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Dammarane Type Saponins of Leaves of *Panax japonicus* C.A. Meyer. (2). Saponins of the Specimens collected in Tottori-ken, Kyoto-shi, and Niigata-ken

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Isolation and structure determination of leaf-saponins of *P. japonicus* C.A. Meyer (Araliaceae) collected in Hiroshima-ken had already been reported. The aglycones of these saponins are represented by 20(*S*)-protopanaxatriol (**7**) and its homologue (modification of the side chain).[4] The present study revealed that the composition of leaf-saponins of this plant significantly depend upon the localities. From the leaves collected in Tottori-ken (T-type), there were isolated two dammarane type saponins, named chikusetsusaponins-LT_5 (**11**) and -LT_8 (**12**), the former of which was also obtained from the leaves collected in Kyoto-shi (K-type). By means of ^{13}C nuclear magnetic resonance spectroscopy and other physical procedures, the structures of **11** and **12** were established to be dammar-24-ene-3β,20*S*-diol-3-(O-β-glucopyranoside)-20-[O-β-glucopyranosyl(1→6)-β-glucopyranoside] and dammar-24-ene-3β,20*S*-diol-12-one-3,20-di(O-β-glucopyranoside), respectively.

On the other hand, the leaves collected in Niigata-ken (N-type) afforded a saponin named chikusetsusaponin-LN_4 (**13**), the structure of which was assigned as dammar-24-ene-3β,20*S*-diol-12-one-3-[O-β-xylopyranosyl(1→6)-β-glucopyranoside]-20-[O-α-arabinopyranosyl(1→6)-β-glucopyranoside]. These saponins, **11**, **12**, and **13** are characteristic of the leaves of T-, K-, and N-types, respectively and were not detected in the leaves collected in Hiroshima-ken (H-type), while the leaf-saponins of H-type were not isolated from the leaves of T-, K-, and N-types.

Keywords——*Panax japonicus*; Araliaceae; dammarane type saponins; ^{13}C NMR of glycosides; CD curve of dammarane-triterpene ketone; chikusetsusaponins-LT_5, -LT_8, and LN_4; chemotaxonomy

In continuation of the chemical studies on Ginseng constituents, dammarane type saponins of the aerial parts of *Panax ginseng* C.A. Meyer,[2] *P. japonicus* C.A. Meyer (=*P. pseudo-ginseng* subsp. *japonicus* Hara[3])[4] and *P. pseudo-ginseng* subsp. *himalaicus* Hara[5] have been reported. The specimen of *P. japonicus* from which ginsenoside-F_1 (**1**),[2a] chikusetsusaponins-L_5 (**2**), -L_{9a} (**3**), and -L_{10} (**4**) were isolated as leaf-saponins, was collected in Kake-cho, Hiroshima-ken (tentatively named H-type). Recently, we had opportunities to collect the specimens of this plant grown near Mt. Daisen, Tottori-ken (named T-type) and Kitayama, Kyoto-shi (named K-type) and found that the thin-layer chromatographic (TLC) pattern of methanolic extract of T-type leaves, being similar to that of K-type leaves, was significantly different from that of H-type. Further, the methanolic extract of the leaves collected at Tashirodaira, Niigata-ken (named N-type) also showed a different TLC pattern from those of H-, T-, and K-types (see Fig. 1). The present paper deals with isolation and structure determination of leaf-saponins of T-, K-, and N-types.

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2) *a*) S. Yahara, O. Tanaka, and T. Komori, *Chem. Pharm. Bull.* (Tokyo), **24**, 2204 (1976); *b*) S. Yahara, K. Matsuura, R. Kasai, and O. Tanaka, *ibid.*, **24**, 3212 (1976).
3) H. Hara, *Journ. Jap. Bot.*, **45**, 197 (1970).
4) S. Yahara, R. Kasai, and O. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **25**, 2041 (1977).
5) O. Tanaka and S. Yahara, *Phytochemistry*, **17**, 1353 (1978).

Leaf Saponins of T- and K-Types

Methanolic extract of the leaves collected in the west-foot of Mt. Daisen was separated as described in the experimental part, affording a crude glycoside-fraction (**G**) which was subjected to hydrolysis with crude nariginase[6] to give a crystalline prosapogenin (**5**). Acid hydrolysis of **5** gave glucose and a complex mixture of ether-soluble substances which seemed to be modified aglycones formed from an acid-unstable genuine aglycone.

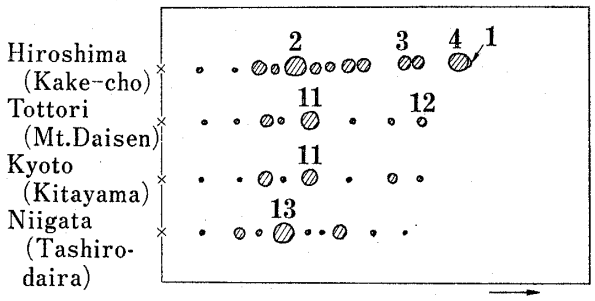


Fig. 1. Thin-Layer Chromatogram of *Panax japonicus* Leaf Saponins on Kieselgel H

Solvent: $CHCl_3$: MeOH: H_2O 65: 35: 10 (lower phase). Detection: H_2SO_4.

The infrared (IR) spectrum of **5** (KBr) showed a carbonyl band at 1703 cm^{-1} and its circular dichroism (CD) curve (in MeOH) exhibited negative Cotton effect ($[\theta]^{17}$ −3046 at 286 nm, indicating the presence of a keto-function. A number of saponins having acid-unstable dammarane type aglycone such as 20(*S*)-protopanaxadiol (**6**) and 20(*S*)-protopanaxatriol (**7**) have been isolated from *Panax* spp.[2,4,5,7,8,9] It has been known that CD (or ORD) curves of 3-keto derivatives of dammarane type triterpenes exhibit positive Cotton effect,[10] while those of 6- and 12-keto derivatives show negative Cotton effect at 305 and 285 nm, respectively.[8] The CD curve of **5** mentioned above is almost superimposable on that of a model compound, 3-O-acetyl-12-keto derivative (**8**) derived from betulafolenetriol (**9**),[11] being obviously different from those of the 3- or 6-keto derivatives.

^{13}C nuclear magnetic resonance (NMR) spectroscopy is now essential technique for the structure studies on plant glycosides, especially isoprenoid-glycosides having acid-unstable aglycones. For the purpose of its application to studies on *Panax* saponins, stereochemistry of β-glucosylation effect[12] and assignments of carbon chemical shifts of dammarane type triterpenes[13] have been explored. The ^{13}C NMR spectrum of **5** showed a carbonyl carbon signal at δ 210.9 (ref. that of **8** appears at δ 211.5). Appearance of one proton signal due to an anomeric proton of a β-glucoside at δ 5.07 (1H, d, J=7.5 Hz) in the ^1H NMR spectrum and the inspection of the sugar-carbon resonance-region of the ^{13}C NMR spectrum (Table I) revealed that **5** must be a mono-β-glucopyranoside. The anomeric carbon signal of **5** appeared at unusually high field (δ 98.3), indicating that **5** must be a β-glucopyranoside of a tertiary alcohol.[12] This was also supported by the presence of a signal as singlet at δ 81.4 due to a quarternary carbon bearing an oxygen function.[12,13] In comparison of the carbon resonances attributable to the aglycone moiety of **5** with those of 20-O-β-glucopyranosyl-20-(*S*)-protopanaxadiol(=compound K[14]) (**10**) and with those of **8**, signals assignable to C-1∼7, 10, 21, 23 27, and other methyl groups of **10** were observed at the similar positions in the

6) H. Kohda and O. Tanaka, *Yakugaku Zasshi*, **95**, 246 (1975).
7) O. Tanaka, M. Nagai, T. Ohsawa, N. Tanaka, K. Kawai, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **20**, 1204 (1972); M. Nagai, T. Ando, N. Tanaka, O. Tanaka, and S. Shibata, *ibid.*, **20**, 1212 (1972); T. Ohsawa, N. Tanaka, O. Tanaka, and S. Shibata, *ibid.*, **20**, 1890 (1972).
8) M. Nagai, O. Tanaka, and S. Shibata, *Tetrahedron*, **27**, 881 (1971).
9) S. Sanada, N. Kondo, J. Shoji, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **22**, 421 and 2407 (1974).
10) C. Djerassi, J. Osiecki, and W. Closson, *J. Am. Chem. Soc.*, **81**, 4587 (1959).
11) R. Kasai, K. Shinzo, and O. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **24**, 400 (1976).
12) R. Kasai, M. Suzuo, J. Asakawa, and O. Tanaka, *Tetrahedron Lett.*, **1977**, 175. (Stereochemistry of β-glucosylation effects).
13) J. Asakawa, R. Kasai, K. Yamasaki, and O. Tanaka, *Tetrahedron*, **33**, 1935 (1977). (Carbon chemical shifts assignments of dammarane type triterpenes).
14) I. Yosioka, T. Sugawara, K. Imai, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 2418 (1972).

spectrum of **5**, while the resonances due to C-8, 9, 11, 12, 14, and 15 of **8** were found to be present at the similar positions in the spectrum of **5**.

Because the glycosyl bonding at 20-*tert*-hydroxyl group of dammarane type triterpenes is quite unstable, mass spectra of acetylated or trimethylsilylated saponins of this type show no fragment ion bearing the 20-O-glycosyl moiety, exhibiting a fragment peak such as ion **A** in Chart 1.[15] The mass spectrum of acetylated **5** showed a peak with highest mass number at *m/e* 482, which could be assigned to the ion **A-1** in Chart 1.

These evidences strongly suggested that **5** would be formulated as 12-keto derivative of **10**. This was finally confirmed by preparation of **5** from **10**. The 12β-hydroxyl group of **10** is rather hindered than other hydroxyl groups by the presence of 20-O-glucosyl group,[9] being expected to be remained unacetylated under mild reaction condition. Acetylation of **10** with acetic anhydride and pyridine at 5° followed by oxidation with CrO_3-pyridine complex and subsequent saponification of the resulting keto-acetate yielded a crystalline

Table I. ^{13}C NMR Chemical Shifts[e]

	Aglycone moieties						Sugar moieties						
	10	8	5	12	11	13		10	5	12	11	13	
C-1	39.4	34.6	40.5[a]	40.9[a]	40.4[a]	40.4[a]	3-Glc 1			106.9	106.4	106.4	
2	28.0	23.5	27.9	26.5	26.3	26.3	2			75.7	75.2[c]	75.4	
3	78.0	78.0	77.9	88.3	88.5	88.7	3			79.1[c]	78.3[d]	78.5[c]	
4	39.4	38.0	39.4	39.7	39.4	39.6	4			71.8	71.6	72.0	
5	56.3	51.2	56.3	56.1	56.3	56.2	5			78.4[c]	77.8[d]	76.4[d]	
6	18.7	18.6	18.9	18.6	18.4	18.3	6			63.0	62.7	69.8	
7	35.0	34.3	34.9	34.8	34.7	34.6	Xyl 1					105.2	
8	39.9	41.0	41.0	40.9	40.9	40.9	2					74.3	
9	50.2	56.5	56.3[b]	56.1[b]	56.1[b]	56.2[b]	3					77.5	
10	37.2	37.0	38.0	37.5	37.5	37.5	4					70.9	
11	30.7	41.6	40.2[a]	40.2[a]	40.0[a]	40.2[a]	5					66.6	
12	70.2	211.5	210.9	211.2	210.8	211.3	20-Glc 1	98.1	98.3	98.4	98.2	98.2	
13	49.2	54.4	55.0[b]	54.8[b]	54.8[b]	55.0[b]	2	74.9	75.5	75.7	75.2[c]	75.4	
14	51.7	55.8	56.3	56.1	56.1	56.2	3	78.8[b]	78.9[c]	78.6[c]	77.8[d]	78.3[c]	
15	30.7	32.0	32.3	32.3	32.2	32.1	4	71.2	72.1	71.8	71.6	72.0	
16	26.5	25.7	24.5	24.6	24.4	24.4	5	78.0[b]	77.4[c]	77.9[c]	76.3	76.0[d]	
17	51.3	44.4	42.7	42.6	42.6	42.7	6	62.5	63.1	63.0	70.3	69.8	
18	16.2[a]	17.4[a]	17.7[c]	17.9[c]	17.8[c]	17.8[c]	Glc 1				105.0	104.2	1 Ara(Pyr)
19	15.9[a]	17.4[a]	17.0[c]	17.0[c]	17.0[c]	17.0[c]	2				74.7[c]	72.0	2
20	83.3	73.3	81.4	81.3	81.4	81.5	3				77.8[d]	73.8	3
21	22.3	26.6	22.4	22.4	22.2	22.3	4				71.6	68.3	4
22	35.9	39.9	39.2[a]	38.8[a]	39.0[a]	39.0[a]	5				77.8[d]	65.4	5
23	23.2	23.1	23.9	24.0	23.9	23.9	6				62.7		
24	125.8	126.0	125.7	125.8	125.8	125.8							
25	130.8	130.8	130.7	130.8	130.7	130.8							
26	25.7	24.6	25.7	25.7	25.6	25.7							
27	17.7[a]	15.9[a]	16.3[c]	16.6[c]	16.5[c]	16.5[c]							
28	28.6	28.0	28.5	28.0	28.0	28.0							
29	16.2[a]	21.7	16.3[c]	16.3[c]	16.1[c]	16.3[c]							
30	17.2[a]	15.9[a]	16.0[c]	15.9[c]	15.9[c]	16.0[c]							
Ac		21.0											
		170.1											

Chemical shifts of aliphatic β-xylopyranoside, α-arabinopyranoside and β-D-glucopyranoside: see previous paper.[4]
a, b, c, d) Values any vertical colomn may be reversed although those given here are preferred.
e) FT NMR conditions: spectral width: 4 and 6.25 KHz; acquition time: 0.5 and 0.3 sec.; number of data points: 4096; recycle time: 1 sec.; number of recycle time: 1000—10000; pulse flipping angle: 90°.

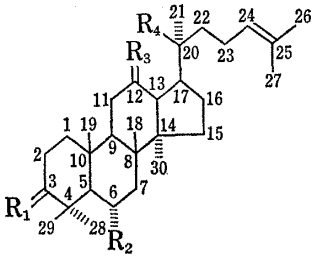
15) T. Komori, O. Tanaka, and Y. Nagai, *Org. Mass Spectr.*, **9**, 744 (1974); R. Kasai, K. Matsuura, O. Tanaka, S. Sanada, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **25**, 3277 (1977).

compound, which was identical with **5** by comparison of TLC, CD curve, IR and ^{13}C NMR spectra.

The crude glycoside fraction (**G**) (*vide supra*) was subjected to the separation by droplet counter current chromatography (DCCC) followed by column chromatography on silica gel afforded two new saponins, named chikusetsusaponins-LT_5 (**11**) and -LT_8 (**12**).

Acid hydrolysis of **12** gave glucose. The ^1H NMR spectrum of **12** showed two anomeric proton signals at δ 4.95 and 5.08 (1H each, d, J=7 Hz, β-glucosyl linkage). The IR spectrum (KBr) of **12** exhibited a carbonyl absorption band at 1700 cm^{1-} and the CD curve of **12** was almost superimposable on that of **5**.

In comparison with the spectrum of **5**, the ^{13}C NMR spectrum of **12** showed signals due to one additional set of β-glucopyranosyl unit, whose anomeric carbon signal appeared at δ 105.0 being characteristic of 3-O-β-D-glucopyranosyl linkage of dammarane type triterpenes.[12]



Glc=glucopyranosyl
Xyl=xylopyranosyl
Ara=arabinopyranosyl

1: R_1=—OH, R_2=OH R_3=—OH R_4=O—Glc(β)
H(α) H(α)

2: R_1=—OH, R_2=OH, R_3=—OH, R_4=O—Glc(β)(6→1)—Ara(β)(4→1)—Xyl(β)
H(α) H(α)

4: R_1=—OH, R_2=OH, R_3=—O—Glc(β), R_4=OH
H(α) H(α)

5: R_1=—OH, R_2=H, R_3=O, R_4=O—Glc(β)
H(α)

6: R_1=—OH, R_2=H, R_3=—OH, R_4=OH
H(α) H(α)

7: R^1=—OH, R_2=OH, R_3=—OH, R_4=OH
H(α) H(α)

8: R_1=—H, R_2=H, R_3=O, R_4=OH
O-Ac

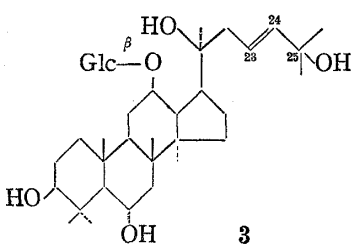
9: R_1=—H, R_2=H, R_3=—OH, R_4=OH
OH H(α)

10: R_1=—OH, R_2=H, R_3=—OH, R_4=O—Glc(β)
H(α) H(α)

11: R_1=—O—Glc(β), R_2=H, R_3=O, R_4=O—Glc(β)(6→1)—Glc(β)
H(β)

12: R_1=—O—Glc(β), R_2=H, R_3=O, R_4=O—Glc(β)
H(β)

13: R_1=—O—Glc(β)(6→1)—Xyl(β), R_2=H, R_3=O, R_4=O—Glc(β)(6→1)—Ara(α)
H(β)



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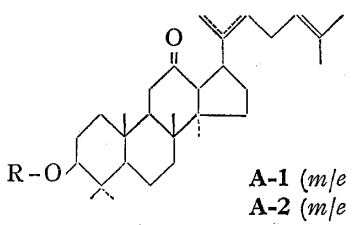

A

A-1 (*m/e* 482) R=-Ac
A-2 (*m/e* 770) R=-Glc(-Ac_4)
A-3 (*m/e* 986) R=hexose(-Ac_3)-pentose(-Ac_3)

Chart 1

With regard to the resonances due to the aglycone moiety, on going from **5** to **12**, the signal due to C-3 of **5** (δ 77.9) was displaced by +10.4 ppm (at δ 88.3) and the signal assignable to C-2 of **5** (δ 27.9) was shielded by −1.4 ppm by the β-D-glucosylation effect,[12] while other carbon resonances remained almost unshifted. These evidences led to formulate **12** as 3-O-β-glucopyranoside of **5**. In the mass spectrum of acetylated **12**, appearance of a peak at *m/e* 770 which can be assigned as ion **A-2** also supports this formulation.

Acid hydrolysis of **11** afforded glucose. The IR spectrum of **11** (KBr) exhibited a carbonyl absorption band at 1700 cm^{-1} and its CD curve was almost superimposable on those of **5** and **12**. The ^1H NMR spectrum of **11** showed three anomeric proton signals due to β-glucosyl linkage at δ 4.87 (1H d, J=8 Hz) and 5.06 (2H d, J=8 Hz). Comparison of ^{13}C NMR spectrum of **11** with that of **12** indicated that the signals attributable to the aglycone moiety of **11** appeared at the almost same positions as those of **12**. With respect to sugar-carbon region, signals assignable to three sets of β-glucopyranosyl unit appeared in the spectrum of **11**. It is noted that one of the three C-6 carbon signals (triplets) of these glucosyl units was deshielded by about +7 ppm (at δ 70.3), demonstrating the presence of a β-genitobiosyl moiety (=6-O-β-glucopyranosyl-β-glucopyranosyl) in **11**.[16] The mass spectrum of acetylated **11** showed a fragment peak at *m/e* 770 which can be formulated as **A-2**, revealing that the gentiobiosyl moiety of **11** must be present not at the 3-hydroxyl group but at the 20-*tert*-hydroxyl group. It follows now that **11** can be formulated as dammar-24-ene-3β, 20*S*-diol-12-one-3-(O-β-glucopyranoside)-20-[O-β-glucopyranosyl (1→6)-β-glucopyranoside]as illustrated in Chart 1.

From the K-type leaves collected at Kitayama, Kyoto-shi, **11** was isolated and identified but **12** could not be isolated in a pure state though the TLC of its methanolic extract suggested the presence of a small amount of **12**.

A Leaf-saponin of N-Type

A glycoside-fraction of the methanolic extract of leaves of N-type(collected at Tashirodaira, Niigata-ken) was subjected to column chromatography on silica gel and then on polyamide, affording a main saponin named chikusetsusaponin-LN_4 (**13**).

Acid hydrolysis of **13** gave glucose, xylose, and arabinose. The IR spectrum of **13**(KBr)-showed a carbonyl band at 1703 cm^{-1}. Comparison of the CD curve and the ^{13}C NMR spectrum of **13** with those of **11** and **12** disclosed that **13** has the same 3,20-diglycosylated aglycone as that of **11** and **12** (Table I). The ^{13}C NMR spectrum of **13** also revealed that the sugar moiety of **13** consists of β-xylopyranosyl, α-arabinopyranosyl, and two of 6-substituted β-glucopyranosyl units, *i.e.* the 6-carbinol carbon signals (triplets) of both glucosyl units were displaced downfield by about +7 ppm (at δ 69.8) from its position of unsubstituted β-glucopyranosides.[16] The mass spectrum of acetylated **13** showed a peak at *m/e* 986, which must be represented by the ion **A-3** in Chart 1. Methylation of **13** followed by methanolysis of the resulting permethyl ether yielded methyl 2,3,4-trimethylxylopyranoside, methyl 2,3,4-trimethylarabiopyranoside, and methyl 2,3,4-trimethylglucopyranoside. These evidences indicated the presence of 6-O-β-xylopyranosyl-β-glucopyranosyl and 6-O-α-arabinopyranosyl-β-glucosyl units in **13**.

It has been reported that the 20-O-glycosyl linkage of dammarane type saponins is very unstable, being hydrolyzed only by heating with aqueous acetic acid.[17] A solution of **13** in 50% aqueous acetic acid was heated at 80°, affording prosapogenin- and oligosaccharide-fractions. The former yielded glucose and xylose on acid hydrolysis, while acid hydrolysis of the latter gave glucose and arabinose. It follows now that **13** can be represented by dammar-24-ene-3β,20*S*-diol-12-one-3-[O-β-xylopyranosyl(1→6)-β-glucopyranoside]-20-[O-α-ara-

16) T. Usui, N. Yamaoka, K. Matsuda, K. Tsuzimura, H. Sugiyama, and S. Seto, *J.C.S. Perkin I*, 1973, 2425.

17) S. Shibata, T. Ando, and O. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **14**, 1157 (1966).

binopyranosyl(1→6)-β-glucopyranoside]. This saponin (**13**) was not isolated from the leaves of T- and K-types, while saponins (**11, 12**) were not present in the leaves of N-type. The TLC patterns of methanolic extracts of the leaves collected at the following places were very similar to that of N-type: Ozenuma, Gumma-ken; Shimoshinkawa, Toyama-ken; Sosori-cho, Ishikawa-ken; Yasumiya Aomori-ken; and Teine-yama and Horonuka, Hokkaido.

The saponins (**11, 12, 13**) are the first examples of the dammarane-type glycosides having 12-keto-function in the aglycone moiety and it is notable that such a type of saponins have not been detected in the leaves of H-type, and that the TLC of methanolic extracts of the leaves collected in Fukuoka-ken, Shimane-ken, Ehime-ken, Mie-ken, Nara-ken, Nagano-ken, Tochigi-ken, Tokyo-to, and Miyagi-ken showed the similar patterns to that of H-type regardless of the season of collection. No remarkable difference was observed in the rhizome-saponins[18] between H-, T-, K-, and N-types. From the chemotaxonomical view point, further study on the leaf-saponins of this plant is under progress.

Experimental

NMR spectra were taken on JEOL PFT-100 spectrometer in C_5D_5N using TMS as an internal standard (^1H NMR at 100 MHz and ^{13}C NMR at 25.15 MHz). MS were taken at 75 eV on JEOL 01-SG-2 spectrometer. Acetylation of saponins for MS determination and identification of the resulting monosaccharides after acid hydrolysis of saponins were all referred to the previous paper.[2a] Melting points were taken on a micro-hot-stage and uncorrected.

Extraction of the Leaves of T-Type——The leaves were collected at Sannosawa near Mt. Daisen in August. The dried leaves (70 g) were extracted with MeOH. After evaporation of the solvent, the residue was suspended in H_2O and the aqueous suspension was washed with ether and then extracted with *n*-BuOH (saturated with H_2O). The BuOH-extract was concentrated to dryness and an aqueous solution of the residue was passed through a column of polyamide. The eluant was concentrated to dryness, affording a crude glycoside-fraction (**G**) (5 g).

Enzymatic Hydrolysis of the Crude Glycoside-Fraction (G)——A solution of **G** (2.5 g) and crude naringinase (3 g) in 0.2 M phosphate buffer (500 ml, pH 4.0) was incubated at 40° for 5 days. The reaction mixture was extracted with *n*-BuOH (saturated with H_2O) and the extract was concentrated to dryness. The residue (2.1 g) was chromatographed on silica gel by eluting with $CHCl_3$–MeOH (10: 1) affording fractions 1—5. The fraction 2 was acetylated with Ac_2O–C_5H_5N in the usual way to give an acetate, colorless crystals, mp 242—244° (from MeOH), (520 mg). A solution of this acetate (200 mg) in 10% KOH–MeOH (20 ml) was allowed to stand at room temperature for 2 hr. The reaction mixture was diluted with H_2O and then extracted with ether. The ether solution was washed with H_2O, dried, and concentrated to dryness yielding the prosapogenin (**5**) as white powder, $[\alpha]_D^{25}$ +32.2° (c=0.19, MeOH). *Anal.* Calcd. for $C_{36}H_{60}O_8 \cdot 1/3H_2O$: C, 68.97; H, 9.76. Found: C, 69.16; H, 9.47. CD (c=0.06, MeOH) $[\theta]^{17}$ (nm): −3046 (286) (negative maximum).

Preparation of 5 from 10——A solution of **10** (100 mg) in Ac_2O (1.5 ml) and C_5H_5N (3 ml) was allowed to stand at 5° for 24 hr. After working up in the usual way, the resulting acetate (IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3530 (OH), 1760 (acetyl)) (130 mg) was oxidized with CrO_3 (300 mg) and C_5H_5N (20 ml) on standing at room temperature overnight. After working up in the usual way, the resulting keto-acetate was saponified with 10% KOH in MeOH (30 ml) on standing at room temperature for 2 hr. The reaction mixture was diluted with H_2O, dried, and concentrated to dryness. The residue was chromatographed on silica gel by eluting with $CHCl_3$–H_2O (10: 1), yielding a keto-glucoside (30 mg), $[\alpha]_D^{12}$ +29.5° (c=0.31, MeOH), white powder: *Anal.* Calcd. for $C_{36}H_{60}O_8 \cdot 1/3H_2O$: C, 68.97; H, 9.76. Found: C, 68.93; H, 9.41. This was identical with **5** by comparison of IR, ^{13}C NMR, and other physical constants.

Separation of Leaf-Saponins of T-Type——The aforementioned crude glycoside-fraction (**G**) (2.5 g) was subjected to DCCC: solvent system $CHCl_3$–MeOH–iso-PrOH–H_2O (5: 6: 4: 1), stationary phase: lower layer and mobile phase: upper layer, affording fractions-1-7. The fraction-5 gave **11**, colorless crystals, mp 265—270° (from H_2O), $[\alpha]_D^{25}$ +4.5° (c=0.53, MeOH), yield: 2.0%. *Anal.* Calcd. for $C_{48}H_{80}O_{18} \cdot H_2O$: C, 59.85; H, 8.57. Found: C, 59.73; H, 8.49. CD (c=0.09, MeOH) $[\theta]^{17}$ (nm): −3683 (284) (negative maximum).

The fraction-6 was further chromatographed on silica gel by eluting with $CHCl_3$–MeOH–H_2O (22: 4: 1) to give **12**, white powder, $[\alpha]_D^{25}$ +16.9° (c=0.21, MeOH). *Anal.* Calcd. for $C_{42}H_{70}O_{13} \cdot H_2O$: C, 62.97; H, 9.06. Found: C, 62.82; H, 8.87. CD (c=0.05, MeOH) $[\theta]^{19}$ (nm): −3234 (284) (negative maximum), yield 0.1%.

18) T.D. Lin, N. Kondo, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **24**, 253 (1976) and the references cited therein.

Extraction and Separation of Leaves of K-Type——The leaves (65 g) were collected at Kitayama, Kyoto-shi in August. The dried leaves were extracted with MeOH and the MeOH-extract was separated in the same way as that of T-type leaves, affording **11**, colorless crystals, mp 265—268° (from H_2O), $[\alpha]_D^{12}$ +4.6° (c=0.65, MeOH), IR ν_{max}^{KBr} cm^{-1} 1700 (C=O), yield 0.5%. The identification with a sample of **11** isolated from the T-type leaves was achieved by comparison of MS, ^{13}C NMR, and other physical and chemical properties.

Extraction and Separation of Leaves of N-Type——The leaves were collected at Tashirodaira, Niigata-ken at the beginning of Octover. The dried leaves (7 g) were extracted with MeOH. A suspension of the MeOH extract in H_2O was washed with ether and then extracted with n-BuOH (saturated with H_2O). The BuOH extract (660 mg) (crude glycoside-fraction) was chromatographed on silica gel by eluting with $CHCl_3$–MeOH–H_2O (300: 110: 11) to give fractions-1—5. The fraction-4 was further chromatographed on polyamide by eluting with H_2O affording **13**, white powder, $[\alpha]_D^{15}$ −12.3° (c=0.65, MeOH)in a yield of 1.4%, CD (c=0.10, MeOH), $[\theta]^{17}$ (nm): −3440 (284) (negative maximum). *Anal.* Calcd. for $C_{52}H_{86}O_{21}$: C, 59.64; H, 8.28. Found: C, 59.69; H, 7.85.

Methylation of **13** followed by methanolysis and identification of the resulting methylated-monosaccharides were carried out in the same way as those of the structure studies reported previously.[2a,4]

Partial Hydrolysis of 13 Followed by Acid Hydrolysis——A solution of **13** (6 mg) in 50% aqueous AcOH (10 ml) was heated at 80° for 1 hr. The reaction mixture was diluted with H_2O and then extracted with n-BuOH (saturated with H_2O). The BuOH- and H_2O layers were concentrated to dryness and both of the residues were hydrolyzed by heating at 90° for 3 hr with 8% HCl in dioxan–H_2O (1: 1) (5 ml), respectively. In the hydrolysate from the BuOH layer (prosapogenin fraction), xylose and glucose were identified and in that from the H_2O layer (oligosaccharide fraction), arabinose and glucose were identified; GLC of monosaccharides: see previous paper.[2a,4]

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