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Study on Saponins of Rhizomes of *Panax pseudo-ginseng* subsp. *himalaicus* Collected at Tzatogang and Pari-la, Bhutan-Himalaya

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Saponins of rhizomes of two specimens of *Panax pseudo-ginseng* subsp. *himalaicus* collected at high-altitude areas of Bhutan (Tzatogang and Pari-la: tentatively named specimens T and P) were investigated. With regard to oleanolic acid saponins, two new saponins, pseudo-ginsenosides-RT_1 and -RP_1, were isolated along with the known saponins, chikusetsusaponins IVa and V, from both specimens. It seems significant that chikusetsusaponin IV was not detected in these specimens, in contrast to Japanese and Chinese *P. japonicus* and *P. pseudo-ginseng* subsp. *himalaicus* collected at lower-altitude areas of Bhutan, all of which contained chikusetsusaponin IV as one of the major saponins, whereas they lacked pseudo-ginsenosides-RT_1 and -RP_1. As regards dammarane-saponins, ginsenosides-Rb_1, -Rd, -Rg_1 and a new saponin, pseudo-ginsenoside-RT_3, were isolated from specimen T, while ginsenosides-Rb_1, -Rd, -Re, -Rg_1 and -F_2, gypenoside-XVII and pseudo-ginsenoside-RT_3 were isolated from specimen P. Characteristic saponins having the ocotillol-type side chain were also isolated; pseudo-ginsenoside-F_{11} and three new saponins, pseudo-ginsenosides-RT_2, -RT_4 and -RT_5, from specimen T and pseudo-ginsenoside-RT_4 from specimen P.

Keywords——*Panax pseudo-ginseng* subsp. *himalaicus*; Himalayan *Panax*; Araliaceae; oleanolic acid saponin; dammarane-saponin; ocotillol-type saponin; pseudo-ginsenoside; chikusetsusaponin; ginsenoside; ^{13}C-NMR

A variety of wild *Panax* spp. (Araliaceae), which are morphologically related to each other, are distributed from Japan to the Eastern Himalayas through the South-Western Province of China:

Japan: *P. japonicus* C. A. MEYER = *P. pseudo-ginseng* WALL. subsp. *japonicus* (MEYER) HARA (Japanese name: Chikusetsu-ninjin or Tochiba-ninjin).

China: *P. japonicus* (Chinese name: Zhao-shen, Zhujie-shen or Zhujie-sanchi); *P. japonicus* C. A. MEYER var. *major* C. Y. WU *et* K. M. FENG; *P. japonicus* C. A. MEYER var. *stipuleanatus* H. T. TSAI *et* K. M. FENG; *P. zingiberensis* C. Y. WU *et* K. M. FENG and several other spp.

Himalayas: *P. pseudo-ginseng* WALL. subsp. *himalaicus* HARA; *P. pseudo-ginseng* WALL. subsp. *himalaicus* HARA var. *angustifolius* (BURK.) LI; *P. pseudo-ginseng* WALL. subsp. *himalaicus* HARA var. *bipinnatifidus* (SEEM.) LI.

These *Panax* spp., tentatively named group I, commonly have a horizontally elongated rhizome in contrast to *P. ginseng* C. A. MEYER (Ginseng), *P. quinquefolium* L. and *P. notoginseng* (BURK.) F. H. CHEN, all of which have a carrot-like root with a small rhizome (tentatively designated as group II). With regard to saponin composition, the major saponins of the roots of group II consist of a number of dammarane-saponins,[1] while from the

rhizomes of Japanese *P. japonicus* (group I), large amounts of oleanolic acid saponins, chikusetsusaponin V (**1**) (=ginsenoside-Ro), *etc.*[2] were isolated along with the characteristic dammarane-saponins, chikusetsusaponin III (**2**), *etc.*[3]

As a member of the third Botanical Expedition to Eastern Himalaya, one of the authors, Tanaka, collected several specimens of Himalayan *Panax* spp. in Bhutan for a comparative study of the saponin composition.[4] The specimen of *P. pseudo-ginseng* subsp. *himalaicus* collected at Khosa (elevation above sea level: 1800 m) has a bamboo-like horizontally creeping rhizome which is similar to that of *P. japonicus*. From rhizomes of this specimen (tentatively named specimen K), large amounts of oleanolic acid saponins, **1** and chikusetsu-saponins IV (**3**) and IVa (**4**), which have already been isolated from rhizomes of Japanese *P. japonicus*, were isolated.[5] Ginsenoside-Rb_1 (**5**), one of the major dammarane-saponins of *P. ginseng* roots was also isolated from the same rhizomes, while saponins of other Himalayan specimens have remained uninvestigated owing to the poor availability of the materials. Recent developments in techniques for separation and structure elucidation have made it possible to investigate glycosides from a limited amount of plant materials, prompting us to study saponins of the rhizomes of the other Himalayan specimens.

In contrast to *P. japonicus* and specimen K, the rhizome of the specimen of *P. pseudo-ginseng* subsp. *himalaicus* (tentatively designated as specimen T) collected at Tzatogang (elevation above sea level: 3100 m) has elongated and slender internodes just like a string of Buddhist beads (juzu in Japanese), being morphologically similar to that of Chinese *P. japonicus* var. *major*. The methanolic extract of the rhizomes of specimen T (30 g) was subjected to chromatography on a column of highly porous polymer to give a saponin fraction, which was separated by repeated chromatography on a silica gel column and on a reverse-phase column to give twelve saponins I—XII in the yields shown in Table I. Of these, saponins III, VII and X were respectively identified as known dammarane-saponins, ginsenosides-Rg_1 (**6**), -Rd (**7**) and -Rb_1 (**5**), which are major saponins of Ginseng roots.[6] Saponin VI was identified as pseudo-ginsenoside -F_{11} (**8**), an ocotillol-type saponin which has previously been isolated from leaves of specimen K and also from leaves of *P. quinquefolium*.[7] Saponins VIII and XII were identical with **1** and **4**, oleanolic acid saponins previously isolated from rhizomes of *P. japonicus* and specimen K.

The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of a new major saponin XI named pseudo-ginsenoside-RT_1 (**9**) showed signals due to an aglycone moiety at almost the same positions as those of **1**, indicating that **9** may be a 3,28-bisdesmoside of oleanolic acid. Glucose, glucuronic acid and xylose were identified in the acid hydrolysate of **9**. On alkaline saponification, **9** afforded 1,6-anhydroglucose (**10**) and a prosapogenin (**11**), of which the latter was identical with the prosapogenin already obtained from hemsloside Ma2 (**12**), a saponin of *Hemsleya* spp.[8] It is known that β-glucosyl esters of di- and triterpene carboxylic acids afford **10** on alkaline saponification. Based on these results, **9** was formulated as the β-glucosyl ester of **11**. The ^{13}C-NMR spectrum of **9** is consistent with this formulation.

Saponin IX was identical with the prosapogenin (**11**) which was also isolated from rhizomes of the specimen collected between Gasa and Pari-la (specimen P, *vide infra*), and was designated as pseudo-ginsenoside-RP_1.

A new saponin IV (**13**) was named pseudo-ginsenoside-RT_3. On acid hydrolysis, **13** yielded glucose and xylose and its ^{13}C-NMR spectrum indicated that **13** is a bisdesmoside of 20(*S*)-protopanaxatriol having glycoside linkages at both the 6- and 20-hydroxyl groups. It has been reported that because of the instability of the glycosyl linkage on the 20-*tert*-hydroxyl group of dammarane-type triterpenes, electron impact-mass spectrum (EI-MS) of acetylated or trimethylsilylated dammarane-saponins exhibits no fragment ion having an intact glycoside-group at C-20. The EI-MS of acetylated **13** showed ions at *m/z* 740 and 741 associated with 6-*O*-xylosyl fragments, **14** and **15**, together with a terminal tetraacetylglucosyl

ion (*m/z* 331) and a terminal triacetylxylosyl ion (*m/z* 259), lacking ions which are characteristic of 6-*O*-glucosyl fragments. It was found that the sugar carbon signals of **13** consisted of those of the 20-*O*-*β*-glucopyranoside moiety of **6** and a *β*-xylopyranoside unit linked with a (*S*)-secondary alcohol. These results led to the formulation of **13** as 6-*O*-*β*-xylopyranosyl-20-*O*-*β*-glucopyranosyl-20(*S*)-protopanaxatriol.

A new saponin V named pseudo-ginsenoside-RT_2 (**16**) afforded glucose and xylose on acid hydrolysis. The EI-MS of acetylated **16** showed a strong ion at *m/z* 143 due to the fragment **17**, which is characteristic of ocotillol-type triterpenes and their saponins such as **8**. In our study[9] on the stereochemistry of ocotillol-type triterpenes, assignment of carbon signals of the triterpenes of this type was investigated, and it was shown that the chemical shift difference of C-24 and -26(or -27) is significantly diagnostic for determination of the chirality of C-24. All of the aglycone carbon signals of **16** were almost superimposable on those of **8**, indicating that **16** has the same aglycone as that of **8** including the chirality of C-24 (24(*R*)), and the glycosyl linkage must be located at the 6*α*-hydroxyl group. The EI-MS of acetylated **16** exhibited ions at *m/z* 259 and 547 associated with the terminal xylosyl-Ac_3 and (xylosyl-glucosyl)-Ac_6 fragments. Recently, the ocotillol-type saponin (24(*S*)) named majonoside R2 (**18**) was isolated from rhizomes of *P. japonicus* var. *major*.[10] The carbon signals of both the sugar and aglycone moieties of **16** were found at almost the same positions as those of **18** except for the signals of C-24 and C-26 (or -27). These results led to the formulation of **16** as the 24-epimer of **18**, as shown in Chart 1.

The remaining new saponins I and II, named pseudo-ginsenosides-RT_4 (**19**) and -RT_5

TABLE I. Comparison of Saponins from Rhizomes of *Panax pseudo-ginseng*, *Panax japonicus* var. *major*, *Panax zingiberensis* and *Panax japonicus*

	Yield %						
	(A) (Specimen T)	(A) (Specimen P)	(B)[10] (Yunnan)	(A)[5] (Specimen K)	(C)[15] (Yunnan)	(D)[16] (Yunnan)	(D)[2,3] (Japan)
C-IVa	1.7	1.8	0.2	0.6	0.03	2.8	+
C-IV	–	–	–	0.3	0.3	3.4	0.4
C-V	0.4	0.1	1.0	7.3	2.1	3.1	5.4
Z-R1	–	–	–	–	0.08	–	–
Pro-C-V	–	0.02	–	–	–	–	–
RT_1	1.5	5.0	–	–	–	–	–
RP_1	0.07	0.1	–	–	–	–	–
C-Ib	–	–	–	–	–	–	+
	Rb_1 (0.3)	Rb_1 (0.05)	Rd (0.7)	Rb_1 (1.1)	Rg_1 (0.6)	Rd (0.04)	C-III (1.2)
	Rd (0.2)	Rd (0.07)	N-R2 (0.03)		Rh_1 (+)	Re (0.1)	Rg_2 (+)
	Rg_1 (0.4)	Re (0.1)	20glc-Rf (0.01)			Rg_1 (0.2)	C-Ia (+)
	RT_3 (0.1)	F_2 (0.02)				Rg_2 (0.05)	
		Rg_1 (1.2)				N-R2(0.02)	
		Gy-XVII (0.03)					
		RT_3 (0.02)					
	F_{11} (0.07)	RT_4 (0.02)	M-R1 (0.07)			F_{11} (0.2)	
	RT_2 (0.09)		M-R2 (0.1)				
	RT_4 (0.08)						
	RT_5 (0.07)						

(A), *P. pseudo-ginseng* subsp. *himalaicus*; (B), *P. japonicus* var. *major*; (C), *P. zingiberensis*; (D), *P. japonicus*. Specimen T, collected at Tzatogang (3100 m, May 27, 1967); Specimen P, collected between Gasa and Pari-la (2600—3550 m, May 14, 1967); Specimen K, collected at Khosa (1800 m, May 10, 1967). C, chikusetsusaponin; Z, zingibroside; N, notoginsenoside; Gy, gypenoside; M, majonoside; Rb_1, Rc, Rd, Re, Rg_1, Rg_2, Rh_1, F_2, 20glc-Rf, ginsenoside; Pro-C-V, prosapogenin of C-V; F_{11}, RP_1, RT_{1-5}, pseudo-ginsenoside; +, very low yield.

(**20**), gave glucose on acid hydrolysis and the EI-MS of their acetates exhibited an ion at *m/z* 143 (fragment **17**) as well as ions at *m/z* 331 and 169 associated with a terminal tetraacetylglucosyl group. The ^{13}C-NMR spectrum (Table II) revealed that the aglycone of **19** is identical with that of **18** (24(*S*)), having a glycosyl linkage at the 6-hydroxyl group, and the sugar carbon signals of **19** appeared at the same positions as those of ginsenoside-Rh$_1$ (**21**, 6-*O*-*β*-glucopyranosyl-20(*S*)-protopanaxatriol),[11] a minor saponin of Ginseng root. It follows that **19** can be formulated as the 6-*O*-*β*-glucopyranoside of 3*β*,6*α*,12*β*,25-tetrahydoxy-(20(*S*),24(*S*))-epoxydammarane. The ^{13}C-NMR spectrum of **20** showed the same aglycone carbon signals as those of **16** and the same sugar carbon signals as those of **19**, leading to the formulation of **20** as the 24-epimer (24(*R*)) of **19**.

Another specimen of *P. pseudo-ginseng* subsp. *himalaicus* collected between Gasa and Pari-la (elevation above sea level: 2600—3550 m), tentatively named specimen P, has a rhizome of similar type to that of specimen T. A methanolic extract of rhizomes of specimen P (62 g) was subjected to repeated chromatography, affording thirteen saponins, **19**, the prosapogenin (**22**) of **1**, ginsenoside-F$_2$ (**23**) previously isolated from Ginseng leaves,[12] **13**, **6**, **11**, **4**, ginsenoside-Re (**24**),[13] **7**, gypenoside XVII (**25**) previously isolated from *Gynostemma pentaphyllum* MAKINO (Cucurbitaceae),[14] **9**, **5** and **1**.

As a part of the China–Japan cooperative studies on Chinese medicinal plants, the present authors have been engaged in comparative studies on the saponin composition of

TABLE II. ^{13}C-NMR Chemical Shifts: Aglycone Moiety (in C_5D_5N)

	1	**9**	**11**	**22**	**13**	**8**	**16**	**18**	**19**	**20**
C-1	38.6	38.7	38.7	38.7	39.5	39.5	39.6	39.6	39.6	39.5
C-2	26.6	26.6	26.7	26.7	27.9	27.7	27.8	27.9	28.0	27.9
C-3	89.3	89.3	89.3	89.1	78.7	78.4	78.8	78.1	78.2	78.2
C-4	39.5	39.5	39.6	39.5	40.3	40.0	40.2	40.3	40.4	40.4
C-5	55.8	55.8	55.9	55.8	61.4	60.9	61.5	61.4	61.6	61.5
C-6	18.4	18.5	18.5	18.5	78.2	74.2	79.4	79.5	78.6	78.6
C-7	33.2	33.1	33.3	33.3	45.4	46.0	44.9	44.9	45.2	45.1
C-8	39.9	39.9	39.7	39.8	41.2	41.1	41.0	41.1	41.1	41.0
C-9	48.0	48.0	48.0	48.0	50.0	50.1	50.5	50.3	50.4	50.6
C-10	36.9	36.9	37.0	37.0	39.7	39.6	39.6	39.6	39.7	39.6
C-11	23.7	23.6	23.8	23.8	31.0	32.4	32.4	32.6	32.5	32.4
C-12	122.6	122.8	122.5	122.5	70.2	71.2	71.2	70.9	70.9	71.2
C-13	144.1	144.1	144.8	144.8	49.2	48.3	48.4	49.2	49.2	48.4
C-14	42.0	42.1	42.2	42.2	51.4	52.2	52.2	52.3	52.2	52.2
C-15	28.1	28.2	28.4	28.2	30.8	31.7	31.7	32.6	32.3	31.7
C-16	23.7	23.6	23.8	23.8	26.6	25.5	25.4	25.8	25.8	25.4
C-17	47.0	46.9	46.7	46.7	51.6	49.4	49.5	49.5	49.6	49.5
C-18	41.6	41.6	42.0	42.0	17.5[a]	17.8[a]	17.8[a]	17.9[a]	17.8[a]	17.9[a]
C-19	46.3	46.2	46.5	46.5	17.6[a]	17.5[a]	17.0[a]	17.2[a]	17.2[a]	17.1[a]
C-20	30.8	30.8	31.0	31.0	83.3	86.7	86.7	87.1	87.1	86.7
C-21	34.1	34.0	34.3	34.3	22.4	27.0[b]	26.9[b]	27.0[b]	27.0[b]	26.9[b]
C-22	32.6	32.5	33.3	33.3	36.1	32.8	32.7	32.6	32.7	32.7
C-23	28.1	27.8	27.9	28.2	23.2	28.8	28.8	28.7	28.7	28.8
C-24	16.7	16.3	16.4	17.0	126.0	85.6	85.6	88.4	88.4	85.6
C-25	15.5	15.5	15.5	15.5	130.9	70.3	70.3	70.0	70.0	70.3
C-26	17.4	17.4	17.4	17.4	25.8	27.2[b]	27.1[b]	26.6[b]	26.6[b]	27.1[b]
C-27	26.1	26.1	26.2	26.2	17.8[a]	27.6[b]	27.7[b]	29.0[b]	29.0[b]	27.7[b]
C-28	176.4	176.4	180.1	180.1	31.6	32.1	31.7	31.7	31.7	31.7
C-29	33.2	33.1	33.3	33.3	16.5[a]	16.9[a]	16.6[a]	16.7[a]	16.3[a]	16.3[a]
C-30	23.7	23.6	23.8	23.8	17.3[a]	18.2[a]	18.1[a]	17.9[a]	17.9[a]	18.0[a]

a, b) Assignments in any column may be interchanged, though those given here are preferred.

Table III. ^{13}C-NMR Chemical Shifts: Sugar Moiety (in C_5D_5N)

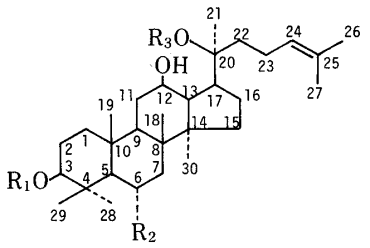
	9	11	22	13	16	18	19	20	21
3-GlcUA 1	105.2	105.2	107.2	—	—	—	—	—	—
3-GlcUA 2	83.5	83.5	75.5	—	—	—	—	—	—
3-GlcUA 3	77.8[a]	77.8[a]	77.8[a]	—	—	—	—	—	—
3-GlcUA 4	73.1	73.2	73.5	—	—	—	—	—	—
3-GlcUA 5	78.0[a]	78.1[a]	78.2[a]	—	—	—	—	—	—
3-GlcUA 6	172.5	172.2	172.9	—	—	—	—	—	—
Xyl 1	107.0	106.9	—	—	—	—	—	—	—
Xyl 2	76.5	76.6	—	—	—	—	—	—	—
Xyl 3	77.4[a]	77.3[a]	—	—	—	—	—	—	—
Xyl 4	71.1	71.1	—	—	—	—	—	—	—
Xyl 5	67.5	67.5	—	—	—	—	—	—	—
6-Glc 1	—	—	—	—	103.5	103.5	106.0	106.0	105.9
6-Glc 2	—	—	—	—	79.9	79.9	75.5	75.5	75.4
6-Glc 3	—	—	—	—	78.8[a]	78.8	80.2[a]	80.1[a]	80.0[a]
6-Glc 4	—	—	—	—	71.3[b]	71.3[a]	71.9	71.9	71.8
6-Glc 5	—	—	—	—	80.4	80.4	79.7[a]	79.7[a]	79.5[a]
6-Glc 6	—	—	—	—	63.0	63.0	63.2	63.2	63.1
Xyl 1	—	—	—	106.6	104.9	104.9	—	—	—
Xyl 2	—	—	—	75.2[a]	75.9	75.9	—	—	—
Xyl 3	—	—	—	79.1[b]	78.1[a]	78.8	—	—	—
Xyl 4	—	—	—	71.1	71.8[b]	71.7[a]	—	—	—
Xyl 5	—	—	—	67.0	67.3	67.3	—	—	—
20-Glc 1	—	—	—	98.3	—	—	—	—	—
20-Glc 2	—	—	—	75.1[a]	—	—	—	—	—
20-Glc 3	—	—	—	79.6[b]	—	—	—	—	—
20-Glc 4	—	—	—	71.7	—	—	—	—	—
20-Glc 5	—	—	—	79.3[b]	—	—	—	—	—
20-Glc 6	—	—	—	62.9	—	—	—	—	—
28-Glc 1	95.7	—	—	—	—	—	—	—	—
28-Glc 2	74.1	—	—	—	—	—	—	—	—
28-Glc 3	79.2[b]	—	—	—	—	—	—	—	—
28-Glc 4	71.1	—	—	—	—	—	—	—	—
28-Glc 5	78.8[b]	—	—	—	—	—	—	—	—
28-Glc 6	62.1	—	—	—	—	—	—	—	—

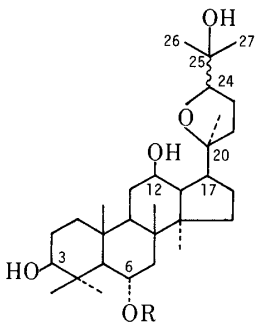
a, b) Assignments in any column may be interchanged, though those given here are preferred. GlcUA, β-D-glucuronic acid; Glc, β-D-glucopyranosyl; Xyl, β-D-xylopyranosyl.

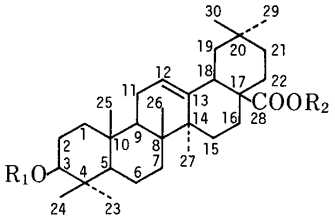
Chinese *Panax* species. The yields of saponins of specimens T and P with those from other related plants are summarized in Table I. It has been reported that the major oleanolic acid saponins of hitherto investigated rhizomes of Japanese, Chinese and Himalayan *Panax* spp. (group I, *vide supra*) consist of **1**, **3** and **4**. It is noteworthy that the Himalayan specimens of the present study, collected at relatively high-altitude, contain a large amount of a new oleanolic acid saponin **9** along with **1** and **4**, but no **3** was detected in either of the specimens. Geographical and taxonomical relationships among these *Panax* species will be further investigated by Chinese and Japanese botanists.

Experimental

General Procedures——Nuclear magnetic resonance (NMR) spectra were taken on JEOL JNM PFT-100 (^{1}H-NMR at 100 MHz and ^{13}C-NMR at 25.15 MHz), JEOL FX-100 (^{1}H-NMR at 99.55 MHz and ^{13}C-NMR at 25.00 MHz) and JEOL GX-270 (^{1}H-NMR at 270 MHz and ^{13}C-NMR at 67.80 MHz) spectrometers in C_5D_5N with







	R_1	R_2	R_3
2:	$-Glc\begin{smallmatrix}^{2}<Glc\\_{6}<Xyl\end{smallmatrix}$	–H	–H
5:	$-Glc^{2}-Glc$	–H	$-Glc^{6}-Glc$
6:	–H	–O–Glc	–Glc
7:	$-Glc^{2}-Glc$	–H	–Glc
13:	–H	–O–Xyl	–Glc
21:	–H	–O–Glc	–H
23:	–Glc	–H	–Glc
24:	–H	$-O-Glc^{2}-Rha$	–Glc
25:	–Glc	–H	$-Glc^{6}-Glc$

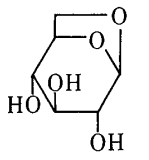
	R	
8:	$-Glc^{2}-Rha$	24(*R*)
16:	$-Glc^{2}-Xyl$	24(*R*)
18:	$-Glc^{2}-Xyl$	24(*S*)
19:	–Glc	24(*S*)
20:	–Glc	24(*R*)

	R_1	R_2
1:	$-GlcUA^{2}-Glc$	–Glc
3:	$-GlcUA^{4}-Ara(f)$	–Glc
4:	–GlcUA	–Glc
9:	$-GlcUA^{2}-Xyl$	–Glc
11:	$-GlcUA^{2}-Xyl$	–H
12:	$-GlcUA\begin{smallmatrix}^{2}<Xyl\\_{3}<Ara(p)\end{smallmatrix}$	–Glc
22:	–GlcUA	–H

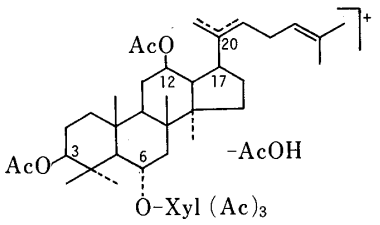
Glc; β-D-glucopyranosyl
Rha; α-L-rhamnopyranosyl
GlcUA; β-D-glucuronic acid

Xyl; β-D-xylopyranosyl
Ara(f); α-L-arabinofuranosyl
Ara(p); α-L-arabinopyranosyl

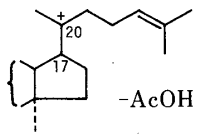
Chart 1



10



14



15

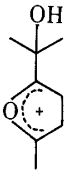


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Chart 2

tetramethylsilane (TMS) as an internal standard.

MS were recorded on JEOL 01-SG-2 and JEOL JMS-DX300 mass spectrometers at 75 and 70 eV, respectively. Trimethylsilylation for MS: A methyl ester (1—2 mg) of each saponin prepared by treatment with CH_2N_2 was heated with *N*-trimethylsilylimidazole (5 drops) in a sealed micro-tube at 80 °C for 2 h. The reaction mixture was diluted with H_2O and then extracted with *n*-C_6H_{14}. The C_6H_{14} layer was washed with H_2O and concentrated to dryness by blowing N_2 gas over it at room temperature. The residue was subjected to MS. Acetylation for MS: A sample of saponin (1—2 mg) was heated with $(CH_3CO)_2O$ (2—3 drops) and C_5H_5N (5—6 drops) in a sealed micro-tube at 80 °C for 2—3 h. The reaction mixture was concentrated to dryness by blowing N_2 gas over it at room temperature and then the residue was subjected to MS.

Optical rotation were measured with a Union automatic digital polarimeter at 20—27 °C in MeOH.

High Performance Liquid Chromatography (HPLC): A Toyo Soda HLC-802UR liquid chromatograph was used.

Plant Materials——The plants were collected by O. Tanaka as a member of the Botanical Expedition to Eastern Himalaya organized by Hiroshi Hara, University of Tokyo in 1967. All specimens were identified by Hiroshi Hara and are deposited in the University Museum of the University of Tokyo.

Extraction and Separation of Specimen T——Dried and powdered rhizomes (30 g) of specimen T collected at May 27, 1967 in Tzatogang (elevation above sea level: 3100 m), Bhutan, were extracted with hot MeOH (300 ml × 6) and then hot 50% MeOH (300 ml × 2) to give an MeOH extract (after evaporation) in a yield of 43%. An aqueous suspension of this MeOH extract was subjected to column chromatography on reverse-phase highly porous polymer (DIAION HP-20, Mitsubishi Chemical Ind., Ltd.) (solvent: H_2O (6 l), MeOH (7 l) and finally $CHCl_3$ (5 l)) to provide

the H_2O eluate (5.7 g), MeOH eluate (crude saponin fraction) (7.1 g) and $CHCl_3$ eluate (130 mg). This MeOH eluate was separated into four fractions, Fr-1 to Fr-4, by column chromatography on silica gel (solvent: $CHCl_3$: MeOH : H_2O (6 : 4 : 1, homogeneous)).

Fr-1 was separated into two fractions, Fr-1a and Fr-1b, by column chromatography on silica gel (solvent: $CHCl_3$: MeOH : H_2O (40 : 10 : 1, 30 : 10 : 1 and finally 20 : 10 : 1, homogeneous)). Fr-1a was further subjected to preparative HPLC on a reverse-phase column of ODS-120A (Toyo Soda) (21.5 mm × 30 cm; mobile phase, 63% MeOH; flow rate, 3.6 ml/min; chart speed, 12 cm/h; injection vol, 1 ml (280 mg/3 ml 63% MeOH); RI range, 4 × 100 mV) to give **19** (yield: 0.08%) and **20** (yield: 0.07%). **19**; colorless needles (from MeOH–H_2O), mp 247—249 °C, $[\alpha]_D^{25}$ +14.4° (c = 1.00, MeOH). *Anal.* Calcd for $C_{36}H_{63}O_{10} \cdot 2/3H_2O$: C, 64.74; H, 9.71. Found: C, 64.48; H, 9.65. **20**; colorless needles (from MeOH–H_2O), mp 211—213 °C, $[\alpha]_D^{25}$ +24.2° (c = 1.05, MeOH). *Anal.* Calcd for $C_{36}H_{63}O_{10} \cdot 3H_2O$: C, 60.90; H, 9.80. Found: C, 61.19; H, 9.43. Fr-1b was further subjected to preparative HPLC on a reverse-phase column of ODS-120A (*vide supra*) (21.5 mm × 30 cm; mobile phase, 60% MeOH; flow rate, 3.6 ml/min; chart speed, 12 cm/h; injection vol, 1—1.5 ml (600 mg/6 ml 60% MeOH); RI range, 2 × 100 mV) and on silica gel (solvent: $CHCl_3$: MeOH : H_2O (55 : 10 : 1, homogeneous)) to give **6** (yield: see Fr-2 of this section), **13** (yield: 0.1%), **16** (yield: 0.09%) and **8** (yield: 0.07%). **13**; a white powder (from EtOH–EtOAc), $[\alpha]_D^{24}$ +30.9° (c = 1.15, MeOH). *Anal.* Calcd for $C_{41}H_{70}O_{13} \cdot 1\frac{1}{4}H_2O$: C, 61.71; H, 9.22. Found: C, 61.92; H, 9.07. **16**; a white powder (from MeOH–EtOAc), $[\alpha]_D^{24}$ +11.1° (c = 0.85, MeOH). *Anal.* Calcd for $C_{41}H_{70}O_{14} \cdot H_2O$: C, 61.17; H, 9.02. Found: C, 61.13; H, 9.12. **8**; a white powder (from MeOH–EtOAc), $[\alpha]_D^{24}$ −12.3° (c = 1.19, MeOH).

Fr-2 was separated into two fractions, Fr-2a and Fr-2b by column chromatography on silica gel (solvent: $CHCl_3$: MeOH : H_2O (30 : 10 : 1 to 15 : 10 : 1, homogeneous)). Fr-2a was further chromatographed on silylated silica gel (LiChroprep RP-8 (Merck)) (solvent: 60% MeOH) to give **6** (yield: 0.4%), a white powder (from EtOH–EtOAc), $[\alpha]_D^{22}$ +31.7° (c = 1.05, MeOH). Fr-2b was further chromatographed on silylated silica gel (*vide supra*) (solvent: 75% MeOH) to give **7** (yield: 0.2%), a white powder (from MeOH–EtOAc), $[\alpha]_D^{22}$ +23.5° (c = 1.14, MeOH).

Fr-3 was recrystallized from H_2O–MeOH, followed by deionization with ion exchange resin (Amberlite MB-3), affording **4** (yield: 1.7%), colorless needles (from MeOH–H_2O), mp 210—212 °C, $[\alpha]_D^{23}$ +10.9° (c = 1.39, MeOH). The mother liquor was chromatographed on silylated silica gel (*vide supra*) (solvent: 75% MeOH) and then subjected to preparative HPLC on a reverse-phase column of ODS-120A (*vide supra*) (21.5 mm × 30 cm; mobile phase, 70% MeOH; flow rate, 8 ml/min; chart speed, 12 cm/h; injection vol, 1 ml (500 ml/5 ml 70% MeOH); RI range, 4 × 100 mV) to give crude **11** and crude **5**. Crude **11**, after repeated column chromatography on silica gel (solvent: $CHCl_3$: MeOH : H_2O (6 : 4 : 1, homogeneous)) and on reverse-phase highly porous polymer (MCI-gel CHP-20P, Mitsubishi Chemical Ind., Ltd.) (solvent: 80% MeOH), followed by deionization with ion exchange resin (Amberlite MB-3), afforded **11** (yield: 0.07%), colorless needles (from MeOH), mp 230—232 °C, $[\alpha]_D^{20}$ +2.1° (c = 1.00, MeOH). Crude **5**, after repeated column chromatography on silica gel (solvent: $CHCl_3$: MeOH : H_2O (6 : 4 : 1, homogeneous)), gave **5** (yield: 0.3%), a white powder (from MeOH–EtOAc), $[\alpha]_D^{20}$ +12.9° (c = 1.09, MeOH).

Fr-4, after repeated column chromatography on silica gel (solvent: $CHCl_3$: MeOH : H_2O (6 : 4 : 1, homogeneous)), followed by deionization with ion exchange resin (Amberlite MB-3), afforded **9** (yield: 1.5%) and **1** (yield: 0.4%). **9**; colorless needles (from MeOH), mp 235—238 °C (dec), $[\alpha]_D^{20}$ +8.4° (c = 0.11, MeOH). *Anal.* Calcd for $C_{47}H_{74}O_{18} \cdot 4H_2O$: C, 56.50; H, 8.27. Found: C, 56.69; H, 8.29. **1**; a white powder (reprecipitated from MeOH–EtOAc), $[\alpha]_D^{22}$ +14.2° (c = 1.16, MeOH).

Identification of the Known Saponins——Each known saponin was identified by thin layer chromatography (TLC) on Kieselgel 60F_{254} (Merck) with $CHCl_3$: MeOH : H_2O (65 : 35 : 10, lower layer) and with $CHCl_3$: MeOH : H_2O (6 : 4 : 1, homogeneous), and by reverse-phase TLC on silica gel plates (RP-8 and RP-18 F_{254} (Merck)) with 60—75% MeOH (detection: H_2SO_4), as well as by ^{1}H-NMR and ^{13}C-NMR spectroscopy, optical rotation measurement and MS (as the acetate or trimethylsilyl ether) in comparison with an authentic sample.

Hydrolysis of a Saponin and Identification of the Resulting Monosaccharides——A saponin (a few mg) was heated with 10% HCl in H_2O–dioxane (1 : 1) in a sealed micro-tube at 80 °C for 2 h. The reaction mixture was concentrated to dryness by blowing N_2 gas over it at room temperature. For analysis by gas liquid chromatography (GLC), the residue was trimethylsilylated by the same procedure as that used for MS (*vide supra*). GLC: On a Shimadzu GC-6A gas chromatograph; glass column of 2% SE-30 on Chromosorb W (AW-DMCS), 2.6 mm × 2 m; detector, FID; injection temperature, 200 °C; column temperature, 180 °C; carrier gas, N_2 (40 ml/min).

Methanolysis of Saponins and Identification of the Resulting Monosaccharides——A saponin (a few mg) was heated with 9.7% dry HCl–MeOH in a sealed micro-tube at 70 °C for 3 h. The reaction mixture was neutralized with Ag_2CO_3 and then filtered. The filtrate was concentrated to dryness by blowing N_2 gas over it at room temperature. For GLC analysis, the residue was trimethylsilylated by the same procedure as that used for MS (*vide supra*). GLC: On a Shimadzu GC-6A gas chromatograph; glass column of 2% SE-30 on Chromosorb W (AW-DMCS), 2.6 mm × 2 m; detecter, FID; injection temperature, 200 °C; column temperature, 160 °C; carrier gas, N_2 (40 ml/min).

Alkaline Hydrolysis of 9——**9** (100 mg) was heated with 10% KOH in MeOH–H_2O (1 : 1) (5 ml) at 80 °C for 3 h. Water was added to the reaction mixture and the whole was acidified to pH 5 with aq. acetic acid and then extracted with 1-BuOH saturated with H_2O to give a prosapogenin solution. The aqueous solution was neutralized with Amberlite MB-3, and the presence of 1,6-anhydroglucose was further confirmed as follows. This aqueous solution

was concentrated to dryness *in vacuo* and the residue was treated with 5% H_2SO_4 at 80 °C for 2.5 h. The reaction mixture was neutralized with Amberlite MB-3 and concentrated to dryness *in vacuo*. In this residue, glucose was identified by TLC and GLC. GLC was carried out under the same conditions as used for hydrolysis of a saponin.

The prosapogenin solution (BuOH layer, *vide supra*) was washed with H_2O and concentrated to dryness *in vacuo*. The residue was chromatographed on silica gel (solvent: $CHCl_3$: MeOH : H_2O (6 : 4 : 1, homogeneous)) to give **11** (32 mg), a white powder (reprecipitated from MeOH–EtOAc), $[\alpha]_D^{27}$ +15.7° (c = 1.19, MeOH).

Extraction and Separation of Specimen P——Dried and powdered rhizomes (62 g) of specimen P collected at May 14, 1967, between Gasa and Pari-la (elevation above sea level: 2600—3550 m), Bhutan, were extracted with MeOH (200 ml × 5) under reflux and the extract was concentrated to dryness *in vacuo*. The residue (21.4 g) was suspended in water (500 ml) and extracted with Et_2O (200 ml × 3) followed with 1-BuOH (200 ml × 4). The aqueous layer was passed through an Amberlite IR-120 resin column and the eluate was extracted with 1-BuOH (200 ml × 4). The 1-BuOH extracts were combined and concentrated to dryness *in vacuo*. The residue (13 g) was subjected to column chromatography on Avicel with $CHCl_3$–MeOH–H_2O (7 : 1 : 1 to 7 : 3 : 1, lower phase) to provide eight fractions: Fr-I (0.6 g), Fr-II (1.0 g), Fr-III (1.7 g), Fr-IV (0.6 g), Fr-V (4.0 g), Fr-VI (2.0 g), Fr-VII (1.5 g) and Fr-VIII (1.6 g).

Fr-I was separated into two fractions, fr-1 and fr-2, by chromatography on Lobar RP-8 (size A, Merck) with 80% MeOH. Fr-1 was purified by silica gel with $CHCl_3$–MeOH–EtOAc–H_2O (2 : 2 : 4 : 1, lower phase) to give **19** (yield: 0.02%). Fr-2 was chromatographed on TSK gel LS-410 (Toyo Soda Co., Ltd., 75% MeOH) to provide **22** (yield: 0.02%) and **23** (yield: 0.02%).

Fr-II was also divided into two fractions, fr-1 and fr-2, by RP-8 (70% MeOH), and fr-1 was rechromatographed on silica gel ($CHCl_3$–MeOH–H_2O (7 : 1 : 0.1) to afford **13** (yield: 0.02%) and **6** (yield: 1.2%). Fr-2 was purified by column chromatography on LS-410 with 75% MeOH to give **11** (yield: 0.1%).

Fr-III was purified by chromatography on RP-8 with 70% MeOH to afford **4** (yield: 1.8%), and Fr-IV was chromatographed on RP-8 (70% MeOH) on silica gel ($CHCl_3$–MeOH–H_2O (7 : 2 : 0.2)) to give **24** (yield: 0.1%), **7** (yield: 0.07%) and **25** (yield: 0.03%).

Fr-V was purified by column chromatography on RP-8 (70% MeOH) to give **9** (yield: 5.0%), and Fr-VI was subjected to column chromatography on RP-8 (70% MeOH) then on LS-410 (65% MeOH) to provide **9** (yield: see Fr-V of this section) and **5** (yield: 0.05%).

Fr-VII was chromatographed on RP-8 (70% MeOH) to afford **1** (yield: 0.1%).

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