[Chem. Pharm. Bull. 36(12)4769—4775(1988)]

Studies on the Saponins of *Lonicera japonica* THUNB.

HIDEAKI KAWAI,[a] MASANORI KUROYANAGI,*,[a] KAORU UMEHARA,[a] AKIRA UENO[a] and MOTOYOSHI SATAKE[b]

School of Pharmaceutical Sciences, University of Shizuoka,[a] 2-2-1, Oshika, Shizuoka-shi 422, Japan and Tsukuba Medicinal Plants Research Station, National Institutes of Hygienic Sciences,[b] Hachimandai, Tsukuba-shi, Ibaraki 305, Japan

(Received May 30, 1988)

From the aerial parts of *Lonicera japonica* THUNB. (Caprifoliaceae), twelve triterpenoidal saponins having oleanolic acid and hederagenin as aglycones, were isolated. The structures of four new saponins, **6**, **9**, **11** and **12**, were established to be 3-*O*-α-L-arabinopyranosyl-28-*O*-[β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl]oleanolic acid, 3-*O*-[α-L-rahmnopyranosyl(1→2)-α-L-arabinopyranosyl]-28-*O*-β-D-glucopyranosyl hederagenin, 3-*O*-[α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl]-28-*O*-[β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl]oleanolic acid and 3-*O*-[α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoyl]-28-*O*-[6-acetyl-β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl]hederagenin, respectively, by means of carbon-13 nuclear magnetic resonance spectroscopy and chemical evidence. The present studies revealed that the saponin compositions of the materials collected at different places were significantly different from each other.

Among these saponins, monodesmosides showed strong hemolytic activity, but bisdesmosides showed weak hemolytic activity.

Keywords——saponin; *Lonicera japonica* ; Caprifoliaceae; hederagenin; oleanolic acid; methyl sugar benzoate; ^{13}C-NMR; hemolysis

The aerial part of *Lonicera japonica* THUNB. (Caprifoliaceae) is used as a folk medicine in Japan (Japanese name Nindou) and China.[1] However, little is known about the constituents of the plant, and saponins have not been reported from the plant. Recently, several saponins were isolated from a European *Lonicera* sp. plant, *L. nigura*, as molluscidal[2] constituents.[3] Therefore, as a part of our work on saponin chemistry, the isolation and structural elucidation of saponins of *L. japonica* were carried out. The materials for the study were collected at three different places, *i.e.*, the Medicinal Plants Garden of the University of Shizuoka (named type A), a Shimogamo-machi (named type B) and Tsukuba Medicinal Plants Research Station (named type C). In the previous paper, the isolation of iridoid glucosides from the type A materials was reported.[4]

From type A material, saponins **4**, **9**, **10** and **11** were isolated, from type B material, saponins **4**, **10** and **12** were isolated, and from type C material, saponins **1**, **2**, **3**, **5**, **6**, **7** and **8** were isolated. The structures of these saponins were elucidated by spectral, especially carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectral, and chemical evidence. The saponin compositions of the materials collected at several places were checked by mean of thin layer chromatographic (TLC) and densitometrical methods. Saponins are generally considered to have hemolytic activity,[5] so the isolated saponins were tested for hemolytic activity.

The isolation of saponins in the case of type A material is illustrated in Fig. 1. The aerial parts were extracted with hot water and methanol (MeOH), successively, and the extracts were treated as shown in Fig. 1. In the course of the purification, treatment with MCI gel HP-20 resin was effective to remove iridoid glucosides, as described in the experimental section. The type B and type C materials were also treated in the same way as type A material (described in the experimental section).

Structures of Saponins

Saponins **1**, **2**, **4**, **5**, **8** and **10** were methanolyzed with 5% HCl–MeOH to give an aglycone along with methyl sugars. The aglycone was identified as hederagenin (**13**) by direct comparison with the authentic sample, and the ^{13}C-NMR spectra of these saponins also showed the presence of a hederagenin moiety. The methyl sugars were analyzed by microanalysis[6,7] using high-performance liquid chromatography (HPLC) and circular dichroism (CD) spectroscopy of per-*p*-bromobenzoates of the methyl sugars. This approach is effective for identification of the kinds of sugars and for determination of D- and L-types of sugars by comparing A-values.[7] From the analysis, the kinds of sugars and their compositions in the saponins were determined. Some of these saponins, such as saponins **5**, **8** and **10**, were hydrolyzed under alkaline conditions to give prosapogenins, such as **1**, **2** and **4**. The ^{13}C-NMR spectra (Table I) of these saponins showed the presence of glucopyranosyl, arabinopyranosyl or rhamnopyranosyl moieties, and also suggested the substitution patterns of sugar moieties. From these data, the structures of the saponins having hederagenin as the aglycon were supposed to be **1**,[3] **2**,[3] **4**,[8] **5**,[3] **8**[3] and **10**,[9] respectively. These structures were confirmed by comparison with the reported data.

Saponins **3** and **7** were also methanolyzed by 5% HCl–MeOH to give oleanolic acid (**14**) as the aglycone along with methyl sugars. Oleanolic acid was identified by comparison with an authentic sample. The methyl sugars were also subjected to microanalysis. The ^{13}C-NMR spectra (Table I) of these saponins showed the presence of oleanoic acid, arabinopyranosyl and glucopyranosyl moieties. From these data, the structures of the saponins were determined to be **3**[3] and **7**,[3] respectively.

Saponin **6** showed the pseudo molecular ions, *m/z* 935 $(M+Na)^+$ and 913 $(M+H)^+$ in the positive fast atom bombardment mass spectrum (FAB-MS) and *m/z* 911 $(M-H)^-$ in the negative FAB-MS. From the MS data and elemental analysis, the molecular formula was concluded to be $C_{47}H_{76}O_{17}$. Saponin **6** was methanolized to give **14** and methyl sugars, which were identified as methyl arabinopyranoside and methyl glucopyranoside in the ratio of 1 to 2. The ^{13}C-NMR spectrum of the sugar moiety of **6** was identical with that of **5** (shown in Table I). From these data the structure of the new saponin **6** was established to be 3-*O*-α-L-arabinopyranosyl-28-*O*-[β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl]oleanolic acid.

Saponin **9** showed the pseudo molecular ions, *m/z* 935 $(M+Na)^+$ in the positive FAB-MS and *m/z* 911 $(M-H)^-$ in the negative FAB-MS. From the MS data and elemental analysis, the molecular formula of **9** was concluded to be $C_{47}H_{76}O_{17}$. Saponin **9** was methanolized to give **13** and methyl sugars, which were identified as methyl arabinopyranoside, methyl rhamnopyranoside and methyl glucopyranoside in the ratio of 1 to 1 to 1. Alkaline hydrolysis of **9** gave a prosapogenin, which was identical with **4**. The ^{13}C-NMR spectrum of **9** showed the presence of a terminal D-glucopyranosyl group linked to C-28 of hederagenin as an ester (δ 95.3). In the negative FAB-MS, fragments at *m/z* 748 $(M-H-glc)^-$, 603 $(M-H-glc-rha)^-$ and 471 $(M-H-glc-rha-ara)^-$, were seen. From these facts, the structure of the new saponin **9** was established to be 3-*O*-[α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl-28-*O*-β-D-glucopyranosyl]hederagenin.

Saponin **11** was assigned the molecular formula, $C_{53}H_{86}O_{21}$, from the elemental analysis. Saponin **11** was methanolized to give **14** and methyl sugars, which were identified as methyl glucopyranoside, methyl arabinopyranoside and methyl rhamnopyranoside in the ratio of 2 to 1 to 1. Alkaline hydrolysis of **11** gave a prosapogenin (**15**). The ^{13}C-NMR spectrum of **15** showed the same signal pattern of the sugar moiety as in **4** and a glucosylation shift at C-3 (δ 88.6) of the oleanolic acid moiety. The ^{13}C-NMR signals of the sugar moiety of **11** were identical with those of **10**. From these data, the structure of the new saponin **11** was established to be 3-*O*-[α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl]-28-*O*-[β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl]oleanolic acid.

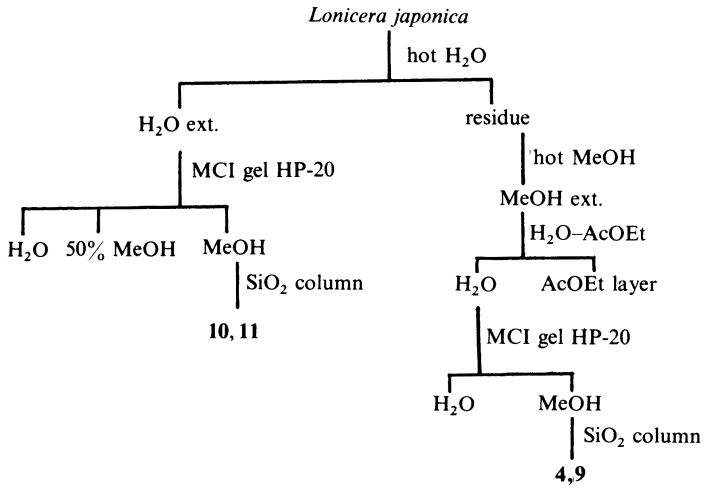
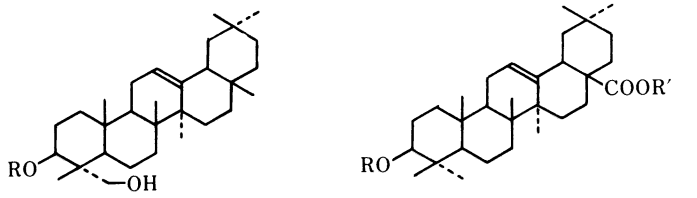


Fig. 1. Purification of Type A *L. japonica*



1:	R = ara	R′ = H	**3**:	R = ara$^{2\text{-}1}$glc	R′ = H
2:	R = ara$^{2\text{-}1}$glc	R′ = H	**6**:	R = ara	R′ = glc$^{6\text{-}1}$glc
4:	R = ara$^{2\text{-}1}$rha	R′ = H	**7**:	R = ara$^{2\text{-}1}$glc	R′ = glc$^{6\text{-}1}$glc
5:	R = ara	R′ = glc$^{6\text{-}1}$glc	**11**:	R = ara$^{2\text{-}1}$rha	R′ = glc$^{6\text{-}1}$glc
8:	R = ara$^{2\text{-}1}$glc	R′ = glc$^{6\text{-}1}$glc	**14**:	R = H	R′ = H
9:	R = ara$^{2\text{-}1}$rha	R′ = glc	**15**:	R = ara$^{2\text{-}1}$rha	R′ = H
10:	R = ara$^{2\text{-}1}$rha	R′ = glc$^{6\text{-}1}$glc			
12:	R = ara$^{2\text{-}1}$rha	R′ = glc$^{6\text{-}1}$glc^{6}–Ac			
13:	R = H	R′ = H			

Chart 1

Saponin **12** showed the pseudo molecular ions, *m/z* 1139 $(M+Na)^+$ in the positive FAB-MS and *m/z* 1115 $(M-H)^-$ in the negative FAB-MS. From the MS data and the elemental analysis, the molecular formula of **12** was established to be $C_{55}H_{88}O_{23}$. Saponin **12** was methanolized to give **13** and methyl sugars, which were identified as methyl glucopyranoside, methyl arabinopyranoside and methyl rhamnopyranoside in the ratio of 2 to 1 to 1. Alkaline hydrolysis of **12** gave a prosapogenin (**4**). The ^{13}C-NMR spectrum of **12** showed the presence of an acetyl group (δ 20.6, 170.6), which was also supported by the presence of the fragment *m/z* 1073 $(M+Na-Ac)^+$ in the positive FAB-MS and *m/z* 1073 $(M-Ac)^-$ in the negative FAB-MS. Deacetylation was carried out under mild alkaline condition to give saponin **10**. This indicated the presence of the acetyl group in **12**. The ^{13}C-NMR spectrum of **12** showed the acylation shift at C-6 (δ 64.5) of the terminal glucopyranosyl moiety. These data indicated that the structure of the new saponin **12** was 3-*O*-[α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl]-28-*O*-[6-acetyl-β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl]hederagenin.

Saponin Compositions of *L. japonica* from Different Sources

The saponins isolated from *L. japonica* contained oleanolic acid and hederagenin as aglycones. These saponins were classified into two types, rhamnosyl-arabinosyl type and

TABLE I. ^{13}C-NMR Spectral Data for Saponins **1**—**13** (22.5 MHz, Pyridine-d_5, δ Values)

		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**15**
3-Ara	C-1	106.5	103.9	104.8	103.8	106.6	107.3	104.7	103.6	103.8	104.0	104.5	104.0	104.4
	C-2	73.1	81.0	80.7	75.6	73.1	72.9	80.7	80.9	75.5	75.9	75.9	75.7	75.7
	C-3	74.7	73.5	73.4	74.0	74.7	74.6	73.4	73.4	74.0	74.1	73.8	74.2	73.7
	C-4	69.6	68.3	68.3	69.4	69.4	69.4	68.2	68.1	69.4	69.3	68.2	69.6	68.2
	C-5	66.8	64.9	64.7	64.9	66.8	66.6	64.9	64.7	65.0	65.2	64.0	64.9	64.1
3-Rha	C-1				101.2					101.2	101.6	101.6	101.5	101.5
	C-2				72.0[a]					71.8[a]	72.2[a]	72.2[a]	72.1[a]	72.1[a]
	C-3				72.2[a]					72.1[a]	72.5[a]	72.4[a]	72.4[a]	72.3[a]
	C-4				73.7					73.7	73.9	73.2	74.0	73.7
	C-5				68.8					68.8	69.0	69.8	68.9	69.6
	C-6				18.3					18.1	18.5	18.7	18.3	18.3
3-Glc	C-1		105.7	105.8				105.8	105.5					
	C-2		76.1	76.2				76.3	76.0					
	C-3		78.2	78.1				78.1[a]	78.1[a]					
	C-4		71.5	71.6				71.6	71.3					
	C-5		78.2	78.1				78.3[a]	78.1[a]					
	C-6		62.6	62.6				62.6	62.4					
28-Glc	C-1					95.7	95.7	95.6	95.4	95.3	95.6	95.7	95.4	
	C-2					73.9	73.8	74.0	73.7	73.7	74.1	73.8	73.8	
	C-3					78.3[a]	78.4[a]	78.3[a]	78.1[a]	78.8[b]	78.3[b]	78.2[b]	78.0[b]	
	C-4					69.6	69.4	69.4	69.2	70.8	69.7	69.6	69.3	
	C-5					78.0[a]	77.9[a]	78.1[a]	78.1[a]	78.4[b]	77.9[b]	77.7[b]	77.8[b]	
	C-6					70.9	70.9	70.9	70.7	61.9	70.9	70.9	71.2	
28-Glc	C-1					105.1	105.1	105.1	104.9		105.1	104.9	105.0	
(Terminal)	C-2					75.1	75.1	75.1	74.9		75.0	74.9	75.0	
	C-3					78.6[a]	78.6[a]	78.7[a]	78.5[a]		78.6[b]	78.5[b]	78.7[b]	
	C-4					71.6	71.6	71.6	71.3		71.5	71.4	70.9	
	C-5					78.3[a]	78.4[a]	78.3[a]	78.1[a]		78.3[b]	78.2[b]	74.8	
	C-6					62.4	62.6	62.6	62.4		62.6	62.5	64.5	
C-3		82.1	82.2	89.0	80.8	82.1	88.8	88.9	82.0	80.7	81.1	88.8	80.9	88.6
C-28		180.3	180.3	180.5	179.9	176.6	176.6	176.6	176.4	176.0	176.5	176.5	176.3	179.9
CO													170.6	
CH_3													20.6	

a, *b*) The values may be interchangeable in each column.

TABLE II. Difference of Saponins in *L. japonica* from Different Sources

	3-*O*-Arabinosyl-rhamnosyl type saponins	3-*O*-Arabinosyl-glucosyl type saponins
Type A	**4** (0.0074), **9** (0.0044), **10** (0.037), **11** (0.011)	
Type B	**4** (0.0044), **10** (0.011), **12** (0.018)	
Type C		**2** (0.013), **3** (0.0016), **7** (0.031), **8** (0.0010)

Values in parentheses are the saponins yields in %.

TABLE III. Hemolytic Activities of Saponins Isolated from *L. japonica*

Compounds	Hemolysis (%)	Compounds	Hemolysis (%)
1	76	**7**	78
2	105	**9**	47
3	108	**10**	67
4	62	**11**	57
5	12	**12**	39
6	−8	**15**	63

glucosyl-arabinosyl type, according to the sugar moieties at C-3. The type A material contained the rhamnosyl-arabinosyl type saponins, **4**, **9**, **10** and **11**. The type B material also contained the rhamnosyl-arabinosyl type saponins, **4** and **10**, and the acetyl derivative **12**. The type C material contained the glucosyl-arabinosyl type saponins, **2**, **3**, **7** and **8**. These results are summarized in Table II.

As mentioned above, the saponin constituents of the type C material were significantly different from these of the type A material. The saponin constituents of the type C *L. japonica* were almost the same as those of a European *Lonicera* sp. plant, *L. nigra*. It is interesting that the compositions of saponins in the same spacies of *L. japonica* from different regions in Japan were significantly different, whereas the composition of saponins of the type C *L. japonica* was almost the same as that of a different species, *L. nigra* in Europa.

We next compared the saponin constituents of several materials collected at different places in Japan by TLC with densitometry. The material from the Medicinal Plants Garden of the University of Shizuoka contained saponins **10** and **11** as the main constituents, but the materials from Shimada and Nara contained only saponin **10**. The materials from Hiroshima and Izu Medicinal Plants Research Station showed the presence of several saponins, which were not identical with those isolated from the types A, B and C. Purchased sample of the crude drug "Nindou" and Tsukuba-shi showed the presence of no saponins.

It is noteworthy that the compositions of saponins in different specimens of *L. japonica* were significantly different.

Hemolytic Activity

Saponins are generally considered to have hemolytic activity, so we examined the hemolytic activity of these saponins of *L. japonica* according to Arima *et al.*[10] Dimethyl sulfoxide (DMSO) was used at the concentration of 8.3%, because of the insolubility of some of the saponins. The results are given in Table III. The monodesmosides, such as **1**—**4**, showed strong hemolytic activity. In particular, **2** and **3**, which have glucosyl-arabinosyl moiety at C-3 showed very strong hemolytic activity. No difference of activity between oleanolic acid saponins and hederagenin saponins was observed. On the other hand, bisdesmosides, such as **5**—**12**, showed relatively weak activity; **5** and **6**, which have an arabinosyl moiety at C-3 and a gentiobiosyl moiety at C-28, showed no hemolytic activity.

Experimental

All melting points were measured on a Yanagimoto micro melting points apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were measured on a JEOL JNM-FX-90 NMR spectrometer at 89.55 and 22.5 MHz, respectively, in pyridine-d_5; chemical shifts are given in ppm with tetramethylsilane as an internal standard. MS were recorded on a JEOL JMS-DX303 mass spectrometer. CD spectra were recorded on a JASCO J-20A automatic recording spectropolarimeter. Optical rotations were determined on a JASCO DIP-140 digital polarimeter. Column chromatography was carried out on Silica gel Type 60 (Merck). TLC was carried out on precoated Silica gel $60F_{254}$ plates (Merck).

Plant Materials——For the isolation of saponins, three different materials, collected at Medicinal Plants Garden of this university (named type A), Shimogamo Nakaizu-machi, Shizuoka-ken and Tsukuba Medicinal Plants Research Station, NIHS, were used.

For the comparison of saponin compositions, six samples, collected in Hiroshima-ken, in Nara-ken, at Shimada-shi, Shizuoka-ken, at the Medicinal Plants Garden of this university, at Izu Medicinal Plants Research Station of NIHS and at Tsukuba-shi, Ibaraki-ken in November 1986, and a commercially available product, were used.

Isolation of Saponins——Fresh aerial parts of *L. japonica* (type A materials) (2.7 kg), collected in October 1986 in the Medicinal Plants Garden of the University of Shizuoka, were crushed and extracted successively with boiling water and boiling MeOH to give a water extract and an MeOH extract. The water extract was passed through MCI gel HP-20 (Mitsubishi Kasei Co. Ltd.) and eluted with water, 50% MeOH and MeOH successively to give a water eluate, a 50% MeOH eluate (29 g) and an MeOH eluate (7.5 g). The 50% MeOH eluate contained iridoid glycosides. The MeOH eluate showed the presence of saponins on TLC. This eluate was repeatedly chromatographed on a silica gel (SiO_2) column using a gradient of $CHCl_3$–MeOH, $CHCl_3$–MeOH–water (65 : 35 : 10, lower phase) and $CHCl_3$–MeOH–water (7 : 13 : 8, lower phase) to give saponins **10** (1 g) and **11** (300 mg). These products were further purified by HPLC (Develosil C-8 column and YMC ODS-7 column) using an acetonitrile-water solvent system. The MeOH extract was concentrated and fractionated between water and ethyl acetate (AcOEt), and the resultant water layer was passed through an MCI gel HP-20 column. The MeOH eluate thus obtained was repeatedly chromatographed on an SiO_2 column as described above to give saponins **4** (200 mg) and **9** (120 mg), which were finally purified by HPLC as described above.

Fresh aerial parts (4.5 kg) of *L. japonica* (type B material), collected in October, 1986, at Shimogamo-machi, Shizuoka-ken, were extracted and separated as in the case of type A material to give saponins **10** (500 mg) and **12** (800 mg) from the water extract and saponin **4** (200 mg) from the MeOH extract.

Fresh aerial parts (3.2 kg) of *L. japonica* (type C material), collected in June, 1986, at Tsukuba Medicinal Plants Research Station of the National Institute of Hygienic Sciences, were extracted and separated as in the case of the type A material to give saponins **5** (1.5 g) and **7** (1 g) from the water extract, and saponins **1** (200 mg), **2** (40 mg), **3** (50 mg), **6** (60 mg) and **8** (30 mg) from the MeOH extract. The type C material collected in June was identical with that collected in October in terms of saponin constituents determined by TLC.

Saponin 1——White powder, mp 221—224 °C (MeOH). $[\alpha]_D$ +61.6° (c=0.44, MeOH). *Anal.* Calcd for $C_{35}H_{56}O_8 \cdot 3/2H_2O$: C, 66.53; H, 9.41. Found: C, 66.66; H, 9.12. ^{13}C-NMR data are given in Table I.

Saponin 2——White powder, mp 247—249 °C (MeOH). $[\alpha]_D$ +9.9° (c=0.44, MeOH). ^{13}C-NMR data are given in Table I.

Saponin 3——White powders, mp 266—268 °C (MeOH). $[\alpha]_D$ +20.7° (c=0.12, MeOH). *Anal.* Calcd for $C_{41}H_{66}O_{13} \cdot 8/5H_2O$: C, 61.88; H, 8.77. Found: C, 61.69; H, 8.83. ^{13}C-NMR data was given in Table I.

Saponin 4——White powder, $[\alpha]_D$ −9.0° (c=0.17, MeOH). *Anal.* Calcd for $C_{41}H_{66}O_{12} \cdot 2H_2O$: C, 62.57; H, 8.97. Found: C, 62.52; H, 8.73. ^{13}C-NMR data are given in Table I.

Saponin 5——White powder, mp 208—212 °C (MeOH). $[\alpha]_D$ +15.1° (c=0.50, MeOH). *Anal.* Calcd for $C_{47}H_{76}O_{18} \cdot 2H_2O$: C, 58.49; H, 8.36. Found: C, 58.34; H, 8.27. ^{13}C-NMR data are given in Table I.

Saponin 6——White powder, $[\alpha]_D$ +9.7° (c=0.2, MeOH). *Anal.* Calcd for $C_{47}H_{76}O_{17} \cdot 5/2H_2O$: C, 53.91; H, 8.23. Found: C, 54.13; H, 8.13. ^{13}C-NMR data are given in Table I.

Saponin 7——White powder, mp 220—223 °C (MeOH). $[\alpha]_D$ +1.8° (c=0.13, MeOH). *Anal.* Calcd for $C_{53}H_{86}O_{22} \cdot 7/2H_2O$: C, 56.22; H, 7.75. Found: C, 56.25; H, 7.81. ^{13}C-NMR data are given in Table I.

Saponin 8——White powder, $[\alpha]_D$ +9.1° (c=0.14, MeOH). *Anal.* Calcd for $C_{53}H_{86}O_{23} \cdot 5/2H_2O$: C, 56.02; H, 8.07. Found: C, 56.04; H, 8.11. ^{13}C-NMR data are given in Table I.

Saponin 9——White powder, $[\alpha]_D$ 4.5° (c=0.2, MeOH). *Anal.* Calcd for $C_{47}H_{76}O_{17} \cdot H_2O$: C, 60.62; H, 8.44. Found: C, 60.62; H, 8.55. ^{13}C-NMR data are given in Table I.

Saponin 10——White powder, $[\alpha]_D$ −14.5° (c=0.14, MeOH). *Anal.* Calcd for $C_{53}H_{86}O_{22} \cdot 3H_2O$: C, 56.37; H, 8.21. Found: C, 56.24; H, 9.12. ^{13}C-NMR data are given in Table I.

Saponin 11——White powder, $[\alpha]_D$ −47.7° (c=0.2, MeOH). *Anal.* Calcd for $C_{53}H_{86}O_{21} \cdot H_2O$: C, 59.09; H, 8.14. Found: C, 58.81; C, 8.11. ^{13}C-NMR data was given in Table I.

Saponin 12——White powder, $[\alpha]_D$ −80.0° (c=0.06, MeOH). *Anal.* Calcd for $C_{55}H_{88}O_{23} \cdot 2H_2O$: C, 56.68; H, 8.07. Found: C, 56.99; H, 8.01. ^{13}C-NMR data are given in Table I.

Methanolysis of Saponins 1—12——A solution of saponins (*ca.* 1 mg) in 5% HCl–MeOH (3 ml) was refluxed for 4 h. The reaction solution was poured into water and passed through HP-20 to give a water eluate and MeOH and eluate. The MeOH eluate was dried and the aglycones were identified by TLC (solvent, $CHCl_3$: MeOH = 10 : 1) and HPLC (column, TSK gel 80Tm, solvent, CH_3CN : water = 80 : 20) comparison with authentic oleanolic acid or hederagenin. The water eluate was further passed through Amberlite IR-45 for neutralization and concentrated to give the methyl sugars fraction.

***p*-Br-Benzoylation of the Methyl Sugars**——The methyl sugars and *p*-bromo-benzoyl chloride (excess) in pyridine (1 ml) were heated at 90 °C overnight. Then the reaction solution was poured into water and extracted with hexane. The hexane solution was washed with water and concentrated. The methyl sugar *p*-Br-benzoates were analyzed by HPLC (column, TSK gel 80Tm; solvent, CH_3CN : H_2O = 90 : 10), and identified by comparison with authentic methyl sugar *p*-Br-benzoates (α-methyl arabinopyranoside per-*p*-Br-benzoate; 7.2 min, α-methyl rhamnopyranoside per *p*-Br-benzoate; 8.8 min, α-methyl glucopyranoside per-*p*-Br-benzoate; 12.8 min).

Determination of the A-Values——Per-*p*-Br-benzoates of methyl sugars were purified on an analytical scale by HPLC and the ultraviolet spectra and CD spectra of the collected peaks were directly measured. The concentrations of the benzoates were calculated from the absorbance and the reported extinction coefficients (57200 for tri-*p*-Br-benzoates and 76400 for tetra-*p*-Br-benzoates). The A-value was calculated from the CD data as follows.

A-value = of first Cotton − of second Cotton

Alkaline Hydrolysis of Saponins 7, 8, 10 and 11——A solution of saponin **7** (1 mg) or **8** (1 mg) in 10 ml of 0.5 N KOH–MeOH solution, was refluxed for 1 h. The reaction solution, was passed through HP-20. The MeOH eluates gave prosapogenins **3** and **2**, respectively. These were identical with the natural **3** and **2** on TLC.

Saponins **10** (50 mg) and **11** (50 mg) were treated as described above to give prosapogenins **4** (21 mg) and **15** (22 mg). ^{13}C-NMR data for **15** are given in Table I.

Deacetylation of Saponin 12——A solution of **12** (1 mg) in 2% KOH–MeOH solution (10 ml) was stirred at room temperature for 30 min. The reaction solution was passed through Amberlite IR-120 for neutralization, and concentrated to give **10**. This product was identical with the saponin **10** by TLC using two different solvent systems, *i.e.*, $CHCl_3$: MeOH : H_2O = 65 : 35 : 10, lower phase and isopropanol : AcOEt : H_2O = 4 : 8 : 5, upper phase).

Test of Hemolytic Activity——Hemolytic activity of the saponins was tested according to Arima *et al.* method.[10] Sheep red blood cells were collected from a commercial sheep red blood cell suspension (Denka Seiken Co., Ltd., Tokyo, Japan) by centrifugation at 1500 rpm for 5 min, washed with 0.86% NaCl solution 3 times and suspended in 100 volumes of NaCl solution. Samples were dissolved in 8.3% DMSO in 0.86% NaCl solution and then an equal volume of the red blood cell suspension was added. After incubation of the mixture for 4 h at 37 °C, the hemolytic activity was determined according to the following equation:

$$\text{hemolytic activity } (\%) = 100 \times (a - c)/(pc - c)$$

pc: absorbance of complete hemolysate in water
c: absorbance of negative control
a: absorbance of sample solution

Acknowledgements The authors wish to thank Dr. H. Kohda, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, and Dr. K. Nishi, Izu Medicinal Plant Research Station, NIHS, for collection of the materials. Thanks are also due to Mrs. H. Kitamura of the Analysis Center of this University for elemental analysis, and JEOL Ltd. for FAB-MS measurement.

References

1) Chiang Su New Medicinal College, "Dictionary of Chinese Crude Drugs," Shanghai Scientific Technologic Publisher, 1977, p. 1194 (in Chinese).
2) A. Marston and K. Hostettmann, *Phytochemistry*, **24**, 639 (1985).
3) B. Domon and K. Hostettmann, *Helv. Chim. Acta*, **66**, 422 (1983).
4) H. Kawai, M. Kuroyanagi and A. Ueno, *Chem. Pharm. Bull.*, **36**, 3664 (1988).
5) J. Hase, K. Kobayashi, K. Mitsui, T. Namba, M. Yoshizaki and T. Tomimori, Symposium Papers, 10th Wakanyaku Symposium, 1977, p. 85.
6) K. Nakanishi, M. Kuroyanagi, H. Nambu, E. M. Oltz, R. Takeda, G. L. Verdine and A. Zask, *Pure & Appl. Chem.*, **56**, 1031 (1984).
7) H-W. Liu and K. Nakanishi, *J. Am. Chem. Soc.*, **104**, 1178 (1982).
8) H. Kimata, T. Nakashima, S. Kokubu, K. Nakayama, Y. Mitoma, N. Yata and O. Tanaka, *Chem. Pharm. Bull.*, **31**, 1998 (1983).
9) M. M. Mukhamedziev, P. K. Alimbaeva, T. T. Gorvits and N. K. Abubakirov, *Khim. Prir. Soedin*, **7**, 153 (1971).
10) A. Kato, K. Ando, G. Tamura and K. Arima, *Cancer Res.*, **31**, 501 (1971).