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Saponins of Plants of *Panax* Species Collected in Central Nepal and Their Chemotaxonomical Significance. I

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Saponins of plants of wild *Panax* species (Araliaceae), collected at Chame and Ghorapani in central Nepal and designated as a subspecies or a variety of *P. pseudo-ginseng*, were investigated. From rhizomes of the specimen collected at Chame, the known dammarane-saponins, ginsenosides-Rg_1 (**1**), -Re (**2**), -Rb_1 (**4**), -Rb_3 (**5**), -Rd (**6**) and gypenoside XVII (**7**) were isolated together with two new saponins, 24(*S*)-pseudo-ginsenoside F_{11} (**3**) and monoacetyl-ginsenoside-Rd (**8**; named pseudo-ginsenoside-RC_1). From rhizomes of the specimen collected at Ghorapanai, **1**—**7**, notoginsenoside-R1 (**11**), quinquenoside-R_1 (**12**), majonoside-R2 (**13**) and malonyl-ginsenoside-Rb_1 (**14**) were isolated. No saponin of oleanolic acid (**16**) was detected in either of the specimens. The specimens of the present study have a horizontally elongated rhizome, being morphologically similar to the *Panax* spp. which distributed from Bhutan in the Eastern Himalaya (*P. pseudo-ginseng* subsp. *himalaicus*) to Japan through the South-West province of China, the rhizomes of which contain a large amount of the common saponins of **16** along with the dammarane saponins. It is chemotaxonomically and pharmacognostically significant that the saponin composition of the specimens of the present study is evidently different from that of these plants, being rather similar to that of roots of *P. ginseng*, *P. quinquefolium* and *P. notoginseng* whose big carrot-like roots contain a number of physiologically active dammarane saponins with only trace levels or none of the saponin of **16**.

Keywords——*Panax* spp.; Araliaceae; Nepal; Himalayan medicinal plant; dammarane saponin; ginsenoside; pseudo-ginsenoside-RC_1; 24(*S*)-pseudo-ginsenoside-F_{11}; Sanchi-Ginseng

Extensive studies on physiologically active saponins of *Panax ginseng* C. A. MEYER and their congeners have been reported.[1] As a part of these studies, comparison of saponin compositions of Japanese,[2,3] Chinese[4-7] and Himalayan[8,9] wild *Panax* spp., which have a close botanical relationship, has been conducted. In this chemotaxonomical study, saponins of a number of *Panax* spp. growing wild in the South-West province of China (Yunnan *etc.*) have been investigated in cooperation with Kunming Institute of Botany, Academia Sinica.[4-7,10] However, with regard to the Himalayan plants, chemical study has been limited to *P. pseudo-ginseng* WALL. subsp. *himalaicus* HARA[11] collected in Bhutan, Eastern Himalaya[8,9] and saponins of specimens from other Himalayan regions have been left uninvestigated. In August, 1983, Namba and his collaborators collected several specimens of wild *Panax* spp. in central Nepal. The present paper deals with a chemical study on the saponin composition of these specimens, and presents some interesting findings from both chemotaxonomical and pharmacognostical viewpoints.

The specimens in the present study were collected at Chame (the eastern foot of Mt. Annapurna; elevation above sea level 2700 m) and Ghorapani (the western foot of Mt. Annapurna; elevation above sea level 2743 m) in August, 1983. Like *P. japonicus* C. A.

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MEYER var. *major* (BURK.) C. Y. WU *et* K. M. FENG (Chinese Zu-Tzi-Shen) and *P. pseudo-ginseng* subsp. *himalaicus* (collected at Tzatogang and Pari-la, Bhutan), these specimens have a horizontally elongated rhizome with slender internodes. Both the specimens have been deposited at the Herbarium of the Institute of Wakan-Yaku, Toyama Medical and Pharmaceutical University. Both the plants can be assigned as a subspecies or a variety of *P. pseudo-ginseng* WALL. and precise taxonomical identification is in progress.

Dried and powdered rhizomes (10.8 g) collected at Chame were extracted with hot methanol and then with hot 50% aqueous methanol. The combined extract was subjected to column chromatography on highly porous polymer, which separated the saponin mixture from other substances. This saponin mixture was separated into five fractions (tentatively named fr. 1—5 in increasing order of polarity) by reversed-phase chromatography. Repeated chromatography of fr. 1 gave ginsenosides-Rg_1 (**1**, yield: 0.19%) and -Re (**2**, yield 0.18%) and a new saponin (**3**, yield: 0.06%). Purification of fr. 2, fr. 3 and fr. 4 by chromatography on silica gel afforded ginsenosides-Rb_1 (**4**, yield: 1.7%), -Rb_3 (**5**, yield: 0.16%) and -Rd (**6**, yield: 2.2%), respectively. Fr. 5 was separated by chromatography on silica gel to give gypenoside XVII (**7**, yield: 0.11%) and a new saponin (**8**, yield: 0.10%). Saponins **1**, **2**, **4**, **5** and **6** are known as the dammarane saponins of *P. ginseng* and other *Panax* spp.,[1] and **7** was first isolated from *Gynostemma pentaphyllum* MAKINO[12] (Cucurbitaceae), being distributed also in plants of *Panax* spp.[1] The identifications of these known saponins were unambiguously confirmed by comparisons of optical rotation, the proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra, mass spectrum (MS) (as the acetate or trimethylsilyl ether) and thin layer chromatographic (TLC) behavior with those of authentic samples.

Glucose and rhamnose were identified in the acid hydrolysate of **3**. The electron impact-mass spectra (EI-MS) of acetylated **3** exhibited ions at *m/z* 561 [(Glc-Rha)Ac_6], 273 [(terminal Rha)Ac_3] and 143 (Chart 1), the latter of which is characteristic of ocotillol-type triterpenes. In the study on the saponin, pseudo-ginsenoside F_{11} (**9**) from leaves of *P. pseudo-ginseng*

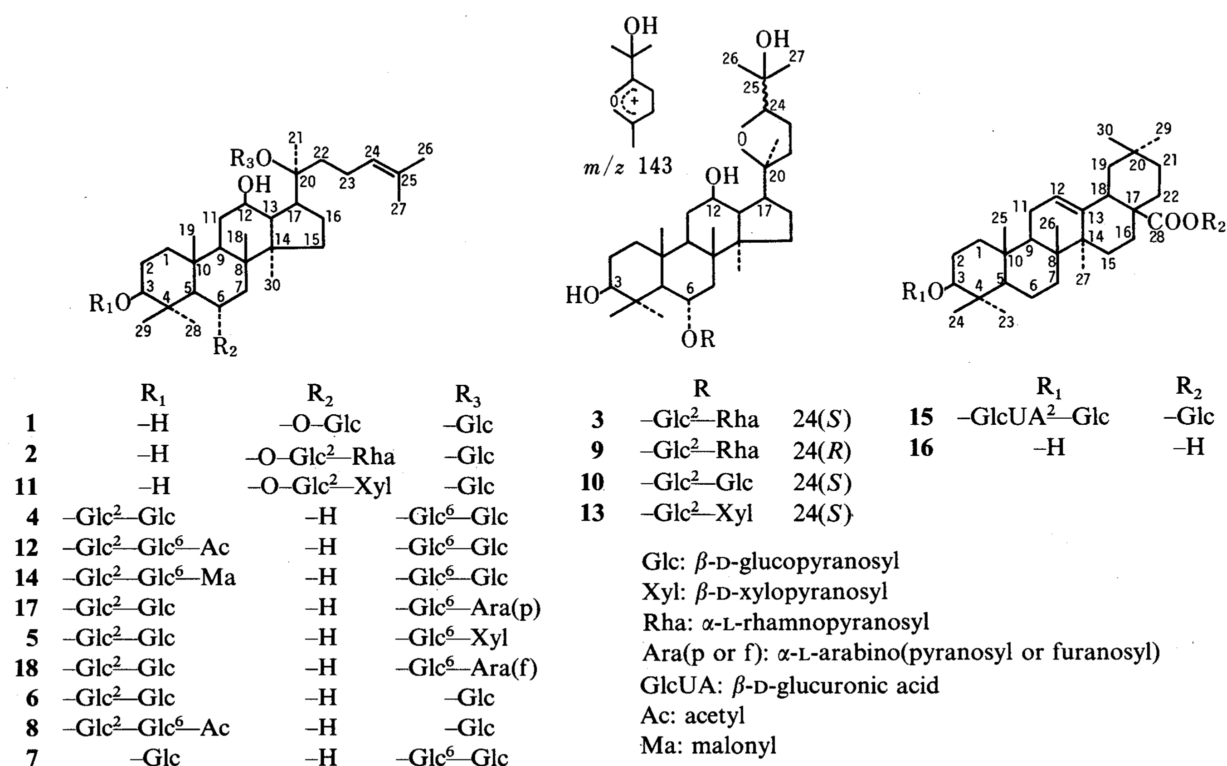

Chart 1

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

	9[13]	**3**	**10**[4]	**6**	**8**
C-1	39.4	39.5	39.7	39.2	39.1
C-2	27.5	27.6	27.8	26.6	26.6
C-3	78.1	78.3	78.0	88.9	89.2
C-4	39.8	39.9	40.3	39.6	39.7
C-5	60.7	60.8	61.5	56.3	56.4
C-6	73.9	74.0	79.9	18.4	18.4
C-7	45.8	45.9	45.0	35.1	35.1
C-8	40.9	41.0	41.2	39.9	40.0
C-9	49.9	49.9	50.4	50.1	50.2
C-10	39.4	39.5	39.7	36.8	36.8
C-11	31.8	32.0	32.4	30.8	30.8
C-12	71.0	70.8	71.0	70.2	70.2
C-13	48.1	49.0	49.2	49.3	49.3
C-14	52.0	52.1	52.3	51.3	51.4
C-15	31.6	32.0	32.4	30.8	30.8
C-16	25.3	25.7	25.8	26.6	26.6
C-17	49.2	49.3	49.5	51.6	51.7
C-18	17.7[a]	17.8[a]	17.9[a]	16.2[a]	16.2[a]
C-19	17.4[a]	17.5[a]	17.3[a]	15.8[a]	15.9[a]
C-20	86.5	87.0	87.1	83.2	83.2
C-21	26.8[b]	26.9[b]	27.1[b]	22.4	22.4
C-22	32.6	32.5	32.4	35.9	36.0
C-23	28.7	28.6	29.0	23.2	23.2
C-24	85.7	88.3	88.4	125.8	125.9
C-25	70.2	70.0	70.1	130.8	130.8
C-26	27.0[b]	26.5[b]	26.7[b]	25.7	25.7
C-27	27.5[b]	28.9[b]	29.0[b]	17.7[a]	17.7[a]
C-28	31.9	32.0	32.4	28.0	27.9
C-29	16.8[a]	16.9[a]	16.8[a]	16.5[a]	16.4[a]
C-30	18.0[a]	17.8[a]	17.9[a]	17.3[a]	17.3[a]

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

subsp. *himalaicus* collected in Bhutan,[13] the carbon signals of ocotillol-type triterpenes have been assigned, being diagnostic for the C-24 configuration. The ^{13}C-NMR spectrum of **3** was compared with that of **9** whose C-24 configuration was established as *R*. As shown in Tables I and II, sugar- and aglycone-carbon signals of both compounds appeared at almost the same positions except for those due to C-24 and -26(or -27) of the aglycone moiety. Further, the carbon signals associated with the aglycone moiety of **3** were observed at almost the same positions as those of majonoside R1[4] (**10**, 24(*S*)), another ocotillol-type saponin from *P. japonicus* var. *major*, except for that of C-6 (Table I). Based on these results, **3** can be formulated as the C-24 epimer of **9**, and is designated as 24(*S*)-pseudo-ginsenoside F_{11}.

Another new saponin, named pseudo-ginsenoside RC_1 (**8**) gave glucose on acid hydrolysis. Its ^{1}H-NMR spectrum showed a signal at δ 2.00 (3H, s) and its ^{13}C-NMR spectrum exhibited signals at δ 170.9 and 20.9, indicating the presence of an acetoxyl group. Mild alkaline saponification of **8** yielded **6**. The EI-MS of its trimethylsilyl (TMSi) ether showed ions at *m/z* 451 [(terminal Glc)$TMSi_4$], 421 [(terminal Glc)$TMSi_3$Ac] and 799 [(Glc-Glc)$TMSi_6$Ac], disclosing the presence of an acetoxyl group in the terminal glucosyl moiety of the 3-*O*-sophorosyl group of **6**. In the ^{13}C-NMR spectra, on going from **6** to **8**, carbon signals due to C-5 and -6 of one of the glucosyl moieties were displaced upfield by 2.7 ppm and downfield by 2.0 ppm, respectively, while other carbon signals remained almost unshifted. It

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

	9[13]	**3**	**6**	**8**
3-Glc				
1	—	—	104.9	104.8
2	—	—	83.2	84.2
3	—	—	78.0[a]	77.9[a]
4	—	—	71.4	71.4
5	—	—	78.0[a]	77.8[a]
6	—	—	62.6	62.7
Glc				
1	—	—	105.7	106.1
2	—	—	76.8	76.6
3	—	—	79.0[a]	79.1[a]
4	—	—	71.4	70.9
5	—	—	78.0[a]	75.3
6	—	—	62.6	64.6
6-Glc				
1	101.6	101.8	—	—
2	79.1[a]	79.2[a]	—	—
3	78.1[a]	78.3[a]	—	—
4	72.1	72.2	—	—
5	78.1[a]	78.3[a]	—	—
6	63.0	63.0	—	—
Rha				
1	101.6	101.8	—	—
2	72.1	72.2	—	—
3	72.4	72.5	—	—
4	73.9	74.3	—	—
5	69.2	69.3	—	—
6	18.6	18.6	—	—
20-Glc				
1	—	—	98.1	98.2
2	—	—	74.9	75.0
3	—	—	78.0[a]	78.1[a]
4	—	—	71.4	71.4
5	—	—	78.0[a]	78.4[a]
6	—	—	62.6	62.7
$\underset{\cdot}{C}H_3CO$	—	—	—	20.9
$CH_3\underset{\cdot}{C}O$	—	—	—	170.9

Glc, β-D-glucopyranosyl; Rha, α-L-rhamnopyranosyl. *a*) Values in any column may be reversed, though those given here are preferred.

follows that the acetoxyl group in **8** must be located at the 6-hydroxyl group of the terminal glucosyl moiety of the 3-*O*-sophorosyl group of **6**.

An extract of rhizomes of the specimen (15.5 g) collected at Ghorapani was separated by the same method as used for the specimen from Chame, affording **1**—**7** in yields of 0.46, 0.12, 0.10, 5.0, 0.09, 0.94 and 0.39%, respectively. Notoginsenoside-R1 (**11**, yield: 0.06%, a major saponin of roots of *P. notoginseng* (Burk.) F. H. Chen,[14] Chinese Sanchi-Ginseng), quinquenoside-R_1[15] (**12**, yield: 0.12%, a minor saponin of roots of *P. quinquefolium* L., American Ginseng) and majonoside-R2 (**13**, yield: 0.10%, a saponin of rhizomes of *P. japonicus* var. *major*,[4] Chinese Zu-Tzi-Shen) were also isolated and identified.

Several unstable malonyl esters of dammarane saponins have been isolated[16] from White Ginseng, which is prepared from roots of *P. ginseng* without steaming. From the more polar fraction of the extract of the Ghorapani specimen, malonyl-ginsenoside-Rb_1 (**14**) was isolated

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TABLE III. Comparison of Saponins from Rhizomes of *Panax pseudo-ginseng* subsp. *himalaicus*, *P. japonicus* var. *major*, *P. zingiberensis*, *P. japonicus* Collected in the Himalayan Region (Tzatogang, Pari-la and Khosa), China (Yunnan) and Japan (Yield %)

	Tzatogang[a,9)]	Pari-la[a,9)]	Yunnan[b,4)]	Yunnan[c,6)]	Khosa[a,8)]	Yunnan[d,5)]	Miyazaki[d,3)]	Japan[d,2)]
Oleanolic acid (**16**) saponins								
C-IVa	1.7	1.8	0.2	0.03	0.6	2.8	—	+
C-IV	—	—	—	0.3	0.3	3.4	1.4	0.4
C-V (**15**)	0.4	0.1	1.0	2.1	7.3	3.1	2.2	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	—	—	—	—	—	—	—	+
Dammarane saponins	Rb_1 (**4**, 0.3) Rd (**6**, 0.2) Rg_1 (**1**, 0.4) RT_3 (0.1)	Rb_1 (**4**, 0.05) Rd (**6**, 0.07) Re (**2**, 0.1) F_2 (0.02) Rg_1 (**1**, 1.2) Gy-XVII (**7**, 0.03) RT_3 (0.02)	Rd (**6**, 0.7) N-R2 (0.03) 20glc-Rf (0.01)	Rg_1 (**1**, 0.6) Rh_1 (+)	Rb_1 (**4**, 1.1)	Rd (**6**, 0.04) Re (**2**, 0.1) Rg_1 (**1**, 0.2) Rg_2 (0.05) N-R2 (0.02)	Rb_1 (**4**, 0.7) Rc (**18**, 0.1) Re (**2**, 0.3) Rg_1 (**1**, 0.4) N-R1 (**11**, 0.05) N-R2 (0.3) Gy-XVII (0.02)	C-III (1.2) Rg_2 (+) C-Ia (+)
Ocotillol saponins	F_{11} (**9**, 0.07) RT_2 (0.09) RT_4 (0.08) RT_5 (0.07)	RT_4 (0.02)	M-R1 (**10**, 0.07) M-R2 (**13**, 0.1)			F_{11} (**9**, 0.2)		

Tzatogang (3100 m), Pari-la (2600—3550 m), Khosa (1800 m): Bhutan, Himalaya. Miyazaki: collected at Miyakonojou, Miyazaki pref., Japan. *a*) *P. pseudo-ginseng* subsp. *himalaicus*. *b*) *P. japonicus* var. *major*. *c*) *P. zingiberensis*. *d*) *P. japonicus*. 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: ginsenosides. F_{11}, RP_1, RT_{1-5}, pseudo-ginsenosides; Pro-C-V, prosapogenin of C-V; +, very low yield.

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TABLE IV. Comparison of Saponins from Rhizomes of *Panax* spp. (Specimens C and G) Collected at Chame and Ghorapani in Central Nepal, and Major Saponins from Roots of *P. notoginseng* and *P. ginseng* (Yield %)

	Specimen C	Specimen G	Sanchi[a,17)]	Ginseng[b,1)]
G-Rb_1 (**4**)	1.7	5.0	1.8	0.47
G-Rb_2 (**17**)	—	—	—	0.21
G-Rb_3 (**5**)	0.16	0.09	—	0.005[c)]
G-Rc (**18**)	—	—	—	0.26
G-Rd (**6**)	2.2	0.94	0.2	0.15
G-Re (**2**)	0.18	0.12	0.15	0.15
G-Rg_1 (**1**)	0.19	0.46	1.9	0.21
Ma-G-Rb_1 (**14**)	—	0.07	—	0.82[16)]
Q-R_1 (**12**)	—	0.12	—	0.015[d)]
P-RC_1 (**8**)	0.10	—	—	—
N-R1 (**11**)	—	0.06	0.16	0.007[d)]
Gy-XVII (**7**)	0.11	0.39	—	—
M-R2 (**13**)	—	0.10	—	—
24(*S*)P-F_{11} (**3**)	0.06	0.10	—	—
G-Ro(=C-V) (**15**)	—	—	—	0.02[c)]

Chame (2700 m), Ghorapani (2743 m): Central Nepal. G, ginsenoside; Ma, malonyl; Q, quinquenoside; P, pseudo-ginsenoside; N, notoginsenoside; Gy, gypenoside; M, majonoside; C, chikusetsusaponin. *a) P. notoginseng. b) P. ginseng. c)* Minor saponin. *d)* Minor saponin of red ginseng.

in a yield of 0.07%. TLC analysis of the extracts suggested the existence of much greater amounts of malonyl saponins in this specimen and also in the specimen collected at Chame, though further investigation could not be done because insufficient material was available, and because of the instability of compounds of this type.

It should be noted that sapogenins of all of the saponins obtained from the specimens of the present study are represented by dammarane triterpenes including the ocotillol-type triterpenes, while no oleanane saponin was detected in either specimen. The relationship between the shape of the underground part and the saponin composition of *Panax* spp. has been reported[1)] to be as follows. Roots and small rhizomes (corms) of *P. ginseng, P. quinquefolium* (American Ginseng) and *P. notoginseng* (Sanchi-Ginseng) which have a large carrot-like root (tentatively classified as group I) contain a large amount of dammarane saponins with either a small amount of the oleanane saponin, chikusetsusaponin V (=ginsenoside-Ro, **15**) or none (Sanchi-Ginseng). In contrast, from the Himalayan region to Japan through the South-West province of China, plants having a large horizontally elongated rhizome, *P. japonicus* (Japanese Chikusetsu-ninjin and Chinese Zhujie-Shen), Chinese *P. japonicus* var. *major* (Zu-Tzi-Shen), Chinese *P. zingiberensis*, Chinese *P. stipuleanatus* and Himalayan *P. pseudo-ginseng* subsp. *himalaicus* (collected in Bhutan), *etc.* (group II) are distributed, and their saponin composition includes a large amount of oleanane saponins (bisdesmoside of oleanolic acid (**16**) such as **15**), together with dammarane saponins which are characteristic of each species as summarized in Table III. It has also been found that saponin composition of small roots of group II is similar to that of the rhizomes. It should be noted that in spite of the morphological similarity to the plants of group II, the specimens of the present study collected in central Nepal contain no saponin of **16**. Further, it seems significant that, regardless of the morphological difference in the underground parts, the saponin composition of the specimens of the present study is rather similar to that of group I, especially to Chinese Sanchi-Ginseng (*P. notoginseng*) in respect to containing **1, 2, 4** and **6** as the major dammarane saponins and lacking **14**, ginsenosides-Rb_2 (**17**) and -Rc (**18**), the later

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two of which are the major dammarane saponins of roots of *P. ginseng* and *P. quinquefolium* (group I). No wild original plant of Sanchi-Ginseng, which is cultivated in Yunnan, China, has been found as yet.

Experimental

General Procedures——Nuclear magnetic resonance (NMR) spectra were taken on 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 tetramethylsilane (TMS) as an internal standard.

MS were recorded on a JEOL 01-SG-2 mass spectrometer at 75 eV.

Trimethylsilylation for MS: A sample of saponin (1—2 mg) 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 rotations were measured with a Union automatic digital polarimeter at 17—23 °C in MeOH.

High Performance Liquid Chromatography (HLC) Equipment: HLC 803D pump (Toyo Soda); detector, RI-8 differential refractometer (Toyo Soda).

Elemental analyses of new saponins **3** and **8** have not been done since we wished to preserve the authentic samples obtained from the limited amount of Himalayan plant materials for future studies.

Extraction and Separation of Saponins from the Specimen Collected at Chame——Dried and powdered rhizomes (10.8 g) were extracted with hot MeOH (80 ml × 4) and then with hot 50% MeOH (80 ml × 2) to give an MeOH extract (after evaporation) in a yield of 40.7%. An aqueous suspension of this MeOH extract was subjected to column chromatography on reversed-phase highly porous polymer (Kogel B-G 4600, Beads 60—80 mesh, Shoko-Tsusho Co., Ltd.) (solvent: H_2O (700 ml), 10% MeOH (700 ml), MeOH (800 ml) and finally $CHCl_3$ (500 ml)) to provide the H_2O eluate (2.8 g), 10% MeOH eluate (125 mg), MeOH eluate (crude saponin fraction) (1.2 g) and $CHCl_3$ eluate (65 mg). This MeOH eluate was separated into five fractions, fr. 1 to fr. 5, by column chromatography on silylated silica gel (LiChroprep RP-8 (Merck)) (solvent: 70% MeOH).

Fr. 1 was chromatographed on silica gel (solvent: $CHCl_3$–MeOH–H_2O (6 : 4 : 1, homogeneous)) and was further separated into two fractions, fr. 1a and fr. 1b by column chromatography on silica gel (solvent: $CHCl_3$–MeOH–H_2O (25 : 10 : 1 and then 6 : 4 : 1, homogeneous)). Fr. 1a was purified by column chromatography on silylated silica gel (*vide supra*) (solvent: 55% MeOH) to give **1** (yield: 0.19%), a white powder (from MeOH–EtOAc), $[\alpha]_D^{19}$ +35.4° (*c*=1.02, MeOH) and **3** (yield: 0.06%), a white powder (from MeOH–EtOAc), $[\alpha]_D^{17}$ −23.5° (*c*=0.34, MeOH). Fr. 1b was further subjected to preparative HLC on a reversed-phase column of ODS-120A (Toyo Soda) (21.5 mm × 30 cm; mobile phase, 55% MeOH; flow rate, 6.3 ml/min; injection vol., 1 ml (48 mg/1 ml 55% MeOH); detector, RI-8) to give **2** (yield: 0.18%), colorless needles (from 50% MeOH), mp 202—204 °C, $[\alpha]_D^{19}$ −1.9° (*c*=1.20, MeOH).

Fr. 2 was further chromatographed on silica gel (solvent: $CHCl_3$–MeOH–H_2O (6 : 4 : 1, homogeneous)) to give **4** (yield: 1.7%), a white powder (from MeOH–EtOAc), $[\alpha]_D^{17}$ +12.1° (*c*=1.38, MeOH).

Fr. 3 was further chromatographed on silica gel (Kieselgel 60 H, Art. 7736, Merck) (solvent: $CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower layer)) to give **5** (yield: 0.16%), a white powder (from MeOH–EtOAc), $[\alpha]_D^{23}$ +10.6° (*c*=0.54, MeOH).

Fr. 4 was further chromatographed on silica gel (solvent: $CHCl_3$–MeOH–H_2O (20 : 10 : 1, homogeneous)) to give **6** (yield: 2.2%), a white powder (from MeOH–EtOAc), $[\alpha]_D^{17}$ +22.6° (*c*=1.31, MeOH).

Fr. 5 was chromatographed on silica gel (solvent: $CHCl_3$–MeOH–H_2O (20 : 10 : 1, homogeneous)) to give crude **8** and **7** (yield: 0.11%), a white powder (from MeOH–EtOAc), $[\alpha]_D^{17}$ +17.9° (*c*=0.43, MeOH). Crude **8** was further chromatographed on silica gel (solvent: $CHCl_3$–MeOH–H_2O (20 : 10 : 1, homogeneous)) to give **8** (yield: 0.10%), a white powder (from MeOH–EtOAc), $[\alpha]_D^{17}$ +20.8° (*c*=0.63, MeOH).

Extraction and Separation of Saponins from the Specimens Collected at Ghorapani——Dried and powdered rhizomes (15.5 g) were extracted with hot MeOH (120 ml × 4) and then with hot 50% MeOH (120 ml × 2) to give an MeOH extract (after evaporation) in a yield of 38.4%. An aqueous suspension of this MeOH extract was subjected to column chromatography on reversed-phase highly porous polymer (*vide supra*) (solvent: H_2O (700 ml), 10% MeOH (700 ml), MeOH (800 ml) and finally $CHCl_3$ (500 ml)) to provide the H_2O eluate (3.7 g), 10% MeOH eluate (44 mg), MeOH eluate (crude saponin fraction) (2.0 g) and $CHCl_3$ eluate (75 mg). This MeOH eluate was separated into five fractions, fr. 1 to fr. 5, by column chromatography on silylated silica gel (*vide supra*) (solvent: 70% MeOH).

Fr. 1 was separated into two fractions, fr. 1a and fr. 1b, by column chromatography on silica gel (solvent: $CHCl_3$–MeOH–H_2O (6 : 4 : 1, homogeneous)). Fr. 1a was further separated into two fractions, fr. 1a-1 and fr. 1a-2, by column chromatography on silica gel (solvent: $CHCl_3$–MeOH–H_2O (25 : 10 : 1 and then 6 : 4 : 1, homogeneous)). Fr.

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1a-1 was further chromatographed on silylated silica gel (*vide supra*) (solvent: 55% MeOH) to give a mixture of **1** and **13**, and **3** (yield: 0.10%), a white powder (from MeOH–EtOAc), $[\alpha]_D^{17}$ $-24.5°$ (c=0.53, MeOH). The mixture of **1** and **13** was subjected to preparative HLC on a reversed-phase column of ODS-120A (*vide supra*) (21.5 mm × 30 cm; mobile phase, 55% MeOH; flow rate, 6.3 ml/min; injection vol., 1 ml (105 mg/1 ml 55% MeOH); detector, RI-8) to give **1** (yield: 0.46%), a white powder (from MeOH–EtOAc), $[\alpha]_D^{19}$ $+35.5°$ (c=1.15, MeOH) and **13** (yield: 0.10%), a white powder (from MeOH–EtOAc), $[\alpha]_D^{23}$ $-5.4°$ (c=0.67, MeOH). Fr. 1a-2 was subjected to preparative HLC on a reversed-phase column of ODS-120A (*vide supra*) (21.5 mm × 30 cm; mobile phase, 55% MeOH; flow rate, 6.3 ml/min; injection vol., 1 ml (70 mg/1 ml 55% MeOH)) to give **11** (yield: 0.06%), colorless needles (from MeOH–H_2O), mp 203—205 °C, $[\alpha]_D^{19}$ $+22.2°$ (c=0.51, MeOH) and **2** (yield: 0.12%), colorless needles (from 50% MeOH), mp 201—203 °C, $[\alpha]_D^{19}$ $-2.0°$ (c=1.01, MeOH). Fr. 1b was purified by column chromatography on silica gel (solvent: BuOH–EtOAc–H_2O (4 : 1 : 2, upper layer)), followed by deionization with ion exchange resin (Amberlite MB-3), to afford **14** (yield: 0.07%), a white powder, $[\alpha]_D^{23}$ $+18.1°$ (c=0.74, MeOH).

Fr. 2 was further chromatographed on silica gel (solvent: $CHCl_3$–MeOH–H_2O (6 : 4 : 1, homogeneous)) to give **4** (yield: 5.0%), a white powder (from MeOH–EtOAc), $[\alpha]_D^{17}$ $+12.9°$ (c=1.26, MeOH).

Fr. 3 was further chromatographed on silica gel (Kieselgel 60 H, Art. 7736, Merck) (solvent: $CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower layer)) to give **12** (yield: 0.12%), a white powder (from MeOH–EtOAc), $[\alpha]_D^{23}$ $+13.6°$ (c=0.73, MeOH) and **5** (yield: 0.09%), a white powder (from MeOH–EtOAc), $[\alpha]_D^{23}$ $+10.0°$ (c=0.39, MeOH).

Fr. 4 was further chromatographed on silica gel (solvent: $CHCl_3$–MeOH–H_2O (20 : 10 : 1, homogeneous)) to give **6** (yield: 0.94%), a white powder (from MeOH–EtOAc), $[\alpha]_D^{17}$ $+22.6°$ (c=1.15, MeOH).

Fr. 5 was further chromatographed on silica gel (solvent: $CHCl_3$–MeOH–H_2O (20 : 10 : 1, homogeneous)) to give **7** (yield: 0.39%), a white powder (from MeOH–EtOAc), $[\alpha]_D^{17}$ $+18.6°$ (c=1.07, MeOH).

Identification of the Known Saponins——Each known saponin was identified by thin layer chromatography (TLC) on Kieselgel 60 F_{254} (Merck) with $CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower layer), $CHCl_3$–MeOH–H_2O (6 : 4 : 1, homogeneous) and $CHCl_3$–BuOH–MeOH–H_2O (20 : 40 : 15 : 20, lower layer) and by reversed-phase TLC on silica gel plates (RP-8 and RP-18 F_{254} (Merck)) with 60—80% MeOH (detection: H_2SO_4), as well as by comparison of the ^1H-NMR and ^{13}C-NMR spectra, optical rotation and MS (as the acetate or trimethylsilyl ether) with those of the corresponding authentic sample.

Hydrolysis of 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, 170 °C; carrier gas, N_2 (40 ml/min).

Saponification of 8——**8** (a few mg) was heated with 5% methanolic KOH (5 drops) at 80 °C for 5 min. The reaction mixture was neutralized with Amberlite MB-3. In this solution, **6** was identified by TLC on Kieselgel 60F_{254} (Merck) with $CHCl_3$–MeOH–H_2O (6 : 4 : 1, homogeneous) and with $CHCl_3$–BuOH–MeOH–H_2O (20 : 40 : 15 : 20, lower layer), and by reversed-phase TLC on silica gel plates (RP-8 F_{254} (Merck)) with 80% MeOH (detection: H_2SO_4).

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