[Chem. Pharm. Bull. 36(10)4177—4181(1988)]

Analysis of Saponins of Wild *Panax ginseng*

HIROYUKI YAMAGUCHI,*,[a] HIROMICHI MATSUURA,[a] RYOJI KASAI,[b] OSAMU TANAKA,[b] MOTOYOSHI SATAKE,[c] HIROSHI KOHDA,[b] HIROMASA IZUMI,[d] MARIKO NUNO,[e] SHIGEKI KATSUKI,[f] SUSUMU ISODA,[g] JUNZO SHOJI,[g] and KATSUMI GOTO[h]

Central Research Laboratories, Wakunaga Pharmaceutical Co., Ltd.,[a] Shimokotachi 1624 Kohda-cho, Takata-gun, Hiroshima 729-64, Japan, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine,[b] Kasumi, Minami-ku, Hiroshima 734, Japan, National Institute of Hygienic Sciences, Tsukuba Medicinal Plant Research Station,[c] Hachimandai, Yatabe-cho, Tsukuba, Ibaraki 305, Japan, Tokyo College of Pharmacy,[d] Horinouchi, Hachioji, Tokyo 192-03, Japan, Faculty of Pharmaceutical Sciences, Teikyo University,[e] Suarashi, Sagamiko-machi, Kanagawa 199-01, Japan, Medicinal Plant Garden, School of Pharmaceutical Sciences, Kitasato University,[f] Kitasato 2-15-1, Sagamihara, Kanagawa 228, Japan, Faculty of Pharmaceutical Sciences, Showa University,[g] 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan, and Garden of Medicinal Plants, Kyoto Pharmaceutical University,[h] Hinobayashi-cho 39, Fushimi-ku, Kyoto 612, Japan

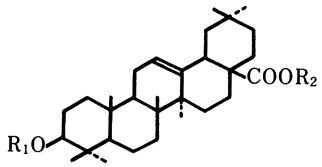
(Received March 14, 1988)

The composition and contents of neutral and acidic saponins of Chinese and Japanese wild ginseng were analyzed by high-performance liquid chromatography and two-dimensional thin layer chromatography. There was no significant difference in the major saponins between wild and cultivated specimens. It is noteworthy that the content of ginsenoside-Ro, the glucuronide saponin of oleanolic acid, is remarkably high in the rhizome and main root of Chinese wild ginseng.

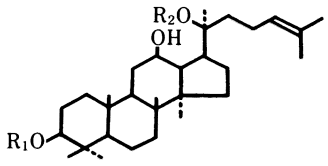
Keywords——ginseng; wild *Panax ginseng*; *Araliaceae*; HPLC; saponin content; ginsenoside; malonyl-ginsenoside; quantitative analysis

Ginseng, the famous oriental plant drug, is the root of *Panax ginseng* C. A. MEYER. This herb grows wild in cool and shady forests extending from Korea and North Eastern China to Far Eastern Siberia. Recently, the wild plant was also discovered near Mt. Fuji, Japan.[1] Because the wild plant is now very rare, it has been cultivated in Korea, China and Japan. From ginseng, a number of characteristic neutral dammarane saponins[2] and a glucuronide saponin of oleanolic acid named ginsenoside-Ro (**1**)[3] have been isolated. The acidic saponin, **1**, was first isolated from rhizomes of *P. japonicus* C. A. MEYER in a high yield by Kondo and Shoji.[4] being named chikusetsusaponin V. Recently, Kitagawa *et al.* reported the isolation of acidic malonyl-dammarane saponins from this drug.[5]

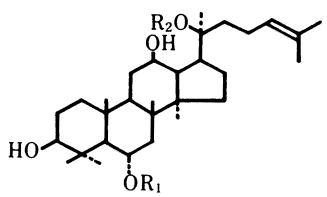
Ginseng saponins have been extensively analyzed by thin layer chromatography (TLC)[6] as well as high-performance liquid chromatography (HPLC),[7] and the evaluation of the cultivated materials as a crude drug has been conducted by analysis of saponins. However, no analytical study on the wild plant has appeared in the literature, mainly due to the much greater cost as compared with the cultivated materials. Recently, we have found that borate ion-exchange HPLC[8] and normal-phase HPLC on a column of new hard spherical hydroxyapatite[9] are useful for the analysis of neutral saponins of ginseng. The analysis of the



G-Ro (**1**)
=Chikusetsu-saponin-V



G-Rb_1 (**2**), G-Rb_2 (**3**)
G-Rc (**4**), G-Rd (**5**)
G-F_2 (**13**)



G-Re (**6**), G-Rf (**7**)
G-Rg_1 (**8**), G-F_1 (**12**)
G-F_3 (**14**)

R_1 = malonyl-sugar
R_2 = sugar
MG-Rb_1 (**9**), MG-Rb_2 (**10**)
MG-Rc (**11**)

G, ginsenoside; MG, malonyl-ginsenoside.
Abbreviations of saponins: see text.

Chart 1

saponins including acidic saponins (*vide supra*) by HPLC was also investigated.[10] By means of the combination of these procedures, we have compared the saponin compositions of long-aged wild ginseng and cultivated material (aged six years) as to the following major saponins (Chart 1): ginsenosides-Rb_1 (**2**), -Rb_2 (**3**), -Rc (**4**), -Rd (**5**), -Re (**6**), -Rf (**7**), and -Rg_1 (**8**) (neutral dammarane saponins), **1**, and malonyl-ginsenosides-Rb_1 (**9**), -Rb_2 (**10**) and -Rc (**11**).

Experimental

Plant Materials——Chinese wild ginseng (specimen CW): Collected in Jilin, North East Province of China, aged thirteen years. Dry weight: rhizome 0.55 g, main root 2.62 g, lateral roots 1.25 g. Japanese wild ginseng[2] (two specimens JW-1 and JW-2): collected near Mt. Fuji, Japan, aged thirteen years.[1] Dry weight: whole roots JW-1 2.11 g, JW-2 2.97 g. Leaves: JW-1 0.71 g. Roots of Chinese cultivated ginseng (CC): Cultivated in Jilin, China for six years and dried without peeling. Leaves of cultivated ginseng: Cultivated at Daikon-jima, Shimane, Japan for four years.

Authentic Samples of Saponins——All of the saponins were obtained during our serial studies on ginseng.[2]

Separation of Saponin Fraction for Analysis[10]——Material (0.5—3 g) was extracted with 70% MeOH (30 ml) for 30 min at room temperature five times to complete the extraction of all of the saponins without decomposition of malonyl-ginsenosides. The combined extracts were concentrated to dryness below 40°C. A suspension of the residue was passed through a column of highly porous polymer, MCI-gel CHP20P (1.0 × 10 cm, Mitsubishi Chem. Ind.), eluted with H_2O (100 ml), 40% MeOH (100 ml) and then MeOH (100 ml). The MeOH eluate was concentrated to dryness below 40°C, affording a saponin fraction which was subjected to analysis.

Two-Dimensional TLC (2D-TLC)——Silica gel: Kieselgel 60 F_{254} 20 × 20 cm (E. Merck, Art. 5554). Solvent 1: A mixture of 30 ml of the lower phase of $CHCl_3$–MeOH–H_2O (65 : 35 : 10) and 3 drops of AcOH. Solvent 2: A mixture of 30 ml of the upper phase of 1-BuOH–AcOEt–H_2O (4 : 1 : 2) and 3 drops of AcOH. A plate was developed with solvent 1 and then with solvent 2 in the two-dimensional mode. Detection: spraying 5% ethanolic H_2SO_4 followed by heating at 110 °C for a few minutes.

HPLC——A Trirotar-III HPLC apparatus (JASCO, Tokyo, Japan) equipped with a Shimadzu SPD-2A variable-wavelength UV detector was used (detection: UV 202 nm).

Condition A: On an octadecyl silica (ODS)-column, Ultron N-C_{18} (4.6 × 150 mm, Chromato Packings Centre, Tokyo). Mobile phase 1, CH_3CN–0.5% H_3PO_4 (20 : 80), for the qualitative and quantitative analysis of **6** and **8**; mobile phase 2, 50 mM KH_2PO_4 in 31% CH_3CN for the qualitative and quantitative analysis of other major neutral and acidic saponins.[10] Flow rate: 1.0 ml/min. Column temperature: 40 °C.

Condition B: On an amino-column, Ultron-NH_2 (4.6 × 250 mm, Chromato Packings Centre, Tokyo). Mobile phase: CH_3CN–1.0% H_3PO_4 (83 : 17). Flow rate: 1.0 ml/min. Column temperature: 40°C.[10]

Condition C: On a newly developed hydroxyapatite column, Pentax PEC 101 (7.5 × 100 mm, Asahi Optical Co., Ltd., Tokyo). Mobile phase: linear gradient from 90% to 70% aqueous CH_3CN in 30 min. Flow rate: 2.0 ml/min. Column temperature: ambient.[9]

Condition D: Borate ion-exchange mode, on an Asahipak ES-502N column (7.6 × 100 mm, Asahikasei Kogyo

Co., Ltd., Tokyo). Mobile phase: 0.25 M H_3BO_3 in 12.5% CH_3CN. Flow rate: 0.5 ml/min. Column temperature: 70°C.[8]

Results and Discussion

Identification of each major saponin in the wild specimens was substantiated by 2D-TLC as well as HPLC in the following four modes (Fig. 1 and 2); on a ODS column (condition A), on an amino column (condition B), on a new type of hydroxyapatite column and in the borate ion-exchange mode. No significant difference in the composition of the major saponins was observed between wild (Chinese and Japanese) and cultivated specimens.

Contents of the major saponins were determined by HPLC (condition A) according to the method of our previous report, as shown in Fig. 2.[10] The result obtained by this procedure was consistent with that obtained under condition B. As shown in Table I, in the case of cultivated ginseng, the saponin contents in the rhizome and the lateral roots of the Chinese wild specimen are evidently higher than in the main root. Malonyl-ginsenosides (**9**—**11**) were also present in wild specimens and the content of **9** in both the Chinese and Japanese wild specimens was somewhat higher than in the cultivated specimen.

It was found that the contents of **1** in the rhizome and the main root of the Chinese wild specimen were remarkably higher than in the cultivated specimen. Chemotaxonomy of *Panax* species has been based on comparison of saponins in the roots of cultivated ginseng (aged six years) with those of rhizomes of a variety of species of *Panax* (*P. japonicus*, *etc.*) growing wild in Japan, the Himalayas and the southwest province of China (aged more than ten years). It was reported that rhizomes of the latter wild *Panax* species contained a large amount of a variety of oleanolic acid saponins such as **1**, being chemotaxonomically distinguished from ginseng root which contains a relatively small amount of **1**.[1] However, the present results

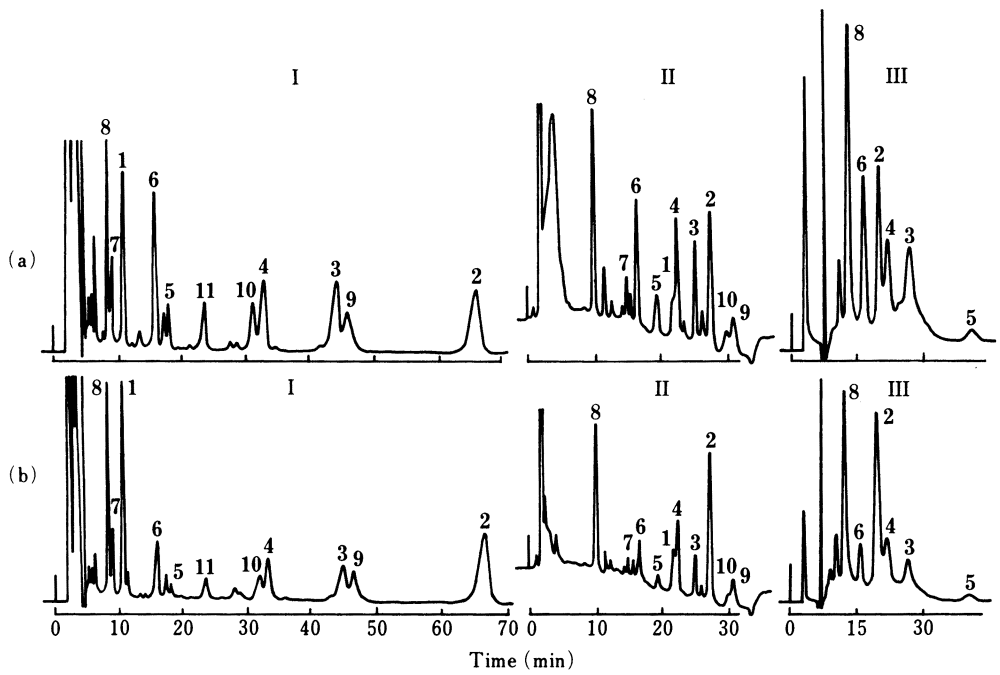


Fig. 1. HPLC Patterns of Cultivated and Wild *Panax ginseng*

(a) Cultivated ginseng, (b) wild *Panax ginseng* (China). I, amino column (condition B); II, hydroxyapatite column (condition C); III, borate anion-exchange column (condition D).

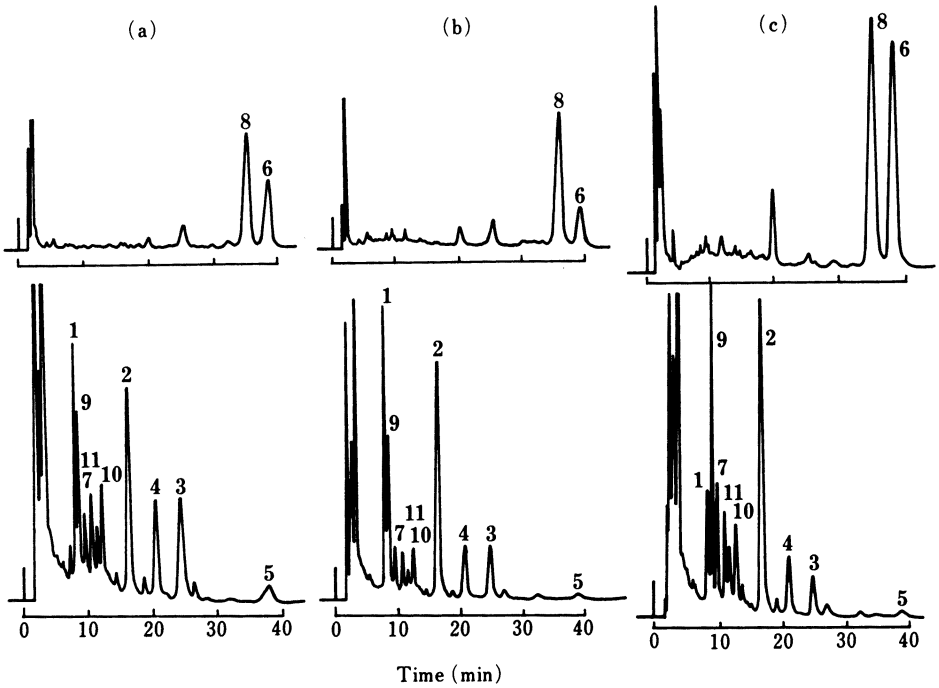


Fig. 2. HPLC Patterns of Cultivated and Wild *Panax ginseng* in Reversed-Phase Mode (Condition A)

(a) Cultivated ginseng, (b) wild *Panax ginseng* (China), (c) wild *Panax ginseng* (Japan). Upper, mobile phase 1 for analysis of **6** and **8**. Lower, mobile phase 2 for analysis of other major saponins.

TABLE I. Comparison of Saponin Contents and Compositions in Cultivated and Wild Ginseng

	CW			CC			JW-1 WR	JW-2 WR	JW-1 LE	CC LE
	R	M	L	R	M	L				
1	3.4	1.1	0.68	1.8	0.50	0.62	0.24	0.38	—	—
2	1.4	1.2	2.9	0.88	0.55	2.0	0.99	1.4	0.02	0.18
3	0.45	0.33	1.7	0.57	0.37	1.8	0.34	0.27	0.20	0.61
4	0.47	0.32	1.5	0.47	0.31	1.5	0.32	0.32	0.12	0.44
5	0.07	0.04	0.49	0.16	0.08	0.52	0.06	0.04	0.77	1.8
6	0.47	0.19	0.87	0.57	0.35	1.4	0.52	0.66	0.24	2.0
7	0.15	0.08	0.17	0.15	0.11	0.17	0.23	0.24	—	—
8	0.45	0.52	0.38	0.38	0.45	0.25	0.46	0.55	1.2	1.6
9	1.3	0.63	1.3	0.69	0.41	1.2	1.2	1.6	0.04	0.11
10	0.40	0.20	0.83	0.42	0.30	1.1	0.50	0.39	0.10	0.20
11	0.34	0.15	0.64	0.35	0.23	0.84	0.37	0.39	0.03	0.05
(%)	8.9	4.8	11.5	6.4	3.7	11.4	5.2	6.2	2.7	7.0

R, rhizomes; M, main roots; L, lateral roots; WR, whole roots; LE, leaves.

suggest that the chemotaxonomical comparison must be reinvestigated based on analysis of the same part of specimens of similar age. The revised chemotaxonomical classification will be reported elsewhere.

Contents of other major saponins in the wild specimens were found to be rather similar to

those of the cultivated specimens.

From leaves of cultivated *P. ginseng*, Yahara *et al.*[11] have isolated **2**—**6**, **8** and ginsenosides-F_1 (**12**), -F_2 (**13**) and -F_3 (**14**), the latter three of which are characteristic of the leaves. In the present study, no significant difference in the saponin composition was observed between the leaves of cultivated and wild (Japanese) specimens. The presence of a small amount of malonyl-ginsenosides was detected, but **1** and **7** were not detected in cultivated of wild (Japanese) specimens. Contents of **2**—**6** and **8**—**11** in the leaves were determined by HPLC not under condition A, but under condition B, because under condition A, the peaks of the above saponins were incompletely separated from each other. Contents of saponins, especially **5** and **6**, in the leaves of cultivated specimens were found to be relatively higher than those of the wild specimens.

Acknowledgements H. Yamaguchi and H. Matsuura are grateful to Dr. T. Fuwa for his encouragement throughout this study.

References

1) H. Kohda, H. Izumi, M. Nuno, S. Katsuki, S. Isoda, K. Gotoh and M. Satake, *Shokubutsu Kenkyu Zasshi*, **61**, 249 (1986).
2) O. Tanaka and R. Kasai, "Progress in the Chemistry of Organic Natural Products," Vol. 46, ed. by W. Herz, H. Grisebach, G. W. Kirby and Ch. Tamm, Springer-Verlag, Vienna, New York, 1984, p. 1; O. Tanaka, R. Kasai and T. Morita, *Abstracts of Chinese Medicines*, **1**, 130 (1986).
3) S. Sanada, N. Kondo, J. Shoji, O. Tanaka and S. Shibata, *Chem. Pharm. Bull.*, **22**, 421 (1974).
4) N. Kondo and J. Shoji, *Yakugaku Zasshi*, **88**, 325 (1968).
5) I. Kitagawa, T. Taniyama, T. Hayashi and M. Yoshikawa, *Chem. Pharm. Bull.*, **31**, 3353 (1983).
6) S. Sanada, J. Shoji and S. Shibata, *Yakugaku Zasshi*, **98**, 1048 (1978); T. M. Lee and A. D. Marderosian, *J. Pharm. Sci.*, **70**, 89 (1981).
7) F. Soldati and O. Sticher, *Planta Medica*, **38**, 348 (1980); H. Kaizuka and K. Takahashi, *J. Chromatogr.*, **258**, 135 (1983); T. Nagasawa, T. Yokozawa, Y. Nishino and H. Oura, *Chem. Pharm. Bull.*, **28**, 2059 (1980).
8) H. Yamaguchi, H. Matsuura, R. Kasai, K. Mizutani, H. Fujino, K. Ohtani, T. Fuwa and O. Tanaka, *Chem. Pharm. Bull.*, **34**, 2859 (1986).
9) R. Kasai, H. Yamaguchi and O. Tanaka, *J. Chromatogr.*, **407**, 205 (1987).
10) H. Yamaguchi, R. Kasai, H. Matsuura, O. Tanaka and T. Fuwa, *Chem. Pharm. Bull.*, **36**, 3468 (1988).
11) S. Yahara, K. Kaji and O. Tanaka, *Chem. Pharm. Bull.*, **27**, 88 (1979); S. Yahara, O. Tanaka and T. Komori, *ibid.*, **24**, 2204 (1976).