HISTOCHEMISTRY. I. GINSENOSIDES IN GINSENG (*PANAX GINSENG* C. A. MEYER, ROOT)

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ABSTRACT.—The location of the ginsenosides, active principles in *Panax ginseng* root tissue, was studied histochemically in order to estimate ginseng quality from the ginsenoside content. When a piece of the root was treated with an ethanol solution of silicotungstic acid, a saponin-detecting reagent, ginsenosides were found to be localized outside of the cambium in the root tissue, i.e., in the periderm and cortex. Since the peel is removed from commercial white ginseng, it contains fewer ginsenosides because the active principle is removed with the peel.

Ginseng (*Panax ginseng* C. A. Meyer, root) has been an important crude drug used in Chinese traditional medicine. Recently, in the field of pharmacognosy, scientific studies have been conducted to elucidate the medicinal value of ginseng, namely, chemical studies on the saponins named ginsenosides Rx (x = o, b_{1-3}, c, d, e, f, 20-gluco-f, g_{1-2}, h_1) and various other components (1–9). Other aspects, such as the pharmacological profile (10–14), the role of the ginsenoside in metabolism of lipids and sugars (15–19) and in RNA and protein synthesis (20–22), are also being investigated. From these studies, the ginsenosides are known to be the active principles in ginseng.

In the present investigation, the ginsenoside content was used as an index of ginseng quality. It would be beneficial to know where in the plant the ginsenosides are found for the purpose of improving the method of ginseng processing. Accordingly, we initiated histochemical studies to find their location in the root. Treatment of a cross section of fresh *Panax ginseng* root with a saponin-detecting reagent revealed that most of the ginsenosides are localized outside of the cambium, i.e., in the cortex, and not in the xylem or pith, the latter two zones comprising most of the root.

MATERIALS AND METHODS

The roots of 1- and 6-year-old *Panax ginseng* C. A. Meyer cultivated in Nagano Prefecture in Japan were used for the experiments.

1) The transverse cut surface on fresh ginseng root was stamped onto a thin layer chromatography (tlc) plate (silica gel $60F_{254}$, Merck), in order to transfer some of the constituents to the plate. Then a 2% (w/v) ethanol solution of silicotungstic acid, a saponin detecting reagent, was sprayed on the tlc plate, and the plate was heated to develop the color.

2) A mucilaginous exudate, originating from the oil canals in the cortex of a fresh ginseng root was dissolved in methanol and applied to a tlc plate. After development with chloroform-methanl-water (6:4:1), the plate was sprayed with the saponin detecting reagent (1% $Ce(SO_4)_2$/ 10% H_2SO_4), heated to bring out the color, and scanned with a densitometer (Shimadzu Dual-Wave-length TLC Scanner CS-910; reflection, linear scan, single wave-length 560nm). Authentic ginsenosides obtained from ginseng were also chromatographed for comparison.

3) Roots were dried under natural conditions, a 2–3 mm thick cross section was sprayed with an ethanol solution of silicotungstic acid and heated to develop the color.

RESULTS AND DISCUSSION

(A) ANATOMICAL CHARACTERISTICS OF THE MAIN ROOT (fig. 1).

A cross section of the ginseng root showed that the outer part of the root is composed of a yellowish-brown periderm, which is easily peeled off to reveal large oil canals distributed in a circle. Small oil canals are also distributed in the

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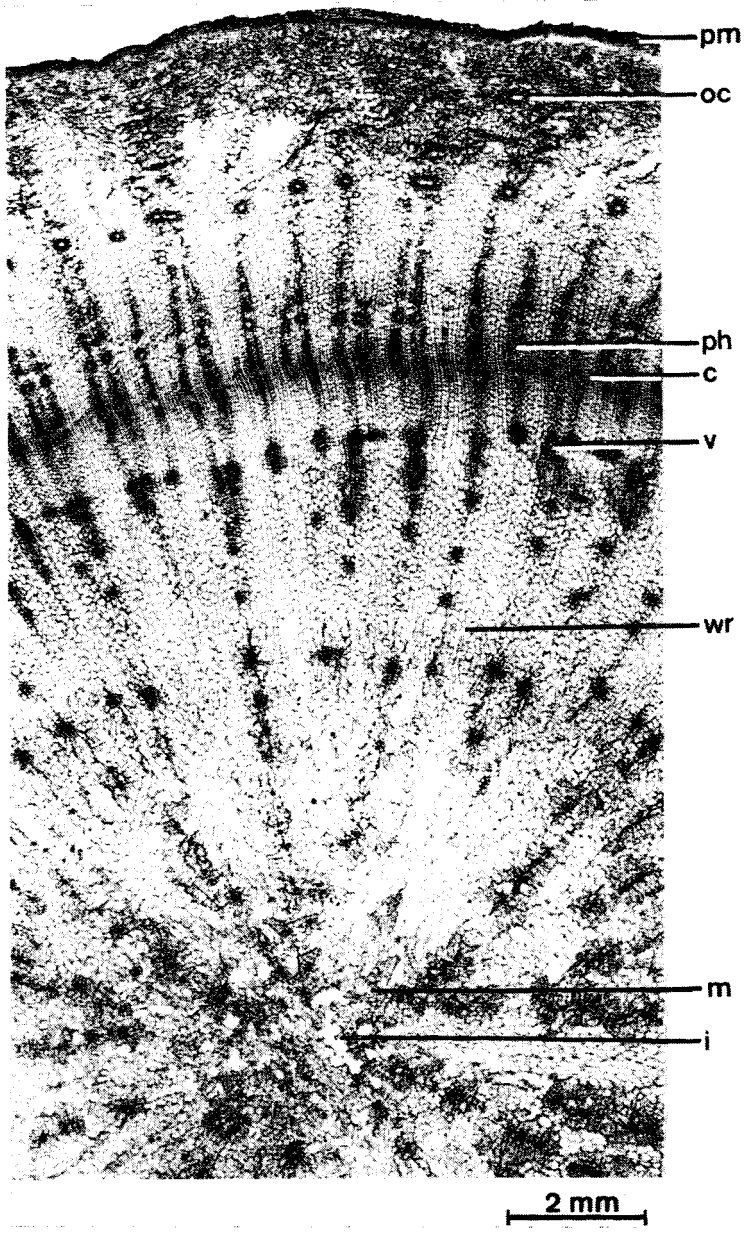

FIG. 1. Transverse section of the main root of the *Panax ginseng* (6 years old). pm: periderm, oc: oil canal, ph: phloem, c: cambium, v: vessel, wr: wood ray, m: pith, i: intercellular space.

phloem. Two to three associated vessels were found arranged radially in the xylem tissue, and large intercellular spaces were observed in the pith.

(**B**) THIN LAYER CHROMATOGRAM FROM APPLICATION OF THE CUT SURFACE OF THE FRESH GINSENG ROOT TO THE PLATE (fig. 2).

By stamping the transverse cut surface of the fresh root tissue on a tlc plate, the constituents of the oil canal (mucilage) in the cortex were transferred to the

plate. Color formation on the plate after spraying with the saponin-detecting reagent suggested that saponin was present in the mucilage.

(C) TLC-PROFILE OF GINSENG MUCILAGE (fig. 3).

A densitometory was used to analyze a thin-layer chromatogram of the mucilage exuding from the oil canals of the ginseng root (tlc-profile analysis) (23). Color spots were observed at corresponding areas on the chromatogram occupied by authentic ginsenosides. The finding that ginsenosides are in the oil canal

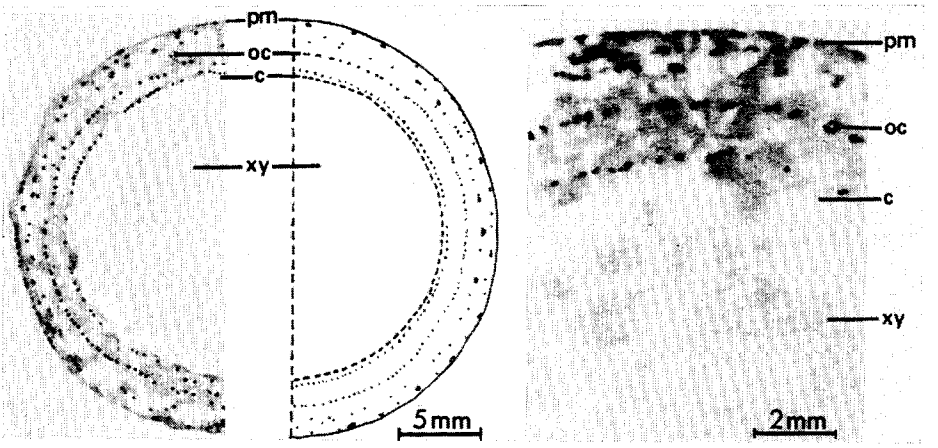

FIG. 2. Distribution pattern of saponins in the cross section of 6-year-old fresh ginseng. Cut surface was stamped in the plate for the tlc and treated with a saponin reagent.

exudate of *P. ginseng* root is in good accord with the reports of the alkaloids of *Papaver somniferum* (24, 25) and diterpenoid irritants of *Euphorbia* species (26, 27) being found in their latices; thus it was not unexpected to find ginsenosides in the oil canal exudate of *P. ginseng* roots.

(D) MAIN ROOT OF A 6-YEAR-OLD GINSENG (fig. 4 and 5).

When a cross section of the main root from 6-year-old ginseng was treated with the ethanol solution of silicotungstic acid, the outermost periderm and oil canals and surrounding zones in the cortex turned purple. The xylem and pith, which comprise most of the main root, were not stained, indicating a lack of detectable quantities of ginsenosides in these areas (fig. 4A).

Treatment of a radial section of ginseng root showed similar results, i.e., oil canals and surrounding zones turned purple (fig. 4B). A tangential section of the root showed a reticulated distribution of oil canals in the cortex (figs. 4C, 5).

From these results, the ginsenosides were considered to be localized in the oil canals and surrounding zones of the cortex of *Panax ginseng* root. Quantitative analyses of ginsenosides in the outer and inner parts of *P. ginseng* root have been reported (30), but a further more detailed paper concerning distribution of ginsenosides in periderm cortex, phloem, and xylem of root will be presented (28). In the root of *Bupleurum* species, saikosaponins have also been found to be distributed mainly in the cortex by Nagoshi *et al.* (29). Such localized distribution of saponins in the outer part of the root tissue may be of interest in the field of plant physiology.

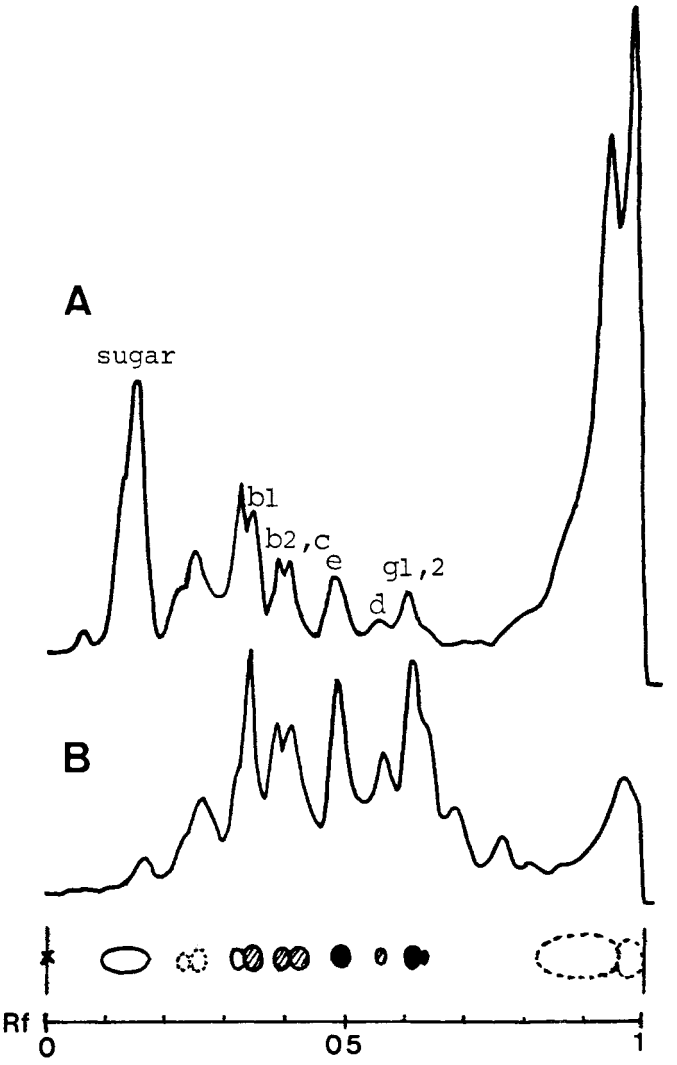

FIG. 3. Tlc-Profile of mucilage exuded from oil canals of the ginseng root.
A: mucilage B: authentic ginsenosides

Commercial white ginseng is made by removing the outer peel of the root, the part which contains the periderm and some parenchyma-containing oil canals. Such processing should result in an appreciable loss of ginsenosides and is not recommended.

(**E**) COMPARISON OF THE ROOT HAIRS FROM 6-YEAR- AND 1-YEAR-OLD GINSENG ROOTS (fig. 6).

Commercial white ginseng, specified as comprising the lateral roots of ginseng (Hige-ninjin in Japanese), consists of lateral roots and root hairs of 6-year-old ginseng. However the roots of 1- or 2-year-old plantlets (Mabiki-ninjin in Japanese) are sometimes found in admixture with 6-year-old roots. Oil canals and

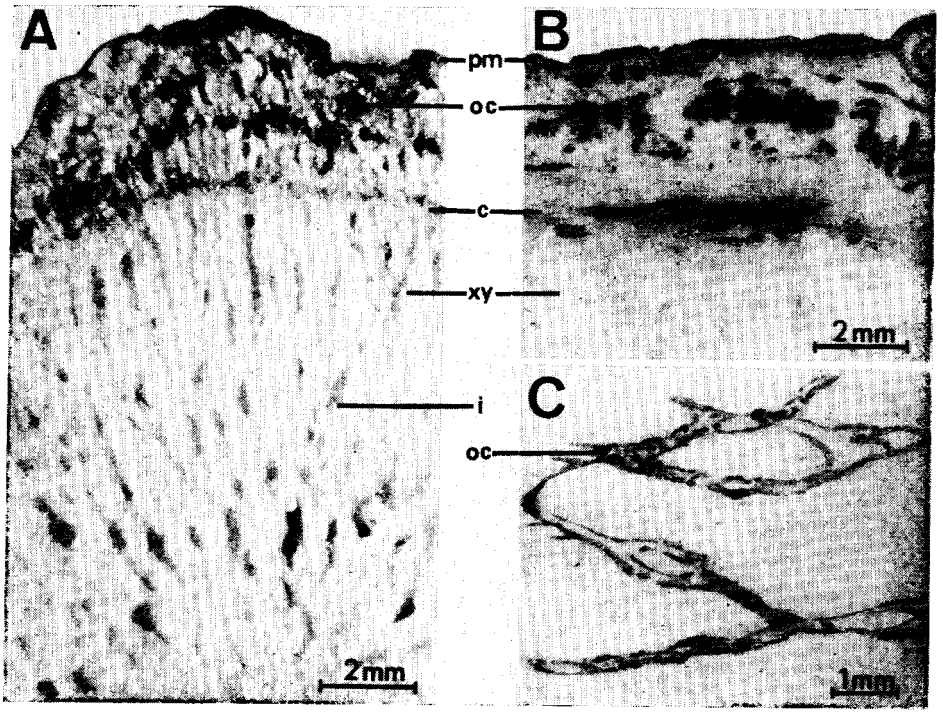

FIG. 4. Color reaction of a ginseng root obtained after silicotungstic acid treatment. 6-year-old ginseng (A: main root, cross section; B: main root, radial section; C: oil canals in main root cortex, tangential section).

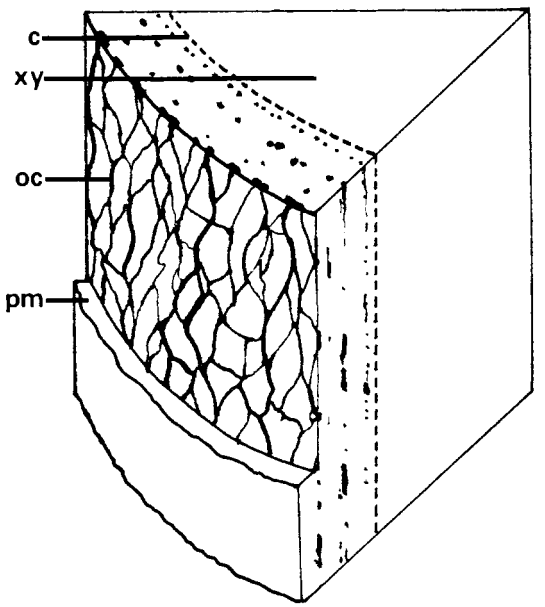

FIG. 5. Oil canals in a ginseng root, three dimensional diagram.

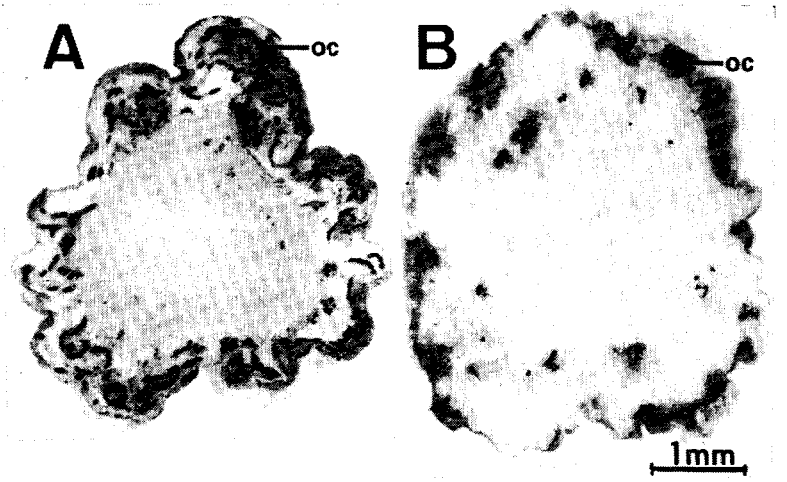

FIG. 6. Color reaction of the cross section of ginseng root.
A: lateral root of 6-year-old ginseng
B: main root of 1-year-old ginseng

their surrounding zones of a cross section of the lateral root from a 6-year-old ginseng turned purple after treatment with silicotungstic acid, as did corresponding areas of the main root (fig. 6A). A cross section of a 1-year-old ginseng root gave only a faint reaction with this reagent (fig. 6B). Considering that the content of saponin is an important factor in determining the quality of ginseng, plantlet ginseng would not be a suitable substitute for the lateral roots from 6-year-old ginseng plants (8).

TABLE 1. Ginsenoside content of commercial ginseng products.

Ginseng products	Ginsenoside content		
	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

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