Ginsenosides in Roots and Leaves of American Ginseng†

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The six major ginsenosides, Rg_1, Re, Rb_1, Rc, Rb_2, and Rd, in roots and leaves of American ginseng have been isolated and quantified by high-performance liquid chromatography. In 4-year-old roots, the main ginsenosides were Re and Rb_1, and together they accounted for >75% of the total ginsenosides. In leaves, the concentration and composition of ginsenosides varied with the maturity of the leaf tissue. One-month-old leaves contained 1.33−2.64 g ginsenoside/100 g dry weight, and the ginsenoside Re accounted for >50% of the total concentration. In mature, 4-month-old leaves, the total ginsenoside content ranged from 4.14 to 5.58 g/100 g dry weight, and the ginsenosides Re and Rd each accounted for ∼40% of the total ginsenosides. The production site of ginseng influenced the ginsenoside contents of roots and leaves. However, few significant correlations were found between root and leaf ginsenosides and between ginsenoside levels and mineral composition of the leaves and soil.

Keywords: *Panax quinquefolium; saponins; ginsenosides; HPLC analysis; soil fertility; leaf tissue nutrient status*

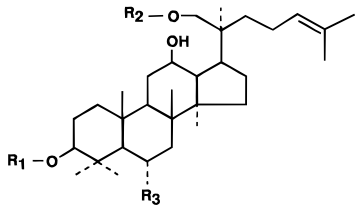
INTRODUCTION

American ginseng (*Panax quinquefolium* L.) is a perennial aromatic herb of eastern North America. It has a long, fleshy root, the shape of which somewhat resembles the human body. Native North Americans used ginseng root as part of their traditional medicine and as an aphrodisiac. Trade in American ginseng began at the beginning of the 1700s, when it was discovered that American ginseng roots possessed properties similar to those of the ginseng from China (*Panax ginseng* C. A. Meyer).

Until quite recently, American ginseng was dug from the wild. As native stands declined, commercial production started in Quebec and later in Wisconsin and Ontario. In British Columbia, commercial ginseng production began in the early 1980s. Presently, >2300 ha of American ginseng, valued at ∼$250 000/ha, are in production in Canada.

The active constituents of ginseng are dammarane saponins, commonly referred to as ginsenosides (Shibata et al., 1985; Tanaka, 1994). The most abundant ginsenosides present in American ginseng are Rb_1, Rb_2, Rc, and Rd, which possess 20(S)-protopanaxadiol as an aglycon; and ginsenosides Rg_1 and Re, which possess 20(*S*)-protopanaxatriol as an aglycon (Figure 1). Several pharmacological properties have been reported for ginsenosides or ginseng, including effects on the central nervous system, tranquilizing and antipsychotic actions, protection from stress ulcers, increased gastrointestinal mobility, antifatigue action, endocrinological effects, enhancement of sex behavior, and acceleration of both metabolism and synthesis of carbohydrates, lipids, and proteins (Tanaka, 1994).

Quantitative differences in total and individual ginsenosides vary depending on the species, such as *P. ginseng* and *P. quinquefolium* (Lewis, 1988); growing environment, such as wild and cultivated (Betz et al., 1984); soil and fertility conditions (Konsler et al., 1990); age of the roots (Soldati and Tanaka, 1984); different parts of the plant (Soldati and Sticher, 1980); and extraction methods (Schulten and Soldati, 1981). Quantitative high-performance liquid chromatography (HPLC) analyses of ginsenosides have been reported by several authors (Soldati and Sticher, 1980; Pietta et al., 1986; Kanazawa et al., 1987). However, almost all of these studies have focused on the analyses of ginsenosides in *P. ginseng*, and no systematic analysis of ginsenosides in American ginseng has been reported.



	R_1	R_2	R_3
R_{b1}	-glc[2→1]glc	-glc[6→1]glc	-H
R_{b2}	-glc[2→1]glc	-glc[6→1]ara(p)	-H
R_c	-glc[2→1]glc	-glc[6→1]ara(f)	-H
R_d	-glc[2→1]glc	-glc	-H
R_e	-H	-glc	-O-glc[2→1]rha
R_f	-H	-H	-O-glc[2→1]glc
R_{g1}	-H	-glc	-O-glc

glc : glucose ara(p) : α-Larabinopyranose ara(f) : α-Larabinofuranose rha : rhamnose

Figure 1. Structures of ginsenosides in ginseng.

The aim of the present investigation was to study the variation of ginsenosides in roots and leaves of American ginseng grown in three different regions of British Columbia, Canada, and to examine the effects of soil fertility and leaf tissue nutrient status on ginsenoside levels in roots and leaves.

MATERIALS AND METHODS

Roots and leaves of 4-year-old American ginseng (*P. quinquefolium* L.) and soil samples were collected in the summer of 1994 from each of nine commercial ginseng fields in British Columbia. The samples consisted of leaves chosen randomly in the middle of June from six plants per sampling site, and samples of leaves, roots, and soil taken in early September.

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Extraction, Purification, and Analysis of Ginsenosides. All the leaf and root samples were refrigerated and then freeze-dried at −65 °C within 3 days of sampling. Freeze-dried tissue was ground in a Wiley mill and stored at −35 °C until analyzed. Each ground, freeze-dried sample (200 mg) was weighed into a PTFE-stoppered tube and sonicated with 5 mL of 80% (v/v) methanol/water for 40 min in a Bransonic ultrasonic bath. The mixture was centrifuged, and the supernate was filtered through a 0.45-μm hydrophilic Durapore membrane filter (Millipore Corp., Bedford, MA). The crude extract was taken to dryness under a stream of nitrogen, redissolved in 10 mL of water, and refiltered. Two and a half milliliters of the aqueous extract was applied to a C_{18} solid-phase extraction cartridge (Waters Chromatography C_{18} Sep-Pak Classic, 360 mg, preconditioned with 2 mL of methanol and 5 mL of water), and the cartridge was washed with 5 mL of 30% (v/v) methanol. Ginsenosides were eluted with 5 mL of methanol into a NH_2 solid-phase extraction cartridge (Waters Chromatography Sep-Pak Vac NH_2, 500 mg, preconditioned with 6 mL of water and 6 mL of methanol). The cartridges were further washed with 1 mL of methanol. The eluate, which contained purified ginsenosides, was taken to dryness under a stream of nitrogen. The dried extract was redissolved by sonication and vortexing in 1.0 mL of 25% (v/v) acetonitrile/water, filtered through a 0.45-μm membrane filter, and immediately analyzed for ginsenoside content by HPLC.

Ginsenosides Rb_1, Rb_2, Rc, Rd, Re, and Rg_1 were measured on a Hewlett-Packard 1084B HPLC with gradient elution and a reversed-phase (RP) column (Spheri-5 RP-18, 4.6 × 220 mm, 5-μm packing, with RP-18 guard column, 4.6 × 30 mm; Brownlee Labs, Santa Clara, CA). Mobile phases were (A) water and (B) acetonitrile (HPLC grade; J. T. Baker Inc., Phillipsburg, NJ), with a flow rate of 1.5 mL/min and the following gradient: 0−16 min, 21.5% B; 16−50 min, 21.5−40% B; 50−52 min, 40−95% B; 52−55 min, 95% B; 55−60 min, 95−21.5% B; and 60−70 min, 21.5% B. Detection was by UV spectrophotometry at 203 nm, with a reference wavelength of 595 nm. Samples were introduced by autoinjector, with a 20- or 50-μL injection volume.

The concentration of ginsenosides was determined from standard curves prepared by injecting different volumes of stock solutions of authentic ginsenosides Rb_1, Rb_2, Rc, Rd, Re, and Rg_1 that were purchased from Carl Roth GMBH (Karlsruhe, Germany).

Leaf and Soil Analyses. Leaf and soil samples were analyzed for nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca) in triplicate, according to standard procedures (AOAC, 1980). Soil samples were also assayed for organic matter (OM) and pH. Nitrate N was extracted with 0.25 N HOAc + 0.015 N NH_4F, and its concentration was determined as nitrite by an automated copper−cadmium reduction procedure and color development by sulfanilamide and *N*-(1-naphthyl)ethylenediamine dihydrochloride. Phosphorus was determined by extraction with 0.25 N HOAc and 0.015 N NH_4F at a 1:10 (v/v) soil:solution ratio, and its concentration was measured on an inductively coupled argon plasma spectrophotometer (ARL 34000). Extractable Ca, Mg, and Na were determined by extraction with a 0.25 N acetic acid and 0.015 N ammonium fluoride solution at a 1:10 (v/v) soil:extractant ratio, and their concentrations were measured on an inductively coupled, argon plasma spectrophotometer.

Statistical Analysis. Statistical data analysis was carried out on a Microvax computer with a GLM procedure, Duncan's New Multiple Range Test, and Pearson's Correlation of SAS (SAS, 1985). Correlation coefficients were calculated between mineral levels in leaf and soil samples and ginsenoside contents of young and mature leaves and roots.

RESULTS AND DISCUSSION

Typical chromatograms of ginseng root, young leaf (l-1), and mature leaf (l-2) extracts and authentic ginsenosides are shown in Figure 2. A highly satisfactory separation of the major ginsenosides of American ginseng including Rg_1 and Re was achieved in <50 min. In agreement with published results (Soldati and Sticher, 1980; Pietta et al., 1986; Kanazawa et al., 1987), the protopanaxtriol derivatives Rg_1 and Re eluted well ahead of the protopanaxadiols Rb_1, Rc, Rb_2, and Rd.

In Tables 1, 2, and 3 are the average contents of the total and individual ginsenosides in young leaves (l-1), mature leaves (l-2), and 4-year-old roots from nine commercial fields in the Vernon-Kamloops-Lilloet region

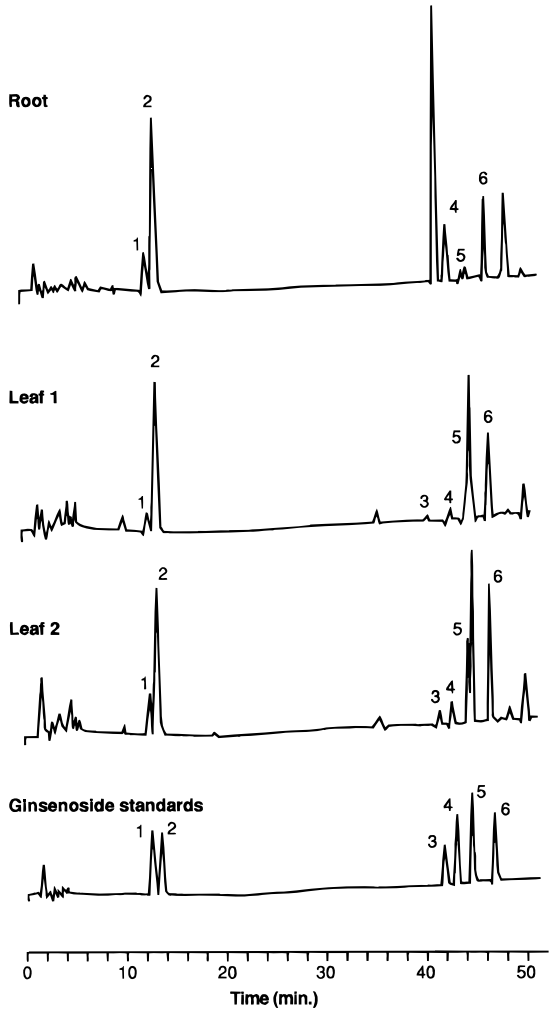

Figure 2. Typical HPLC chromatograms of ginsenosides in American ginseng, monitored at 203 nm: (peak 1) Rg_1; (peak 2) Re; (peak 3) Rb_1; (peak 4) Rc; (peak 5) Rb_2; and (peak 6) Rd.

Table 1. Contents (Grams per 100 g of Dry Weight) of Individual and Total Ginsenosides in Young Leaves of American Ginseng Taken from Commercial Fields in British Columbia in 1994

location	leaf (l-1) ginsenosides[a]						
	Rg_1	Re	Rb_1	Rc	Rb_2	Rd	total
1	0.18a	1.34ab	0.04a	0.07bcd	0.10cd	0.18b	1.80bcd
2	0.13a	0.95cd	0.06a	0.05d	0.08d	0.21b	1.43cd
3	0.19a	1.25abc	0.02a	0.06cd	0.14bcd	0.31ab	1.97bcd
4	0.12a	0.77d	0.04a	0.06cd	0.12cd	0.24b	1.33d
5	0.10a	1.04bcd	0.03a	0.06cd	0.05d	0.15b	1.42cd
6	0.12a	1.04bcd	0.05a	0.12abc	0.23abc	0.35ab	1.90bcd
7	0.16a	1.42a	0.04a	0.15a	0.33a	0.55a	2.64a
8	0.17a	0.94cd	0.02a	0.10bcd	0.26ab	0.52a	2.01bc
9	0.14a	1.14abc	0.06a	0.13ab	0.26ab	0.51a	2.23ab
av	0.13	1.10	0.04	0.09	0.20	0.34	1.86

[a] Means in each column followed by the same letter are not significantly different ($p \leq 0.05$; $n = 6$).

Table 2. Contents (Grams per 100 g of Dry Weight) of Individual and Total Ginsenosides in Mature Leaves of American Ginseng Taken from Commercial Fields in British Columbia in 1994

	leaf (l-2) ginsenosides[a]						
location	Rg_1	Re	Rb_1	Rc	Rb_2	Rd	total
1	0.35a	1.45a	0.13b	0.22a	0.49cd	1.67a	4.32a
2	0.33a	1.78a	0.22ab	0.39a	0.83ab	2.02a	5.58a
3	0.22a	1.63a	0.26ab	0.28a	0.59bcd	1.43a	4.42a
4	0.24a	1.26a	0.31a	0.33a	0.70abc	2.29a	5.12a
5	0.22a	1.38a	0.22ab	0.36a	0.93a	2.02a	5.12a
6	0.35a	1.76a	0.19ab	0.33a	0.50cd	1.00a	4.14a
7	0.25a	1.71a	0.29ab	0.32a	0.50cd	1.81a	4.88a
8	0.26a	1.54a	0.23ab	0.30a	0.42cd	1.63a	4.38a
9	0.26a	1.53a	0.25ab	0.33a	0.66d	1.78a	4.82a
av	0.28	1.56	0.23	0.32	0.62	1.74	4.18

[a] Means in each column followed by the same letter are not significantly different ($p \leq 0.05$; $n = 6$).

Table 3. Contents (Grams per 100 g of Dry Weight) of Individual and Total Ginsenosides in 4-Year-Old Roots of American Ginseng Taken from Commercial Fields in British Columbia in 1994

	root ginsenoside[a]						
location	Rg_1	Re	Rb_1	Rc	Rb_2	Rd	total
1	0.33a	0.87a	0.96bc	0.24a	0.03a	0.26ab	2.69b
2	0.16a	1.35a	0.84c	0.15cd	0.02a	0.27ab	2.79b
3	0.19a	1.03a	0.77c	0.17bcd	0.02a	0.27ab	2.44b
4	0.16a	1.26a	1.46ab	0.17bcd	0.02a	0.23b	3.29ab
5	0.14a	1.23a	1.86a	0.23ab	0.03a	0.40a	3.88a
6	0.19a	1.38a	1.44ab	0.21abc	0.03a	0.31ab	3.56ab
7	0.12a	1.00a	1.09bc	0.17bcd	0.02a	0.25ab	2.65b
8	0.11a	0.84a	1.13bc	0.14d	0.02a	0.29ab	2.52b
9	0.23a	0.96a	1.46ab	0.17bcd	0.02a	0.31ab	3.16ab
av	0.18	1.10	1.22	0.18	0.02	0.29	3.00

[a] Means in each column followed by the same letter are not significantly different ($p \leq 0.05$; $n = 6$).

of British Columbia. In young leaves, the content of total and individual ginsenosides, except for Rg_1 and Rb_1, varied significantly from location to location (Table 1). Location 7 had the highest contents of Rb_2, Rc, Rd, Re, and total ginsenosides. In mature leaves, only the ginsenosides Rb_1 and Rb_2 showed significant differences among locations. All other ginsenosides and the total contents were not affected by production site (Table 2). In 4-year-old roots, production site had a statistically significant effect on the content of Rb_1, Rc, Rd, and total ginsenosides (Table 3). Samples from locations 4, 5, 6, and 9 contained the highest levels of total root ginsenosides, and locations 1, 2, 3, 7, and 8 had the lowest levels. On a dry weight basis, mature leaves contained the highest ginsenoside contents, followed by roots and young leaves. Reported contents of ginsenosides in roots of American ginseng range from 1.70 to 2.56% (Soldati and Sticher, 1980; Lewis, 1988; Konsler et al., 1990). Our values are marginally higher than the published levels. This difference could be the result of differences in the age of plants, growing conditions, and soil fertility. In North America, ginseng roots are traditionally harvested after 4 years of cultivation. In this study, ginsenosides were evaluated at this age, although it has been reported that total content of ginsenosides increases with age of the plant (Soldati and Tanaka, 1984) and with moderate addition of lime to the soil (Konsler et al., 1990).

The amount of ginsenosides extracted from leaves varies with the stage of leaf development. As indicated in Tables 1 and 2, the total ginsenosides (Rb_1, Rb_2, Rc, Rd, Re, and Rg_1) of 1.86% for 1-month-old leaves

Table 4. Correlation Coefficients between Ginsenoside Contents of 1-Month-Old and Mature Leaves of American Ginseng

mature leaf	1-month-old leaf						
	Rg_1	Re	Rb_1	Rc	Rb_2	Rd	total
Rg_1	0.01	0.06	0.55	0.04	−0.06	−0.20	−0.05
Re	0.19	0.33	0.31	0.37	0.39	0.34	0.43
Rb_1	−0.13	−0.24	−0.11	0.17	0.31	0.39	0.09
Rc	−0.77[a]	−0.53	0.43	−0.04	−0.10	−0.04	−0.37
Rb_2	−0.69[a]	−0.38	0.26	−0.55	−0.68[a]	−0.59	−0.67[a]
Rd	−0.33	−0.34	0.09	−0.38	−0.37	−0.27	−0.43
total	−0.02	0.16	−0.43	0.32	0.27	0.23	0.26

[a] Significant at the 0.05 level of probability.

increased to 4.16% in mature leaves. These values are within the range of total leaf ginsenosides reported in the literature. Without any indication of specific time of leaf collection, Konsler et al. (1990) reported values of 2.27−2.92% total ginsenosides (A_1, Rb_2, Rd, Re, and Rg_1), whereas Lui and Staba (1980) found a total of 6.6% (Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, and Rg_2).

In our study, significantly different total ginsenosides from nine locations were mainly due to different levels of Rb_1, Rc, and Rd (Table 3), indicating that these three major components are affected by the growing conditions. The Rb_1 in roots from different locations showed the greatest variation from 0.77 to 1.86%. A similar variation was also reported by Sanada and Shoji (1978), with Rb_1 of 1.57%; by Lewis (1988), with Rb_1 of 0.99%; and by Soldati and Sticher (1980), with Rb_1 of 0.26%.

Correlation coefficients relating ginsenoside contents of young and mature leaves are given in Table 4. Significant correlations were obtained between Rb_2 content of mature leaves and Rg_1, Rb_2, and total ginsenoside content of 1-month-old leaves, and between the Rc level of mature leaves and the Rg_1 content of young leaves. The sign of these coefficients suggests that low levels of ginsenosides in young leaves lead to an increased accumulation of ginsenosides in mature leaves. However, the limited number of significant correlation coefficients between the individual and total ginsenoside contents in leaves at the two stages of maturity indicate that factors other than maturity influence the accumulation of ginsenosides in leaves of American ginseng.

Correlations between ginsenoside contents of leaves and roots were very low. The only significant ($p \leq 0.05$) combinations were Rg_1 of young leaves with Rb_1 ($r = -0.75$) and total ($r = -0.89$) of roots; total ginsenosides of young leaves with Rg_1 ($r = -0.83$) and total content ($r = 0.69$) of roots; and Rb_1 and Rc of mature leaves with Rb_2 ($r = -0.76$) and Re ($r = -0.71$) of roots, respectively. The sign of these coefficients indicates that increases in leaf ginsenosides correspond to lower ginsenoside contents in roots. These findings agree with previous studies (Konsler et al., 1990) that have shown that the relationship between leaf and root ginsenosides contents is generally poor.

Correlation analyses between N, P, K, Mg, and Ca contents of 1-month-old leaves and ginsenoside levels of leaves and roots revealed that the majority of the significant correlations were between ginsenosides and N levels of young leaves. The sign of the coefficients was negative for N and Re, Rb_1, and total ginsenosides of young leaves, and Re of mature leaves, and positive for N and Rb_1 and total ginsenosides of roots. There were no significant correlations between P contents and ginsenosides, whereas K, Mg, and Ca were correlated with Re of young leaves and roots, Rb_2 of roots, and Re

of young leaves, respectively. There were no correlations between N, P, K, Mg, and Ca of mature leaves and ginsenosides of leaves at the same stage of maturity. Contents of N, Mg, and Ca in the soil showed significant negative correlation with Rb_2 ($r = -0.77$), Rb_1 ($r = -0.70$) and Rc ($r = -0.70$), and Rb_1 ($r = -0.79$), respectively. The Mg and Ca content of mature leaves showed significant negative correlation with Re ($r = -0.69$) and Rg_1 ($r = -0.77$) of root, whereas only N content in the soil showed positive correlation with Rd ($r = 0.82$) and total ginsenosides of root ($r = 0.67$).

There is almost no published information on the effects of cultural practices and nutrient status of leaves and soil on ginsenosides of American ginseng, thus no comparison of our results with published data can be made. The relatively strong and consistent correlations between N contents and ginsenosides of leaves and roots, however, indicate that N fertilization of ginseng should be carried out cautiously.

Organic matter, pH, P, and K levels of soil were not significantly correlated with ginsenosides of mature leaves and roots, even though Konsler et al. (1990) noted that soil pH and phosphate levels result in significant changes in the tissue content of certain ginsenosides. The level of N in the soil showed a significant negative correlation with Rb_2 of mature leaves and a positive correlation with Rd and total ginsenosides of root, whereas Mg and Ca levels in the soil showed negative correlations only with Rb_1 and Rc of mature leaves and not with ginsenosides in root. These observations are in agreement with Konsler et al. (1990), who concluded that soil fertility factors, including Mg and Ca, are more closely related to leaf than to root ginsenoside concentrations. Further work is required to better elucidate the effects of soil fertility and timing of application of fertilizers on ginsenosides of American ginseng.

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