HISTOCHEMISTRY II[1]. GINSENOSIDES IN GINSENG (*PANAX GINSENG*, ROOT)

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ABSTRACT.—The location of the ginsenosides, active principle of *Panax ginseng* root, was determined histochemically. Ginsenosides were found localized outside of the root cambium, particularly in the periderm and outer cortex outside of the phloem. From this result the quality, as affected by the processing method of commercial ginseng, was discussed.

In our previous paper (1), we reported that the ginsenosides, active principles of ginseng, are localized in the periderm and cortex and not in the xylem and pith of *Panax ginseng* roots, as determined by a qualitative histochemical method. In the present paper, in order to confirm the distribution of the ginsenosides, the amounts of ginsenosides in various tissues or parts of the ginseng root were determined.

Quantitative analysis of the ginsenosides was performed by gas-chromatographic (glc) determination of trimethylsilyl(TMS)-sapogenols. The amounts of saponins was expressed as ginsenoside Rbc (from peak area of TMS-panaxadiol) or Rg (from TMS-panaxatriol) equivalent values (2). The results thus obtained confirmed our previous finding (1) that the ginsenosides are localized outside of the root cambium. The ginsenoside Rbc content differed in the various parts of the root (e.g. main root, lateral root, adventitious root, etc.) and the total ginsenoside content increased with plant age, as has been reported previously (2, 3, 4, 5, 6). Based on these findings, the processing procedure of fresh ginseng roots for commercial products appears to result in a crude drug of inferior quality.

MATERIALS AND METHODS

The 1- to 6-year-old *Panax ginseng* C. A. Meyer plants used in the study were cultivated at Nagano Prefecture in Japan (fig. 1). The plants were separated into the parts to be analyzed and powdered after drying.

Commercial ginseng products used in the study are shown in table 1.

1) QUANTITATIVE ANALYSIS OF GINSENOSIDE AGLYCONES BY GLC.—The methanol extract of defatted ginseng powder was hydrolyzed with 5% H_2SO_4-ethanol (3:1) on a boiling water-bath for 6 hours, according to the method of Tanaka et al. (2). The ether-soluble aglycone fraction of the hydrolysate was separately treated with alkaline water to remove oleanolic acid (derived from ginsenoside Ro). The residue, which mainly contained panaxadiol (derived from ginsenoside Rbc group saponins) and panaxatriol (derived from ginsenoside Rg group saponins), was treated with *N*-trimethylsilylimidazole for 1 hour at room temperature. TMS products were gas-chromatographed on a Shimadzu GC-6A[detector:FID; column: glass column, 3 mm x 2 m filled with 1.5% SE-30 on chromosorb W(60–80 mesh, AW, CMCS); column temperature: 260°; carrier gas: 10 kg/cm² of He] with digital integrator ITG-4A.

After authentic samples were analyzed, in order to prepare the calibration curves for ginsenoside Rb_1 and Rg_1, glc peak areas of TMS-panaxadiol, derived from Rb_1, and TMS-panaxatriol, derived from Rg_1 were integrated. According to the calibration curves, the ginsenoside Rbc and Rg group saponins were determined by the peak area obtained from the ginseng samples.

2) DENSITOMETRIC PATTERN OF GINSENOSIDES ON TLC PLATE.—One gram of dry ginseng powder was extracted two times with 100 ml of methanol under reflux for 2 hours, and the extract was

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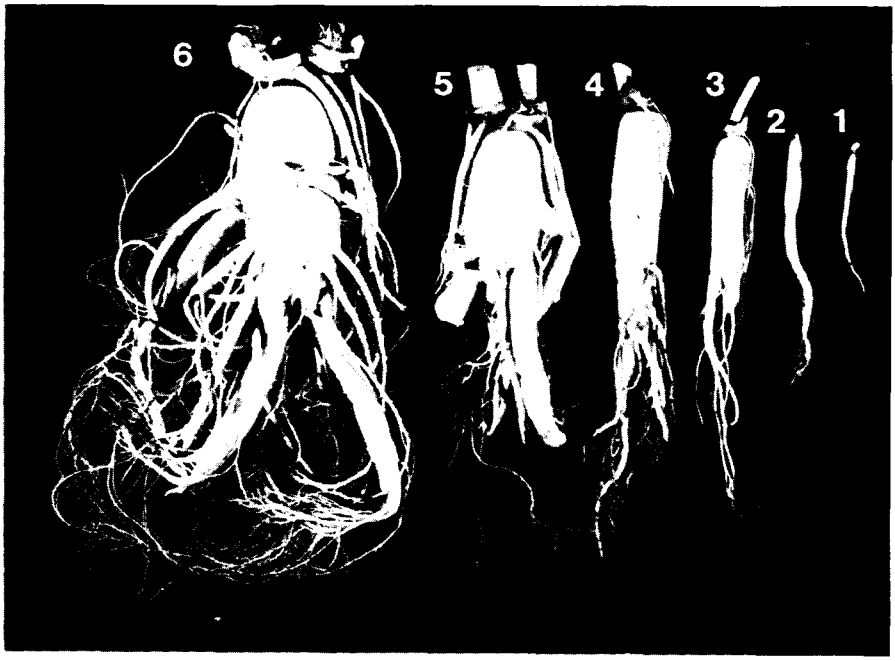


FIG. 1. 1- to 6-year-old roots of *Panax ginseng*.

concentrated under reduced pressure. The residue was treated with 1 ml of methanol. The methanol-soluble portion served as the sample solution for thin-layer chromatography (tlc). Conditions for tlc (tlc-profile analysis) (7, 8) were the same as in our preceding paper (1).

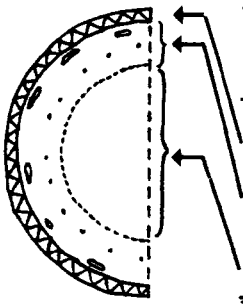
RESULTS AND DISCUSSION

(*A*) *Ginsenoside content in different tissues of the main root of ginseng.* The main root of a 6-year-old ginseng plant was separated into the periderm, cortex,

TABLE 1. *Ginsenoside content of commercial ginseng products.*

ginsenoside content / ginseng products	Calcd. for Rbc (%)	Calcd. for Rg (%)	Total (%)
White Ginseng (Korea, 6-year-old, with peel)	0.5	0.1	0.6
White Ginseng (North Korea, 3-year-old, with peel)	0.4	0.4	0.8
White Ginseng (Korea, Kyoku-jin in Japanese, 4-year-old, without peel)	0.8	1.1	1.9
White Ginseng (Korea, Shoboshi-ninjin or Kiboshi-ninjin in Japanese, 4-year-old, with peel)	1.3	1.2	2.5
White Ginseng (Nagano, Japan, Shoboshi-ninjin or Kiboshi-ninjin, 6-year-old, with peel)	1.0	1.4	2.4
Red Ginseng (Korea, 6-year-old, with peel)	0.7	0.3	1.0
Adventive Root of Ginseng (Korea, Taba-ke in Japanese, 6-year-old, with peel)	2.2	1.7	3.9
Lateral Root of Ginseng (Aizu, Japan, Hige-ninjin in Japanese, 6-year-old, with peel)	1.4	1.0	2.4
Ginseng Peelings (Korea, Jin-pi or San-pi in Japanese, 6-year-old)	2.4	3 4	5 8

and xylem, and the ginsenoside content of each tissue was determined from the glc peak areas of TMS-panaxadiol and TMS-panaxatriol (fig. 2). Ginsenoside



	weigth ratio	Calcd. for Rbc group(%)	Calcd. for Rg group(%)
periderm	1	1.5	0.9
cortex	6	4.6	2.4
xylem	12	0.0	0.0

FIG. 2. Ginsenoside content in various tissues of the main root of the 6-year-old ginseng.

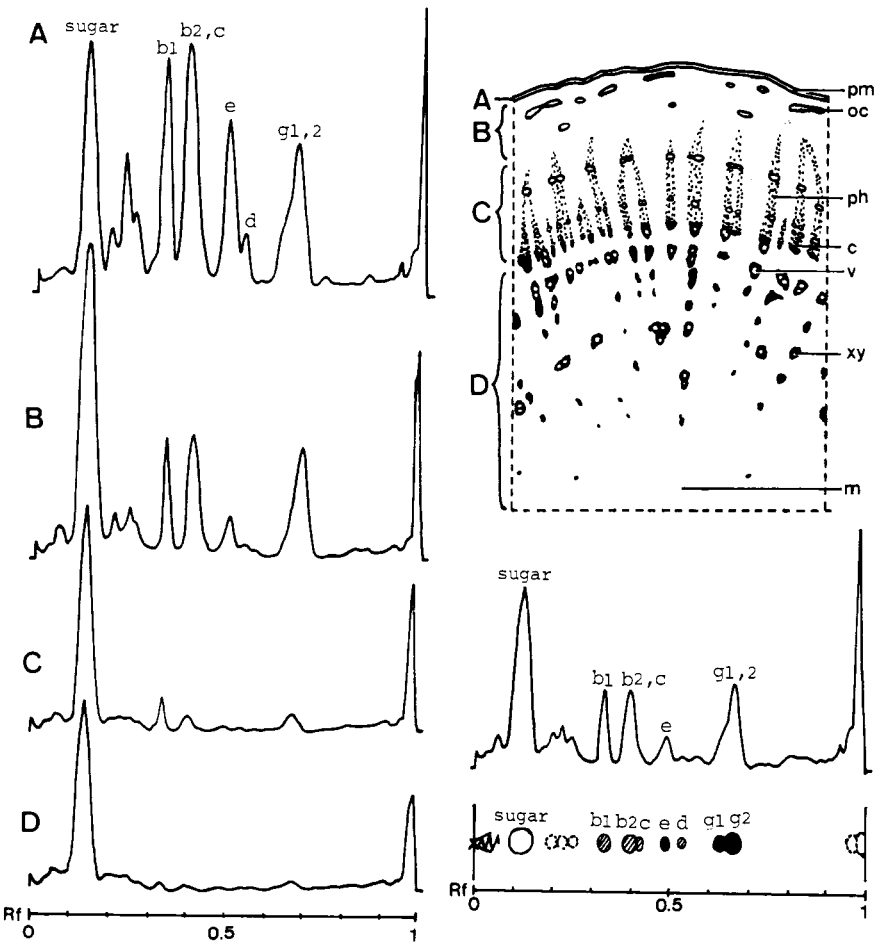


FIG. 3. Tlc-profile of methanol extracts obtained from different tissues of the main root of 6-year-old ginseng.
pm: periderm, oc: oil canal, ph: phloem, c: cambium, v: vessel, xy: xylem, m:pith, w: whole tissue
b_1, b_2, c, e, d, g_1, g_2: ginsenoside Rb_1, b_2, c, e, d, g_1, g_2

content in the periderm and cortex were 2.5% and 7%, respectively. Only traces of ginsenosides could be found in the xylem and pith.

Ginsenoside of four tissues (A, B, C, and D in fig. 3) separated from the main root of a 6-year-old ginseng plant were compared by means of tlc-profiles of the tissues. The clear peaks representing some ginsenosides in the tlc-profile were obtained from the samples taken from the periderm (A), but only slight peaks were obtained in the sample form phloem(C). None was obtained in the sample from xylem and pith(D), which comprise most of the main root.

Thus, the ginsenosides were found localized outside of the cambium and primarily in the periderm and outer cortex. This finding is in agreement with our previous report (1) in which localization of the ginsenosides was investigated histochemically with a saponin detecting reagent.

(B) Ginsenoside content in different parts of the root (fig. 4, 5). The sapogenol composition and content of the rhizome (Rozu in Japanese), main root, lateral root (Hige-ninjin in Japanese), and adventitious root (Taba-ke in Japanese) were determined by glc. The content of the ginsenoside Rg group showed no significant difference in each part (i.e., 1.4–1.9%), which is in accordance with previous reports (4, 5, 6, 7, 8).

Ginsenosides in the leaf (L), stem (S), rhizome (I), main root (II, III), lateral root (IV, V), root hair (VI) and adventitious root (VIII) of the 6-year-old ginseng plant were determined by tlc-profile analysis (fig. 5). The ginsenoside composition of the leaf (L) was considerably different from that of the other parts, and the peaks representing ginsenosides Rd and Rg in the leaf were higher than those in the root, as in previous reports (9, 10). The stem had smaller peaks representing

	Calcd. for Rbc group(%)	Calcd. for Rg group(%)
rhizome	0.8	1.8
adventive root	2.9	1.9
main root	1.9	1.4
lateral root	4.8	1.7

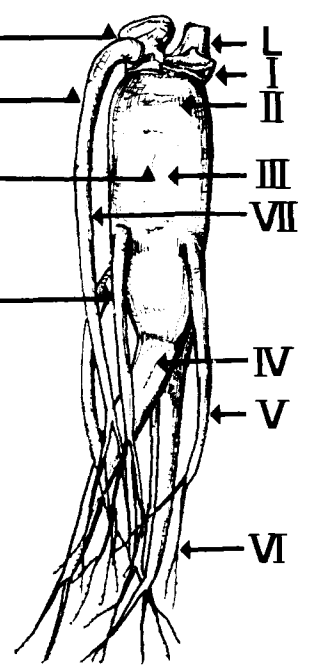


FIG. 4. Ginsenoside content of various parts of a 6-year-old ginseng root.

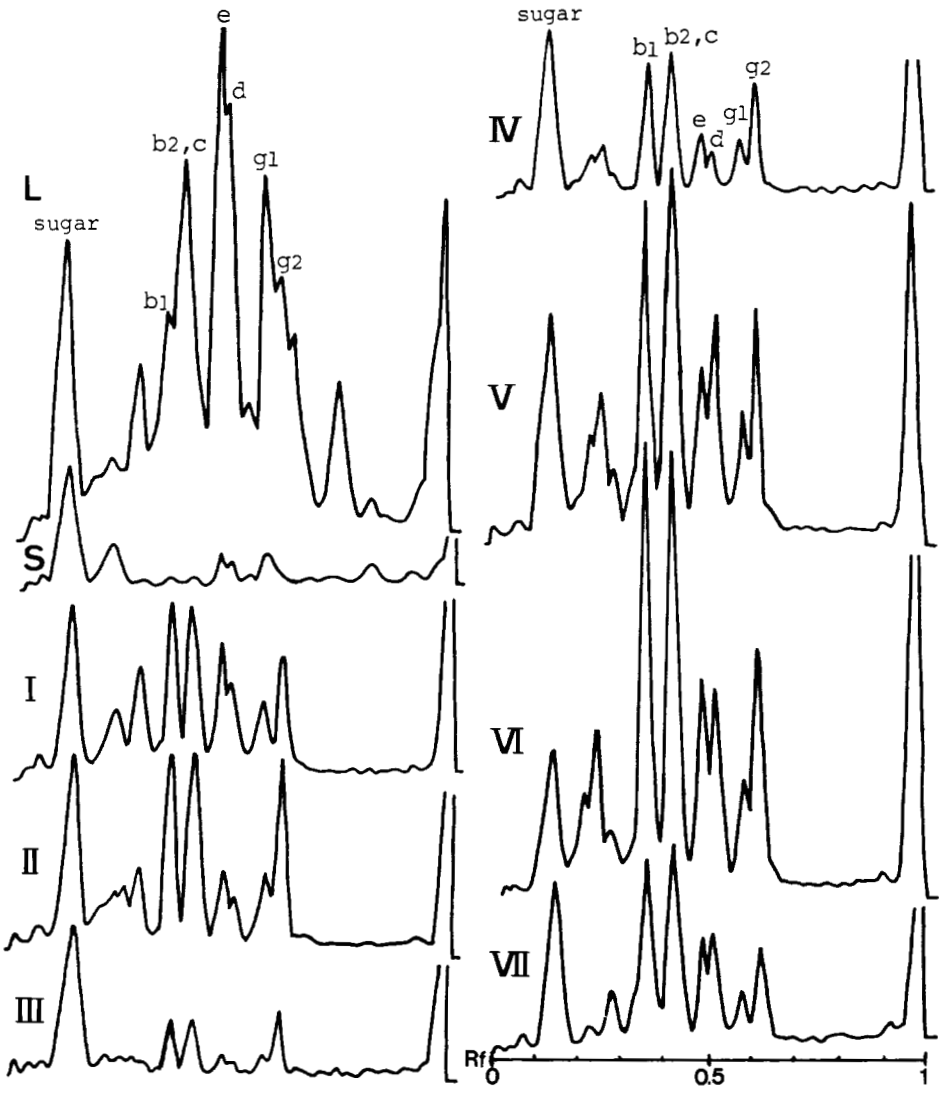


FIG. 5. Tlc-profile of methanol extracts of various parts of a 6-year-old ginseng root. L: leaf, S:stem, I: rhizome, II: main root (upper), III: main root (center), IV, V: lateral root, VI: root hair, VII: adventive root (I VII: see fig. 4).

ginsenosides Rd, Re and Rg_1 than did the root. The tlc-profile of the rhizome (I) was less pronounced than that of the root. It is apparent than thinner roots contain higher amounts of ginsenosides Rb_1, Rb_2 and Rc, compared with the tlc-profile of the main root (II, III), lateral root (IV, V) and root hair (VI).

(*C*) *Ginsenoside content of ginseng plants based on age* (*fig. 6*). A comparison of the tlc-profiles obtained from 1- to 6-year-old ginseng roots showed the ginsenoside composition relative to the growth period (fig. 6). The 1- to 2-year-old roots

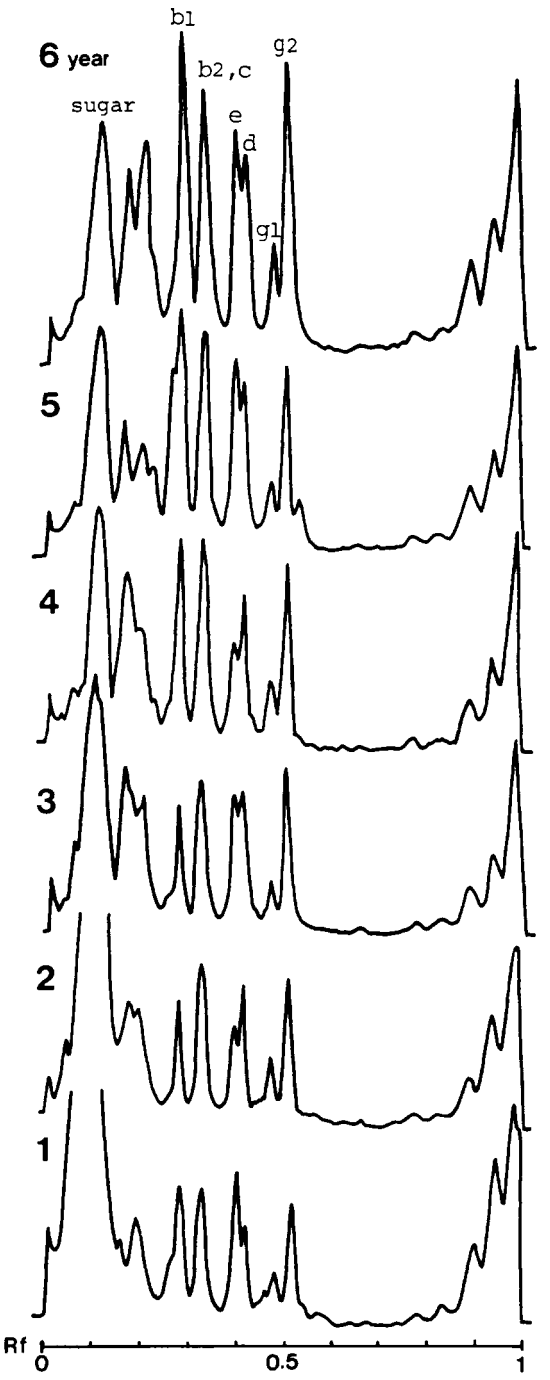


FIG. 6. Tlc-profile of methanol extracts of the ginseng at different ages (1- to 6-year-old).

contained carbohydrates as the major components and ginsenosides as minor components. Higher levels of ginsenosides were observed in 5- to 6-year-old roots than in 1- to 3-year-old ones. It can be stated that 1- or 2-year-old ginseng plantlets (Mabiki-ninjin in Japanese) are unsuitable substitutes for the commercially available lateral roots of 5- or 6-year-old ginseng (Hige-ninjin in Japanese), based on these findings (1).

(*D*) *Ginsenoside content as affected by the processing procedure of the ginseng products.* White ginseng is usually prepared by removing the peel from ginseng root. Commercially available ginseng peel (Jin-pi or San-pi in Japanese) consists mainly of periderm and contains 2.4% of the ginsenoside Rbc group and 3.4% of the ginsenoside Rg group. Removal of the peel during processing may render white ginseng products less desirable due to the reduced ginsenoside content (1).

Red ginseng is made by steaming 6-year-old ginseng roots. The exudate obtained during the steaming process was found to contain 4.1% of the Rbc group and 2.3% of the Rg group of ginsenosides. Thus, the steaming procedure in making red ginseng may also reduce the content of active principles in ginseng.

(*E*) *Ginsenoside content in some commercial ginseng products* (*table 1*). The amount of ginsenosides in some commercial ginseng products was determined by glc analysis. Ginsenoside content varied according to the processing method for ginseng products. The total saponin content was 5.8% for ginseng peelings (Jin-pi or San-pi in Japanese), 3.8% for adventitious roots (Taba-ke in Japanese), and 2.3–2.5% for lateral roots (Hige-ninjin in Japanese) and for ginseng roots with peels (Kiboshi-minjin or Shoboshi-ninjin in Japanese). Ginseng without peels contained only 0.6–0.9% of ginsenosides. This clearly shows that the removal of peels in making white ginseng might affect the quality of the ginseng products.

Recently, some peeled white ginseng products with low ginsenoside content have been marketed in Japan. Thus, one must exercise great care in the selection of commercial ginseng products.

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