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Saponins from *Panax pseudo-ginseng* WALL. subsp. *pseudo-ginseng* HARA Collected at Nielamu, Tibet, China

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The ginseng dammarane saponin, ginsenoside-Rg_1, and the oleanolic acid saponins, chikusetsusaponin-IV and pseudo-ginsenoside-RT_1, were isolated in yields of 0.02, 0.15 and 0.04%, respectively, from roots and small rhizomes of *Panax pseudo-ginseng* WALL. subsp. *pseudo-ginseng* HARA collected at Nielamu, Tibet. A saponin formulated as 3-*O*-α-L-arabinofuranosyl(1→4)-β-D-glucuronide of oleanolic acid was also isolated in a partially purified state. The chemotaxonomical significance of the results is disccussed.

Keywords——*Panax pseudo-ginseng* subsp. *pseudo-ginseng*; Araliaceae; Tibet; saponin; dammarane; ginsenoside-Rg_1; oleanolic acid; chikusetsusaponin-IV; pseudo-ginsenoside-RT_1

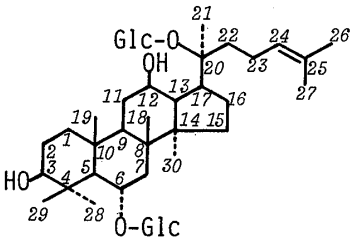
In our series of investigations of the chemical constituents of Ginseng and other *Panax* spp.,[1] comparative studies on the saponin compositions of specimens collected from the Himalaya region (Bhutan, Nepal) through China and Japan to North America have been conducted from chemotaxonomical and pharmacological viewpoints.

In the Himalaya region, various subspecies and varieties of *Panax pseudo-ginseng* WALL. grow.[2] Of these plants, only *P. pseudo-ginseng* WALL. subsp. *pseudo-ginseng* HARA has a carrot-like root with a small rhizome, and it is very rare, while all of the others have a long horizontal rhizome with a small round root, and are abundant in the Eastern and Central Himalaya. The saponin compositions of rhizomes of the latter type collected in Bhutan[3,4] and Nepal[5,6] have been reported. The present paper deals with identification of saponins from underground parts of *p. pseudo-ginseng* subsp. *pseudo-ginseng* collected in Tibet, China.

Since no significant difference in saponin composition was observed between the roots and the small rhizomes in a preliminary comparison of the thin layer chromatograms, both parts (total 35 g) were combined and extracted with hot methanol and then with hot 50% aqueous methanol. The combined extract was subjected to column chromatography on highly porous polymer to give a crude saponin mixture in a yield of 2.8%. This mixture was separated by repeated column chromatography to give four saponins, I-IV. Saponin I (yield: 0.02%) was identified as the dammarane saponin, ginsenoside-Rg_1 (**1**)[7] which is one of the major saponins of the roots of *P. ginseng* C. A. MEYER and has also been isolated from many other *Panax* species. Saponin II was identified as chikusetsusaponin-IV (**2**, yield: 0.15%),[8] a saponin of oleanolic acid (**3**), isolated from rhizomes of *P. japonicus* C. A. MEYER and many other morphologically related plants.[1] Saponin III was identical with pseudo-ginsenoside-RT_1 (**4**, yield: 0.04%),[4] a saponin of **3** isolated from rhizomes of *P. pseudo-ginseng* WALL. subsp. *himalaicus* HARA collected at relatively high altitude in Bhutan (Eastern Himalaya region). Although saponin IV could not be obtained in a completely pure state owing to the low content, the major signals of the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum (in pyridine-d_5) suggested it to be the 3-*O*-α-L-arabinofuranosyl (1→4)-β-D-glucuronide of **3** (**5**), which was obtained by alkaline saponification of **2**.[8] The presence of two additional

minor saponins, probably bisdesmosides of **3**, was also observed. One of these seemed to be identical with stipuleanoside-R_2 (**6**), one of two major saponins from rhizomes of *P. stipuleanatus* H. T. TSAI *et* K. M. FENG,[9] though complete purification and identification could not be done because of the shortage of material.

It has been found that the underground parts of *Panax* spp. having carrot-like roots with relatively small rhizomes (tentatively classified as group I), *P. ginseng, P. quinquefolium L.* (American Ginseng) and *P. notoginseng* (BURK.) F. H. CHEN (Sanchi-Ginseng) contain a large amount of dammarane saponins with either a small amount of saponin of **3** or none (Sanchi-Ginseng).[1] A variety of plants of *Panax* spp. of another type, which have a large horizontally elongated rhizome, grow from the Himalayan region to Japan through the South-West province of China, and are tentatively classified as group II. In contrast to the plants of group I, the underground parts of group II contain a large amount of saponins of **3** together with dammarane saponins,[1] except for the specimens collected near Mt. Annapurna, the rhizomes of which contain dammarane saponins exclusively,[5] like Sanchi-Ginseng. The specimen of the present study was morphologically classified into group I. However the contents of saponins of **3** (**2**, **4** and **5**) in the underground parts are rather higher than that of the dammarane saponin (**1**). This seems significant from the chemotaxonomical viewpoint. It is also noteworthy that the total saponin content in the underground part of this specimen is evidently lower than those of the other *Panax* spp.



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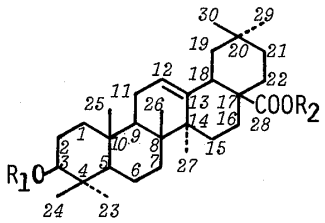
Ara: α-L-arabinofuranosyl
Glc: β-D-glucopyranosyl
GlcUA: β-D-glucuronic acid
Xyl: β-D-xylopyranosyl



	R_1	R_2
2:	–GlcUA^4–Ara	–Glc
5:	–GlcUA^4–Ara	–H
6:	–GlcUA^{3,4}(Glc, Ara)	–Glc
3:	–H	–H
4:	–GlcUA^2–Xyl	–Glc

Chart 1

Experimental

General Procedures——NMR spectra in C_5D_5N, mass spectra (MS), and optical rotations were measured by the same procedures as in the previous paper.[3-5] Each known saponin was identified by comparison of the ^1H- and ^{13}C-NMR spectra, optical rotation and MS (as the acetate or the trimethylsilyl ether) with those of a corresponding authentic sample.

Plant Material——The plant was collected at Nielamu (2100 m), Tibet, in August, 1976 and identified by the members of Kunming Institute of Botany. The specimen has been deposited in the Herbarium of Kunming Institute of Botany.

Extraction and Separation of Saponins——Dried and powdered roots and rhizomes (35 g) were extracted with hot MeOH (300 ml × 3) and then with hot 50% MeOH (300 ml × 2) to give an MeOH extract (after evaporation) in a combined yield of 23.9%. 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; 10% MeOH (2 l), MeOH (1 l) and finally $CHCl_3$ (700 ml)) to provide 10% MeOH eluate (7.0 g), MeOH eluate (crude saponin mixture) (980 mg) and $CHCl_3$ eluate (9 mg). This MeOH eluate was separated into two fractions, fr-1 and fr-2, by column chromatography on silica gel (solvent; $CHCl_3$–MeOH–H_2O (6:4:1, homogeneous)).

Fr-1 was chromatographed on silylated silica gel (Li-Chroprep RP-8, Merck) (solvent; 60% MeOH) and further chromatographed on silica gel (solvent; $CHCl_3$–MeOH–H_2O) (20:10:1, homogeneous) to give **1** (0.02% yield), a white powder (MeOH–EtOAc), $[\alpha]_D^{16}$ + 11.0° (c = 0.31, MeOH) and another crude saponin. This crude saponin was

further treated with ion exchange resin (Amberlite MB-3) to afford **5** (0.01% yield), which was still not completely pure as judged by thin layer chromatography, though the ^{13}C-NMR spectrum supported its formulation.

Fr-2 was chromatographed on silylated silica gel (*vide supra*) (solvent; 60% MeOH) and was further chromatographed on silica gel (solvent; $CHCl_3$–MeOH–H_2O (6:4:1, homogeneous)) to give crude **2** and crude **4**. Crude **2** was further purified by treatment with ion exchange resin (Amberlite MB-3) to afford **2** (0.15% yield), colorless prisms (from MeOH–H_2O), mp 216—218 °C (dec.), $[\alpha]_D^{19}$ −12.0° (c=1.19, C_5H_5N). Crude **4** was further chromatographed on silylated silica gel (*vide supra*) (solvent; 60% MeOH), followed by deionization with ion exchange resin (Amberlite MB-3) to afford **4** (0.04% yield), a white powder (MeOH–EtOAc), $[\alpha]_D^{16}$ +4.0° (c=0.50, MeOH).

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