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New Dammarane Type Saponins of Leaves of *Panax japonicus* C.A. Meyer. (1). Chikusetsusaponins-L_5, -L_{9a} and -L_{10}

Shoji Yahara,[1a] Ryoji Kasai, and Osamu Tanaka[1b]

Faculty of Pharmaceutical Sciences, Kyushu University[1a] and Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine[1b]

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From leaves of *Panax japonicus* C.A. Meyyr, ginsenoside-F_1[7] and three new dammarane type saponins named chikusetsusaponins-L_5(I), -L_{10}(II) and -L_{9a}(III) were isolated. The structures of I, II and III were elucidated mainly by mass, ^{13}C-nuclear magnetic resonance and ^{1}H-nuclear magnetic resonance spectroscopy including partially relaxed Fourier transform method.

Keywords——dammarane type saponin; *Panax japonicus*; Araliaceae; chikusetsusaponins-L_5, -L_{9a}, -L_{10}; C-13 NMR; partially relaxed Fourier transform method; ginsenoside-F_1; MS of trimethylsilyl ether of saponins

In connection with the studies on the saponins of roots of *Panax ginseng* C.A.Meyer,[2,3] Shoji and his co-workers elaborated the isolation of saponins from rhizomes of *P. japonicus* C.A.Meyer,[4] an oriental crude drug "Chikusetsu-ninjin in Japanese", establishing structures of chikusetsusaponins-I,-Ia,-Ib,-III,-IV,-IVa and -V.[5] With regard to the constituents of leaves of this plant, the preliminary work revealed the presence of several saponins by thin-layer chromatography (TLC).[6] In continuation of our studies on the constituents of leaves and flower-buds of *Panax* spp.[7,8] the present authors have been undertaking isolation and structural determination of saponins of *P. japonicus*.

A suspension of the methanolic extract of the dried leaves was washed with ether and then extracted with *n*-butanol. The butanolic solution was passed through a column of polyamide and concentrated to dryness to give a crude saponin-fraction(TLC: see Fig. 1), which was subjected to the separation as shown in Chart 1, affording four saponins I–IV. Of these, a saponin(IV) was proved to be identical with ginsenoside-F_1, which has already been isolated from leaves of *P. ginseng*, being characterized to be 20-O-β-glucopyranosyl-20(S)-protopanaxatriol.[7] Other three new saponins I–III were named as chikusetsusaponins -L_5(I), -L_{10}(II) and -L_{9a} (III) and the structures were established by the mordern procedures of glycoside-chemistry, which have been developed recently by our research group.[9–12]

1) Location: *a*) *Maedashi, Higashi-ku, Fukuoka-shi*; *b*) *Kasumi, Hiroshima-shi.* Correspondence should be addressed to O. Tanaka.[1b]
2) S. Sanada, N. Kondo, J. Shoji, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **22**, 421 (1974); S. Sanada, N. Kondo, J. Shoji, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **22**, 2407 (1974).
3) Y. Nagai, O. Tanaka, and S. Shibata, *Tetrahedron*, **27**, 881 (1971).
4) Prof. Hara proposed to treat this plant as a geographycal subspecies of *P. pseudo-ginseng*, designating as *P. pseudo-ginseng* subsp. japonicus (H. Hara, *Journ. Jap. Bot.*, **45**, 197 (1970)).
5) N. Kondo and J. Shoji, *Yakugaku Zasshi*, **88**, 325 (1968); N. Kondo, J. Shoji, N. Nagumo, and N. Komatsu, *Yakugaku Zasshi*, **89**, 846 (1969); N. Kondo, K. Aoki, H. Ogawa, R. Kasai, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **18**, 1558 (1970); N. Kondo, Y. Marumoto, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **19**, 1103 (1971); T.D. Lin, N. Kondo, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **24**, 253 (1976).
6) S. Shibata, T. Ando, O. Tanaka, Y. Meguro, K. Soma, and Y. Iida, *Yakugaku Zasshi*, **85**, 753 (1965).
7) S. Yahara, O. Tanaka, and T. Komori, *Chem. Pharm. Bull.* (Tokyo), **24**, 2208 (1976).
8) S. Yahara, K. Matsuura, R. Kasai, and O. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **24**, 2312 (1976).
9) J. Asakawa, R. Kasai, K. Yamasaki, and O. Tanaka, *Tetrahedron*, accepted.
10) R. Kasai, M. Suzuo, J. Asakawa, and O. Tanaka, *Tetrahedron Letters*, accepted.
11) R. Kasai, K. Matsuura, O. Tanaka, S. Sanada, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), "in press."
12) H. Kohda and O. Tanaka, *Yakugaku Zasshi*, **95**, 246 (1975).

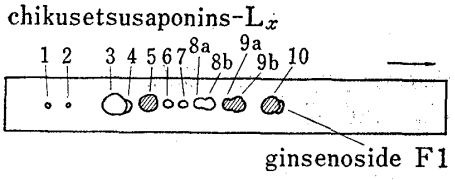


Fig. 1. Thin-Layer Chromatogram of *Panax japomicus*

Leaves saponins on Kieselgel H.
Solvent: the lower phase of $CHCl_3$–MeOH–H_2O (65: 35: 10).
Color reagent: 10% H_2SO_4.

Assignments of ^{13}C-nuclear magnetic resonance (CMR) signals of Ginseng sapogenins and their related dammarane type triterpenes have been established[9] and CMR spectroscopy of isoprenoid β-D-glucopyranosides have also been investigated.[10] Under the consideration of these studies, comparison of CMR spectrum of I with that of IV revealed that the carbon resonances of the aglycone moiety of I appear at the almost same positions as those of IV. This indicated that I must be a glycoside of 20(*S*)-protopanaxatriol (V)[3] and a location of its glycosyl linkage should be limited to the C–20 hydroxyl group of V (Table I). This was supported by the occurrence of a fragment peak (*m/e* 675 (VI)) in the mass spectrum (MS) of pertrimethylsilyl (TMS) ether of I (TMS–I), since it has been found that in the MS of TMS-derivatives of dammarane-type-saponins, fragment ions having the intact 20-O-(TMS-sugar) moiety can not be detected.[11]

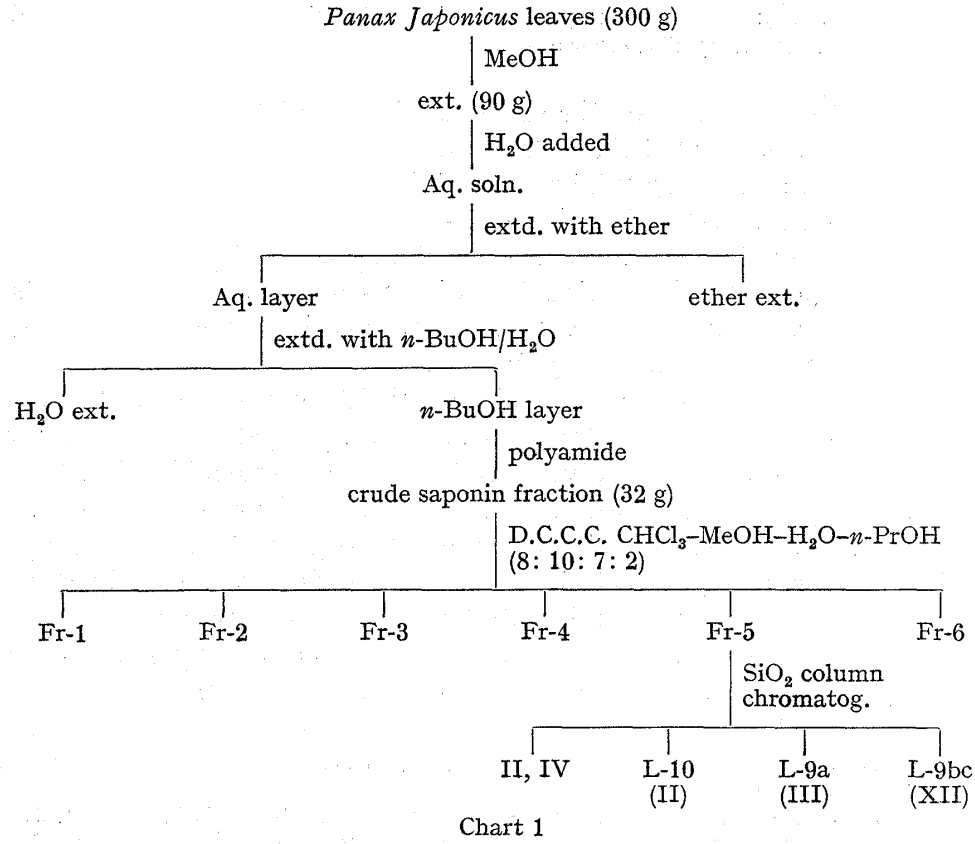


Chart 1

Efficient hydrolysis of Ginseng saponins with crude preparation of hesperidinase was reported.[12] On hydrolysis with this enzyme, I yielded glucose, arabinose, xylose and V along with a partially hydrolyzed product, IV. Further, MS fragment ions of TMS–I at *m/e* 1003 (VII: $(TMS)_3$pentose-$(TMS)_2$pentose-$(TMS)_3$-hexose$^+$), 625 (VIII: $(TMS)_3$pentose-$(TMS)_3$pentose$^+$) and 349(IX: $(TMS)_3$pentose$^+$) as well as a characteristic peak at *m/e* 757 (X) due to the fragmentation of $(TMS)_3$pentose-$(TMS)_2$pentose$^{1\text{–}6}$$(TMS)_3$hexose unit[13] demonstrated that the sugar moiety of I can be represented by xylose—arabinose$^{1\text{–}6}$glucose— or arabinose—xylose$^{1\text{–}6}$glucose—.

13) N.K. Kochetokov, O.S. Chizhov, and N.V. Molodtsov, *Tetrahedron*, **24**, 5587 (1968); J.P. Kamerling, J.F. Vliegenthart, J. Vink, and J.J. DeRidder, *Tetrahedron*, **27**, 4275 (1971).

TABLE I. ^{13}C Chemical Shifts[a)]

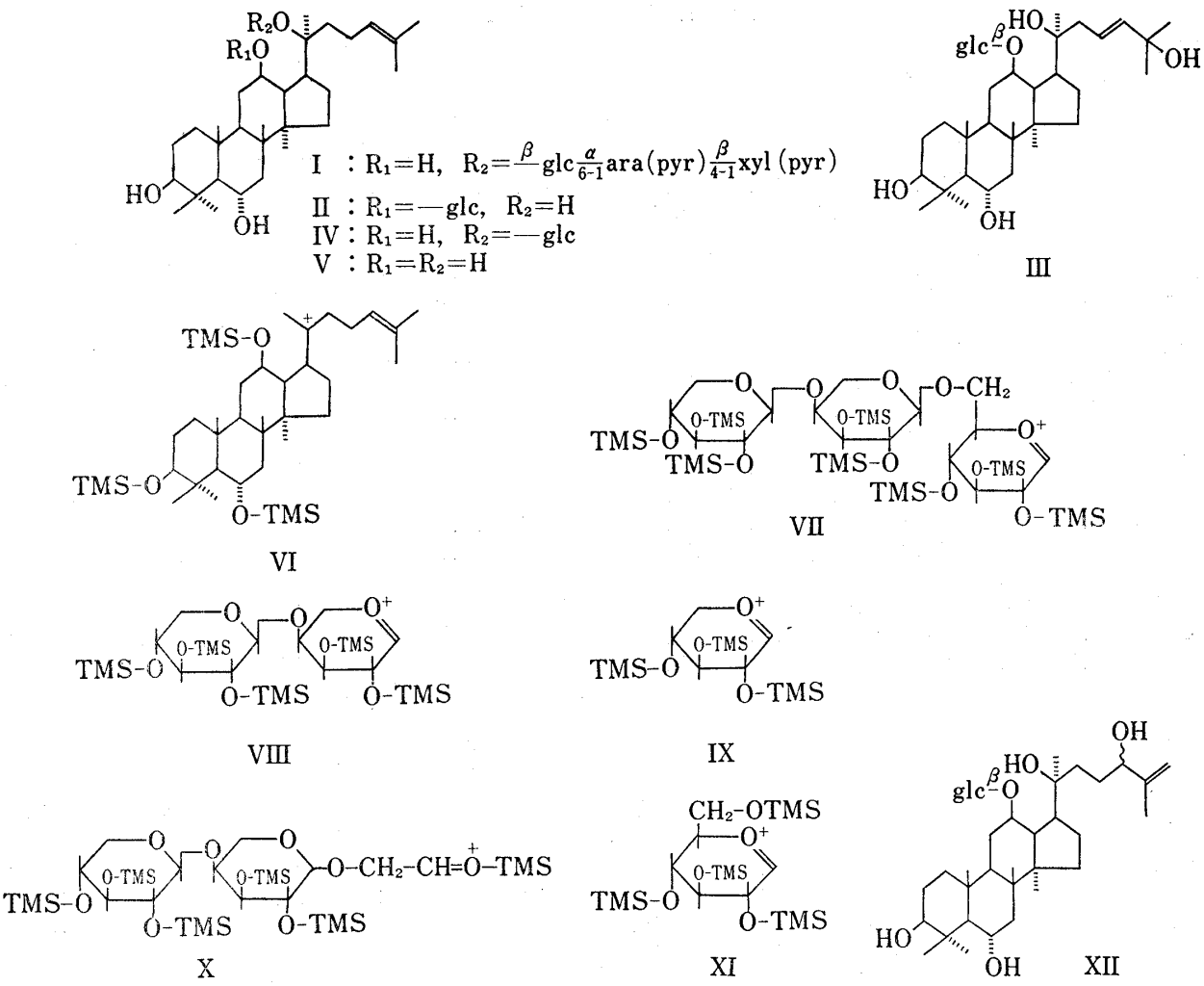
	Aglycone moieties							Aglycone moieties					
	V	IV	I	II	III	XII		V	IV	I	II	III	XII
C-1	39.3	39.2	39.3	39.0	39.0	39.0	C-19	17.4[c)]	17.3[c)]	17.4[c)]	17.2[c)]	17.5[c)]	17.4[c)]
C-2	28.0	27.9	28.0	27.9	28.0	28.0	C-20	72.9	83.2	83.4	73.1	73.3	73.2
C-3	78.3	78.4[b)]	78.4	78.3[b)]	78.3[b)]	78.4[b)]							(72.9)
C-4	40.2	40.1	40.2	40.2	40.3	40.3	C-21	26.9	22.3	22.2	26.6	27.6	27.0[d)]
C-5	61.7	61.6	61.7	61.6	61.7	61.7	C-22	35.7	35.9	36.1	36.3	40.8	32.2
C-6	67.6	67.6	67.7	67.5	67.6	67.6							(32.5)
C-7	47.4	47.3	47.4	47.1	47.2	47.2	C-23	22.9	23.1	23.1	22.8	123.5	30.0
C-8	41.1	41.1	41.2	41.0	41.1	41.0							(30.4)
C-9	50.1	49.8	49.9	49.7	49.8	49.9	C-24	126.2	125.8	125.8	126.4	141.4	75.9
C-10	39.3	39.2	39.3	39.3	39.4	39.4							(76.2)
C-11	31.9	30.8	30.8	27.9	28.0	28.0	C-25	130.6	130.8	131.0	130.5	69.8	109.7
C-12	70.9	70.2	70.1	78.3[b)]	78.3[b)]	78.6[b)]							(110.0)
C-13	48.1	48.9	49.1	46.2	46.7	46.4	C-26	25.8	25.7	25.8	25.8	31.2	150.1[d)]
C-14	51.6	51.3	51.3	52.0	52.1	52.0	C-27	17.7	17.7	17.9	17.6	31.2	18.6
C-15	31.3	30.5	30.8	31.1	31.2	31.3							(18.2)
C-16	26.8	26.6	26.6	26.6	26.9	26.9	C-28	31.9	31.8	31.9	31.8	31.8	31.9
C-17	54.6	51.6	51.3	54.1	53.4	54.2	C-29	16.4[c)]	16.4[c)]	16.5[c)]	16.4[c)]	16.4[c)]	16.4[c)]
C-18	17.5[c)]	17.3[c)]	17.4[c)]	17.2[c)]	17.5[c)]	17.4[c)]	C-30	17.0	17.3	17.4	17.2	17.2	17.4

		Sugar moieties						
		M-G[e)]	IV	F-3[16)]	I	II	III	XII
β-Gluco-pyranosyl	C-1	105.5	98.0	98.0	97.9	100.4	100.2	100.1
	C-2	74.9	74.9	74.9	74.6	75.1	75.1	75.1
	C-3	78.3	78.9[b)]	79.1	79.1	78.3[b)]	78.3[b)]	78.3[b)]
	C-4	71.6	71.4	71.8	71.7	70.9	71.1	71.1
	C-5	78.3	78.0[b)]	76.6	76.6	77.7[b)]	77.4[b)]	77.3[b)]
	C-6	62.7	62.9	69.1	69.7	62.8	62.4	62.4
α-Arabino-pyranosyl	C-1			104.5	104.8			
	C-2			72.1	72.7			
	C-3			74.0	73.7			
	C-4			68.5	78.4			
	C-5			65.5	65.5			
		P-C[15)]						
β-Xylo-pyranosyl	C-1	106.4			106.7			
	C-2	75.1			75.2			
	C-3	77.9			78.4			
	C-4	70.8			70.8			
	C-5	66.9			67.2			

a) δ ppm from internal TMS in C_5D_5N; JEOL JNM-PFT-100NMR spectrometer at 25.15 MHz; concentration: 0.1—0.4M; temperature: 25°; using 10 mm tubes; FT NMR conditions; spectral width: 4KHz; pulse flipping angle: 45° or 90°; acquistion time: 0.5 sec; number of data points: 4096; recycle time: 1-2 sec: number of recycle: 1000—30000.
b, *c*) Values in any vertical column may be reversed although those given here are preferred.
d) An expected sub-peak which is attributabe to the C-24-epimer, could not be identified. This would be due to its overlapping on the other signal. ( ): sub-peak due to the C-24-epimer.
e) Methyl β-D-glucopyranoside.[10)]

In CMR spectroscopy of straight chain oligosaccharides, it was reported that carbons of terminal monosaccharide units have much longer spin-lattice relaxation times(T_1) than those of the inner units and by means of partially relaxed Fourier transform (PRFT) method, carbon resonances due to individual monosaccharide units can be distinguished from each other.[14)]

14) A. Allerhand and D. Doddrell, *J. Am. Chem. Soc.*, **93**, 2777 (1971).



The inspection of the PRFT spectra of I (Fig. 2) as well as its comparison with the signals of the β-xylopyranoside,[15] the α-arabinopyranoside,[16] the α-arabinofuranoside[17] and the β-glucopyranoside[10] disclosed that the terminal monosaccharide unit(longer T_1 value) of I must be attributable to β-xylopyranoside. The allocation of a set of signals with medium T_1 values were inconsistent with the expected spectrum for 4-substituted α-arabinopyranoside, in which the sigmals due to the C–4 of arabinose should be displaced downfield, while those of C–3 and –5 must be shifted upfield from their corresponding positions in the spectrum of the unsubstituted α-arabinopyranoside.[10,16,17] Further, signals with shorter T_1 values in this region can be reasonably assigned to those of the 6-substituted β-glucopyranoside.[18] It follows now that I can be formulated as 20-O-[β-xylopyranosyl-(1→4)-α-arabinopyranosyl-(1→6)-β-glucopyranosyl]-20(*S*)-protopanaxatriol. This was confirmed also by the following evidence; the

15) With regard to carbon signals of the β-xylopyranoside in C_5D_5N, the CMR spectrum of akebia saponin-Pc, 3-O-[β-xylopyranosyl-(1→3)-α-arabinopyranosyl]-hederagenin (R. Higuchi and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **24**, 1021 (1976)) in C_5D_5N was referred. The authors are grateful to Prof. Kawasaki for his kind donation of a sample of this compound.

16) With regard to carbon signals of the α-arabinopyranoside in C_5D_5N, the CMR spectrum of ginsenoside-F_3[7] was referred.

17) P.A.J. Gorin and M. Mazurek, *Carbohydrate Research*, **48**, 171 (1976); P.A.J. Gorin and M. Mazurek, *Can. J. Chem.*, **53**, 1212 (1975), and the references cited therein.

18) T. Usui, N. Yamaoka, K. Matsuda, K. Tuzimura, H. Sugiyama, and S. Seto, *J.C.S. Perkin I*, **1973**, 2425; P. Colson and R. King, *Carbohydrate Research*, **47**, 1 (1976).

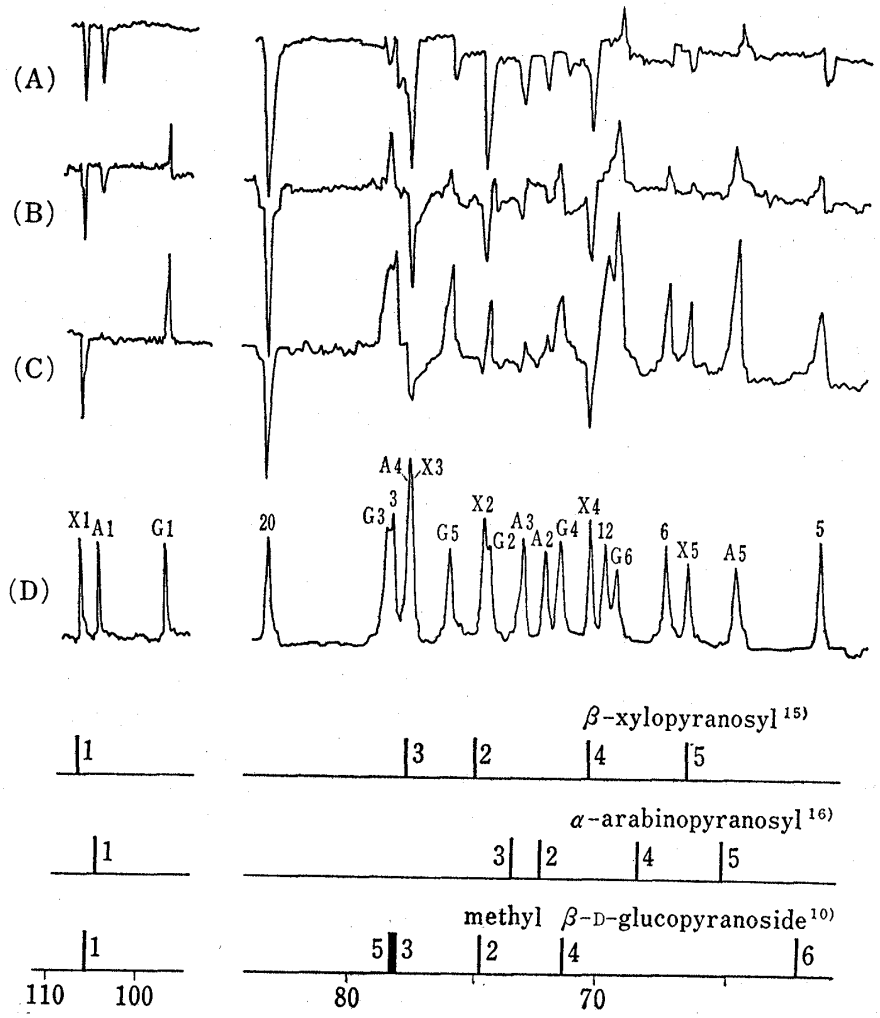


Fig. 2. Proton-decoupled Carbon-13 Fourier Transform NMR Spectra of 0.2 M Chikusetsusaponin-L_5 (I) at 60°

(A—C) PRFT spectra; The intervals (sec) are; (A): 0.09, (B): 0.12, (C): 0.14, with a recycle time of 3.6 sec. (D) Normal Fourier transform spectrum using a recycle time of 3.6 sec.

δ ppm from internal TMS in C_5D_5N.

permethyl ether of I (IR: no OH absorption in CCl_4; ^1H NMR(PMR): δ 4.14(1H doublet, J=6.0 Hz, anomeric H) and 4.33(2H doublet, J=7 Hz, anomeric H×2 in $CDCl_3$) was subjected to methanolysis yielding methyl 2,3,4-tri-O-methylxylopyranoside, methyl 2,3-di-O-methylarabinopyranoside and methyl 2,3,4-tri-O-methylglucopyranoside.

The enzymatic hydrolysis of II yielded glucose and V. Comparison of the CMR spectrum of II with that of methyl β-D-glucopyranoside revealed that II must be a monoglucopyranoside of V. This was supported by the presence of one anomeric proton signal at δ 5.21(1H doublet, J=7.5 Hz in C_5D_5N) in the PMR spectrum of II as well as occurrence of a fragment ion at m/e 331 (base peak, tetraacetylhexosyl ion) in the MS of peracetylated II.

Location of the β-glucopyranosyl linkage in II was elucidated by comparison of the CMR spectrum of II with of that V. On going from V to II, the carbon resonance of 12–C was displaced downfield by +7.4 ppm and those of 11–C and 13–C were shielded by −4.0 and −1.9 ppm, respectively, while other carbon signals due to the aglycone moiety were remained unshifted. Referring to the stereochemistry of the glucosylation shift of aliphatic alcohols,[10] these observations led unequivocally to assign the structure of II as 12-O-β-glucopyranosyl-20(S)-protopanaxatriol. It is notable that this is the first example of occurrence of a 12-O-glycosylated dammarane type triterpene in nature.

The MS of TMS-chikusetsusaponin-L_{9a} (TMS–III) exhibited a molecular ion at *m/e* 1230 ($C_{36}H_{54}O_{10}(TMS)_8$) and a terminal TMS-hexosyl ion(*m/e* 451, XI). As shown in Table I, the CMR spectrum of III showed eight quartets(methyls) and carbon resonances of III appeared at the almost same positions as those of the corresponding signals of II except for signals due to the side chain carbons (22-, 23-, 24-, 25- 26- and 27-C). In comparison with the spectrum of II, the presence of an additional tertiary hydroxyl group and a *trans*-disubstituted double bond (-CH_2-CH=CH-$\underset{|}{C}$- type) in III was demonstrated by its CMR signals at δ 69.8(singlet), 123.5(doublet) and 141.4(doublet) as well as PMR signals at δ 5.81(1H doublet, J=16 Hz) and 6.15(1H multiplet, appeared as doublet(J=16 Hz) on irradiation at δ 2.35), respectively. These evidences led to propose the structure for III as shown in Chart 2. This was substantiated by the derivation of III from II.

On photosensitized oxidation followed by reduction of the resulted hydroperoxides with $NaBH_4$,[19] II yielded a tertiary alcohol (A) and a secondary alcohol (B). The former was proved to be identical with III by comparison of CMR and TLC. In the CMR spectrum of the latter alcohol (B), carbon signals appears at the almost same positions as those of the corresponding signals of II except for those of the side chain carbons (22-, 23-, 24-, 25-, 26- and 27-C) and its resonances assignable to -H$\underline{C}_{(24)}$–OH (δ 75.9 (doublet)) and C=$\underline{C}H_2$(δ 109.7 (triplet)) are accompanied by sub-peaks, δ 76.2 (doublet) and 110.0 (triplet), respectively, indicating that the alcohol(B) must be a mixture of C–24 epimers(XII). Although the separation of this mixture into the each epimer has not been successful as yet, the same mixture was isolated also from the leaves, being tentatively named as chikusetusaponin-L_{9bc}.

Since TLC of the methanolic extract of the fresh leaves exhibited the almost same pattern as that of the dried leaves, any of the saponins of the present report are not artifacts formed during the storage of the leaves.

Experimental

NMR spectra were taken on JEOL-PFT-100 NMR spectrometer using TMS as an internal standard (PMR at 100 MHz and CMR at 25.15 MHz). MS were taken at 75 eV on JEOL O1-SG-2 spectrometer.

Acetylation of saponins for MS determination, enzymatic hydrolysis of saponins, identification of the resulted monosaccharides after hydrolysis, TLC of saponins and sapogenins are all referred to the previous paper.[7]

Trimethylsilylation of Saponins for MS Determination——To a sample (*ca.* 1 mg) in a small test tube were added two or three drops of N-trimethylsilyl imidazole. The tube was sealed and heated at 80° for 1 hr. After cooling and diluting with a few drops of H_2O, the reaction mixture was extracted with *n*-hexane. The hexane extract was washed with H_2O and concentrated to dryness by blowing N_2 gas. The residue was dried *in vacuo* at room temperature overnight and subjected to MS determination without further purification. It has been proved that this TMS-reagent is strong enough to form a *tert*-OH-TMS-derivative.[11]

Extraction and Separation of Saponins——The plant material was collected at Kake-cho, Yamagata-gun, Hiroshima-ken at 30th, May, 1975. The dried leaves (300 g) was extracted with MeOH and a suspension of the MeOH extract (90 g) in H_2O was washed with ether and then extracted with *n*-BuOH. The BuOH-layer was passed through the column of polyamide, and concentrated to dryness affording a crude saponin mixture (32 g) which was subjected to D.C.C.C. separation as illustrated in Chart 1.

Column chromatography of Fr. 2 (Chart 1) on silica gel (solvent CH_2Cl_2: MeOH: H_2O (370: 85: 8, homogeneous) followed by recrystallization from MeOH–H_2O–EtOAc gave I, colorless needles, mp 193—195°, $[\alpha]_D^{20}$ +23.0° (c=0.35, MeOH), yield 0.7%. *Anal.* Calcd. for $C_{46}H_{78}O_{17}\cdot H_2O$: C, 59.97; H, 8.75. Found: C, 59.47; H, 8.41.

Repeated column chromatography of Fr. 5 (Chart 1) on silica gel (solvent $CHCl_3$: MeOH: H_2O (60: 12: 1 homogeneous)) furnished the separation into II, III, XII and a mixture of II and IV. This mixture which showed no C=O absorption in IR (KBr), was acetylated with Ac_2O in C_5H_5N under the usual condition and the crude acetylated mixture was chromatographed on silica gel (solvent $CHCl_3$: ether (19: 1)) yielding acetylated II and IV, both of which were saponified with 5% KOH in MeOH, respectively, affording II and IV. Identification of IV (yield 0.01%) with ginsenoside-F_1 was performed by comparison of PMR, TLC and MS of the acetate with those of an authentic sample.

19) N. Ikekawa, A. Ohta, M. Seki, and A. Takahashi, *Phytochemistry*, **11**, 3037 (1972).

II: Yield 0.2%, white powder, $[\alpha]_D^{24}$ +42.0° (c=0.15, MeOH), *Anal.* Calcd. for $C_{36}H_{62}O_9 \cdot 1^1/_2H_2O$: C, 64.93; H, 9.84. Found: C, 64.87; H, 9.39.

III: Yield 0.1%, white powder, $[\alpha]_D^{23}$ +14.0° (c=0.51, MeOH), *Anal.* Calcd. for $C_{36}H_{62}O_{10} \cdot 1^1/_2H_2O$: C, 63.41; H, 9.61. Found: C, 63.48; H, 9.29.

XII: Yield 0.2%, white powder, $[\alpha]_D^{23}$ +12.1° (c=1.01, MeOH), *Anal.* Calcd. for $C_{36}H_{62}O_{10} \cdot H_2O$: C, 64.26; H, 9.58. Found: C, 64.16; H, 9.00.

Permethylation Followed by Methanolysis of I[20]——A mixture of NaH (200 mg) and DMSO (4 ml) was heated at 70° for 1 hr under N_2 and to this mixture was added a solution of I (150 mg) in DMSO (3 ml). After stirring at room temperature for 1 hr, MeI (10 ml) was added to this mixture and the solution was further stirred at room temperature for 3 hr. After dilution with H_2O, the reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried, and concentrated to dryness. Chromatography of the residue on silica gel (solvent $CHCl_3$: MeOH (70: 1)) gave the permethyl ether of I (50 mg), IR (in CCl_4): no OH absorption. This permethyl ether (5 mg) was dissolved in 5% HCl-MeOH and the solution was refluxed for 2 hr. After working up in the usual way, the hydrolysate was subjected to GLC, being proved to contain methyl 2,3,4-tri-O-methylxylopyranoside, methyl 2,3-di-O-methylarabinopyranoside and methyl 2,3,4-tri-O-methylglucopyranoside. Condition of GLC: On a glass culumn 2 mm×2 m packed with 20% butan-1,4-diol succinate on Chromosorb WAW, column temp-155°, N_2 1.0 kg/cm^2.

Photosensitized Oxidation of II——A solution of II (200 mg) and Rosebengal (20 mg) in iso-PrOH (50 ml) was irradiated by a fluorescent lamp under a stream of O_2 for 10 days. The reaction mixture was passed through a column of active charcoal to remove the pigment and concentrated. A solution of the residue and $NaBH_4$ (170 mg) in a mixture of ether: MeOH (1: 1) (20 ml) was stirred at room temperature for 2 hr. After addition of AcOH and then a mixture of *n*-BuOH: H_2O, the BuOH layer was concentrated to dryness and the residue was chromatographed on silica gel (solvent $CHCl_3$: MeOH: H_2O (60: 12: 1 homogeneous)), yielding an alcohol (A) (22 mg) and a mixture of alcohols (B) (48 mg), which were proved to be identical respectively with III and XII by comparison of TLC, PMR and CMR.

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20) S. Hakomori, *J. Biochem.*, **55**, 205 (1964).