3
oleanolic acid	H	H	H
hederagenin	H	OH	H
II	ara	OH	H
III	glc	OH	H
IV	rham$\frac{1\ 2}{}$ara	H	H
V	rham$\frac{1\ 2}{}$ara	OH	H
VI	ara	OH	glc
VII	H	OH	rham$\frac{1\ 4}{}$glc$\frac{1\ 6}{}$glc
VIII	glcUA	H	H
IX	6-*O*-Me-glcUA	H	H
X	glcUA	OH	H
XI	6-*O*-Me-glcUA	OH	H
XII	ara	OH	rham$\frac{1\ 4}{}$glc$\frac{1\ 6}{}$glc
XIII	rham$\frac{1\ 2}{}$ara	OH	rham$^{1}_{4}$ acetyl$_{6}$ > glc$\frac{1\ 6}{}$glc
XIV	rham$\frac{1\ 2}{}$ara	OH	rham$\frac{1\ 4}{}$glc$\frac{1\ 6}{}$glc

ara, α-L-arabinopyranosyl; glc, β-D-glucopyranosyl; rham, α-L-rhamnopyranosyl

glcUA, β-D-glucuronopyranosyl; 6-*O*-Me-glcUA, 6-*O*-methyl-β-D-glucuronopyranosyl

Chart 1

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

Aglycone moiety	Ole.[a]	Hed.[b]	III	VI	VII	VIII	IX	X	XI		
C-1	39.0	38.9	38.7	39.0	38.9	38.7	38.7	38.6	38.7		
2	28.1	27.6	25.9	26.1	27.7	26.7	26.6	26.0	26.0		
3	78.2	73.7	82.3	82.1	73.7	89.2	89.3	82.2	82.4		
4	39.4	42.9	43.5	43.6	42.9	39.6	39.6	43.5	43.5		
5	55.9	48.8	47.8	47.7	48.7	55.9	55.9	47.6	47.6		
6	18.8	18.7	18.3	18.3	18.7	18.5	18.5	18.2	18.2		
7	33.3	33.0	33.0	33.0	32.9	33.3	33.3	33.0	32.9		
8	39.8	39.8	39.8	40.1	39.9	39.8	39.8	39.8	39.8		
9	48.2	48.2	48.2	48.3	48.2	48.1	48.1	48.1	48.2		
10	37.5	37.3	37.0	37.1	37.3	37.1	37.0	36.9	36.9		
11	23.8	23.8	23.8	24.0	23.9	23.8	23.8	23.8	23.8		
12	122.7	122.7	122.7	123.1	123.1	122.7	122.7	122.7	122.7		
13	145.0	145.0	145.1	144.3	144.3	145.0	145.0	145.0	145.0		
14	42.2	42.2	42.2	42.2	42.2	42.2	42.2	42.2	42.2		
15	28.4	28.4	28.3	28.3	28.3	28.3	28.4	28.4	28.4		
16	23.8	23.8	23.8	23.5	23.4	23.8	23.8	23.8	23.8		
17	46.7	46.7	46.7	47.1	47.1	46.7	46.7	46.7	46.7		
18	42.1	42.0	42.0	41.8	41.7	42.1	42.0	42.0	42.0		
19	46.6	46.5	46.5	46.3	46.3	46.6	46.5	46.4	46.5		
20	31.0	31.0	31.0	30.8	30.8	31.0	31.0	31.0	31.0		
21	34.3	34.3	34.3	34.1	34.1	34.3	34.3	34.3	34.3		
22	33.3	33.3	33.3	32.6	32.6	33.3	33.3	33.3	33.3		
23	28.8	68.2	64.9	64.6	68.1	28.3	28.2	64.6	64.5		
24	16.6	13.1	13.6	13.6	13.0	17.0	16.9	13.6	13.6		
25	15.6	16.0	16.1	16.3	16.1	15.5	15.5	16.1	16.1		
26	17.4	17.5	17.5	17.6	17.6	17.4	17.4	17.4	17.4		
27	26.2	26.2	26.2	26.1	26.1	26.3	26.2	26.2	26.2		
28	180.3	180.4	180.4	176.7	176.7	180.4	180.3	180.4	180.4		
29	33.3	33.3	33.3	33.2	33.2	33.3	33.3	33.3	33.3		
30	23.8	23.8	23.8	23.7	23.7	23.8	23.8	23.8	23.8		
										Me glcUA Me ester[c]	
3-*O*-Sugar moiety										*β*	*α*
C-1			106.0	106.8		107.4	107.4	106.3	106.5	106.3	102.2
2			75.9	73.2		75.6	75.4	75.5	75.5	74.9	73.4[d]
3			78.8[d]	74.8		78.0	78.0	77.9	77.9	77.8	74.8[d]
4			71.8	69.8		73.5	73.2	73.4	73.2	73.2	73.3[d]
5			78.4[d]	66.8		78.3	77.2	78.1	77.3	77.3	73.4[d]
6			62.9			173.2	171.0	173.0	170.9	170.8	171.4
COOMe							52.1		52.0	52.1	52.1
28-*O*-Sugar moiety									(C_1-OMe)	57.2	55.6
glc-1				95.9	95.8						
2				74.3	73.9						
Inner 3				79.4	78.8						
4				71.3	71.0						
5				79.1	78.1						
6				62.4	69.3						
glc-1					104.9						
2					75.4						
Outer 3					76.5						
4					78.3						
5					77.1						
6					61.4						
rham-1					102.4						
2					72.7[d]						
3					72.8[d]						
4					73.9						
5					70.2						
6					18.4						

a) Ole.: oleanolic acid. *b*) Hed.: hederagenin. *c*) Me glcUA Me ester: methyl glucuronopyranoside methyl ester. *d*) Assignments may be reversed in each column.

HN-saponin H (VII), a white powder (MeOH–AcOEt), mp 198—202 °C (dec.), $[\alpha]_D$ −3.3 °, was methanolyzed to yield hederagenin, methyl glucoside and methyl rhamnoside. On alkaline hydrolysis with 0.5 N KOH, VII gave hederagenin. The ^{1}H-NMR spectrum of VII exhibited three anomeric proton signals at 4.96 (d, J=7.1 Hz), 5.84 (s) and 6.22 ppm (d, J=6.8 Hz), and the ^{13}C-NMR spectrum of VII showed three anomeric carbon signals at 95.8, 102.4 and 104.9 ppm. These data indicated that VII was hederagenin monodesmosyl ester with a trisaccharide moiety at the C-28 position. Comparison of the ^{13}C-NMR spectrum of VII with that of Kizuta saponin K_{10} (XII)[4a,d] suggested that VII was hederagenin 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester. Finally, VII was identified by direct comparison of the IR, ^{1}H-NMR and ^{13}C-NMR spectra with those of an authentic sample[4d] obtained by partial acid hydrolysis of Kizuta saponin K_{12} (XIV).

HN-saponin I (VIII), colorless needles (MeOH), mp 264—267 °C (dec.), $[\alpha]_D$ +20.4 °, was methanolyzed to give oleanolic acid, methyl glucuronopyranoside methyl ester and methyl glycoside of glucurono-6,3-lactone. The ^{1}H-NMR spectrum of VIII exhibited one anomeric proton signal at 5.03 (d, J=6.8 Hz), and the ^{13}C-NMR spectrum of VIII showed one anomeric carbon signal at 107.4 ppm. These findings indicated that VIII was the β-glucuronopyranoside of oleanolic acid. Comparison of the ^{13}C-NMR spectrum of the 6′-*O*-methyl ester derivative (IX) of VIII with those of oleanolic acid and methyl glucuronopyranoside methyl ester showed that IX was oleanolid acid 3-*O*-β-D-glucuronopyranoside 6′-*O*-methyl ester.[6,7] Consequently, VIII is oleanolic acid 3-*O*-β-D-glucuronopyranoside.

HN-saponin K (X), colorless needles (MeOH), mp 224—227 °C (dec.), $[\alpha]_D$ +22.6 °, was methanolyzed to yield hederagenin, methyl glucuronopyranoside methyl ester and methyl glycoside of glucurono-6,3-lactone. Comparison of the ^{1}H- and ^{13}C-NMR spectra of X and its 6′-*O*-methyl ester derivative (XI) with those of hederagenin, methyl glucuronopyranoside methyl ester, VIII and IX established the structure of X to be hederagenin 3-*O*-β-D-glucuronopyranoside.

The isolation of VI and VII from nature is reported for the first time in this paper, although VI has been obtained from Akebia seed saponin D,[8] and VII from Kizuta saponin K_{12} (XIV),[4d] by partial hydrolysis. It has also become evident that the saponin constituents in the stem and bark of *H. nepalensis* differ from those of *H. rhombea, i.e.*, the former contains only oleanane-type saponins, while the latter contains both oleanane-type and dammarane-type saponins. This is of interest from a chemotaxonomical point of view.

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 25 MHz, respectively, with a JEOL JNM-FX-100 spectrometer in pyridine-d_5 solution, and chemical shifts are given as δ (ppm) with tetramethylsilane as an internal standard (s, singlet; d, doublet; br, broad). IR spectra 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 a glass column (2 m × 4 mm i.d.) packed with 5% SE-30 on Chromosorb W (60—80 mesh); column temperature, programmed from 150 °C (20 min hold) to 240 °C at 5 °C/min (GLC-1), 180 °C (GLC-2) or 280 °C (GLC-3). TLC was performed on precoated Silica gel 60F_{254} plates and spots were detected by spraying dil. H_2SO_4 followed by heating.

Isolation——The stem and bark of *Hedera nepalensis* (dried, 1.4 kg) collected in Central Nepal in August, 1983, were extracted with hot MeOH three times. The MeOH extractive was concentrated to dryness under reduced pressure. The water-soluble portion of the resulting residue was successively extracted with Et_2O, AcOEt and BuOH. The AcOEt layer was concentrated and the residue (6 g) was chromatographed on silica gel (600 g) with a gradient of $CHCl_3$–MeOH (MeOH 0—5%) and then with $CHCl_3$–MeOH–H_2O (25:3:0.3 → 25:4:0.4 → 25:5:0.5 → 25:6:0.6 → 25:7:0.8 → 25:8:1) to give Frs. 1—7. Recrystallizations of Frs. 1, 2 and 4 gave I (40 mg), II (210 mg) and V (120 mg), respectively. Fraction 3 (170 mg) was rechromatographed on silica gel (30 g) with a gradient of $CHCl_3$–MeOH (MeOH 0—10%) to give a mixture of III and IV. This mixture (20 mg) was rechromatographed on silica gel (10 g) with a gradient of $CHCl_3$–BuOH (BuOH 0—20%) and then with $CHCl_3$–BuOH–H_2O (25:10:0.5) to give III

(10 mg) and IV (3.7 mg). Fraction 5 was crystallized from dil. MeOH to give crude VIII. An absorption band at 1600 cm^{-1} in the IR spectrum of crude VIII indicated that it was a carboxylate, but this was considered to have been formed during the chromatography. Crude VIII was dissolved in 0.1 N H_2SO_4 in 60% dioxane and a large excess of water was added. Then, the total solution was concentrated to the original volume under reduced pressure to afford precipitates, which were crystallized from MeOH to give pure VIII (10 mg). Fraction 6 (200 mg) was treated with 0.1 N HCl–MeOH (5 ml) at room temperature for 20 h. The reaction mixture was neutralized with Ag_2CO_3 and the precipitates were filtered off. The filtrate was concentrated and the residue was chromatographed on silica gel (40 g) with $CHCl_3$–MeOH–H_2O (25 : 1 : 0.05 → 25 : 1.5 : 0.07 → 25 : 2 : 0.1) to give the monomethyl ester of VIII (IX, 35 mg) and the monomethyl ester of X (XI, 110 mg). Fraction 7 was crystallized from MeOH to give crude X, which was then treated with 0.1 N H_2SO_4 in 60% dioxane in the same manner as described for crude VIII to give pure X (15 mg). The BuOH layer was concentrated and the residue (8.6 g) was chromatographed on silica gel (900 g) with $CHCl_3$–MeOH–H_2O (25 : 3 : 0.3 → 25 : 6 : 0.7 → 25 : 8 : 1 → 25 : 9 : 1.3 → 25 : 10 : 1.6 → 25 : 11 : 1.9) to give Frs. 8—11. Fraction 8 (90 mg) was chromatographed on silica gel (10 g) with a gradient of $CHCl_3$–MeOH (MeOH 0—12%) to give crude VI (60 mg), which was then rechromatographed on silica gel (20 g) with a gradient of $CHCl_3$–BuOH (BuOH 0—50%) to afford pure VI (30 mg). Fraction 9 (220 mg) was chromatographed on silica gel (20 g) with a gradient of $CHCl_3$–BuOH (BuOH 0—50%) to give crude VII (40 mg), which was then rechromatographed on silica gel (10 g) with a gradient of AcOEt–BuOH (BuOH 0—40%) to give pure VII (20 mg). Fraction 10 (700 mg) was rechromatographed on silica gel (230 g) with $CHCl_3$–BuOH–H_2O (1 : 5 : 0.45) to give XII (400 mg) and XIII (30 mg). Fraction 11 contained XIV (1.4 g).

HN-saponin A (I)——A solution of I (20 mg) in 10% HCl–MeOH (2 ml) was heated under reflux on a water bath for 3 h. The reaction mixture was neutralized with Ag_2CO_3. The precipitates were filtered off and the filtrate was concentrated to give the residue, which was crystallized from MeOH to give an aglycone as colorless needles (10 mg). The aglycone fraction was shown to contain campesterol (t_R 13 min 55 s, trace), stigmasterol (t_R 14 min 48 s) and β-sitosterol (t_R 16 min 48 s) by GLC-3. The mother liquor of crystallization was examined by TLC [solv., $CHCl_3$–MeOH–H_2O (25 : 11 : 2)] and GLC-1 (as the trimethylsilylether derivative), which revealed the presence of methyl glucoside. ^{13}C-NMR: 102.8 (C-1′), 78.7 (C-3′), 78.5 (C-3), 78.3 (C-5′), 75.5 (C-2′), 71.9 (C-4′).

HN-saponin D_1 (III)——Colorless needles (from MeOH), mp 231—233 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 3380, 1695, 1100—1000. ^{1}H-NMR: 0.93 (6H), 0.98 (3H), 1.00 (3H), 1.02 (3H), 1.25 (3H) (each s, *tert*-Me × 6), 5.14 (1H, d, J = 7.1 Hz, C_1-H of glucose unit), 5.48 (1H, br s, C_{12}-H). ^{13}C-NMR: Table I. A solution of III (5 mg) in 10% HCl–MeOH (2 ml) was heated under reflux on a water bath for 2 h. The reaction mixture was worked up in the same manner as described for I to give an aglycone (2 mg) as colorless prisms; this product was identified as hederagenin by direct comparison of the TLC behavior [solv., $CHCl_3$–MeOH–H_2O (100 : 8 : 1); toluene–HCOOH–HCOOEt (5 : 1 : 4)] and IR spectra. The sugar portion was examined by TLC [solv., $CHCl_3$–MeOH–H_2O (25 : 11 : 2); BuOH–AcOH–H_2O (4 : 1 : 2)] and GLC-1 (as trimethylsilylether derivative), which showed the presence of methyl glucoside (t_R 29 min 10 s, 30 min 00 s).

HN-saponin F (VI)——Colorless needles (from dil. MeOH), mp 202—205 °C (dec.), $[\alpha]_D^9$ +36.0° (c = 0.5, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3380, 1720, 1070. ^{1}H-NMR: 0.89 (9H), 0.97 (3H), 1.14 (3H), 1.19 (3H), (each s, *tert*-Me × 6), 4.98 (1H, d, J = 6.8 Hz, C_1-H of arabinose unit), 5.44 (1H, br s, C_{12}-H), 6.33 (1H, d, J = 7.1 Hz, C_1-H of glucose unit). ^{13}C-NMR: Table I. VI (5 mg) was methanolyzed and worked up in the same way as described for III to give hederagenin (1.2 mg) (identified by TLC, IR) and a sugar fraction, which was shown to contain methyl arabinoside (t_R 8 min 46 s, 9 min 10 s) and methyl glucoside (t_R 29 min 10 s, 30 min 00 s) by GLC-1 (as the trimethylsilylether derivatives). A solution of VI (10 mg) in 0.5 N KOH (1 ml) was heated on a boiling water bath for 0.5 h. The reaction mixture was neutralized with 0.5 N H_2SO_4 and then extracted with a mixture of AcOEt and BuOH (2 : 1). The organic layer was concentrated and the residue was crystallized from dil. MeOH to give a prosapogenin (4 mg) as colorless needles; this product was identified as compound II by direct comparison of the TLC behavior [solv., $CHCl_3$–MeOH–H_2O (25 : 6 : 0.7)] and IR and ^{1}H-NMR spectra. The TLC behavior [solv., $CHCl_3$–MeOH–H_2O (25 : 8 : 1.2); BuOH saturated with water] and IR, ^{1}H-NMR and ^{13}C-NMR spectra of VI coincided with those of an authentic sample obtained on partial hydrolysis of Kizuta saponin K_{12}.[4d]

HN-saponin H (VII)——A white powder (from MeOH–AcOEt), mp 198—202 °C (dec.), $[\alpha]_D^9$ −3.3° (c = 3.00, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 1730, 1100—1000. ^{1}H-NMR: 0.87 (6H), 1.01 (3H), 1.05 (3H), 1.13 (3H), 1.17 (3H) (each s, *tert*-Me × 6), 1.65 (3H, d, J = 6.1 Hz, C_5-Me of rhamnose unit), 4.96 (1H, d, J = 7.1 Hz, C_1-H of outer glucose unit), 5.42 (1H, br s, C_{12}-H), 5.84 (1H, s, C_1-H of rhamnose unit), 6.22 (1H, d, J = 6.8 Hz, C_1-H of inner glucose unit). ^{13}C-NMR: Table I. VII (5 mg) was methanolyzed and worked up in the same way as described for III to give hederagenin (1 mg) (identified by TLC, IR) and a sugar portion which was shown to contain methyl glucoside (t_R 29 min 10 s, 30 min 00 s) and methyl rhamnoside (t_R 10 min 19 s) by GLC-1 (as the trimethylsilylether derivatives). A solution of VII (10 mg) in 0.5 N KOH (2 ml) was heated on a boiling water bath for 0.5 h. The reaction mixture was neutralized with 0.5 N H_2SO_4. The precipitates that appeared were collected by filtration and crystallized from MeOH to give a product (3 mg) as colorless prisms; this was identified as hederagenin by direct comparison of the TLC behavior and IR spectra. The TLC behavior [solv., $CHCl_3$–MeOH–H_2O (25 : 11 : 2); BuOH saturated with water] and IR, ^{1}H-NMR and ^{13}C-NMR spectra of VII coincided with those of an authentic sample obtained on partial acid

hydrolysis of Kizuta saponin K_{12}.[4d]

HN-saponin I (VIII)——Colorless needles (from MeOH), mp 264—267 °C (dec.), $[\alpha]_D^9$ +20.4° (c=0.80, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 1720 (broad), 1100—1000. ^{1}H-NMR: 0.81 (3H), 0.98 (12H), 1.32 (6H) (each s, *tert*-Me×7), 5.03 (1H, d, J=6.8 Hz, C_1-H of glucuronic acid unit), 5.46 (1H, br s, C_{12}-H). ^{13}C-NMR: Table I. VIII (5 mg) was methanolyzed and worked up in the same way as described for III to give oleanolic acid (2.5 mg) (identified by TLC, IR) and a sugar fraction which was shown to contain methyl glucuronopyranoside methyl ester [t_R 13 min 24 s (both α and β)] and methyl glycoside of glucurono-6,3-lactone [t_R 6 min 05 s (α, trace), 6 min 48 s (β)] by GLC-2 (as the trimethylsilylether derivatives), 6′-*O*-Methyl ester (IX) of VIII: colorless needles (from MeOH), mp 195—197 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 3400, 1725, 1700, 1100—1000. ^{1}H-NMR: 0.81 (3H), 0.97 (9H), 1.01 (3H), 1.31 (6H) (each s, *tert*-Me×7), 3.73 (3H, s, $C_{5'}$-COOMe), 4.98 (1H, d, J=7.3 Hz, C_1-H of methyl glucuronate unit), 5.46 (1H, br s, C_{12}-H). ^{13}C-NMR: Table I.

HN-saponin K (X)——Colorless needles (from MeOH), mp 224—227 °C (dec.), $[\alpha]_D^9$ +22.6° (c=1.00, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 1720 (shoulder), 1690, 1100—1000. ^{1}H-NMR: 0.94 (9H), 0.99 (3H), 1.26 (6H) (each s, *tert*-Me×6), 5.24 (1H, d, J=6.6 Hz, C_1-H of glucuronic acid unit), 5.45 (1H, br s, C_{12}-H). ^{13}C-NMR: Table I. X (5 mg) was methanolyzed and worked up in the same way as described for III to give hederagenin (2 mg) (identified by TLC, IR) and a sugar fraction which was shown to contain methyl glucuronopyranoside and methyl glycoside of glucurono-6,3-lactone by GLC-2 (as the trimethylsilylether derivatives). 6′-*O*-Methyl ester (XI) of X: colorless needles (from MeOH), mp 210—213 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 3400, 1730, 1690, 1100—1000. ^{1}H-NMR: 0.92 (9H), 1.00 (6H), 1.24 (3H) (each s, *tert*-Me×6), 3.69 (3H, s, $C_{5'}$-COOMe), 5.19 (1H, d, J=7.1 Hz, C_1-H of methyl glucuronate unit), 5.46 (1H, br s, C_{12}-H). ^{13}C-NMR: Table I.

Identification of HN-saponins B (II), D_2 (IV), E (V), M (XII), N (XIII) and P (XIV)——Compounds II, V, XII, XIII and XIV, were identified as Kizuta saponins K_3, K_6, K_{10}, K_{11} and K_{12}, respectively, based on the results of methanolysis and alkaline hydrolysis and by direct comparison of the TLC behavior [solv., $CHCl_3$–MeOH–H_2O (25:8:1.2, 25:12:2.5); BuOH saturated with water] and IR, ^{1}H-NMR and ^{13}C-NMR spectra with those of corresponding authentic samples.[4a,d] In the same manner, compound IV was identified as prosapogenin CP_2 isolated from the alkaline hydrolysate of crude saponin of *Clematis chinensis*.[5] The conditions of methanolysis and alkaline hydrolysis were the same as for III and as for VI, respectively. Compound XIII (20 mg) was treated with 0.1 N KOH (2 ml) at room temperature for 20 h. The reaction mixture was neutralized with 0.1 N H_2SO_4 and extracted with BuOH. The BuOH Layer was washed with water and concentrated to give a product (12 mg), which was identified as XIV by direct comparison of the ^{1}H- and ^{13}C-NMR spectra.

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