The Pharmacologically Active Constituents of White and Red Ginseng Root

by Dennis V.C. Awang, PhD, FCIC and Michael Z.C. Li, MD, MSc

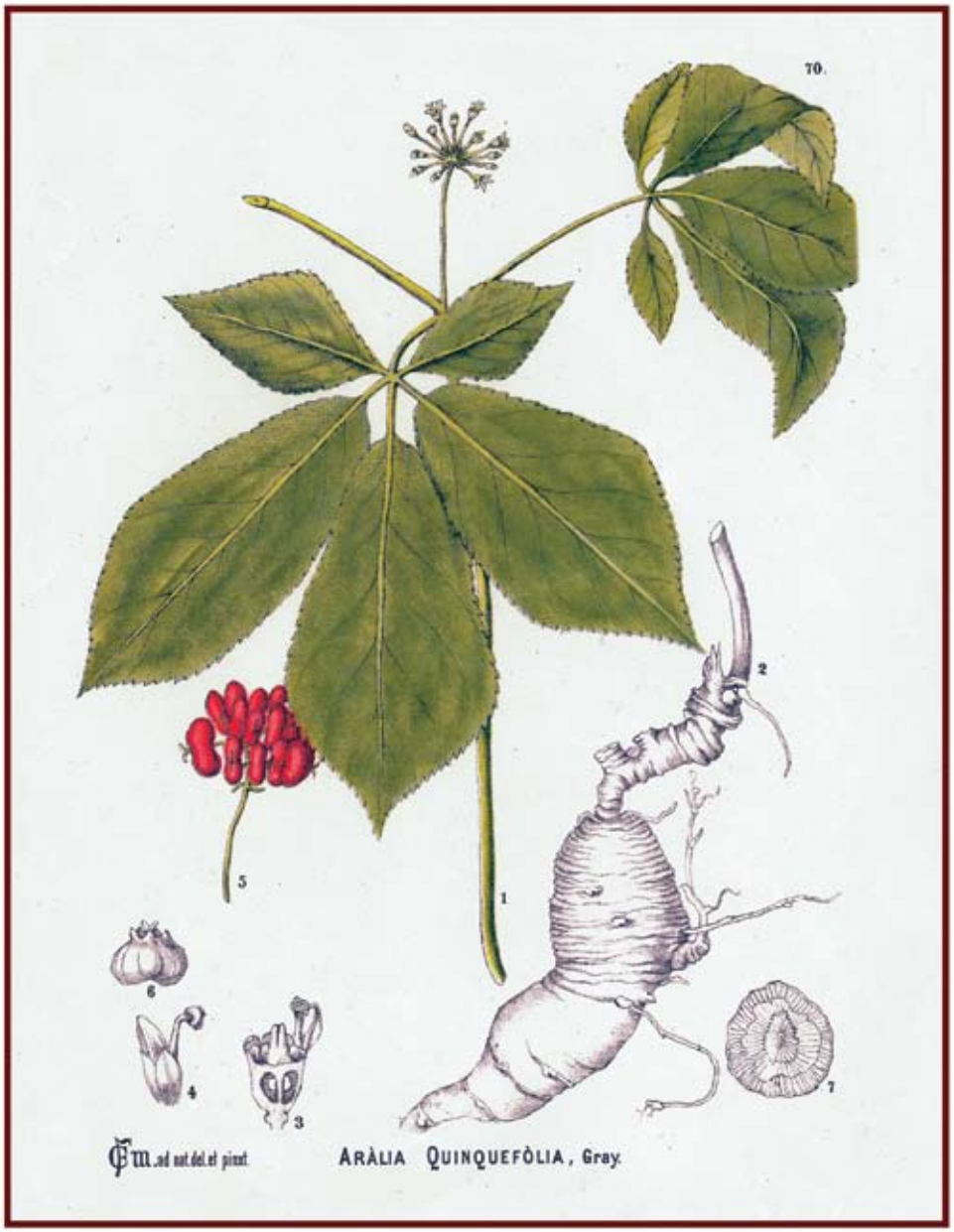


Illustration of **American Ginseng** *Panax quinquefolius* from *American Medicinal Plants: An Illustrated and Descriptive Guide to the American Plants Used as Homeopathic Remedies; Their History, Preparation, Chemistry and Physiological Effects* by Charles F. Millspaugh (1887).
Image courtesy of Steven Foster

This paper is dedicated to the memory of Professor Ying-jie Chen (1936-2003) of Shenyang Pharmaceutical University, PRC, who died during preparation of a book extensively covering the chemistry and pharmacology of ginsenosides.

Summary

Numerous studies of a much varied nature have been conducted on the roots of Asian (Chinese or Korean) ginseng (*Panax ginseng* C.A. Meyer, Araliaceae). Steamed and dried (red) Asian ginseng has been recognized as being appreciably more biologically active than the raw unprocessed (often scraped or peeled) and dried (white) ginseng root in some notable respects. For example: free-radical scavenging, antioxidant, "anxiolytic-like," and anti-tumor promoting activities. Much less investigation has been directed towards American ginseng (*P. quinquefolius* L.). However, in recent years Canadian researchers have demonstrated its activity in treating Type 2 diabetes and developed a special patented polysaccharide fraction that has shown effectiveness in the prevention and treatment of upper respiratory tract infection.

Ginsenosides, the dammarane-type triterpene saponin constituents of *Panax* species, are generally considered the main active components of the plant roots used for medicinal purposes. The normally analyzed neutral ginsenosides vary in level and ratio among the different *Panax* species, and they are accompanied in unprocessed root by acid derivatives of malonic acid. These derivatives are readily hydrolyzed by the steaming process, which converts "white" ginseng to "red" ginseng (actually caramel-colored). The steaming process also converts original ginsenosides to partially deglycosylated derivatives that have enhanced anti-cancer activity (e.g., ginsenosides Rg3, Rg5, and Rh2). The other main pharmacological activities claimed for ginseng extracts, as well as for individual ginsenosides, have been antioxidant (Rb1), calcium channel inhibition (Rf), immunomodulation (Rg1), neuroprotection (Rb1), and platelet inhibition. Diacetylene constituents (usually referred to incorrectly as "polyacetylenes") have also been implicated in platelet inhibition and in tumor inhibition activities, while polysaccharides have been claimed to be involved in cytoprotection, immunomodulation, and tumor inhibition actions.

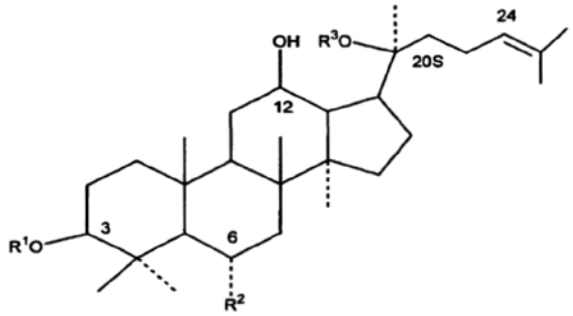
Introduction

Of the 3 prominent commercial ginsengs, Asian ginseng (*Panax ginseng*) is perhaps the most revered of traditional Chinese medicines. It is native to China, Russia, and Korea, and most of the commercially exported supply comes from China and Korea. While the use of Asian ginseng is widely believed to span at least 2000 years, Chinese use of American ginseng (*P. quinquefolius*) began only about 300 years ago. American ginseng is native to eastern North America and is mostly cultivated today in the provinces of British Columbia and Ontario in Canada and in the state of Wisconsin in the United States. Commercial cultivation of American ginseng in China was started in 1980. Sanchi or tienchi ginseng (*P. notoginseng* [Burkill] F.H. Chen ex C.Y. Wu & K.M. Feng) is popular in Asia where it is used as a hemostatic agent for blood-regulating purposes and also as a cardiotonic treatment.

American vs. Asian Ginseng: Ginsenoside Levels and Profiles

Neutral Ginsenosides

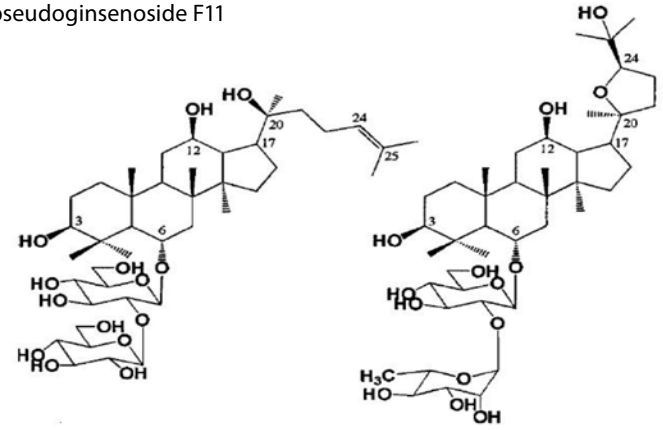
The root of these 2 most commercially prominent *Panax* species have been found to contain more than 30 triterpene dammarane-type saponins (mostly neutral ginsenosides) to which are attributed most of ginseng's pharmacological activities. Quantitative analysis for ginsenosides characterized as Rx—according to their mobility on thin-layer chromatographic (TLC) plates, with polarity decreasing from index "a" to "h"—has focused on the neutral ginsenosides Rb1, Rb2, Rc, Rd (derivatives of protopanaxadiol), and Re, Rf, Rg1, and Rg2 (derivatives of protopanaxatriol) (see Figure 1). Earlier methods of analysis, such as colorimetry and gas chromatography, are non-specific and led to exaggerated and incorrect results, as compared to high-performance liquid chromatog-



Ginsenoside	R^1	R^2	R^3	Molecular formula	Molecular weight
-R_{b1}	Glc-^{2}Glc-	H	Glc-^{6}Glc-	$C_{54}H_{92}O_{23}$	1109
-R_{b2}	Glc-^{2}Glc-	H	Ara(p)-^{6}Glc-	$C_{53}H_{90}O_{22}$	1079
-R_c	Glc-^{2}Glc-	H	Ara(f)-^{6}Glc-	$C_{53}H_{90}O_{22}$	1079
-R_d	Glc-^{2}Glc-	H	Glc-	$C_{48}H_{82}O_{18}$	947
-R_e	H	Rha-^{2}Glc-O-	Glc-	$C_{48}H_{82}O_{18}$	947
-R_f	H	Glc-^{2}Glc-O-	H	$C_{42}H_{72}O_{14}$	801
-R_{g1}	H	Glc-O-	Glc-	$C_{42}H_{72}O_{14}$	801

Figure 1. Prominent protopanaxadiol and protopanaxatriol glycosides from *P. ginseng* and *P. quinquefolius*

Figure 2. Structures of ginsenoside Rf and 24(R)-pseudoginsenoside F11



raphy (HPLC) values. HPLC has been employed since 1980[1] and was later refined using a programmed elution technique with photodiode array detection to allow separation of all 8 major ginsenosides in one analytical run.[2]

In view of the historical variation in analytical techniques employed, the published analytical data for ginsenoside content of ginseng roots are inconsistent and cannot be pooled. In addition, earlier data apply not only to reliably identified raw material but also to commercial material of questionable species and plant part identities. This situation makes it difficult to accurately establish the superiority of ginsenoside content within *P. quinquefolius* root over the root of *P. ginseng*, as is widely appreciated.[3]

However, analysis of dried 4-year-old roots of American ginseng taken from cultivated commercial fields in 9 different locations in British Columbia, Canada, in 1994 revealed an average of 3.00% (2.44-3.88) total for the 6 major ginsenosides (i.e., Rb1, Rb2, Rc, Rd, Re, and Rg1).[4] Rf is absent from *P. quinquefolius* and Rg2 is present at such low levels in both *P. quinquefolius* and *P. ginseng* that ginsenosides Rb1 and Re together accounted for better than 75% of the total ginsenoside complement. Another study reported an average of 4.04% from a total of the 8 main ginsenosides in 3 cultivated American ginseng roots.[5] However, Court et al indicate approximately 8% total ginsenosides in 4-year-old American ginseng roots when taking into consideration the content of malonyl ginsenosides (see later under **Acidic Ginsenosides**). The ratio of Rb1 to Rg1, often used as one basis for distinguishing between the two *Panax* species, ranged from 2.91 to 13.28, with an average of 7.54. In yet another study, 23 purported American ginseng root samples exhibited a ratio of 7.69, while the ratio found for 20 Asian ginseng samples was 1.30; the ratio for *P. ginseng* usually falls between 3 and 1.[1]

The data from analysis of *P. ginseng* roots are more sparse, with only two publications standing out, one dealing with commercial products and the other with cultivated 5-year-old

Asian Ginseng *Panax ginseng*. Photo ©2008 Steven Foster

Figure 3. Ginsenoside Ro

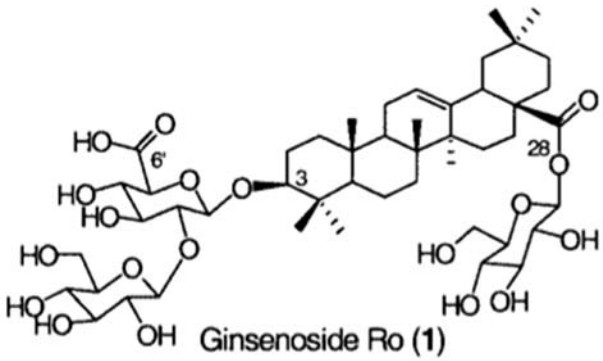
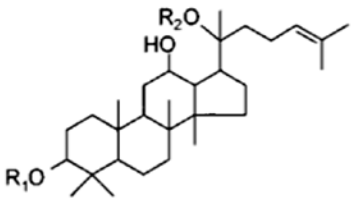


Figure 4. Malonyl ginsenosides



Ginsenoside	R_1	R_2	M
mRb_1	-Glc^2-Glc^6-malonyl	-Glc^6-Glc	1194
mRb_2	-Glc^2-Glc^6-malonyl	-Glc^6-Ara(p)	1164
mRc	-Glc^2-Glc^6-malonyl	-Glc^6-Ara(f)	1164
mRd	-Glc^2-Glc^6-malonyl	-Glc	1032

roots from China.[5] The latter publication reported an average of 1.49% from a total of the 8 main ginsenosides plus the oleanolic ginsenoside Ro in 7 samples (1.27% without Ro), while the former publication reported an average of 1.47% of the 8 main ginsenosides from 8 purported *P. ginseng* root samples in hard gelatine capsules (1 sample was totally devoid of ginsenosides). It should be noted that one has no assurance of what part of the root/rhizome is employed in preparation of commercial root products. Further, the average values found from 20 4-year-old *P. ginseng* plants from Korea were 1.348, 3.532, and 6.148% from the main root, lateral roots, and "root hairs" (rootlets).[1] However, neither an average for the total root content nor ratios of the respective root parts were determined. In 1995, HPLC was used to evaluate the ginsenoside content of more than 60 commercial products, purportedly from various *Panax* species, as well as from a variety of roots and tissue culture samples derived from *P. ginseng* root.[6] Widely differing levels and proportions of ginsenosides were observed. The results from this analysis of commercial products emphasize the need for careful attention to quality control.[6]

Distinguishing American and Asian Ginsengs

The pure root of American ginseng can be really distinguished from the pure root of Asian ginseng by HPLC analysis. American ginseng is devoid of ginsenosides Rf and Rg2, while Asian ginseng contains relatively low concentrations of Rf and Rg2. A not uncommon problem involves either substitution or adulteration of the more expensive American ginseng with sun-dried Asian ginseng.[7] Scientists at Shenyang Pharmaceutical University in the People's Republic of China developed a TLC method by which, at 4° C, ginsenoside Rf was revealed in Asian ginseng and the ocotillol-Type 24(*R*)-pseudoginsenoside F11 was revealed in American ginseng[8] (see Figure 2). Scientists at the University of Illinois in Chicago subsequently developed an HPLC procedure with evaporative light scattering detection for determination of 24(*R*)-pseudoginsenoside F11.[9] Gas chromatographic-mass spectrometric (GC/MS) analysis has revealed that American ginseng contains more than 0.1% of 24(*R*)-pseudoginsenoside F11, which is over 1000 times the content found in Asian ginseng (less than 0.0001%).[10]

Application of the TLC procedure to 43 samples of purported "*Radix quinquefolium* and its preparations" purchased in China showed that 18 samples (44.3%) were either *P. ginseng* or mixtures of *P. ginseng* and *P. quinquefolius*.[8]

Acidic Ginsenosides

Of the more than 30 ginsenosides so far identified in *Panax* spp., 5 are acidic, bearing carboxylic acid functions. Four of these are mono-esters of the dicarboxylic acid, malonic acid (one carboxylic acid function condensing with the primary alcoholic group at C-6 of the terminal glucosyl ring attached to C-3 of the triterpene skeleton); malonyl (m) Rb1, mRb2, mRc and mRd occur in both American and Asian ginseng, as does the 5th acidic ginsenoside, Ro, an oleanolic acid derivative. Originally identified as chikusetsu saponin V in the rhizome of Japanese ginseng (*P. japonicus* [T. Nees] C.A. Meyer), Ro bears a carboxylic acid function at C-6 of the proximal glucose function at C-3 of the triterpene ring system[11] (see Figure 3).

The malonyl ginsenosides (see Figure 4) are more polar and water soluble than the neutral ginsenosides to which they are readily converted by hydrolysis, accelerated by heat, and catalyzed by both acid and base. As such, no malonyl ginsenosides have been detected in steam-processed ginseng root (red ginseng) or cooked Chinese *shihchu* ginseng.[3] HPLC protocols normally employed in the estimation of ginsenoside content do not measure the acidic malonyl ginsenosides and therefore underestimate total ginsenoside values.[12]

HPLC procedures using reversed-phase C18 columns and gradient elution systems, such as those published by Samukawa et al,[13] allow separation and estimation of 22 major and minor, both neural and acidic ginsenosides. Kitagawa et al[14] compared white and red ginseng prepared from the same *P. ginseng* roots and noted appreciable concentrations of the 4 malonyl ginsenosides in white, but not in red ginseng. Chuang et al[3] analyzed 10 samples of white Asian ginseng collected from herb shops throughout Taiwan and characterized the samples by comparing features of external appearance and histological anatomy. The levels of the 4 malonyl ginsenosides and their ratios to their neutral progenitors were considerably

lower than those found in the 2 samples of American ginseng examined. For example, an average of 3.0 was found for the ratio of Rb1 to mRb1 in the Asian samples, while values of 0.90 and 0.80 were found for the two 4-year-old American roots grown in Ontario, Canada. Between completion of the 1st and 4th years of growth, the total content of 6 of the main ginsenosides (minus Rf and Rg2) increased from approximately 3% to almost 8%, of which over 50% was due to combined Rb1 and mRb1 ginsenosides.[15]

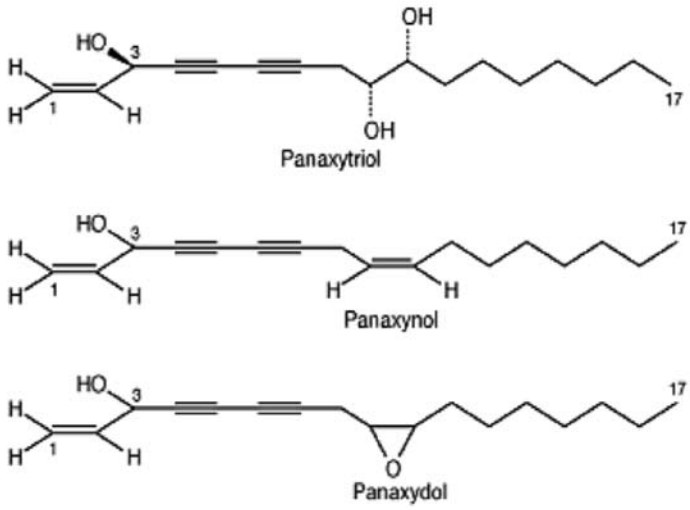
Court et al[16] estimated the malonyl ginsenosides indirectly by first determining the neutral ginsenosides, hydrolysing the acidic ginsenosides with 5% potassium hydroxide, and then re-analyzing for neutral ginsenosides. A more recent study determined that extraction of ginseng roots with liquid ammonia was about twice as efficient as extraction with methanol/water (60:40; v/v) and effected efficient conversion of mRb1, mRb2, mRc, and mRd to their corresponding neutral ginsenosides.[17]

It is worth noting that considerable variability of ginsenoside content occurs in ginseng raw roots, as well as root products. As Court has observed, considering the established differences in content of main root, lateral roots, and rootlets, it is essentially important to carefully mix powdered whole root "prior to filling non-assayed capsules with measured amounts of powdered root."[1,18,19] Even with carefully standardized extraction methods and estimation of total ginsenosides, Soldati and Sticher found up to 3-fold variation in individual ginsenoside levels within 3 lots of extract standardized to roughly 4% total ginsenosides and within 4 lots of extract standardized to 7.2-7.5% total ginsenosides (extracts were in soft gelatin capsules).[1] Such variation could conceivably have an impact on pharmacological activity.

Other Pharmacologically Active Ginseng Constituents

While it is widely believed that ginsenosides are the main active constituents of ginseng roots, responsible for a wide range of biological activities,[7] other constituents, notably polysaccharides, polypeptides, diacetylenes (erroneously referred to as "polyacetylenes"), and alkaloids, have also been implicated in a variety of activities.

Figure 5. Ginseng diacetylenes



The isolated diacetylenes—mainly panaxytriol, panaxynol, and panaxydol (see Figure 5)—show cytotoxic, antiplatelet, and anti-inflammatory effects, respectively.[2] The ginsenans PA, PB, S-IA, S-IIA and some other polysaccharides exhibit immunological activity such as reticuloendothelial system potentiation, effects on the body's unspecific immune system, and alkaline phosphatase-inducing activity.[2] Lee et al found that ginsan, a purified lectin-free acidic polysaccharide from *P. ginseng* (molecular weight about 150,000), stimulated the proliferation of B-cells and T-cells and the cytotoxicity of spleen cells to a wide range of tumor cells *in vitro.* The ginsan also activated macrophages to generate nitrogenous intermediates and become *in vivo* active against B16 melanoma cell lines found in the benzo[a]pyrene-induced autochthonous lung tumor model.[20] Recently, pre-treatment with ginsan (25µg/kg) was shown to protect mice from lethality induced by *Staphylococcus aureus* challenge.[21] Panaxans have been identified in 2 series from *P. ginseng*, namely, A-E from Chinese or Korean roots[22] and Q-V from Japanese roots.[23] (Note: Panaxans are peptidoglycans, i.e., polymers composed of polysaccharide and peptide [condensed amino acid] chains.) All these panaxans showed dose-dependent hypoglycemic activity in normal and alloxan-induced diabetic mice when administered by intraperitoneal injection, but were ineffective when given orally. Presumably this occurred because the high polymer glycans are unlikely to be absorbed from the gastrointestinal tract, probably due to degradation by peptidase and glycosidase enzymes secreted by the gut and by colonic microflora in the large bowel. Quinquefolans A-C from American ginseng have also shown marked blood-glucose-lowering effects when administered to both normal and alloxan-induced hyperglycemic mice.[24] Wang et al have also noted a hypoglycemic effect of ginseng polypeptides.[25,26]

Anti-carcinogenic Activity

Shibata et al demonstrated that partially deglycosylated ginsenosides produced during the steaming process, but also by metabolic transformation through the agency of human intestinal bacteria, have enhanced biological activity, particularly anti-carcinogenic. This is likely the result of increased bioavailability of the degraded original ginsenosides.[27] Prominent among such ginsenosides detected in Korean red ginseng are Rh1, Rh2, Rg3, and Rg5; Rh1 is derived from protopanaxatriol (PPT) ginsenosides, such as Rg1, while Rh2, Rg3, and Rg5 are derived from protopanaxadiol (PPD) ginsenosides (see Figure 6). Other degraded raw ginseng ginsenosides, produced by hydrolysis, isomerization at C-20, and dehydration, are present in relatively minute quantities. Shibata cites other researchers as establishing that Rg3 inhibits *in vitro* cancer cell invasion and metastasis; Rh2 inhibited human cancer cell growth in nude mice. Yun identified Rh1, Rh2, Rg3, and Rg5 as the major saponin components in Korean red ginseng; Rg3 and Rg5 showed statistically significant reduction of lung tumor incidence, while Rh2 had a tendency to prevent non-organ-specific cancer in humans.[28]

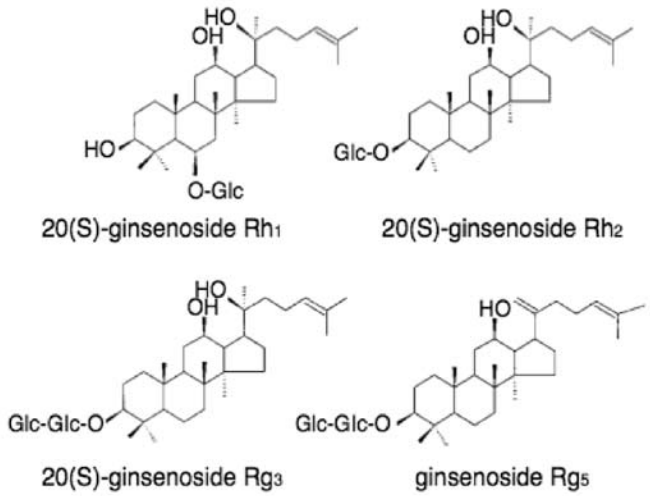
Park et al demonstrated the cytotoxicity of dammarane-type glycosides from steam-processed *P. ginseng*—namely Rg3, Rg5, Rk1, Rs4, and Rs5, having 50% growth inhibition concentrations of 41, 11, 13, 37, and 13 microM, respectively[29]—against SK-HeP-1 hepatoma cancer cells.[30] A recent double-blind placebo-controlled trial of so-called "Sun Ginseng" (high-pressure steam-processed *P. ginseng*) claimed the treatment to be beneficial in improving quality of life in cancer patients, mainly those suffering from gynecologic or hepatobiliary cancer.[31] However, the authors of the study report neither the details of preparation of the tested item nor the vehicle of administration. Regarding chemical composition, the publication states that Sun Ginseng contains different types of ginsenosides, such as Rs4, Rs5, Rs6, and Rs7 (structures not presented), which were not mentioned in the previously cited publication. The latter study claimed that the levels of ginsenosides F4, Rg3, and Rg5 (absent from raw ginseng) are progressively elevated with increasing steam temperature. Rg3 and Rg5 attained values of 39% and 19%, respectively, of total ginsenosides at 120° C under autoclaving for 2 hours.[32] Of the 3 products, when steamed at 100, 110, and 120° C, the last was most potent in the ability to induce endothelium-dependent relaxation.

Steam-processed North American ginseng has not been subjected to chemical or biological evaluation, but an *in vitro* study with MCF-7 breast cancer cells showed that a standardized extract of unprocessed *P. quinquefolius* root synergistically inhibited cell growth when combined with standard chemotherapeutic agents.[33]

Anti-diabetic Clinical Trials

In 1995, the late E.A. Sotaniemi and coworkers reported that treatment with an unspecified ginseng extract—presumably from *P. ginseng* root—improved fasting glycemia and long-term glycemic control, as assessed by glycosylated hemoglobin (HbA_{1c}) in 36 Type 2 diabetic subjects.[34] However, the results of that study were ambiguous because of significant weight loss differences between treatment groups, as well as poorly described statistics. In 2000, Tetsutani and coworkers reported that treatment with a Korean red ginseng extract decreased HbA_{1c} in 34 patients with Type 2 diabetes, as compared to controls.[35] However, subject selection, allocation to treatment, statistics, and follow-up of the study were poorly described. In the years 2000 and 2001, Vuksan, Sievenpiper, and coworkers published the results of 5 randomized, placebo-controlled clinical trials with American ginseng whole root for glycemic control, summarized and discussed in a 2005 publication.[36] American ginseng reduced post-prandial glycemia from 9.1% to 38.5%, doses of 1 to 9 g being equally efficacious from 0 to 120 minutes before the glucose challenge in diabetic subjects—without interaction with background antihyperglycemic medication—but glycemia only being reduced in nondiabetic subjects if the treatment was administered at least 40 minutes before the glucose challenge. A subsequent trial with a different batch of American ginseng from the same supplier was unsuccessful to reproduce the post-prandial effects observed with the original batch, an outcome attributed to "a depressed ginsenoside profile."[37] Further-

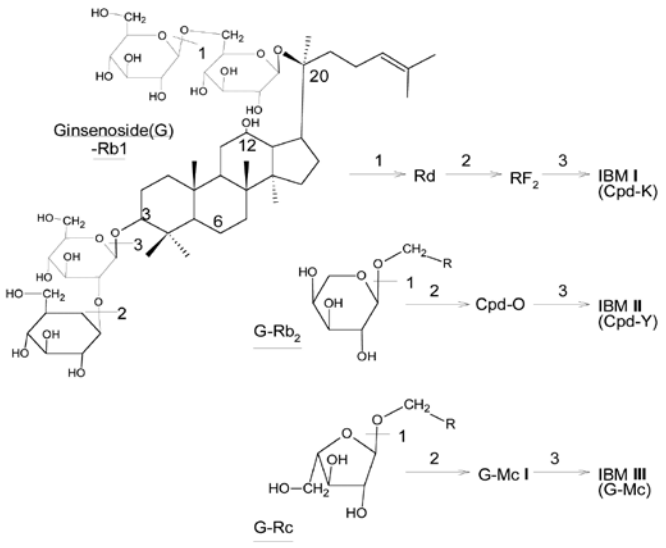
Figure 6. Main ginsenoside products of steamed *P. ginseng* root



American Ginseng *Panax quinquefolius*. Photo ©2008 Steven Foster

Asian Ginseng *Panax ginseng*. Photo ©2008 Steven Foster

Figure 7. Pathways of intestinal bacterial hydrolysis for protopanaxadiol ginsenosides



more, the authors of the study opine that "Although ginsenoside differences are probably driving effects, other components such as quinquefolans (peptidoglycans) and ginsenans (glycans) might share some responsibility." A third batch of American ginseng exhibited acute post-prandial glycemic-lowering efficacy similar to the first, as did Vietnamese ginseng (*P. vietnamensis* Ha & Grushv.), whereas Japanese ginseng (*P. japonicus*), Asian red (steamed root), and Sanchi ginseng (*P. notoginseng*) had null effects; Asian and American wild ginsengs raised glycemia.[38]

Vuksan et al demonstrated that 12 weeks of supplementation with 6 g/day of a selected Korean red ginseng (*P. ginseng*) rootlets product, as an adjunct to conventional antidiabetes therapy, maintained good glycemic control and improved plasma glucose and insulin regulation safely beyond usual therapy in patients with well-controlled Type 2 diabetes.[39] However, clinical efficacy as assessed by HbA_{1c} was not demonstrated, and future studies using intention-to-treat analysis are needed to determined whether this treatment is efficacious as a monotherapy in people with less well controlled diabetes. The total ginsenoside contents reported for 6 and 8 of the main ginsenosides in the efficacious American ginseng root and Korean red ginseng rootlet products were 3.21 and 1.92%, respectively, while that of the ineffective American ginseng root was 1.66%. However, efficacy of these products and the varied glycemic activities of the 5 different *Panax* species[38] might be easily reconciled with either the levels of individual ginsenosides or the various ratios calculated, such as protopanaxadiol (PPD): protopanaxatriol (PPT) ginsenosides, Rb1: Rg1, Rb1: Rc and Rg1: Re. For example, the ratio Rb1:Rg1, the most prevalent PPD and PPT ginsenosides, respectively, for American ginseng was 15.3 and only 0.94 for Korean red. Also, while ginsenoside Rb2 has been found to be the most effective ginsenoside for decreasing blood glucose levels in streptozotocin-induced diabetic rats by intraperitoneal injection,[40] its level in ginseng root was relatively low, being 0.06 and 0.25% in American and Korean red rootlet, respectively. Additionally, while ginsenoside Re has been identified as a prominent active principle in *P. ginseng* berry extract—at a level similar to that in efficacious *P. quinquefolius*—and found to exert significant anti-hyperglycemic effect intro-peritoneally in obese diabetic mice,[41] its level in the efficacious Korean red ginseng rootlet material was only 0.03% and was not a significant predictor of glycemic lowering efficacy in multiple regression models applied by Sievenpiper and associates. Finally, while ginsenoside Rg1 has been shown to decrease blood glucose in resting mice by 16% when administered by stomach intubation, its level was highly variable in the various ginsengs tested, being 0.13 and 0.43 in efficacious American and Vietnamese ginsengs, but 1.74 in ineffective Sanchi ginseng.[38]

Of the other non-ginsenoside constituents considered as potential contributors to ginseng's antihyperglycemic activity, the peptidoglycan panaxan B has been shown to have significant effect in mice.[42] However, as indi-

cated earlier, a reservation remains regarding the potential activity of peptidopolysaccharides by oral administration because peptidase and glycosidase enzymes secreted by gut and colonic microflora in the large bowel would likely degrade peptidoglycans to their base sugar and amino acid units. It should also be noted that analysis for glycans and peptidoglycans has not been applied to the various ginseng root samples tested for antihyperglycemic activity. However, it is interesting to note that Han et al used an alcan blue dye complex formation to confirm that the polysaccharide yield from Korean red ginseng was 3 times greater than from fresh root, which is likely due to hydrolysis of lignin-bound polysaccharides.[43] Furthermore, main roots yielded more polysaccharide than rootlets, polysaccharides being located mainly in the cortex and cambium.

Variability and Standardization

Vuksan and coworkers have stated that "In the absence of adequate standardization to support therapeutic indications, one cannot be assured of reproducible results with other [than tested] sources."[37] Vuksan also warns against "standardization that is without basis"[38] and emphasizes the "need to develop a basis for standardization that ties the composition of herbs to efficacy."[36]

Ginsenoside Metabolism

Towards the end of the last century, research on ginsenosides focused on their metabolism by intestinal bacterial flora. Notably, Korean and Japanese researchers demonstrated that ginsenosides containing two (bisdesmosides) or more glycoside moieties (e.g., the PPT ginsenoside Re) are not appreciably absorbed from the gastrointestinal tract.[12,44] Both PPD and PPT ginsenosides, such as the dominant Rb1 and Rg1, are largely eliminated unchanged. Only deglycosylated derivatives attain appreciable levels in plasma, following intestinal bacterial metabolism (IBM): Rb1 to compound K (IBM I) via Rd (see Figure 7) and Re to Rh1 via Rg1 (see Figure 8). So, while Re from *P. ginseng* berry is an effective antidiabetic agent when injected in mice,[42] it is highly likely that its effectiveness would be considerably reduced by oral administration. If an effective oral human dose of Asian ginseng berry extract could be achieved, the true active principle would likely be ginsenoside Rh1, which incidentally is produced from steamed (red) ginseng root. The earlier observed antihyperglycemic ineffectiveness of white Asian and Asian red ginseng root[38] may have been largely due to reduced ginsenoside content (as compared to Asian ginseng rootlets).

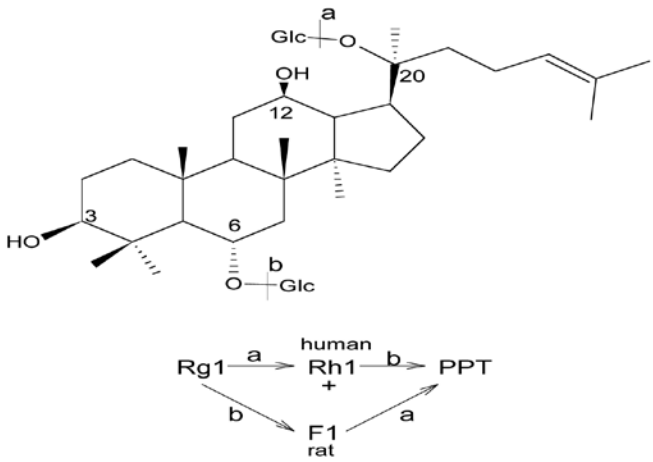
A thorough examination of the composition and antihyperglycemic activity of American red ginseng is sorely needed.

Immunomodulatory Effects

In vitro

A variety of immunological effects have been demonstrated *in vitro* for ginseng root extracts, fractions, and

Figure 8. Intestinal bacterial metabolism of ginsenoside Rg1



American Ginseng *Panax quinquefolius*. Photo ©2008 Steven Foster

isolated principles.[45] Alcohol-insoluble fractions are composed almost exclusively of polysaccharides and oligosaccharides and are much more potent than alcohol-soluble fractions composed of ginsenosides.[46] Most of the *in vitro* studies have been conducted with polysaccharide-rich extracts or isolated polysaccharides from *P. ginseng* root.[46,47] Only 2 studies have been conducted from isolated polysaccharides[48] and active polysaccharide fractions from *P. notoginseng*.[49] One study has been conducted from a proprietary aqueous extract (CVT-E002) of *P. quinquefolius*.[46]

The ethanol-insoluble fraction of an aqueous extract of *P. ginseng* was found to induce proliferation of splenocytes and to generate activated killer cells.[50] The activated killer cells neutralized both NK cell-sensitive and insensitive tumor target cells without MHC-restriction. Two extracts of *P. ginseng* have also been found to activate components of cell-mediated immunity, increasing the phagocytosis index along with phagocytosis fraction.[51]

Two acid polysaccharides isolated from *P. ginseng* root extract, named ginsenan PA and ginsenan PB, exhibited similar reticuloendothelial system (RES), anti-complementary, and alkaline-phosphatase-inducing activity.[52] Pectic polysaccharides from the root of *P. ginseng* have been found to exert potent gastric cytoprotective and anti-ulcer effects.[53]

Immunostimulating activity of *Panax* has also been observed in polysaccharide-containing material extracted from the *leaves* of *P. ginseng*.[52] Rhamnogalactouronan II isolated from the leaves enhanced macrophage Fc receptor expression.[54] An anti-ulcer pectic polysaccharide has also been characterized from leaves of *P. ginseng*.[55]

In one study, immunomodulating polysaccharides were isolated from *P. notoginseng*. In another study, a RES-active polysaccharide, sachinan A,[48] and 4 distinct homogeneous active polysaccharide fractions[49] were isolated from the roots of *P. notoginseng*. In the latter study, one fraction showed strong anti-complementary activity, two fractions significantly induced the production of interferon-γ in the presence of concanavalin-A, and all fractions induced the production of TNF-α, using human serum and antibody-sensitized sheep red blood cells.

One *in vitro* study with a *P. quinquefolius* extract (the aforementioned proprietary product CVT-E002) found stimulation of the proliferation of normal mouse spleen cells, of which the

Left Photo: Author Dr. Dennis Awang inspecting American Ginseng (*Panax quinquefolius)*.
Right photo: Author Dr. Michael Li holding an American Ginseng (*Panax quinquefolius)*.

major responding subpopulation was identified as B lymphocytes. CVT-E002 also activated peritoneal exudates macrophages, which lead to enhanced interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and nitric oxide (NO) production. In addition, CVT-E002 stimulated *in vivo* immunoglobulin G (IgG) production in treated mice.[46] A subsequent *ex vivo* study using C57 BL/6 mice demonstrated that CVT-E002 could significantly increase Con-A-induced IL-2 and IFN-γ productions in spleen cells in a dose-dependent manner.[56]

Ginsenoside Rg1 has been tested for stimulatory effect on immune function of lymphocytes in the elderly.[57] Rg1 was found to stimulate proliferation of lymphocytes and to increase the fluidity of lymphocyte membrane in the aged.

Clinical Studies

During the cold and flu seasons of 2000 and 2001, CV Technologies, Inc. (Edmonton, Alberta, Canada) conducted 2 Phase II randomized, double-blind, placebo-controlled clinical trials (RCT) with their proprietary American ginseng root aqueous extract, CVT-E002.[56,58] The trials compared CVT-E002 with placebo in preventing acute respiratory illness (ARI) in institutionalized older adults (> 60 yrs). The preparation was described as containing 80% poly-furanosyl-pyranosyl-saccharides and 10% protein in a 2004 publication of the results of the combined Phase II trials.[59] The primary endpoint was clinically confirmed ARI, which is defined as the onset of respiratory symptoms (cough, sore throat, nasal or sinus congestion, runny nose) plus one additional respiratory or constitutional symptom (fever, headache, fatigue, myalgia, etc.). The secondary endpoints included severity and duration of respiratory illness, laboratory confirmed respiratory illness or influenza, and severity and duration of influenza. Although the odds for developing ARI defined by symptoms alone were not significantly reduced in the treatment group, the odds ratio for laboratory-confirmed ARI was statistically significant. Secondary endpoint analysis also revealed a statistically significant increase in laboratory-confirmed influenza illness and laboratory-confirmed ARI due to influenza and respiratory syncytial virus, amounting to an 89% lower relative risk for ARI in the CVT-E002 group as compared to the placebo group.

A later study using an extract standardized to contain 90% poly-furanosyl-pyranosyl-saccharides, commercially termed COLD-fX®, was conducted with institutionalized adults aged 65 years or older.[59] The frequency and duration of ARI during the first 2 months of the study was similar in the 2 groups, but during the following 2 months significantly fewer subjects in the COLD-fX group (32%) reported ARI compared to the placebo group (62%). The duration of symptoms during these latter 2 months was significantly shorter in the COLD-fX group than the placebo group (5.6 days in the COLD-fX group vs. 12.6 days in the placebo group). The authors of these studies concluded that COLD-fX supplementation to immunocompetent seniors during an early "cold and flu" season could help to reduce the frequency and duration of respiratory symptoms related to ARI: "a safe natural therapeutic means for the prevention of ARI in healthy seniors."

In the year intervening between the two above publications, an RCT was conducted at the onset of the influenza season with 323 healthy adults aged 18-65 years who had a history of at least two colds in the previous year.[47] Results from the RCT showed a 13% reduction in the absolute risk of getting recurrent colds that meet the Jackson criteria. The mean number of Jackson-verified colds per person was significantly less in the ginseng-derived saccharide-treated group than in the placebo group.[60] Fewer subjects in the saccharide group than in the placebo group reported contracting at least 1 cold during the study, but the difference was not statistically significant. However, there was a significant difference in the recurrence of colds, with 10% in the saccharide group having more than one cold compared with 23% in the placebo group. Ginseng-treated subjects had less severe symptoms and were sick for fewer days. However, while this study was of excellent methodological quality and reported a 13% reduction in the risk of contracting a cold, it does not seem particularly clinically valuable. More promising perhaps is that "the total symptom score was 31% lower and the total number of days on which symptoms were reported was 34% less in the COLD-fX group than in the placebo group over the 4-month intervention period."

Presumably, based on the evaluation of clinical data (i.e., 10 years of research, including 7 clinical trials submitted by CV Technologies), Health Canada ruled that the company can continue to market the COLD-fX product as "helping to reduce the frequency, severity and duration of cold and flu symptoms by boosting the immune system."[61]

Conclusion

While there is ample clinical evidence in support of the beneficial influence of American ginseng in treating Type 2 diabetes and preventing cold and flu attacks, comparable data on behalf of Asian ginseng are wanting. *In vitro* and animal studies also support an anti-cancer potential for steam-processed (red) *P. ginseng* root and an extract of *P. quinquefolius* root has been shown synergistically to inhibit the growth of MCF-7 human breast cancer cells. Overall, much more effort needs to be expended toward standardization of ginseng preparations so as to ensure reliable repeatable therapeutic effect. HG

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