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GINSENG
The Genus *Panax*

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PREFACE TO THE SERIES

There is increasing interest in industry, academia and the health sciences in medicinal and aromatic plants. In passing from plant production to the eventual product used by the public, many sciences are involved. This series brings together information which is currently scattered through an ever increasing number of journals. Each volume gives an in-depth look at one plant genus, about which an area specialist has assembled information ranging from the production of the plant to market trends and quality control.

Many industries are involved such as forestry, agriculture, chemical, food, flavour, beverage, pharmaceutical, cosmetic and fragrance. The plant raw materials are roots, rhizomes, bulbs, leaves, stems, barks, wood, flowers, fruits and seeds. These yield gums, resins, essential (volatile) oils, fixed oils, waxes, juices, extracts and spices for medicinal and aromatic purposes. All these commodities are traded worldwide. A dealer's market report for an item may say "Drought in the country of origin has forced up prices".

Natural products do not mean safe products and account of this has to be taken by the above industries, which are subject to regulation. For example, a number of plants which are approved for use in medicine must not be used in cosmetic products.

The assessment of safe to use starts with the harvested plant material which has to comply with an official monograph. This may require absence of, or prescribed limits of, radioactive material, heavy metals, aflatoxin, pesticide residue, as well as the required level of active principle. This analytical control is costly and tends to exclude small batches of plant material. Large scale contracted mechanised cultivation with designated seed or plantlets is now preferable.

Today, plant selection is not only for the yield of active principle, but for the plant's ability to overcome disease, climatic stress and the hazards caused by mankind. Such methods as *in vitro* fertilisation, meristem cultures, and somatic embryogenesis are used. The transfer of sections of DNA is giving rise to controversy in the case of some end-uses of the plant material.

Some suppliers of plant raw material are now able to certify that they are supplying organically-farmed medicinal plants, herbs and spices. The Economic Union directive (CVO/EU No 2092/91) details the specifications for the **obligatory** quality controls to be carried out at all stages of production and processing of organic products.

Fascinating plant folklore and ethnopharmacology leads to medicinal potential. Examples are the muscle relaxants based on the arrow poison, curare, from species of *Chondrodendron,* and the antimalarials derived from species of *Cinchona* and *Artemisia*. The methods of detection of pharmacological activity have become increasingly reliable and specific, frequently involving enzymes in bioassays and avoiding the use of laboratory animals. By using bioassay linked fractionation of crude plant juices or extracts, compounds can be specifically

targeted which, for example, inhibit blood platelet aggregation, or have antitumour, or antiviral, or any other required activity. With the assistance of robotic devices, all the members of a genus may be readily screened. However, the plant material must be fully authenticated by a specialist.

The medicinal traditions of ancient civilisations such as those of China and India have a large armamentarium of plants in their pharmacopoeias which are used throughout South East Asia. A similar situation exists in Africa and South America. Thus, a very high percentage of the World's population relies on medicinal and aromatic plants for their medicine. Western medicine is also responding. Already in Germany all medical practitioners have to pass an examination in phytotherapy before being allowed to practise. It is noticeable that throughout Europe and the USA, medical, pharmacy and health related schools are increasingly offering training in phytotherapy.

Multinational pharmaceutical companies have become less enamoured of the single compound magic bullet cure. The high costs of such ventures and the endless competition from me too compounds from rival companies often discourage the attempt. Independent phytomedicine companies have been very strong in Germany. However, by the end of 1995, eleven (almost all) had been acquired by the multinational pharmaceutical firms, acknowledging the lay public's growing demand for phytomedicines in the Western World.

The business of dietary supplement in the Western World has expanded from the Health Store to the pharmacy. Alternative medicine includes plant based products. Appropriate measures to ensure the quality, safety and efficacy of these either already exist or are being answered by greater legislative control by such bodies as the Food and Drug Administration of the USA and the recently created European Agency for the Evaluation of Medicinal Products, based in London.

In the USA, the Dietary Supplement and Health Education Act of 1994 recognised the class of phytotherapeutic agents derived from medicinal and aromatic plants. Furthermore, under public pressure, the US Congress set up an Office of Alternative Medicine and this office in 1994 assisted the filing of several Investigational New Drug (IND) applications, required for clinical trials of some Chinese herbal preparations. The significance of these applications was that each Chinese preparation involved several plants and yet was handled as a **single** IND. A demonstration of the contribution to efficacy, of **each** ingredient of **each** plant, was not required. This was a major step forward towards more sensible regulations in regard to phytomedicines.

My thanks are due to the staff of Harwood Academic Publishers who have made this series possible and especially to the volume editors and their chapter contributors for the authoritative information.

Roland Hardman

PREFACE

Twenty three years have passed since a baffled journalist persuaded me to summarise the proceedings of a conference in London concerning a Chinese plant called ginseng. Twelve years earlier I had encountered ginseng in Canada when some Chinese immigrants sought advice on the problems they had encountered in their unsuccessful attempts at the cultivation of ginseng under prairie conditions. Appetite whetted, I sought information everywhere but soon discovered that there was only limited data available. Ginseng had never appeared on the shelves of the chemists' shops in which I had worked from 1937 onwards, it rarely appeared in the literature of the day and was not found in the standard pharmacopoeias and textbooks of pharmacognosy. Many claims were made concerning the multitudinous merits of this Chinese wonder drug but little or no reliable scientific evidence was presented to justify such observations. As allopathic medicine moved inexorably forward my western-trained pharmacologist colleagues dismissed ginseng along with many other pharmacognostical drugs. Yet today ginseng is well known, being found in most pharmacies, supermarkets and health food stores, is advertised freely on the communications Internet and World Wide Web and many ginseng books of a popular type can be purchased in high street bookshops. A more detailed survey of the scientific literature is needed to stress the potential and the limitations of ginseng.

For this book the problem of language when faced with literature in Chinese, Korean and Japanese was solved by consulting research students from the Far East, by use of translations provided in particular by Pharmaton S.A., Lugano, Switzerland and by access to English abstracts in journals such as *Biological Abstracts, Chemical Abstracts, Excerpta Medica* and *Review of Aromatic and Medicinal Plants*. Fortunately significantly more of the Far Eastern literature is now presented in English.

I am indebted to the library staff at the University of Bradford, West Yorkshire, John Moores University, Liverpool, the North-East Wales Institute, Wrexham, the Picton Library, Liverpool and the Royal Pharmaceutical Society of Great Britain, London for the help given me in my search for scientific data. I am also grateful to Dr David Cutler and his colleagues Dr David Frodin and Anna Lynch at the Royal Botanic Gardens, Kew for help in unravelling the many species names currently in use.

My thanks are especially tendered to Doctors Eva Cellárová and Katarina Kimáková of the Department of Experimental Botany and Genetics, Faculty of Science, P.J.Šafárik University, Košice, Slovakia for their contribution concerning the artificial culture of *Panax* spp. and to Pharmaton S.A., Lugano, Switzerland for permission to reproduce the illustrations employed in this work.

Finally I must thank my old friend Dr Roland Hardman who cheered me up when the going was rough and helped me in so many ways and my wife for her tolerance and understanding.

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1. INTRODUCTION

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During the 20th century many plants have been investigated in order to assess their potential value as new medicinal agents or as sources of new organic molecules that could be used in contemporary medicine or could act as templates for the synthesis or semi-synthesis of potentially useful therapeutic compounds. Examples include *Catharanthus roseus* G.Don., the Madagascan periwinkle, source of the indole alkaloids vinblastine and vincristine which have been successfully used in the treatment of Hodgkin's disease (malignant lymphadenoma), *Rauvolfia serpentina* Benth., the Himalayan snakeroot, source of the alkaloids reserpine and ajmaline that have been employed in the medication of stress, hypertension and cardiac oedema and *Taxus* spp., certain yews, that are the source of taxol, a compound of potential use in the treatment of cancers and especially breast cancer.

Among such plants is ginseng, the collective name for a group of plants esteemed by the Chinese for more than 5000 years, but never really accepted in western medicine and therefore soon forgotten by the western world until its reinvestigation as an alleviating agent or cure for the ills of modern stressful lifestyles.

True ginseng, *Panax ginseng* C.A.Meyer, is a small, inconspicuous, shade-loving, perennial shrub attaining a height of about 60 cm and belonging to the ivy family Araliaceae (Fig. 1.). The generic name *Panax* was derived from the Greek "$\pi\alpha\gamma$" and "$\alpha\chi\varepsilon o\mu\alpha\iota$" meaning "all-heal" or "all-cure" and reflected the popular, traditional use of the plant as a panacea. The specific name *ginseng* or *schinseng* is a transliteration of the Chinese names "Jin-chen", "Jen-schen", "Ren-shen", "Schin-sen" or "Schan-shen" (wild mountain ginseng) and relates to the anthropomorphic appearance of the subterranean parts of the plant, the vague resemblance of the mature roots to the human form. Cultivated or garden ginseng is known locally as "Yuan-shen".

According to the old Doctrine of Signatures or Similitudes, a theory apparently derived independently in many parts of the world, a plant would by its colour, shape and characteristics indicate its potential medicinal uses (Court, 1985). Thus ginseng with its man-like appearance was quickly accepted as a tonic, a cure-all with particular value as an aphrodisiac and a treatment for impotence and loss of sexual drive. The more anthropomorphic the better and the price rose accordingly.

The Chinese, the early Egyptians and the Hindus independently believed in their different ways that the world and all that was in it was constructed from a small number of basic indivisible units existing in harmony. In living beings it was believed that imbalance of such units led to ill health. Therefore the quality of life depended on the balance or imbalance of many factors.

Figure 1. A mature *Panax ginseng* plant with red berries.

In China the Taoist philosophy (*ca.* sixth century B.C.) stated that good health and longevity depended on the quality of one's life, good quality being achieved by personal effort and high ethical standards. "Tao" literally means "The Way" and, in particular, the way of nature. The Yin and Yang theory was

developed simultaneously with the Han Dynasty concepts of Confucianism (*ca.* 206 B.C.–24 A.D.). Confucius or K'ung Fu-tse, philosopher, social reformer and teacher, who lived 551–479 B.C., propagated a creed known as "The Way of Humanity" or "Confucianism", a code of ethics advocating exemplary moral standards based on filial piety and brotherly respect. It was during the Western Han Dynasty (*ca.* 298–238 B.C.) that Confucianism was adopted as state orthodoxy.

The Yin and Yang theory suggested that good health depended on the balance of Yin and Yang. Yin, meaning standstill, was passive and dark as the shady side of the hill and thus included death, the darker aspects of life, the moon, the earth, night and darkness, water and damp, cold, etc. as well as other negative and feminine subjects; Yang, on the other hand, meaning motion, was active and light as the sunny side of the hill, therefore embraced life as well as the sunnier aspects of life including the sun itself, heaven, day, fire, heat, light, dryness, creation and other positive and masculine aspects. As sure as light changed to darkness and winter changed to spring so, it was argued, the ever-changing balance of Yin and Yang controlled all natural phenomena. Hence excess Yin, being cold, caused chills and colds and excess Yang, being hot, promoted fevers.

In association with Yin/Yang balance the Chinese also believed in the doctrine of the five elements, wood, fire, earth, metal and water, the five viscera, the heart (controls pulse and spirit), the lungs (control skin and the animal spirit or ghost), the liver (controls muscles and soul), the kidneys (control the bones and the will) and the spleen (controls the flesh and ideas) and the five flavours, salty hardening the pulse, bitter withering the skin, pungent knotting the muscles, sour toughening the flesh and sweet causing aches in the bones. The five element theory or quinary (Table 1.1) was further extended to include the grains, fruits, vegetables, animals, odours, climates, musical notes, etc.

Against this complicated philosophical background the early Chinese medical schools considered ginseng as "Spirit of the Earth" or "Man-Essence", the essence or elixir of the earth crystallised in human form and responsible for the healing virtues of the plant. The underlying philosophy of Eastern medicine

Table 1.1. The Quinary

ELEMENTS	wood	fire	earth	metal	water
BODY	tendons	pulse	muscle	skin/hair	bones
VISCERA	liver	heart	spleen/pancreas	lungs	kidney/bladder
SENSES	eye	tongue	mouth	nose	ears
TASTES	sour	bitter	sweet	sharp	salty
SMELL	rancid	scorched	fragrant	putrid	rotten
ENERGY	dry	hot	wet	pungent	cold
EMOTIONS	anger	joy	sympathy	sadness	fear
CLIMATE	wind	heat	humidity	dryness	cold
SEASONS	spring	summer	late summer	autumn	winter
POINTS	east	south	centre	west	north
PLANETS	Jupiter	Mars	Saturn	Venus	Mercury

was, and still is, the treatment of the patient as a whole, not as an isolated disease condition, coupled with prophylaxis, that is, obeying the axiom that prevention is far better than cure. Therefore the medical texts of those times e.g. *Shen-nung Pen-ts'ao Ching* (*ca.* 200 A.D.) that listed some 365 plant drugs, *Ming-I Pieh-lu* (*ca.* 500), *Chia-yu Pen-ts'ao* (1057) and *Pen-ts'ao Kang-mu* (1596) that included nearly 1900 drugs of animal, vegetable and mineral origin, recommended ginseng as an excellent tonic medicine which could maintain the body in good health, induce rejuvenation and retard the inevitable process of ageing. This was due to the restoration of Yang establishing the healthy Yin/Yang balance in the five visceral areas. Ginseng was therefore employed in the treatment of conditions such as defective memory, gastrointestinal disturbance and debility states. As the treatment of illness comprised the rebalancing of Yin/Yang forces, the herbal plants were evaluated for their Yin or Yang properties. Thus *P. ginseng,* a tonic medicine, was classified as having Yang properties and *P. quinquefolium* L. had Yin properties and was used to "cool" the body system and so treat "hot" conditions such as fevers, sore throats and infections. In addition Chinese traditional medicine classified its herbs in three groups, mild, moderate and curative. Under such classification ginseng was considered a mild drug invigorating the body, strengthening the visceral organs, tranquillising the spirit, countering nervous debility, promoting resistance to infection, improving vision and increasing mental and physical performance.

An early Chinese medical document, now residing in the British Museum, London, indicates the use of ginseng in the formulation of "love potions". During the Liang Dynasty (*ca.* 500 A.D.) the occurrence, harvesting and morphological characteristics of ginseng were described and in the T'ang Dynasty (618–905 A.D.) ginseng was considered a royal plant. That ginseng was much valued is confirmed by the observation in the Sung Dynasty (926–1126) that the price of ginseng was determined by its weight in silver. Not surprisingly, therefore, in eastern medicine ginseng is a very important drug even today.

Ginseng was and often still is used in Chinese medicine in polypharmaceutical mixtures. Many old formulations are presented in the works of Harriman (1973), Hou (1978), Fulder (1993) and Reid (1995) and involve plants such as:-

Kan tsao or liquorice root (*Glycyrrhiza uralensis* Fisch.=Chinese or Manchurian liquorice; *G. glabra* L.=European or Russian liquorice, family Leguminosae),
Gui zhi, Chinese cinnamon or cassia bark (*Cinnamomum aromaticum* Nees= C. *cassia* Nees ex Bl., family Lauraceae),
Shuan tsao ren, wild Chinese jujube or red date (*Zyzyphus jujube* Mill., family Rhamnaceae),
Pai shu or atractylodes thistle root (*Atractylis macrocepbala* or *A. ovata*) and *kang shu* or Chinese atractylodes root (*A. chinenesis* or *A. lancea* (Thunb.) DC.), family Compositae,
Xie bai or Chinese chives bulbs (*Allium macrostemon=A. sativum* L., family Liliaceae),
Mai-men-tung or creeping lilyturf root (*Ophiopogon spicatus=Liriope spicata*),
Sheng jiang or ginger root (*Zingiber officinale* Rose., family Zingiberaceae),

Tzi su ye or perilla leaf (*Perilla frutescens* (L.) Britton, family Labiatae).
Wu-wei-tzu or Chinese magnolia vine fruit (*Schizandra chinensis* (Turczaninow) Baillon, family Schisandraceae),
Xuan shen or figwort (*Scrophularia nodosa* L., family Scrophulariaceae),
Fu-ling or tuckahoe or hoelen (*Pachyma cocos=Poria cocos=Macrohyproia extensa,* a saprophytic basidiomycete fungus growing on the roots of certain conifers of the genera *Pinus* and *Cunninghamia*) and
Sang ye or Russian mulberry root (*Morus tartarica,* family Moraceae).

The mixed herbs are usually taken as decoctions prepared by adding boiling water and boiling to a specified reduced volume. The action of the supporting herbal medicines may include one or more of the functions flavouring, restorative, tonic, curative or supplementary. In addition the action of the supporting drugs may be positive or synergistic, improving the action of the ginseng, or negative or antagonistic, cancelling some of the unwanted actions of the mixture. Although the effect of many ancient formulae can be rationally explained using modern phytochemical, pharmacological and medical knowledge, it is more likely that the original formulations were empirically devised by trial and error rather than by application of ancient medical theory.

Early western medicine developed independently and quite differently, having no obvious contact with the philosophy of the Far East although developed with some understanding of earlier Egyptian medicine (*ca.* 3000–1200 B.C.) and Assyrian medicine (*ca.* 1900–391 B.C.). The initial Greek concepts of holistic medicine propounded by Hippocrates (*ca.* 460- *ca.* 377 B.C.) formed a logical approach to clinical medicine. Unlike the Chinese who had performed little dissection or surgery and used common body organ names to describe areas of functional activity such as digestion, elimination, heat generation, etc., the Greeks based their medical ideas on the structure and functions of precise body organs discovered by the study of the anatomy of man and many other animal species. Later it was replaced by the rigid theory devised by Galen (*ca.* 130–201 A.D.), the Greek physician to the Roman gladiators at Pergamon near Ephesus. Galen's ideas included the early Pythagorean theory of the four elements,

fire=hot+dry	air=hot+moist
water=cold+moist	earth=cold+dry

the Hippocratean concept of four humours or body fluids associated with distinct parts of the body,

blood=hot+moist	phlegm=cold+moist
yellow bile=hot+dry	black bile=cold+dry

and his own theory of the four temperaments of man,

melancholy	sanguine
choleric	phlegmatic.

Illness was considered due to imbalance of these concepts and the aim of medical treatment was the return to homoeostasis or normality. Galen's dogmatic yet erroneous theory was taken seriously by later leaders of the medical profession although his own reputedly excellent practice was probably more due to empirical observation than application of a theory. Nevertheless the theory held sway well into the 18th century; it dominated many of the early dispensatories and pharmacopoeias and undoubtedly held up the progress of European medicine. Although Galen used a very wide range of plants from Europe and Asia, ginseng did not appear in any of the formularies and ginseng was not apparently classified in the Galenical style.

As Galen's hypothesis was successfully challenged, it declined in importance. European medicine as practised by the physicians adopted the Paracelsian ideas of chemical medicine and was dominated in the 17th and 18th centuries by the so-called Humoralism of the Eclectics, the use of venesection (blood-letting), mercurial and antimonial purgatives, bitter bark (from South American *Cinchona* spp.) and opium, drastic treatments for already debilitated patients. Nevertheless the European apothecaries, who operated from shops and were the forerunners of today's pharmaceutical profession, did not usually employ such methods. Instead they used the polypharmaceutical admixtures of mainly plant drugs either as powders, infusions and decoctions or aqueous alcoholic tinctures and extracts and close inspection of old prescription books and medical practice daybooks (1750–1900) coupled with modern insight into plant chemistry and pharmacology reveals that the formulations arrived at by empirical methods were probably effective in ameliorating the patients' conditions although cures were usually not possible as disease states were poorly understood (Court, 1988, 1996a). Ginseng, however, has not appeared in any of the many old prescription books and shop records that I have personally examined.

Although trade betweeen Europe and China had commenced in the Eastern Han Dynasty (25–220 A.D.), no mention of ginseng appeared until *ca.* 1000 when Ibn Cordoba, a Moorish adventurer, returned to Spain with a cargo including ginseng. After initial enthusiasm, interest in ginseng rapidly declined. In 1294 Marco Polo returned to Europe with further supplies of ginseng but the combination of the remoteness of the far East and the marked differences in the two medical philosophies resulted in ginseng having little impact on European medicine.

Despite the cultivation of ginseng in China and Japan from *ca.* 1600 onwards and in Korea and North America from *ca.* 1750 onwards, it did not appear in the early European herbals and pharmacopoeias with the exception of the Württemberg Pharmacopoeia, 1741. Wienmann reported in 1757 that many European apothecaries kept ginseng although often only as a rarity. In Britain Tobias Smollett, surgeon and novelist (1721–1771), wrote in his final masterpiece *"The Expedition of Humphrey Clinker"* (1771) of a letter between Mathew Bramble and Dr. Lewis. Wrote Bramble *"By your advice, I sent to London a few days ago for half a pound of ginzeng, though I doubt much, whether that which comes from America is equally efficacious with what is brought from the East Indies. Some years ago a friend of mine paid sixteen guineas for two ounces*

of it; and, in six months after, it was sold in the same shop five shillings the pound. In short we live in a vile world of fraud and sophistication". This suggests that American and Eastern ginsengs were available in London in the late 18th century although there was doubt concerning quality.

In Theophilus Redwood's *Gray's Supplement to the Pharmacopoeia* published in London in 1848 reference to Ginseng mentions *Panax quinquefolium* (Linn.) and suggests China and North America as sources. According to Gray the root is cordial, alexiterial and aphrodisiac with a dose of 1 to 2 drachms (60 to 120 grains or 4 to 8 grammes) administered by chewing or slicing and preparation as a tea and often confounded with *nin sing*. A cordial was defined as a preparation possessing warm and stimulating properties, capable of exciting animal energies and generally given to elevate the spirits; an alexiterial was an antidote or preservative against contagion or poison and an aphrodisiac was then, as now, used to arouse sexual desire. In the same reference Lindley described ginseng thus:-*"Root an agreeable bitter sweet, with some aromatic pungency; has a prodigious reputation among the Chinese as a stimulant and restorative, under the name of "Ginseng"; by Europeans and Americans considered nothing more than a demulcent approaching liquorice in its properties; this, however, requires further investigation, for we cannot believe that all the Chinese say, believe, and practise, is fabulous or imaginary"*

Despite Lindley's caution ginseng was not listed in most of the materia medica or pharmacognosy textbooks published in the 19th century. In Flückiger and Hanbury's textbook (1879) American ginseng *(P. quinquefolium)* is very briefly described as a spindle shaped root which may occasionally be encountered as an adulterant of the North American drugs senega or rattlesnake root (*Polygala senega* L., family Polygalaceae), a stimulant and expectorant, and serpentaria or Virginian snakeroot rhizome (*Aristolochia serpentaria* L., family Aristolochiaceae), a local and general stimulant and tonic. There was no mention of the value of American ginseng itself.

Significantly American ginseng, not fitting readily into the established galenical ideas of the western-trained medical profession, was traded to Hong Kong or exported to Europe rather than being used indigenously by settlers in the United States and Canada. As early as 1704 Michael Sarrasin, who had arrived in Quebec as a medical adviser on behalf of King Louis XIV, had encountered the little shrub *Panax quinquefolium* in forests near Quebec City. Samples sent to France in the belief that the roots were a reliable aphrodisiac proved ineffective. Today we know that the dominant chemical agent in the roots is a sedative (ginsenoside Rb_1) and that little of the stimulant agent (ginsenoside Rg_1) is present. At the same time, on the other side of the world a French Jesuit priest, Father Pierre Jartoux, a map-maker in northern China, discovered the medicinal virtues of ginseng by living among the indigenous Chinese people. Jartoux's 1713 report to the Royal Society in London evoked considerable interest because it suggested that ginseng might be found in areas of Canada where the mountainous, forested habitat closely resembled that in China. This stimulated Father Joseph Francis Lafitou, a missionary amongst the native Canadian Iriquois tribe, to successfully seek out this wonder drug.

He soon discovered that it was known in Iroquois medicine as *"garentoquen"*, a name referring to its man-like appearance (Harriman, 1973).

Ginseng became an important article of Canadian commerce in the period 1720–1750, being gathered by all and sundry for export via Paris to China. Inevitably the quality of the roots gathered by the itinerant harvesters was extremely variable. No control was exerted over the age of the roots garnered, no rules were laid down concerning effective drying of the roots and no cultivation attempts were undertaken with the object of reseeding and conservation. Therefore the wild stocks were soon depleted. At the same time the Chinese challenged the quality of the extremely variable batches of ginseng that they were importing at much inflated prices.

Inevitably the Canadian trade declined but, simultaneously, an American export trade developed as it was realised that ginseng grew wild in the forested areas of the north-eastern states and subsequently, during the period 1750–1890, ginseng was being gathered freely from the Atlantic seaboard to the Mississippi River and especially in the shady hardwood forests on the Allegheny and Appalachian Mountains as far south as the 35th parallel. Although the ginseng areas in America were much greater than those in Canada, it was obvious that supplies would deplete unless conservation measures were adopted. In 1886 George Stanton, a retired New York tinsmith, set up a Chinese Ginseng Farm. Realising that other attempts to cultivate ginseng had failed miserably, Stanton decided that he would attempt to mimic natural growth conditions. Using woodland soil for the ginseng beds, artificial shade that resembled the natural woodland shade conditions, adequate ventilation and drainage of the beds and fertiliser prepared from mulched forest leaves he successfully grew crops of ginseng. Many others, who attempted to make a rapid fortune by cultivating ginseng root, failed because they did not reproduce the natural conditions that the plant favoured and, in many cases, were not pleased to patiently cultivate a plant for up to 7 years, especially when facing problems of drought and disease. Cultivation, especially in Minnesota, Wisconsin, Michigan and Ohio, reached a peak in about 1920 and trade steadily declined in the 1930's until complete disruption by the Second World War in 1939.

The peak year for American ginseng export was 1862 when no less than 282.5 tonnes of dried roots collected from wild sources were traded to Canton and Hong Kong. About 68 tonnes were cultivated annually in the Depression period (1929–1934). Harriman (1973) reported that many of the ginseng farms became derelict in the 1930's and 1940's and that the annual trade in ginseng post-war, mainly to Oriental markets, was about 75 tonnes. Sadly in 1973 *Panax quinquefolium* was listed in CITES (Convention on International Trade in Endangered Species) as a species in danger of extinction in the wild unless serious efforts were made to preserve and propagate the plants. Fortunately research involving this species has been instigated and continued, especially in the Far East, using carefully cultivated crops and indigenous American cultivation has increased steadily in Canada and the United States.

In Europe and America challenges to the Galenic ideas of medical practice came both from the traditional empirical school of herbal medicine which, as a

result of trial and error, had been practised successfully by the wise women, tribal doctors, travelling quacks, etc. and from the emergence of iatrochemistry (chemical as opposed to herbal medicine). Greater advances in the understanding of chemistry from the 18th century onwards and the new science of pharmacology or the action of chemical entities on living systems from the 19th century onwards have produced the modern sytem of rational medicine where cause and effect are related. Therefore western medicine is today mainly allopathic, using well defined natural or synthetic chemical substances for the suppression of symptoms or the treatment of specific and demonstrable pharmacological phenomena. Many of the new allopathic synthetic medicines have been dramatically effective in the battle against life-threatening diseases e.g. the sulphonamides and synthetic penicillins versus pneumonia and other bacterial infections. Unfortunately, despite the indisputable triumph of modern medicines in producing an extended, healthy and useful lifespan, there have been several well advertised incidents of dramatic and damaging side-effects due to synthetic drugs e.g. thalidomide, neomycin, Opren, etc. There are also problems due to the gradually developing resistance of some invading organisms to allopathic medicines; antibiotic and antimalarial drug resistances are typical examples of conditions caused by the injudicious use of modern medicines. As a result of adverse publicity, the use of herbal medicines worldwide has undergone a renaissance prompted by a revolt against synthetic allopathic medicines, partly because of the alleged side-effects and partly in the widespread but erroneous belief that natural products must be safer to use. Neither view is totally correct but in today's society the consumer does require products, allopathic or herbal, that are dependable.

Understanding ginseng has produced a clash between very different philosophies of medicine but the public interest in oriental and herbal medicines and the need to find new and effective treatments for many troublesome conditions including, in particular, stress states has stimulated research efforts worldwide.

In the early 20th century ginseng was rarely found in the pharmaceutical whole-salers' catalogues and therefore seldom encountered in the community pharmacy. Yet by the 1970's ginseng was appearing on the pharmacy and drugstore shelves. Today the market for ginseng in Europe and America is considerable. For example, in 1994 the United States Medicinal Herb Import Statistics revealed that 496.59 tonnes of cultivated ginseng roots valued at about $6,721,522 and 28.84 tonnes of wild ginseng roots valued at about $319,317 were imported. In the reverse direction about 1088.57 tonnes of American ginseng roots valued at about $76,000,000 were exported to the Orient. Sales of ginseng products, which are regarded as food supplements not required to meet the stringent safety and efficacy standards of the Food and Drug Administration, exceed $300,000,000 annually in the United States. Reports and advertisements for commercial ginseng and ginseng products also appear prominently and abundantly on the international Internet and World Wide Web. As a result of such commercial demand, much research is now in progress and a very large number of publications have appeared during the past three decades including some 4000 research publications, several useful books

(Harriman, S., 1973; Dixon, P., 1976; Hou, J.P., 1978; Lucas, R., 1978; Fulder, S., 1980, 1993 and 1996) and frequent reviews (Sonnenborn, 1987; Baldwin *et al.,* 1986; Court, 1986, 1996b; Tang and Eisenbrand, 1992).

Contemporary research, mainly undertaken in Korea, Japan, China and Russia, has concentrated on six principal areas:-

1) the biology of *Panax* species and the methods of conservation of existing wild populations,
2) the propagation and cultivation of the plants in various parts of the world,
3) a vast amount of fundamental phytochemical research on ginseng plant constituents has been undertaken and sophisticated methods of separation, evaluation and standardisation have been developed,
4) techniques of tissue and callus culture; significantly plant cell biotechnology has been successfully used for only three commercial processes, the production of shikonin from *Lithospermum erythrorhizon,* purpurin from *Rubia akane* and ginsenosides from *Panax ginseng* (Alfermann and Petersen, 1995),
5) the medicinal properties and related pharmacological characteristics of plants and their extracts and purified isolated chemical constituents,
6) carefully controlled clinical trials to prove therapeutic value.

The prolific output of research findings continues unabated.

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2. THE GENUS *PANAX*

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The traditional interest in plants as sources of "natural" medicines prompted the grouping of closely related species under common names. Thus the name "ginseng" is loosely applied to a range of plants from the Araliaceous genus *Panax* although some other non-related "ginseng" species are also encountered in commerce e.g. *Eleutherococcus senticosus* Maxim, family Araliaceae (Siberian ginseng), *Pfaffia paniculata* Martius, family Amaranthaceae (Brazilian ginseng) and *Rumex hymenosepalus* Torrey, family Polygonaceae (Wild red desert ginseng or American wild red ginseng).

The botanical characteristics including the microscopical details of the principal ginsengs were reviewed by Thompson in 1987. Subsequently more information has emerged concerning minor Asiatic species that are employed in folk medicines in tribal areas. Nevertheless there are problems in the nomenclature of the *Panax* species. The species names are liable, in the words of colleagues at the Royal Botanic Gardens, Kew, to "a very wide interpretation". Currently the genus is being revised by Wu and other botanists and further changes of nomenclature and synonymy can be expected.

Ginseng is a member of the plant family Araliaceae, phylogenetically one of the oldest plant families having evolved in the Cretaceous period, some 65 to 100 million years ago, when the giant reptiles had just disappeared and the angiosperms, the flowering plants, were becoming established. Two areas of speciation were important, tropical America and Indo-Malaysia. Fossil evidence indicates the occurrence of Araliaceous species in Alaska in the Upper Cretaceous period (over 65 million years ago) and the Palaeocene period (65–55 million years ago). Fossils of *Panax* species were found in Colorado dating from the Oligocene period, some 38 million years ago. The bicentric generic distribution pattern prompted Hu (1978) to observe that genera with separated distribution were considered to be of "great antiquity" and therefore *Panax* species could be regarded as "living fossils".

The Araliaceae is a family now comprising some 70 genera and 750 woody species varying in habit from trees and shrubs to lianes and perennial herbs and still associated with two particular centres of speciation, North America and South East Asia. Although most species occur in tropical and semitropical areas, a few species are found as thorny deciduous shrubs in temperate areas. Typically the leaves are alternate and may be compound or decompound. The family is characterised by umbellate inflorescences usually comprising pentamerous regular flowers with inferior ovaries. The ovules are solitary and pendulous in each locule and the fruit is drupaceous with a bright red exocarp and usually 2 to 5 oblong seeds.

The family Araliaceae comprises 3 tribes differentiated by petal shape and aestivation (the arrangement of the petals in the bud). Ginseng is a member of the tribe Aralieae, the petals being broad based and somewhat imbricate in the bud (i.e. somewhat overlapping at the tip or side). A feature of the ginseng family is the species variable occurrence of secretory canals in the cortex, phloem and medulla.

Confusion of the related genera *Aralia* and *Panax* occurs in the early literature and herbaria because Joseph Pitton de Tournefort (1656–1708) had classified the genera under the common name *Aralia*. *Panax* was later correctly described as a separate genus by the eminent Swedish botanist Carl von Linné or Carolus Linnaeus (1707–1778) using the American species *Panax quinquefolium* as the lectotype species and its monograph appeared in *Species Plantarum* published in 1753. Hu (1978) stated that the genus *Panax,* based on *Panax quinquefolium* L., was characterised by "species with an underground morphogenetic point, an aerial shoot, a whorl of digitately compound leaves, serrate, double serrate, orpinnatifid-serrate leaflets, terminal umbellate inflorescence, small flowers, 5 petals, inferior ovary, and fleshy red or orange fruits containing 2–5 pyrenes".

The species of the *Panax* genus demonstrate a typical bicentric distribution. In North America ginseng plants can occur in a range from 70°–90° W longitude and 34° to 47° N latitude (Thompson, 1987), an area embracing the southern part of the Canadian provinces of Quebec and Ontario to the north and in the United States of America the spine of the Appalachian Mountains down to Georgia, Mississippi and Arkansas in the south and eastwards to the edges of the Great Plains. In eastern Asia the range extends from 85° to 140° E longitude to 22° to 48° N latitude (Thompson, 1987), an area including China with north east India, Nepal and Bhutan to the west, Burma, Laos and Vietnam to the south and Manchuria, Korea and Japan to the west.

Panax species can be grouped according to their rhizome and root characteristics although rhizome characteristics do vary according to the altitude at which the plants are growing. Thickened nodes and thinner internodes are observed in plants at higher elevations and at lower levels the nodes are less pronounced and the internodes thicker. Such variations have tempted some authors to define variants as new species or subspecies. The fleshy primary root may or may not be persistent and if the tap root degenerates adventitious roots may develop from the rhizome in some species. Species possessing creeping rhizomes and fibrous root systems are regarded as primitive taxa and those with an erect rhizome and fleshy root are considered as derived taxa. Such *Panax* species with erect rhizomes and persistent fleshy roots include *P. ginseng, P. pseudoginseng, P. quinquefolium, P. trifolium, P. vietnamensis, P. wangianum* and *P. zingiberensis*. Typical roots are pale yellowish buff in colour.

As morphological variations are not reliable criteria for the differentiation of species, considerable debate occurs concerning the true status of species and varieties. Cytogenetic study of some ginseng species has revealed that *P. ginseng, P. japonicum* and *P. quinquefolium* plants are tetraploid, i.e. the nuclei contain four times the haploid unpaired set of chromosomes. In contrast *P. pseudoginseng=P. notoginseng* and *P. trifolium* are characterised by a diploid

state i.e. the nuclei contain twice the haploid unpaired set of chromosomes. Typically the somatic chromosome number for the diploid species is n=24 and for the tetraploid plants n=48 (Thompson, 1987).

Nevertheless there is still some doubt concerning the status of the wild Asiatic species or varieties and many scientific journals and particularly abstracting journals refer to *P. ginseng [P. pseudoginseng]* suggesting synonymy. The difficulties of differentiation by traditional macroscopical and microscopical morphological examinations prompted studies establishing the 18S ribosomal ribonucleic acid (RNA) gene sequences of extracted total deoxyribonucleic acid (DNA) from roots of *P. ginseng, P. japonicus* and *P. quinquefolium*. In 1995 Shaw and But investigated the DNA from dried and fresh roots of the three species, *P. ginseng, P. notoginseng* and *P. quinquefolium*, amplifying by the arbitrarily-primed polymerase chain and random-primed polymerase chain reactions. Resultant fingerprints of *P. ginseng* and *P. quinquefolium* proved to be consistent irrespective of source or age of the sample. As substitutes and adulterants yielded different fingerprints the method has applications for quality control. Roots of *P. ginseng* and *P. quinquefolium* were shewn to be more closely related to each other than to *P. notoginseng*.

DNA is formed of nucleotides, products of the combination of one molecule of phosphoric acid, one molecule of the 5-carbon sugar 2-deoxy-D-ribose and one molecule of one of the four bases adenine and guanine (purines) or thymine and cytosine (pyrimidines); the resultant nucleotides are adenylic acid, guanylic acid, thymidylic acid and cytidylic acid respectively. The DNA skeleton comprises two chains of alternate sugar and phosphate units twisted around each other in a double spiral or double helix formation with a base attached to each sugar and the two chains are held together by hydrogen bonding of the bases. DNA occurs mainly in the cell nucleus and controls the formation of the cytoplasmic nucleic acid, ribonucleic acid (RNA), by a process named *transcription*. RNA differs from DNA by the presence of the sugar D-ribose ($C_5H_{10}O_5$) instead of 2-deoxy-D-ribose ($C_5H_{10}O_4$) and the base thymine is replaced by the base uracil. As the base sequence in DNA replicates itself accurately and provides in code the genetic pattern of a particular species, the transcribed and edited RNA, which controls protein synthesis in the cytoplasm, will also be of diagnostic interest.

Therefore the 18S ribosomal RNA regions were amplified by the polymerase chain reaction to facilitate sequence determination. In each species the DNA skeleton comprised 1809 base pairs with different gene sequences. Different base substitutions were recorded at nucleotide positions 497, 499, 501 and 712 and it was possible to exploit the technique in order to clearly identify commercial root samples (Fushimi *et al.,* 1996). Using PCR-RFLP (polymerase chain reaction—restriction fragment length polymorphism) coupled with MASA (mutant allele specific amplification), the differences of the 18S rRNA gene sequences were explored for the same three species. In this case the PCR product of each species on the 18S rRNA gene was digested with the restriction enzymes *Ban*II and *DdeI;* the resultant fragments gave unique electrophoretic profiles for each species. The extracted DNA of each species was amplified by PCR using a designed species-specific oligonucleotide primer. The anticipated sizes of the

fragments specific to each species were detected only when the optimum temperature and reaction time for annealing and extension were met (MASA analysis). Both methods were used on the three species drugs and produced similar results to those for the corresponding original plants. The gene sequences of the three *Panax* species comprised 1259 base pairs and that of *P. quinquefolium* varied markedly at nucleotide position 102 (Fushimi *et al.*, 1997). Further work should offer a better understanding of species differentiation in the Araliaceae.

The work of Wen and Zimmer (1996) considered the sequences of internal transcribed spacers and the 5.8S coding region of the nuclear ribosomal DNA repeat for 12 species of *Panax* in the hope of establishing phylogenetic relationships. The North American species *P. quinquefolium* and *P. trifolium* were distinct, the former probably being more closely related to the eastern Asiatic species. The monophyly of the three important medicinal species *P. ginseng, P. notoginseng* and *P. quinquefolium* which had been suggested by earlier workers was not supported by current data although the close phylogenetic relationship of the genera *Panax* and *Aralia* was confirmed. The authors did, however, warn that a discrepancy between the sequence divergence pattern and the phylogenetic pattern encountered in their work did emphasise the need for caution in using sequence divergence data alone when investigating biogeographical patterns. As the Himalayan area and central and western China are the current centres of diversity of the genus *Panax* and the species located there appear closely related with low internal transcribed spacer sequence divergence it is suggested that rapid evolutionary radiation of the plants may have produced the diverse variations of the species.

Panax ginseng C.A.Meyer; Chinese, Korean or Oriental ginseng.

This species is the most important commercially and is widely cultivated in China and Korea. Because of the wide use of the plant with resultant overharvesting, wild populations that once spread from central China, to Siberia and Korea are now scarce and are found principally in mixed broadleaf and coniferous forest areas in Manchuria. *P. ginseng* was found in the mixed forest areas in Heilongjiang, Jilin Kirin (Manchuria), Liaoning (adjoining North Korea) and northern Hopei (Hu, 1976) and the occurrence of rare wild populations in the Long White Mountain area of northeastern China and neighbouring Korea were reported also by Hu (1978). Typical roots are persistent, thick, fleshy, fusiform and cream to pale yellowish buff in colour; the primary root is frequently irregularly branched (Fig. 2). Older roots are wrinkled due to the annual contractile activity that maintains the position of the dormant bud at soil level. Root shape varies markedly according to the soil environment. Heavy stony soils cause short, thickened primary roots with many thickened secondary roots. Light, sandy soils produce longer, straight, carrot-like tap roots with less secondary branching. Fresh roots possess a strong taste that is bitter yet also sweet and a prominent, characteristic aroma that is gradually lost on storage. The rhizome or underground stem, which is normally unbranched, usually bears adventitious roots which may become thick and fleshy. In wild *P. ginseng* the rhizome may be elongated but cultivated plants yield short, thick, compact,

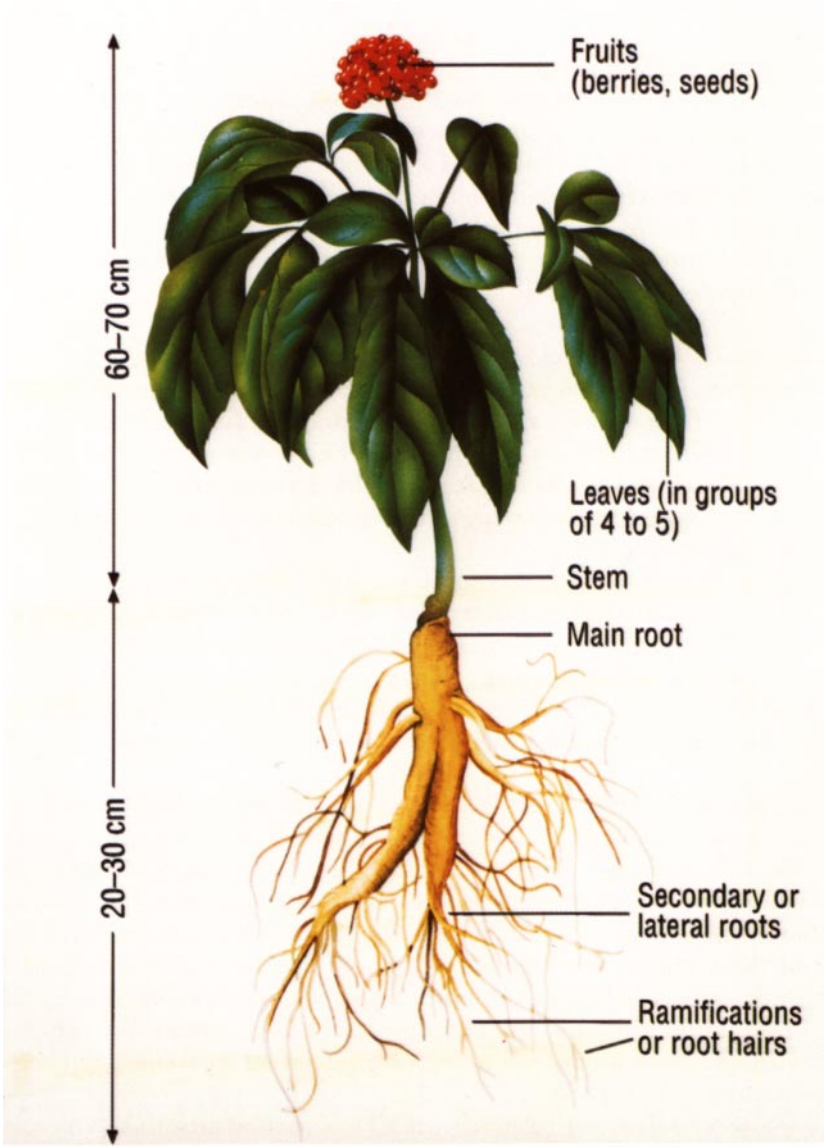

Figure 2. *Panax ginseng.* General habit of the mature plant.

erect rhizomes (Baranov, 1966; Thompson, 1987). Rhizomes bear leaf scars the number of which gives an indication of the age of the plant. The leaves, also known as prongs, may be 3–6 in number in a whorl and are palmately compound, verticillate (whorled) and petiolate, the petioles being 8–15 cm long, slender and terete (smooth and rounded). Each leaf comprises 5, or occasionally 3–7, leaflets, the basal pair being smaller than the upper leaflets. Typical leaflets are serrate, cuneate (wedge-shaped) at the base, acuminate (tapering to a point)

at the apex and bear some stiff hairs along the marginal veins. The floral arrangement or inflorescence is an umbel, a flower cluster in which the flower stalks are of almost equal length and arise from a common centre. The fruit stalk or peduncle of the umbel varies from 7–20 cm long and the terminal umbel comprises 4–40 flowers dependent on the age and growth environment of the plant. The small flowers of ginseng expand in June-July and are about 2–3 mm across. Each consists of a 5-toothed green calyx, 5 yellowish-green entire petals, 5 short stamens with oblong anthers and a bifid style and stigma with an inferior ovary. The fruit is about the size of a common pea; initially it is green but reddens as it matures to form a fleshy drupe containing 2–3 white seeds which are often called pyrenes.

Panax japonicum C.A.Meyer=*P. pseudoginseng* Wall, subsp. *japonicus* Hara; Japanese ginseng. Chikusetsu ginseng. Also the subspecies *P. japonicus* var. *major* (Burk.) Wu and Feng and *P. japonicus* var. *bipinnatifidus* (Seem.) Wu and Feng. Zhujieshen or Rhizoma Panacis japonici, the dried rootstocks of *P. japonicus,* and zhuzishen or Rhizoma Panacis majoris, the dried rootstocks of *P. japonicus* var. *major* or *P. japonicus* var. *bipinnatifidus* are officially listed in Volume I of the Chinese Pharmacopoeia, 1985.

Preferring a warmer climate, this species is found in areas stretching from Japan through temperate and subtropical parts of China to the borders of northern India and Nepal. Indigenous to the mountain areas of Japan it is known locally as *nin-jin* or *chikusetsu nin-jin*. The aerial plant closely resembles *P. ginseng* but the rhizome is thick and horizontal with short internodes and resembles bamboo. Being more bitter than *P. ginseng* roots, Japanese ginseng roots are less valued commercially.

Panax notoginseng (Burk.) F.H.Chen; *P. pseudoginseng* Wallich; Sanchi ginseng. Several subspecies of wild Asiatic origin are recognised including subsp. *himalaicum* (Burk.) Wu and Feng (not currently listed in the Kew Index), subsp. *japonicum* (Nees) Hara (not listed in the Kew Index), subsp. *pseudoginseng* (not listed in the Kew Index), var. *japonicus* (C.A.Meyer) Hoo and Tseng, var. *angustifolium* (not listed in the Kew Index), var. *bipinnatifidum* (not listed in the Kew Index), var. *elegantior* (Burk.) Hoo and Tseng, var. *notoginseng* (Burk.) Hoo and Tseng, var. *wangianus* (Sun) Hoo and Tseng (Thompson, 1987). Sanchi or sanqi, Radix Notoginseng, the dried roots of *P. notoginseng,* is listed in Volume I of the official Chinese Pharmacopoeia, 1985.

This species is indigenous to northeast China particularly in the mountainous areas of the provinces of Heilongjiang, Jilin Kirin (Manchuria), Liaoning (adjoining North Korea) and northern Hopei (Hu, 1976). Its ecological range and therefore potential cultivation does extend westwards to include Nepal, northern India, northern Burma and southeastern Tibet. Therefore it has been reported in the Sichuan (Sze-chwan) and Kiang-su provinces of China, North Vietnam, the forested slopes of the Himalayas and northern India.

The growth habit of this species resembles that of *P. ginseng*. Typical roots are fleshy, firm, obconical or shortly cylindrical, smooth skinned and yellowish

green to brownish yellow in colour. Roots are usually about 2–4 cm long and 1–2 cm diameter. Var. *elegantior* possesses a slender rhizome with elongated internodes and enlarged nodes giving the appearance of a string of pearls, hence the common name Pearl Ginseng. The root of *P. notoginseng* tastes bitter initially but the after-taste is sweetish.

Panax quinquefolium L. American ginseng.
Although indigenous to North America, this slow growing, perennial, herbaceous species rarely occurs in Western medicinal products but is used as crude drug in the Far East. *P. quinquefolium* is distributed in the eastern temperate forest areas of North America from southern Quebec to Minnesota in the north to Oklahoma, the Ozark Plateau and Georgia in the south. American ginseng is not found in the prairie regions and its distribution density in the eastern areas is variable due to overharvesting and habitat destruction (Carpenter and Cottam, 1982; Thompson, 1987). This species has been reported in 33 states but it is now considered as a threatened species in 16 states and as an endangered species in a further 10 states. Wild mountain ginseng from Wisconsin, Pennsylvania and New York States is considered the most desirable commercially although commercial American ginseng is usually cultivated.

P. quinquefolium resembles *P. ginseng* in growth habit, attaining a height of about 25–50 cm. The thick, spindle-shaped, fleshy root is persistent, up to about 10 cm length and 2.5 cm thickness and often transversely wrinkled. Older roots are usually forked or branched producing the characteristic anthropomorphic appearance so exaggerated in earlier drawings of ginseng roots. Correctly dried roots lose about 60 per cent of their initial weight and are firm and solid with a slight aromatic odour and the taste is bitter at first with a sweetish after-taste. Average dried roots weigh 30–60 g and rarely exceed 150 g. The rhizome is erect. The young seedling usually produces a whorl of 3 palmately compound leaves, the leaf is borne on a 2–10 cm petiole and possesses 3 large upper and 2 small lower leaflets as in *P. ginseng*. From the third year onwards the plant usually produces 3–5 compound leaves. The smaller leaflets are normally up to 5 cm long and the larger leaflets attain up to 10 cm in length. The leaves are bright green in the summer and turn yellow in the autumn. The umbel of greenish-white flowers is borne on a single stalk 5–12.5 cm long. The fruits are bright red drupes which are often miscalled "berries" and appear conspicously after about three years growth. The rapid differentiation of *P. quinquefolium* and *P. ginseng* roots by genomic fingerprinting has been successfully investigated using the arbitrarily primed polymerase chain reaction (AP-PCR) technique (Cheung *et al.*, 1994).

Panax trifolium L.Dwarf American ginseng or groundnut ginseng.
This species is hardier than *P. quinquefolium*. It occurs in an area embracing Nova Scotia and Ontario in Canada and spreading eastwards to Minnesota and southwards to Kentucky, Tennessee, North Carolina and northern Georgia. *P. trifolium* is a rapidly growing species with vegetative proliferation between April and June. It can tolerate wetter soil conditions and stronger light intensities

than *P. quinquefolium*. It is characterised by a small, globose root attaining a diameter of 1–2 cm. The vertical rhizome is short and straight with short internodes. The smooth, hairless, annual aerial stem differs from stems of the other common species being cylindrical at the base and polygonal towards the apex. The palmately compound leaves are typical of *Panax* spp. (Thompson, 1987). Although not considered desirable commercially, *P. trifolium* may occur as an adulterant.

Panax vietnamensis Ha. et Grushv. Vietnamese ginseng.
One of the lesser species, *P. vietnamensis* is found in forests in Central Vietnam in the wide area south of Da Nang at latitude 15° N and 108° E at elevations of 1700–2000 m above sea level. This species is characterised by a mainly unilocular ovary with one style and the fruits bear a black spot on the apex when ripe and are usually one-seeded (85 per cent), the seeds being kidney-shaped (Nguyen Thoi Nham et al., 1995).

Panax zingiberensis Wu and Feng. Ginger ginseng.
The plant is apparently restricted to the southeastern area of Yunnan Province. The root system comprises numerous fleshy tuberous roots arising from a short rhizome.

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3. THE GROWTH AND CULTIVATION OF GINSENG

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Wild ginsengs were originally gathered from many Asiatic and North American sources but continuous overharvesting has now considerably reduced the number of potential gathering areas. Despite this reduction, some wild material is still available commercially. There is, however, no real evidence to substantiate the widely-held folklore belief that the wild product is eminently superior to the carefully cultivated drug. Therefore worldwide supplies are now obtained largely from commercial plantations and considerable knowledge of the problems of cultivation is available.

Ginsengs are plants with very specific growth requirements. They cannot tolerate strong sunlight and much prefer shady positions in deep, predominantly hardwood rather than coniferous woodland where there is also protection from extremes of frost, snow, rain and high winds. Under normal conditions the plants extract natural nutrient materials from the soil in considerable quantities and indigenous growers claim that a decade or more is necessary for soil recovery prior to further ginseng growth. For this reason carefully controlled cultivation is necessary if commercial quantities of good quality ginseng are to be available.

Cultivation offers the advantages that there can be close control of growing conditions with rational replacement of essential nutrients, diseased plants can be detected and treated appropriately, pests such as slugs and snails and predators scavenging the fruits can be restricted, experienced workers can be trained to collect the right part of the plant at the correct time, storage conditions for the harvested crop can be regulated, quality control can be undertaken at source and research trials can be conducted. On the debit side, disadvantages of closely standing crops include the damaging effects of natural phenomena such as fire, flood, typhoons, tornadoes, etc., and the rapid spread of fungal and insect infestations.

Four species, *Panax ginseng, P. notoginseng, P. quinquefolium* and *P. japonicum,* have become the subject of extensive research trials and many reports are available especially from Chinese, Japanese and Korean sources. *P. ginseng* cultivation has been studied comprehensively in China and Korea and *P. quinquefolium* production has been investigated both in its native North America and in the Far East. Sanchi ginseng (*P. notoginseng* (Burk.) F.H.Chen=*P. pseudoginseng* Wall. var. *notoginseng* Hoo and Tseng)) is the subject of much current research in its native China. Chikusetsu or Japanese ginseng (*P. japonicus* C.A.Meyer=*P. pseudoginseng* Wall, subsp. *japonicus* Hara) is cultivated in Japan where the rhizome is employed as a substitute for Korean ginseng.

The ginseng plant is a very slow growing perennial propagated from seed. Seeds are obtained from the ripe fruits of healthy 5-year old plants in September or October before the roots are harvested. The small, flat, white disc-shaped seeds are kept moist and carefully planted out in prepared beds (Figs 3 and 4).

Although ginseng plants grow in relatively rough climatic conditions, the air temperature must vary only within the range 0–25° C throughout the year and the plants require protection from direct sunlight. Therefore cultivated plants are normally grown in the shade of tall, forest trees or under specially constructed shelters with thatched roofs that permit about 25 per cent of the available rain to seep through. Plantations are best sited on cool north or northeast facing slopes, never on south facing slopes and preferably in natural woodland. In northern and north-eastern China ginseng grows in areas of the lime trees *Tilia mandshurica* Rupr. and *T. mongolica* Maxim. In North America ginseng favours areas where basswood (*T. americana)*, oak (*Quercus* spp.), hickory (*Carya* spp.), beech (*Fagus* spp.) and maple (*Acer* spp.) grow. Such trees indicate suitable areas for ginseng cultivation, areas where the soil is rich and damp but not wet or muddy. The growing beds are allowed to remain fallow for one year before planting and are treated with a mulch of ginseng leaves although other applications such as leaf mould and horse manure can be used. Rice hull mulch and peat can be used to maintain the soil bed water content. Artificial fertilisers

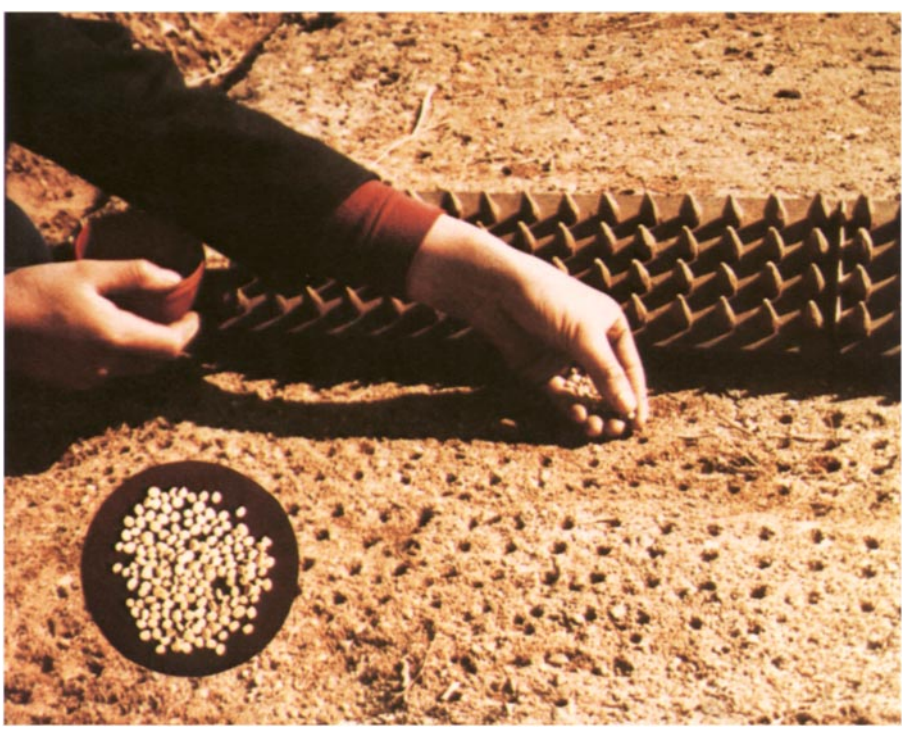

Figure 3. Planting out of ginseng seeds in prepared beds and (inset) ginseng seeds.

Figure 4. Preparation of drills and planting of individual seeds in carefully prepared beds.

are not favoured. The soil should be dug to a depth of 45 cm several times before planting (Harriman, 1973; Hu, 1977).

Seeds planted in the autumn and kept moist in the ground will produce seedlings in about 18 months. Nevertheless a problem with ginseng seeds is the slow emergence of the embryo from the dormant state. Indigenous ginseng farmers in China and Korea had discovered the traditional methods of pre-sowing treatment of seeds by trial and error but their methods have proved unsatisfactory for modern horticulture. Typically with American ginseng *(P. quinquefolium)* approximately two years are required for normal seedling growth. The ripe seed at the time of fruit maturation contains abundant endosperm but the embryo is poorly developed. During the first year the stored food permits slow development of the embryo in the seed and the amylase, catalase and peroxidase enzyme activities are low. Normally about 60 per cent of seeds will germinate in the spring of the second year and then, as development is more rapid, there is a steady increase in enzyme activity. Seeds can continue to germinate up to 5 years after sowing. For ginseng seeds there are three after-ripening phases. The first phase is the formation of the morphologically entire embryo from the rudimentary embryo observed in the ripe seed. The second phase comprises the elongation of the embryo from about 0.2 to 0.3 mm length to up to about 5 mm in August or September of the year following fruit ripening. It is at this stage that the leathery endocarp splits along the sutures of the seedcoat; this stage is referred to as “cracking”.

Despite the development of the embryo the endocarp may not dehisce and the third phase, germination, is therefore incomplete due to an apparent physiological block. Choi and Takahashi (1977) noted that this physiological block can be overcome by moist chilling of seeds at 2° to 5° C (equally applicable to *P. ginseng* and *P. quinquefolium*). Stratification, the method of treating moist seeds at low temperatures (0° to 10° C) for a period of time, stimulates more rapid embryo development. Under natural conditions the cold period of winter ensures that the seed germinates in the spring but for cultivation purposes the moist seeds are usually packed in horticultural flats and stacked either in the open during winter months or in cold-storage rooms. Seeds stratified immediately upon ripening sprouted after about 8 months but those stored for 4 months in dry conditions before stratification required 19 months for similar development (Baranov, 1966). In Korea, Kwon and Lee (1997) examined *P. ginseng* seeds undergoing stratification at 4° C for 16 weeks. Free cytokinins, compounds stimulating cell division, were found in highest concentration (5.06–8.89 μg/g seed in butanol extracts) in seeds chilled for 2–4 weeks but concentrations gradually decreased during subsequent weeks. Total gibberellin-like substances, also extracted in butanol and responsible for cell enlargement and elongation, increased slowly during the stratification process but, after emergence of the radicle, gradually decreased. The Hong Kong group of Ren *et al.* (1997) similarly investigated the hormone changes in *P. quinquefolium* seeds during the after ripening period under controlled stratification conditions. The seeds were stratified in sequence at 18–20° C for 80 days, at 8–13° C for 54 days and at 0–5° C for 88 days, the water content of the stratification medium (sand) being maintained at about 10 per cent. Initially the gibberellic acid (GA_3) and indole acetic acid (IAA) levels in the endosperm were low, slowly increasing to small peaks in 164 and 144 days respectively. However both increased remarkably by the 198th day. Similar changes occurred in the embryo. The naturally occurring cytokinin zeatin (6-(4-hydroxy-3-methylbut-2-enyl)aminopurine) probably stimulated cell division in the developing embryos and shewed a pattern of content low-high-low paralleling the embryo growth rate. Abscisic acid, a natural inhibitor of gibberellins and cytokinins, occurred in high concentration in the embryo and endosperm before chilling at 0–5° C, but thereafter decreased to a very low concentration by the end of the stratification period thus permitting increased giberellin and cytokinin activity. Further work by the Chinese group Huang *et al.* (1998) described the presence of the germination inhibitors acetic acid, butanoic (butyric) acid, isobutanoic acid and 1-phenyl-ethanone in the fruit pulp of *P. quinquefolium* and suggested that this could explain why the embryo of the seed was still immature when the seed itself attained maturity. Recent work by the Korean team of Kwon *et al.* (1998), investigating *P. ginseng* seeds, confirmed that the cytokinin content increased significantly during stratification reaching a maximum by the end of stratification but was very low when the radicle emerged. The major endogenous cytokinin was dihydrozeatin (36–60 per cent of total cytokinins); dihydrozeatin riboside and trans-zeatin riboside increased steadily during stratification and chilling, *trans-zeatin* riboside becoming the most abundant cytokinin in the germinating seeds. Cis-zeatin

and cis-zeatin riboside were similarly detected. It was concluded that these cytokinins were closely involved in the germination of the seeds and that dihydrozeatin riboside and possibly *trans-zeatin* riboside were the principal cytokinins involved.

Germination proper occurs as the radicle emerges through the membraneous testa and the split endocarp. The radicle grows downwards as the plumule with its epicotyl protected between the cotyledons elongates upwards prompted by the enlargement of these structures. In late April or early May the seedling develops a single shoot a few cms high with a composite spray of three small, oval leaves and the cotyledons, having completed their task of nourishing and protecting the rising epicotyl hook as it pushes its way upwards through the soil, gradually die away. The petiole varies in length dependent on the nature and thickness of the surface leaf mould or mulch but is usually 4 to 10 cms. A solitary bud at the base of the leaf petiole differentiates into shoot and bud primordia for the next year's vegetative growth. The growth of the aerial shoot is determinant, that is, it is ultimately limited by the cessation of meristematic activity. Therefore the aerial growth dies down in the autumn and the roots, rhizomes and developing buds for the next season remain dormant until the following spring. The preformed primordium that produces the aerial growth is well enough developed by late summer to reveal the number of leaves and leaflets and, if present, the inflorescence that will emerge in the following spring. All subsequent growth is limited to the development of the preformed primordia in each year of the life of the plant (Baranov, 1966; Thompson, 1987).

Seedlings may be transplanted when they are one or two years old. It is usual to transplant in the autumn or spring when the young plants are leafless. Lateral roots are removed and the main roots positioned vertically to a depth of about 15 cm, the roots being surrounded with sphagnum moss to maintain a moist environment. Seedlings are transplanted usually about 20–25 cm apart.

In the second year two or three compound sprays or prongs of five leaflets arise, extending like parasols from the rootstock. This growth also dies down in the fall or autumn and in the following year a stem some 20–25 cm high develops with characteristically three compound leaves each subdivided into five leaflets and the plant bears flowers for the first time. Thus the plants are dormant during the winter period; the stimulus that initiates growth in the next spring is not fully understood but low temperature or chilling is apparently important. Working with one-year-old American ginseng plants Konsler (1984) noted that 100 per cent emergence could be obtained if the roots were maintained at 0° to 9° C for 75 to 90 days. In his experiments the total dormancy averaged 126.5 days, roughly four months, the days to aerial growth emergence being inversely proportional to the number of days of cold treatment. This agreed with the subsequent work of Lee *et al.* (1985) who suggested that the dormancy requirement was a temperature range of 0° to 10° C for 100 days, the preferred temperature being 5° C for 100 days for 3-year-old roots that were subsequently grown at 15° C.

Flowers and fruits are often removed in order to stimulate vegetative growth. In successive years the plant grows to a height of about 60 cm with a crown of dark green, verticillate leaves. Under cultivation conditions the root mass grows

in a linear manner and there is often a direct correspondence between the number of prongs and the age of the plant e.g. 2 prongs at 2 years, 3 prongs at 3 years, although 5 prongs is rarely exceeded even after prolonged cultivation. Lewis and Zenger (1982) noted that for the slower growing wild *P. quinquefolium* plants there was also a linear relationship between age and the number of prongs but it was not annual.

The flowers are inconspicuous, small and green and normally arise in the third and subsequent years. The single peduncle or flower stalk arises at the junction of the whorl of compound leaves and elongates to lift the multipedicelled umbel of flowers above the leaflets. The umbel is similar to that of the related Araliaceous plant *Hedera helix* L., common ivy. Konsler (1984) observed that *P. quinquefolium* immature inflorescences could be clearly discerned in northern plants as the aerial growth emerged in the spring but in southern plants the immature inflorescence remained obscure until the peduncle elongated. Flowering usually occurs from late May to early August for American *P. quinquefolium* and during June and July for Oriental *P. ginseng*.

The inflorescence produces a cluster of bright red fruits commonly referred to as berries, individual berries being about 1 cm in diameter (Fig. 5). True berries are succulent fruits in which the mesocarp and inner endocarp remain succulent but ginseng fruits are drupes because the innermost endocarp is hard and leathery. The drupes contain 1–3 flat, white disc-shaped seeds. The plants are usually grown for about 5 or 6 years before harvesting roots and seeds. Although it is believed that the fruits falling to the ground naturally are the best

Figure 5. Ginseng plants bearing typical clusters of red berries.

source of seeds, fruits can be collected in the autumn and then spread for several days in the shade to permit the skin and pulp to blacken. The seeds can then be removed by rubbing the fruits and must be stored moist, usually in sand. Dry seeds will not germinate and it is essential to protect all seeds from predators such as birds and rodents.

The roots are described botanically as contractile roots; such roots possess a mechanism for the annual repositioning of the regenerative buds essential for growth in the following season. The short vertical rhizome, an underground stem or rootstock, is sometimes known as "the neck" and this rhizome grows upwards during each growing season. As the regenerative buds arise at the tips of the rhizomes, it is necessary that the roots contract, pulling the rhizome and buds downwards and thereby keeping the buds at soil level; the rate of contraction balances the rate of upward growth (Baranov, 1966).

The *P. ginseng* roots are harvested in September or early October; this time is ideal as the roots are firm thus permitting careful cleaning by hand (Fig. 6). Earlier, in spring or summer, the roots are soft and less easy to process. The size of the roots is age-dependent; 5-year old commercial roots are about 10 cm in length and 2.5 cm in diameter whereas 10-year old examples would be about 25 cm long. Lateral roots are up to 20 cm in length and 5 to 10 mm diameter. It has been claimed that ginseng *(P. ginseng)* will grow for hundreds of years and a substantiated example of a 400-year old root was reported by Grushvitzky (1959). Nevertheless for commercial purposes 5 or 6 years is practicable, the plant being large enough and the yield of desirable chemical constituents optimal.

Figure 6. The cleaning of *Panax ginseng* roots by hand.

Careful analysis of ginseng roots by high performance liquid chromatography (HPLC) indicated an increase in total saponins in the main root up to the 5th year of growth, an increase related to marked weight development; in the 5th year there was a slight decrease before a significant increase in the 6th year. Lateral roots shewed a marked increase in the 3rd year followed by a decrease in subsequent years. The highest yield of ginsenoside-Re was found in the lateral roots. In rhizomes the yield was highest in the 2nd and 3rd years and the content of ginsenoside-Ro was greater than elsewhere in the plant (Yamaguchi *et al.*, 1988). Subsequent work by Samukawa *et al.* (1995) also using HPLC shewed that the saponin content of the roots increased for 3 years but decreased in the fourth year and increased again in the fifth and sixth years, justifying the commercial decision (Samukawa *et al.,* 1995).

Ginseng plants are sensitive to temperature changes, freezing occurring at from -3.5° to -9.6° C for P. *quinquefolium* seeds, dependent on water content, and roots can withstand -5° C for 24 hr without damage but -10° C for only 5 hr will cause serious damage. Varying low temperature was more damaging than constant low temperature and therefore the chances of such damage are greater during the thawing period in early spring than in winter (Lee and Proctor, 1996).

The brownish-white harvested roots are carefully cleaned to remove adherent soil. Without further treatment the roots will not remain in good condition for more than 10 days under normal autumnal conditions; therefore the roots are dried slowly in the sun to yield air-dried white ginseng. Alternatively roots can be dried at 15.5°–27° C in airy, heated sheds, the temperature being gradually raised in a few days to 32° C as the roots dry. Drying times vary according to root diameters and the roots lose up to two thirds of their weight in moisture. After drying the roots are sorted according to size and quality, and, in good laboratories, checked for the absence of bacteria, yeasts and mould fungi, tested for the absence of harmful pesticide residues and examined for possible aflatoxin presence. Further quality control should, and often does, include suitable identity tests for the presence of major chemical constituents (e.g. the ginsenosides) and some quantitative assessment before sale. White ginseng can also be obtained commercially with the surface "skin" removed by careful peeling. Dried white ginseng, if stored carefully, can retain saleable quality for about 12–15 months.

Red ginseng indicates an alternative method of preservation of ginseng. The cleaned roots are sterilised by steam treatment at a temperature of 120° to 130° C for 2 to 4 hours. Sugars present in the roots partially caramelise causing the characteristic red-brown colouration, hence the name "Red Ginseng". The saponin content of red ginseng is increased during processing and Samukawa *et al.* (1995) further observed that in terms of total saponin content the best 6-year old red ginseng came from Korean sources followed by Japanese and Chinese products. The heat treatment increases the hardness of red ginseng roots and also produces chemical artefacts. Carefully stored red ginseng will retain its quality for 2–3 years.

White and red lateral roots are also marketed and all ginseng products require careful storage to prevent rodent damage, bacterial and mould growth and beetle infestation. Commercial pressure has stimulated research into better

methods and techniques for the cultivation of the large quantities of good quality ginseng needed to satisfy the increasing worldwide market.

From the 1960's onwards Japanese and Russian scientists have investigated the use of the gibberellins as growth promoting agents for ginseng. Gibberellins comprise a series of closely related substances obtained from fungi of the genus *Gibberella*. In 1926 the Japanese researcher Kurosawa studied the sterile cell-free filtrates obtained from the fungus *Gibberella fujikuroi* (Sawada) Wollenweber, a fungus causing the pale, spindly growth of rice, and noted that application of the filtrate to rice seedlings caused marked growth stimulation. More than 25 such growth hormones or gibberellins are now known and are all closely related to the commercially available gibberellic acid GA_3. Gibberellins normally occur widespread in plants and operate in conjunction with plant auxins (e.g. indole-3-acetic acid) in growth processes such as cell elongation and enlargement and, in some cases, stimulate the production of hydrolytic and proteolytic enzymes essential to seed germination.

The Japanese horticulturists Ohsumi and Miyazawa (1960) experimented with ginseng seeds soaked in various concentrations of gibberellic acid and incubated in sandbeds and they noted that there was improved growth of the embryos and better germination rates. The number of seeds germinating improved from 50–70 per cent to 90–100 per cent and optimum results were obtained after 25 hour treatment of seeds with 0.05 per cent to 0.1 per cent gibberellic acid solution. The Russian botanists Grushvitskii and Limari (1965) also studied the effect of gibberellic acid on ginseng seeds and observed that the first stage of after-ripening, the dormancy time before germination controlled by cold conditions, could be reduced from 4 months to 2 months and therefore the period of time required for preparation of seeds before sowing could be reduced from 8 to 6 months.

Although other plant growth substances such as the cell enlargement promoting auxins I.A.A. (indole-3-acetic acid), naphthal epiacetic acid and 2,4-D (2,4-dichloro-phenoxyacetic acid) and the cell division stimulating cytokinin kinetin (6-furfuryl adenine) have been investigated, the general conclusion is that gibberellic acid is the best ginseng growth stimulator so far discovered. Certainly Kuribayashi *et al.* (1971) advocated use of 24 hours immersion of seeds in a 100 p.p.m. aqueous solution of gibberellic acid coupled with temperature lowering to 2°–15° C for about 10 days, the optimum germination temperature being 10° C. In a subsequent work Kuribayashi and Ohashi (1975) used kinetin solution to stimulate germination, the minimum concentration required was 25 p.p.m. for 24 hr treatment or 50 p.p.m. for 12 hr and germination was further accelerated when treated seeds were kept at 5°–10° C for 20 days.

Many other plant growth hormone treatments have been studied with the object of accelerating enzyme activity thus increasing decomposition of the stored nutrients in the endosperm with a resultant breakdown of starch and non-reducing sugars in the endosperm and an increase in reducing sugars. Consequently earlier germination occurred and enzymic activity was increased especially in the second year when rapid growth took place (Li *et al.*, 1994a). In particular, gibberellin treatment (soaking in 100 p.p.m. solution for 24 hr prior

to cultivation) caused higher activities of catalase, peroxidase and acid phosphatase in 8-week-old plantlets and accelerated the plant development (Fang *et al.,* 1992).

Sruamsiri *et al.* (1995) investigated the growing of ginseng in Japan and Northern Thailand. For successful cultivation it was realised that photoperiod control, low temperature treatment, growth regulator addition and light intensity limitation were essential. At the Nong Hoi Experimental Station, situated 1000 m above sea level in Northern Thailand, the Chiang Mai University agriculturists submitted ginseng seeds to 24 hours soaking in either 100 p.p.m. gibberellic acid solution or water. The soaked seeds were sown in moist sand layered between clay in pots before placing in a shaded house with an ambient temperature of about 10°–30° C. Stratification for 2 months resulted in opening of the seed coat in 24.0 per cent of the gibberellic acid treated seeds; by 3 months this had increased to 36.8 per cent and in 4 months to 54.4 per cent. The water-soaked seeds revealed no opening of the seed coats after 3 months and a mere 4 per cent after 4 months. Complete germination was not achieved in these experiments so the team next investigated the effect of low temperature storage. Healthy seeds without opened seed coats from the first experiment were again soaked in 100 p.p.m. gibberellic acid solution or water, mixed with moist sand and stored in a refrigerator at 5° C. On monthly examination of the seeds it was apparent that seeds soaked in water and gibberellic acid solution responded similarly; significantly by the end of 2 months stratification 57–59.4 per cent of the seeds shewed open coats, a percentage that increased to 64–77.8 per cent in the 3rd month and in more than half of such seeds the radicle was already protruding. Therefore it was concluded that germination was more efficiently prompted by cold treatment than by gibberellic acid treatment.

Once established the growth of the young ginseng plants is also controlled by other factors including soil nature and pH, trace metals in the soil, light intensity, light colour, etc.

Ginseng grows best in good, natural woodland soil which is preferably well drained, rich, sandy loam. Wei *et al.* (1985) demonstrated that humic soils produced only slightly better yields of total and individual glycosides e.g. in *P. ginseng* grown in farmland soil 3.44–5.50 per cent total saponins were obtained and in humic soil 4.69–6.81 per cent were found. In a 6-year field growth survey the Korean group of Lee *et al.* (1989) reported that the yield of 6-year old roots was 2.4, 2.13 and 1.44 kg/3.3 m^2 in clay loam, loam and sandy loam respectively. They also noted that clay loam produced an overall plant loss rate of 33.6 per cent over 6 years and a greater stem length and diameter whilst sandy loam yielded a higher plant loss rate of 51.6 per cent and smaller stems. Soil aggregation and porosity were slightly greater in 6 year old plots when compared with 2 year old plots and plant survival and yield was significantly correlated with soil clay contents and porosity.

Ginseng growers have noted that ginseng plants do not favour chemical fertilisers or mixtures rich in nitrates and in Korea rice straw, barley straw and corn stem mulches have been shewn to be equally efficient in producing 6-year-old roots and to be better than green manure (Lee *et al.,* 1990). Pine tree leaves

have also been recommended as a mulch (Kim and Kim, 1991) and there was no apparent difference in the growth of the plants but the flavour components were enhanced. Some growers prefer to use unleached wood ashes and other fertilisers used include decomposing leaves mixed with soybean, cotton seed or peanut pressing residues and horse, chicken and even human manures (Hu, 1977). Normally winter mulches are 10–12.5 cm deep in order to protect crowns from cold, frosty conditions and thinner layers are employed to keep soil moist during dry spells. There is a positive correlation of the root yield of sugars and saponins with the organic matter content of the soil but a decrease in available phosphorus and exchangeable cations in the soil is desirable (Kim *et al.,* 1995). Russian workers have suggested the use of zeolites as fertilisers. Zeolites are hydrous aluminosilicate minerals containing barium, calcium, potassium and sodium ions and derived from feldspars obtained worldwide from natural rocks. The open framework structure permits easy diffusion of gases, ions and molecules and zeolites are important catalysts in organic chemical reactions. Applied to ginseng seeds zeolites stimulated germination and increased stratification and, spread on soils with peat, protected seedlings from rot infestations, encouraging growth, development and yields of roots, flowers and seeds (Pushkina *et al.,* 1996).

Trace element studies have indicated that essential elements in soil include aluminium, calcium, magnesium, nitrogen and sulphur in a greater proportion than usual, corresponding with the composition of the greyish-brown forest soil together with the organic matter normally found in such soil. The inorganic nitrogen content increased in 2nd and 3rd year ginseng plots (up to 100–120 p.p.m.) but decreased to 75, 34 and 25 p.p.m. in the 4th, 5th and 6th years respectively and was more variable in sandy soils. The soil phosphorus (P_2O_5), potassium, calcium and magnesium contents varied little with plant age (Lee *et al.,* 1989) but calcium and magnesium intake is reduced by application of nitrogen/phosphorus/potassium fertilisers (Li *et al.,* 1994b). Nevertheless Ma *et al.* (1990), growing *P. quinquefolium,* had concluded that the highest plant mass was obtained when a solution containing nitrogen 100 p.p.m., phosphorus 25 p.p.m. and potassium 250 p.p.m. was applied to the soil. Later work also involving *P. quinquefolium* (Chen *et al.,* 1996) recommended ammonium hydrogen phosphate ($(NH_4)_2HPO_4$) fertiliser to increase the yield of roots by up to 30 per cent, the fertiliser being applied annually when the leaves were fully expanded at the early fruiting stage. Urea foliar spray also increased plant yield and ginsenoside content. The Canadian group Proctor *et al.* (1996) demonstrated the morphoregulatory value of thidiazuron (TDZ or N-phenyl-N^1-1,2,3-thiadiazol-5-yl-urea) used as a soil drench (2.20 p.p.m.) or a foliar spray (0.22 p.p.m.). The treatment, which was appropriately applied to *P. quinquefolium* greenhouse grown seedlings and 3-year old plants, had economic potential. For greenhouse-grown seedlings foliar sprays or soil drenches of TDZ (0.22 or 2.20 p.p.m.) increased stem length and diameter and shoot and root weight and a single foliar application of 62.5 or 125 p.p.m. TDZ to 3-year old field-grown plants 3 months before harvesting increased the root biomass by 19–23 per cent. Thickened secondary roots developed on the upper part of the tap root and adventitious buds formed on the shoulders of 3-year old roots.

Such buds produced shoots after a period of dormancy and subsequently multi-stemmed plants. In addition, dormancy could be reduced by gibberellic acid GA_3 treatment prior to planting out.

The most recent field trials with *P. ginseng* and nitrogen top dressing (Gao *et al.,* 1997) confirmed that excess nitrogen retarded plant growth and overall ginseng yield because the nitrate reductase level in ginseng is low and consequently the absorbed nitrate, not being promptly reduced, accumulates in the tissues.

Studying 1-year-old American ginseng plants in water culture Ren *et al.* (1993) noted that zinc concentrations were important and critical. Thus below 0.05 p.p.m. zinc deficiency caused inhibition of root growth with sparse fibrous root development and abnormally small leaves. Above 0.5 p.p.m. zinc toxicity is demonstrated by absence of fibrous roots, yellowing of the roots and leaves that are very small and chlorotic or totally absent. In the optimal 0.1 to 0.3 p.p.m. range saponin production was normal but zinc deficiency or excess resulted in reduced synthesis and and therefore lowered deposition of saponins.

Experiments with variable pH levels in soil have clearly established that ginseng plants prefer acid conditions. In strongly alkaline soils and even at pH 7 or pH 8, few plants survive and further studies at pH 3-pH 8 confirmed that ginseng prospered at pH 4-pH 6 (Hou, 1978).

Ginsengs are shade-loving plants. Significantly the leaves of shadier rows of ginseng plants contain more chlorophyll, carotenes and xanthophyll than leaves of plants grown in sunnier situations. Therefore trials were undertaken to discover the optimum light requirements for satisfactory growth (Kuribayashi *et al.,* 1971). It was concluded that for *P. ginseng* no plants would survive 4 months exposure to 50 per cent or 100 per cent sunlight. However at 5 per cent or 10 per cent transmittance (3000 to 6000 lux) most plants survived. Later work by Lee (1988b), also using *P. ginseng,* confirmed that for 3-year-old plants 5, 10 and 20 per cent transmittance shade did not produce significant differences in root length, stem length, stem diameter and leaf area although root diameter increased markedly in shade of 10 and 20 per cent transmittance. For 6-year-old plants, the largest root diameters and heaviest roots were obtained under 20 per cent transmittance although root length was not affected.

Other effects of strong light are the reduction of leaf area, increase in the transpiration rate, reduction in the size but not the number of stomata and reduction of the total amount of chlorophyll present, factors collectively leading to poorer roots as the light increases (Lee, 1988a).

Although thatch shade is commonly used (Fig. 7) and often preferred especially for white ginseng roots (Kim *et al.,* 1990), the protection of ginseng plants from strong sunlight can be effected with coloured polyethylene net; red and blue fourfold polyethylene net provided good light intensity for ginseng growth but the red net increased air temperature and prompted early defoliation despite increasing the photosynthetic rate. Blue and black shade also produced an increased photosynthetic rate but root production and saponin yield were significantly increased with the red and blue nets, blue being the overall best option (Mok *et al.,* 1994).

Figure 7. Protection of cultivated ginseng plants from strong sunlight by use of thatched shelters.

Ginseng plants are cultivated using various mulches including ricestraw mulch. Unfortunately such mulch is a suitable habitat for the field slug *Deroceras varians* A.Adams. In the Korean ginseng fields the slugs normally lay eggs from April to June with a few being deposited as late as September. During the summer months the young slugs mature prior to wintering below the moist soil surface and emerging to repeat the cycle in the following April. Ginseng plants, especially 3 to 5-year old plants, are particularly damaged during the egg-laying period. Adult slugs can be eliminated using 5 per cent ethoprop granules or 6 per cent metaldehyde bait, and Bordeaux mixture (copper sulphate 600 mg/mL with quick lime 1200 mg/mL) spray is particularly effective after infestation (Kim *et al.,* 1990). Coincidentally and after 6 years experience Bordeaux mixture had also been preferred and recommended for spraying American ginseng foliage infested with *Alternaria panax* Whetzel blight (Wilson and Runnels, 1944). It was suggested that the maximum interval between sprays should not exceed 14 days and addition of calcium arsenate would give increased protection against chewing insects. Another pest on Korean ginseng is the snail *Acusta despecta siebddiana* which also flourishes in rice straw mulch and, particularly in the period May to July, can cause up to 10 per cent damage to the total number of plants. Metaldehyde bait is a useful control agent (Kim, 1992).

The soft roots of ginseng are particularly attractive to some nematodes, the eelworms or round worms characterised by slender, unsegmented bodies. Ahn *et al.* (1981) observed that the organic pesticides Terbufos and Mocap offered better control of root-knot nematode than Carbofuran alone or mixtures of

these pesticides. None of these compounds transferred off-flavours to the ginseng plants. Another pest damaging the roots is the potato rot nematode *Ditylenchus destructor*. Treatment with non-fumigant nematocides e.g. ethoprop or aldicarb were effective, ensuring a high survival rate in 4-year-old plants (Ohh *et al.*, 1986). *Meloidogyne hapla* root-knot nematodes have also been reported attacking *Panax notoginseng* roots in Yunnan Province in China (Hu *et al.*, 1997).

Fungus infected soil bearing the spores of *Rhizoctonia solani* can cause damping-off of emerging seedlings of *P. ginseng*. Dipping of the seeds in Toclofos-Me solution (1000 p.p.m. for 3 hr) before sowing coupled with soil drenching at 300 g/ha in mid-April will protect the emerging seedlings and the treatment remained effective in the soil for about 32 days (Yu *et al.*, 1989).

Powdery mildew caused by *Erysiphe* spp. can infect Asian ginseng and has recently been reported occurring on American ginseng plants, the infection being characterised by extensive, superficial, white mycelia. Damaged leaves turn yellow and fall prematurely (Sholberg *et al.*, 1996). The species *E. panacis*, a new species and a teleomorph of the powdery mildews found on other Araliaceous species, has been discovered on *P. ginseng* collected in Changchun in the Jilin Province of China and has been described in detail by Bai and Liu (1998).

American ginseng *(P. quinquefolium)* plants occurring in ginseng-growing gardens in British Columbia were carefully monitored for incidence of fungal pathogens. During 4 years 235 samples of roots and 25 of leaves were found to be infested with *Pythium altimum* (35.6 per cent of total isolates), *Fusarium spp*. (30.5 per cent), *Rhizoctonia solani* AG4 (19.6 per cent) and *Cylindrocarpon destructans* (9.8 per cent). Diseased leaf and petiole tissues were attacked by *Phytophthora cactorum, Alternaria panax, A. alternata* and *Botrytis cinerea* and seedling roots were diseased *in vitro* by *P. ultimum, F. solani, R. solani* and *P. cactorum* in that order. On detached leaves the most common pathogenic fungi were *P. cactorum, B. cinerea* and *A. alternata* (Punja, 1997). It was also suggested that chemical control by metalaxyl and biological control with *Bacillus cereus-complex* (GB10) could counter *Phytophthora* leaf blight and root rot under field conditions (Li *et al.*, 1997).

Keeping roots free from disease is imperative. Zhao *et al.* (1997) analysed ginseng roots with a red coating disease, comparing normal healthy and diseased roots. In diseased roots the content of ginsenosides, starch, other carbohydrates and amino acids was reduced although the levels of reducing sugars and pectin were increased. Levels of the metals aluminium, iron and manganese were higher in the diseased roots. As the diseased roots were deficient in essential phytochemicals the commercial market value was likewise reduced.

Careful observation of standing and stored crops ensures detection of problems of mould, slug, worm and nematode infestation and suitable counter-measures can be applied. Nevertheless further problems can be caused by larger natural predators such as gnawing rodents, moles and chipmunk ground squirrels attacking the roots and birds and chipmunks consuming the ripe fruits. Vigilance and appropriate counter measures are therefore essential.

Commercial cultivation of ginseng species has been clearly shewn to be practicable and desirable and now provides the bulk of the ginseng roots available in worldwide markets. Ongoing research will undoubtedly provide further pointers to improved methods and techniques yielding better pharmacological agents for the future.

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4. TISSUE CULTURE OF GINSENG

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The agricultural production of ginseng roots requires growing periods of 4 to 7 years and makes great demands on climate and soil. Ginseng plants collected from their natural habitats have become so scarce that a wild specimen may be sold for thousands of dollars. Therefore as a biotechnological alternative, ginseng tissue culture was adapted to *in vitro* conditions and is providing us with the methods for the large-scale production.

Sources of Explants and Culture Conditions

The first clone of ginseng tissue culture was derived from the root of a four year old plant by Butenko and her colleagues (1964). Since that time, different parts of mature ginseng plants and seeds have been used as sources of explants and tissue cultures (Table 4.1).

Tissue cultures are usually cultivated in the dark at 22°–25° C or under a 16 hr photoperiod at 25–26/22° C

Callus Cultures

Callus cultures serve as a source for the establishment of cell suspensions or other types of tissue and organ cultures as experimental systems for the study of their potential for secondary product formation and biotransformation capacity.

Organogenesis

Most of the data reported refers to plantlet differentiation via somatic embryogenesis, however, differentiation via shoot and root formation has also been published.

Differentiation of shoots

Ginseng calli grown on a medium with 2,4-D can serve as a source for shoot differentiation after transferring to the medium without 2,4-D on which a rigid and compact callus with high potential for organizing apices is formed. If the compact calli are transferred to the medium with kinetin, they actively generate green shoots (Furuya *et al.*, 1986). Odnevall *et al.* (1989c) reported on shoot formation from peeled seeds of ginseng on a half strength MS medium with GA_3 and BA.

Table 4.1. Culture conditions and morphogenetic response of ginseng tissue cultures

Source of explant	*Basal medium*	*Growth regulators [mg/l]*	*Morphogenetic response*	*Reference*
Root	White's	• 2,4-D [1.0] coconut milk [100.0 ml/l]	• callus	Butenko *et al.*, 1964
Root Stem Petiole Leaf Anthophore	MS	• 2,4-D [1.0] kinetin [0.5]	• callus rhizoids	Butenko *et al.*, 1968
Root	MS	• 2,4-D [1.0] adenin [1.0] kinetin [1.0] coconut milk [100.0ml/l]	• organogenesis	Butenko *et al.*, 1968
Root	MS	• 2,4-D [5.0] kinetin [2.0]	• callus	Chang & Hsing 1980
Root Leaves Petioles	MS revised	• 2,4-D [1.0]	• callus root initials	Jhang *et al.*, 1974
Root	MS modif.	• 2,4-D [1.0] kinetin [0.1] • kinetin [0.1] • IBA [1.0]	• callus • shoot formation • rooting	Furuya *et al.*, 1986
Root	MS modif.	• IBA [2.0] kinetin [0.1]	• callus	Takagi *et al.*, 1993
Root Leaf lamina Petiole	RM	• NAA [2.0] kinetin [0.5]	• embryogenic callus	Čellárová *et al.*, 1992
Stem		• NAA [0.1]	• axenic root culture	
		• IBA [2.0] kinetin [0.1]	• axenic root culture	
Seed	MS modif.	• 2,4-D [1.0] NAA [1.0] • GA_3 [1.0] BA [1.0]	• callus • shoot formation	Odnevall *et al.*, 1989

Table 4.1. *continued*

Source of explant	*Basal medium*	*Growth regulators [mg/l]*	*Morphogenetic response*	*Reference*
Flower buds	MS	• 2,4-D [0.8] NAA [2.0] BA [2.5]	• somatic embryogenesis	Shoyama *et al.*, 1988
	1/2MS	• GA_3 [0.5] BA [0.5]	• embryo germination	
		• GA_3 [0.5] BA [2.5]	• multiple shoot formation	
		• NAA [1.0]	• rooting of the shoots	
Zygotic embryos	MS	• 2.4-D [1.0] kinetin [0.01]	• embryogenic callus	Arya *et al.*, 1991
Zygotic embryos	MS	• 2,4-D [0.1]	• somatic embryogenesis	Arya *et al.*, 1993
		• 2,4-D [1.0] BA [1.0] kinetin [0.5]	• secondary embryogenesis	
		• kinetin [1.0]	• shoot formation	
		• kinetin [1.0] GA_3 [1.0]	• rooting	
	1/2MS	• BA [1.0] GA_3 [1.0] kinetin [1.0]	• plantlet formation	

Differentiation of roots

Rhizoids are the most frequent organoids differentiated in ginseng callus cultures (Fig. 8). They occur in calli of different origin.

The formation of rhizoids is influenced by exogenous plant growth regulators and light/dark conditions. Plant growth factors affect especially the beginning of rhizogenesis. The shortest induction time for initiation of rhizoid formation has been observed under the influence of NAA or IBA with kinetin or IBA alone (Furuya *et al.*, 1986, Cellárová *et al.*, 1992). In the dark the rhizoids were formed continuously without any loss of morphogenetic potential during long term cultivation. Rhizoids elongated vigorously without branching in response to NAA. A combination of IBA and kinetin resulted in elongation as well as in the formation of branch roots and subsequent differentiation of the former rhizoids into callus that gave rise to new rhizoids.

Morphology of rhizoids differentiated *in vitro* was studied by Cellárová *et al.* (1992). Usually they are about 2 cm long and form white hairy protuberances that resemble the adventitious roots often differentiated in callus cultures of

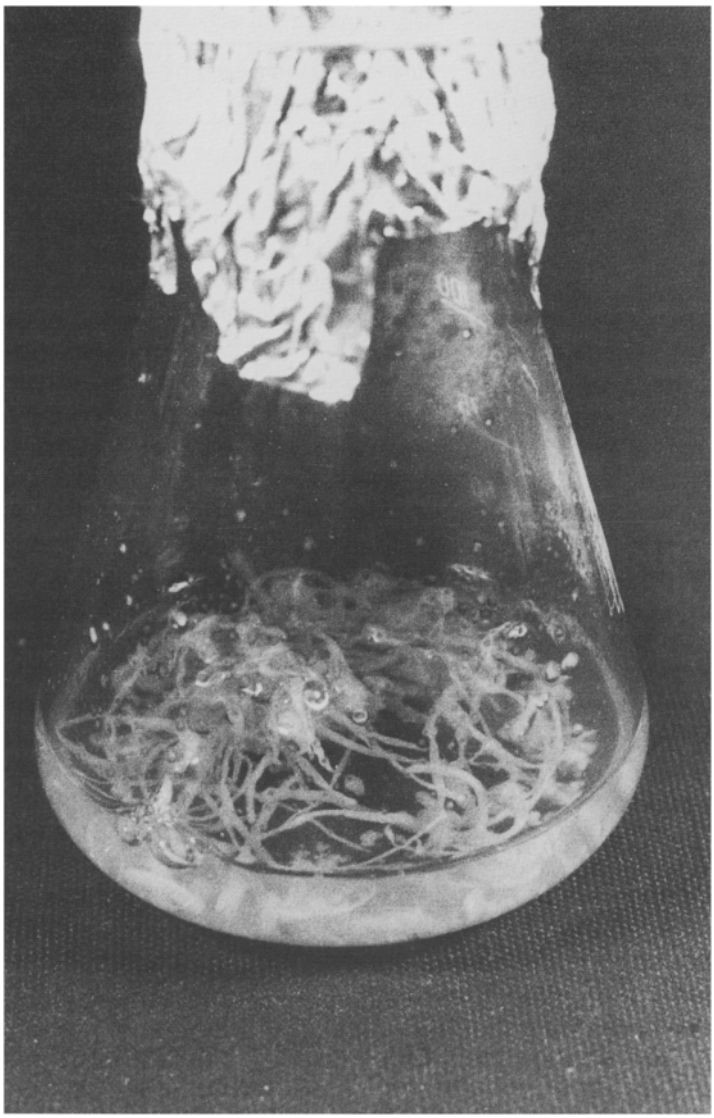

Figure 8. Culture of *Panax ginseng* rhizoids.

any other plant species. Rhizoids arose from a small group of mitotically active cells present in callus cultures. Usually the conical apical part of a rhizoid comprises a centre of dividing cells. The epidermis is composed of a monolayer of small and closely connected cells, although a typical root cap has not been observed. The presence of thin walled cells around the apical part of rhizoids predicts their protective and absorptive function. Ground tissues in the cortex and a central cylinder are formed by iso-diametric parenchymatous cells and small intercellular spaces. Parenchymatous tissue sometimes contains storage

elements such as starch grains. Conductive tissues in the central cylinder are reduced. They form a continual conductive system along the whole rhizoid and connect with tracheal elements in callus cultures.

Somatic embryogenesis and plant regeneration

The suggestion of somatic embryogenesis in ginseng tissue cultures was first made by Butenko and her colleagues (1968) but at that time all attempts to achieve the regeneration of whole ginseng plants from isolated embryo-like structures failed. Pursuing their work on the culture of ginseng root tissues Chang and Hsing (1978) obtained embryoids from root derived callus in defined conditions. Regeneration systems via somatic embryogenesis were established in root callus (Chang and Hsing, 1980), in zygotic embryo callus (Lee *et al.*, 1990) and in protoplast derived callus (Arya *et al.*, 1991). High-yield and short period embryogenesis via callus induced by the culture of young flower buds on the MS medium supplemented with 2,4 D in the dark was developed in 1988 by Shoyama *et al.* Later, Arya *et al.* (1993) reported a rapid somatic embryo formation, obtained due to secondary and tertiary embryogenesis. Embryogenic callus was initiated from immature zygotic embryos of ginseng and the somatic embryos were multiplied by adventitious embryogenesis, their growth and development being dependent on growth hormones in the medium.

Formation of embryoids has also been observed in callus cultures derived from leaf lamina after 6 month cultivation on the basal medium supplemented with NAA and kinetin (Cellárová *et al.*, 1992). Histological analysis of embryogenic calli showed the presence of embryo like structures in various stages of development and many anomalous structures such as two embryoids of the same origin in different stages of development, meristemization of torpedo stage embryoids and asymmetrical development of cotyledons. Further development required a change of culture conditions.

Immature zygotic embryos and the cotyledonary segments of mature zygotic embryos of ginseng actively produced somatic embryos on MS basal medium without any growth regulators (Choi and Soh, 1996a, 1996b), but intact embryos, embryos with half of the cotyledons removed and excised plumules and radicles did not produce any somatic embryos. Such somatic embryos from cotyledon segments arose only near the excised portion at the cotyledonary base. The polar somatic embryogenesis from the cotyledon base was not affected irrespective of the segment sizes of the cotyledons. However, the frequency of somatic embryos formation and their growth rate were highly influenced by the size of segments. The authors concluded that the somatic embryogenesis from excised or wounded cotyledonary segments occurred by the complementary action of both wound response and tissue polarity. When a small needle prick or incision was made on the surface of the cotyledonary segments, the somatic embryos were formed near the wounded portion as well as at the basal excised portion. Somatic embryogenesis did not occur on or near the wounded or excised portion situated in the acropetal direction of cotyledon although the same wounding response was observed on those sites as well.

Embryoids isolated from the callus and subcultured either on half-strength or B5 medium supplemented with BA and GA_3 develop into plantlets. Chang and Hsing (1980) showed *in vitro* flowering of plantlets regenerated from mature root callus. The apical meristem located between two cotyledons transformed into a peduncle terminated by a simple umbel with 3 to 15 flowers. The regeneration procedure described by Shoyama *et al.* (1988) using half strength

Figure 9. Shoots differentiated from embryoids of leaf origin of *Panax ginseng*.

MS medium with GA_3 and BA was suitable also for embryoids of reproductive (Shoyama *et al.*, 1988) and vegetative (Cellárová *et al.*, 1992) origin (Fig. 9).

About 90 per cent of the pollen grains were fertile. Arya *et al.* (1991) described for the first time an efficient procedure for isolation, culture and regeneration of protoplasts into flowering plantlets through somatic embryogenesis. A 4-year old embryonic cell line of *Panax ginseng* capable of regeneration was used. Most plantlets produced more than two epicotyls.

However, further experiments on the genetic uniformity of plantlets produced in such a manner are needed. Asaka *et al.* (1993) developed a technology to induce embryoids by a moderate high temperature treatment from multiple shoots of *P. ginseng*. These embryoids were formed on the surface of the differentiated tissue. Normal plantlets were regenerated from the embryoids by transplanting them on hormone free medium.

Direct somatic embryogenesis from cultured primary somatic embryos capable of plant formation without intervening callus phase was obtained by Arya *et al.* (1993). Cotyledonary stage somatic embryos developed shoot axes when transferred to MS medium supplemented with kinetin and later roots were formed when they were transferred to MS medium with kinetin and GA_3. Secondary somatic embryos also formed plantlets in a one step process on half strength MS medium supplemented with BA or kinetin and GA_3.

Somatic embryogenesis induced *in vitro* is a process which is considered to be a biotechnological alternative to cover the demand for ginseng plants which has significantly increased over recent years. However, when somatic embryos are differentiated from callus, they may not always be able, due to numerous structural abnormalities, to germinate and form normal plantlets. This problem can be overcome when ginseng seeds are used as sources of zygotic embryos. Germinating zygotic embryos give rise to somatic embryos which are able to grow directly into plants. This procedure significantly shortens the regeneration process and provides as many as thousands of ginseng plants by culturing just one seed.

Cryopreservation of Ginseng Explants

With regard to the production of specific compounds, however, cell cultures often show a remarkable instability. The cryopreservation of plant cells is still a routine laboratory method but it is necessary to optimize the individual steps of the cryopreservation procedure. Butenko *et al.* (1984) who cryopreserved ginseng cells for the first time, used a pretreatment which combines low temperatures (+4° C) with a high sucrose concentration (20 per cent). The use of sucrose as a preculture additive and a cryoprotectant seems to be advantageous as it is not toxic for the cells even at very high concentration.

Seitz and Reinhard (1987) developed a cryopreservation procedure for *P. ginseng* cell cultures which makes long term storage of selected strains possible. Sorbitol was used as a short term preculture additive with or without supplementary DMSO as a cryoprotectant. Cell strains of similar appearance but different ginsenoside productivity do not differ in their response to the cryopreservation method used.

Yoshimatsu *et al.* (1996) successfully cryopreserved ginseng hairy root segments obtained by infecting petiole segments with *A. rhizogenes*. For cryopreservation, a vitrification method was applied. The hairy roots regenerated from cryopreserved root tips grew well and showed the same ginsenoside productivity and patterns as control hairy roots cultured continuously at 25° C. Moreover, the PCR analysis proved the presence of T-DNAs in the regenerated hairy roots.

Genetic Transformation of Ginseng

It has been clearly demonstrated that Ti plasmid present in *Agrobacterium tumefaciens* and Ri plasmid from *A. rhizogenes* cause the transformation of plant cells by introducing their T-DNA into genomic DNA of plant cells.

The Ri plasmid is the causative agent of so-called hairy root disease. Hairy root cultures are able to synthesize a variety of plant secondary products. This is of special interest in medicinal plants that synthesize and accumulate pharmaceutically important metabolites in their roots.

The first report of the induction of hairy roots in *P. ginseng* and the establishment of the culture followed by the infection with *A. rhizogenes* was demonstrated by Yoshikawa and Furuya (1987). The ginseng hairy root cultures grew more rapidly and produced saponins more effectively than the ordinary cultured roots obtained by hormonal control. Production of ginseng saponins, such as ginsenosides Rb and Rg in the hairy roots and ordinary cultured roots, as determined by TLC, was comparable. The total saponin contents per dry mass were 0.35–0.95 per cent for the transformed hairy roots and 0.38–0.91 per cent for the ordinary cultured roots. The highest contents were obtained when both hairy and ordinary roots were grown on a medium supplemented with growth regulators such as IBA and kinetin. Inomata *et al.* (1995) achieved the highest growth rate of ginseng hairy roots in batch culture under the effect of BA which also increased the ginsenoside production. Ginsenoside production by hairy root cultures, analyzed by HPLC, was reported by Ko *et al.* (1989); the results were comparable with those of Yoshikawa and Furuya (1987) (0.47–0.82 per cent). These authors found that the production of ginsenoside Rb_1 reached a maximum level in an early stage of culture in contrast to the yields of Rg_1 and Re which reached their maxima at a later stage of culture. Recently these authors reported on the cultivation of hairy root clone HRB-15 in a 3 l bubble type bioreactor (Ko *et al.,* 1996). They established a unique two-step process of hairy root culture to maximize biomass and secondary metabolites. The ginsenoside synthesis was enhanced by yeast elicitation.

Hairy root cultures were further used for biotransformation studies. Kawaguchi *et al.* (1990) reported on the biotransformation of digitoxigenin by *P. ginseng* hairy roots and found that biotransformation involved esterification and high glycosylation ability. As a result, five new components and seven previously reported components were isolated as biotransformation products of digitoxigenin. Among the new compounds three esters (digitoxigenin stearate, digitoxigenin palmitate and digitoxigenin myristate) and two glucosides (3-epidigitoxigenin β-D-gentiobioside and digitoxigenin β-D-sophoroside) were determined.

Further attempts were aimed at the continuous production of glycosides by hairy root cultures cultivated in a bioreactor (Yoshikawa *et al.,* 1993). As a result, a continuous glycosylation of *(RS)*-2-phenylpropionic acid (PPA) was determined during two months. PPA was converted to (RS)-2-phenylpropionyl β-D-glucopyranoside at a 71 per cent conversion ratio, and to (2RS)-2-O-(2-phenylpropionyl)-D-glucose (8 per cent), (2S)-2-phenylpropionyl 6-O-β-D-xylopyranosyl- β-D-glucopyranoside (10 per cent) and a myo-inositol ester of (R)-2-phenylpropionic acid (5 per cent). Moreover, about half of the conversion products were excreted.

Lee *et al.* (1995) have established an efficient transformation system using *P. ginseng* cotyledonary explants and *A. tumefaciens* strain LBA 4404 harbouring the binary vector pBI121 carrying the CaMV 35S promoter-GUS gene fusion and the neomycin phosphotransferase gene (NPTII) as a selectable marker. All embryos derived from kanamycin-resistant calli exhibited a GUS-positive response which appeared in petiole, stem, root and flower in more than 50 per cent of regenerants, which was proved with X-gluc treatment and by Southern blot analysis using a DIG-labeled GUS NOS poly(A) probe. The mitotic stability of the GUS expression in protoplast-derived regenerants was determined by PCR and X-gluc treatment. The PCR method revealed the presence of the GUS gene in 92 per cent of regenerants and the X-gluc treatment in 78 per cent of them.

This *Agrobacterium*-mediated transformation system may be of practical use for introducing single-gene mediated traits, such as herbicide, insect, and disease resistance into this species.

Production of Secondary Metabolites by *in vitro* Culture

The first report on the production of ginsenosides in ginseng callus was published in 1970 by Japanese authors (Furuya *et al.,* 1970). They detected by TLC and column silica gel chromatography analysis a large amount of ginsenoside Rg and a small amount of ginsenoside Rb in ginseng callus derived from petiole of cultivated ginseng grown on MS medium without glycine and supplemented with 1 mg/l 2,4-D. Later Furuya *et al.* (1973) found that the kind and amount of saponins in the callus of the same origin are about the same as in the ginseng root. By means of TLC and column chromatography analysis they isolated the ginsenosides Rb_1 and Rg_1, panaxadiol, panaxatriol and oleanolic acid. Simultaneously they obtained a mixture of phytosterols consisting of a large amount of β-sitosterol and a small amount of campesterol and stigmasterol. The presence of ginsenosides Rb_1 and Rg_1 was confirmed by NMR.

The effect of auxins on saponin production in ginseng callus was studied by Furuya *et al.* (1983a) who found that the saponin production depends on the presence of 2,4-D in the medium. Habituated calli growing without plant growth regulators showed significantly lower capacity for saponin production than normal cells. When the media with 2,4-D-requiring and habituated calli were supplemented with IAA, the production of saponins in both calli was not significantly affected. Khodalovskaya *et al.* (1995) reported on the promoting effect of 4-chlorine phenoxyacetic acid (4-CPA) on the biosynthesis of ginsenosides

in ginseng callus. Furthermore, it was found that semicarbazide and particularly thiosemicarbazide inhibited the production of phytosterols and promoted the biosynthesis of saponins in the presence of mevalonic acid (Furuya *et al.*, 1983b, Linsefors *et al.*, 1989). Odnevall and Björk (1989a) who studied the relationship between the morphological state and ginsenoside formation found that the maximum ginsenoside production occurred in tissue cultures consisting of cell aggregates and differentiated roots on a medium supplemented with 2,4-D and kinetin under light conditions after 2–4 days in the stationary phase. The dynamics of the biosynthesis of ginsenosides during a one growth cycle of callus cell culture of ginseng was studied by Konstantinova *et al.* (1995) who found that maximum ginsenoside accumulation occurred on the 50th–80th day of subculture. Ginsenoside accumulation in ginseng root cultures depended on the carbon source (Odnevall and Bjrk, 1989b). Maximum ginsenoside content (5.2–5.7 mg/g dry weight) was determined under effect of sucrose and fructose.

Further work was aimed at the study of saponin production in ginseng cell suspension cultures cultured in media with different plant growth regulators (Furuya *et al.*, 1983c). It was shown that the growth in rotary shaking cultures was about 1.8 times greater than in reciprocal cultures, while the saponin production was about the same, and the most effective hormonal condition was the combination of IBA with kinetin. Russian authors (Bulgakov *et al.*, 1996) have isolated a cephazolin-resistant cell line Ic-Ceph which produced 2.3 times greater amounts of ginsenosides during three years than their non-selected counterparts.

Biotransformation ability of ginseng root and callus cultures was studied by Furuya *et al.* (1989). They found that root cultures are able to convert (RS)-2-phenylpropionic acid into (RS)-2-phenylpropionyl β-D-glucopyranoside, (2RS)-2-O-(2-phenylpropionyl)-D-glucose, (2S)-2-phenylpropionyl 6-O-β-D-xylopyranosyl-β-D-glucopyranoside and a myo-inositol ester of (R)-2-phenylpropionic acid. The total conversion reached 100 per cent at day three. Compared with the root culture, callus culture showed lower glycosylation ability. As shown by Ushiyama *et al.* (1989), root cultures of ginseng are also able to convert aromatic carboxylic acid into glucose and/or sophorose substituted conjugates. Ginseng cell cultures are able to transform digitoxigenin into nine compounds including a new compound digitoxigenin β-D-glucoside malonyl ester (Kawaguchi *et al.*, 1996).

The adoption of plant cell cultures as an industrial process depends greatly on economics. Lipsky (1992) reported on the mathematical model for the functional relationship between the nominal costs of biomass and secondary metabolites and the plant cell growth characteristics in a multicycle growth system for *Panax ginseng* grown in various types of bioreactors.

The largest market segment for ginseng cell cultures is believed to be the food and beverage industry because such products do not require long study trials and have mass appeal. Furthermore, the content of ginsenoside in such products needs not be verified and such production costs can be reduced. The only notable application to the food and beverage industry is probably the production of ginseng cell mass used in the manufacture of ginseng drinks in

Japan. China and Southeast Asia will be the target market for ginseng-containing drinks and fast foods in the near future.

Abbreviations

B5—Culture medium (Gamborg *et al.*, 1968)
BA—6-Benzylaminopurine
CaMV 35S promoter—Cauliflower mosaic virus 35S promoter
2,4-D—2,4-Dichlorophenoxyacetic acid
DIG—Digoxigenin
DMSO—Dimethylsulfoxide
GA_3—Gibberellic acid
GUS—β-glucuronidase
HPLC—High pressure liquid chromatography
IAA—Indole-3-acetic acid
IBA—Indole-3-butyric acid
MS—Culture medium (Murashige & Skoog, 1962)
NAA—α-phthaleneacetic acid
NOS—Nopaline synthetase
PCR—Polymerase chain reaction
Ri—Root-inducing plasmid from *Agrobacterium rhizogenes*
RM—Culture medium (Linsmaier & Skoog, 1965)
T-DNA—Transferred DNA of the Ti plasmid
Ti—Tumour-inducing plasmid from *Agrobacterium tumefaciens*
TLC—Thin layer chromatography
X-gluc—X-glucuronide

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5. THE PRINCIPAL ACTIVE CHEMICALS IN *PANAX* SPECIES

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One of the earliest recorded chemical investigations of a *Panax* species was published in 1854 when Rafinesque commented on a camphor-like substance named panacene which he had isolated from an extract of the root of the American ginseng *P. quinquefolium* L. In the same year Garriques reported the occurrence of panaquilon, a yellowish amorphous compound with the molecular formula $C_{32}H_{56}O_{14}$ also obtained from the roots of *P. quinquefolium*. Panaquilon demonstrated typically glycosidal characteristics being soluble in water and alcohol and possessing a sweetish taste. On acid hydrolysis panaquilon yielded a water-insoluble substance which he called panacon.

Investigating Manchurian ginseng roots in a similar manner in 1889, the Russian chemist Davydow also recovered a glycoside resembling panaquilon. Early in the 20th century Japanese scientists commenced the investigation of Japanese and Korean ginsengs. Inoue in 1902 isolated a saponin glycoside from Japanese ginseng *(chikusetsu ninjin)* and three years later Fujitani published an account of the recovery of a relatively pure, white, crystalline compound also called panaquilon from extracts of Japanese and Korean ginseng roots. Further progress was achieved in 1906 when Asahini and Taguchi described a noncrystalline compound obtained from an alcoholic root extract; this compound was found to be a saponin hydrolysing into an aglycone or sapogenin and a glycone identified as glucose (Hou, 1978).

A decade later Professor Kondo's research team studied aqueous, methanolic and ethereal extracts of ginseng roots. The aqueous fraction yielded mucilage and inorganic compounds, the methanolic extract sucrose, some nitrogenous substances and a saponin glycoside, and the ether extract an oily material. Steam distillation of this oily fraction yielded two products, a light, yellowish volatile oil and a brown non-volatile residue. From the volatile oil the camphor-like panacene was recovered, and the non-volatile fraction contained phytosterol and fatty acids. Study of the saponin fraction, using 7 per cent alcoholic hydrochloric acid as the hydrolysing agent, produced two compounds, a crystalline panax-sapogenol and an amorphous panaxsapogenol. Closer investigation confirmed that the saponin glycoside had a molecular weight of 876 and comprised a molecule of panax-sapogenol linked to two molecules of glucose and one molecule of pentose. Kondo's team also observed that cultivated Japanese and Korean ginsengs produced similar chemical compounds (Kondo and Tanaka, 1915; Kondo and Yamaguchi, 1918; Kondo and Amano, 1920).

Abe and Saito in 1922 and Yonekawa in 1926 obtained a relatively pure glycoside, ginsenin, from Korean ginseng root and Kotake in 1930 isolated a glycoside called panaxin, which did not demonstrate typical haemolytic characteristics, but hydrolysis with methanolic 50 per cent sulphuric acid produced a prosapogenin, α-panaxin, $C_{38}H_{66}O_{12}$, which could be further hydrolysed with fuming hydrochloric acid to yield glucose and a chlorinated aglucone, $C_{30}H_{53}O_3Cl$ (Hou, 1978). Many years later it was confirmed that Garriques' panaquilon (1854), Yonekawa's ginsenin (1926) and Kotake's panacin (1930) were indeed identical and that panacon (Garriques, 1854), the non-crystalline compound with a melting point exceeding 270° C reported by Asahina and Taguchi in 1906 and α-panaxin (Kotake, 1930) were identical with the prosapogenin actually melting at 330° C (Fujita, Itokawa and Shibata, 1962).

Nevertheless further progress on the isolation and characterisation of ginseng glycosides was very slow. Unlike the alkaloids which could be readily isolated as salts and had been discovered in quick succession after the first such compound, morphine in opium, had been isolated by the German apothecary Wilhelm Sertürner in 1817, glycosides presented much greater difficulties being usually high molecular weight, sugar-linked compounds that would readily hydrolyse and so lose part or the whole of the solubilising side-chains which were often composed of mixed sugars. Therefore progress in the isolation and characterisation of the complex glycosides in the pharmaceutically important species of the genera *Digitalis,* the fox-gloves yielding cardenolide glycosides, *Drimia,* the squills producing bufodienolide cardiac glycosides and *Panax,* the ginsengs containing triterpenoid saponin glycosides, was restricted until the development of efficient new separation techniques including, in particular, chromatographic methods, and sophisticated, diagnostic, instrumental techniques such as spectrometry from 1960 onwards.

In the period up to the 1960's other workers had discovered in various *Panax* species sugars such as glucose, arabinose, sucrose and rhamnose, sterols such as β-sitosterol and stigmasterol, high molecular weight fatty acids (panax acids), fatty acid esters and the triterpenoid genin oleanolic acid (Lin, 1961). However it was not only the development of new analytical techniques that stimulated research into the phytochemistry of ginseng. In Russia (1957) Professor Israel Brekhman and in Bulgaria (1959) Professor Wesselin Petkov had independently studied the pharmacological features of ginseng and, although their ideas were not universally accepted by pharmacologists, they did prompt chemists in Russia and Japan to research the chemistry of the controversial *Panax* species.

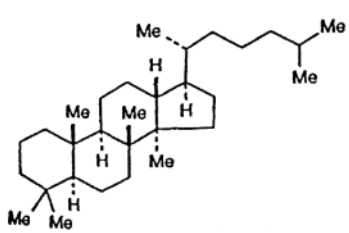
It is generally considered that the most important compounds occurring in ginseng roots form a complex series of closely related triterpenoid saponin glycosides, sugar-linked chemicals possessing the property of lowering the surface tension of water with consequent soap-like frothing or lather formation on shaking; hence the name "saponin". In addition typical saponins possess a bitter-sweet taste and are sternutatory as such compounds irritate nasal mucous membranes with consequent sneezing. Saponins are toxic to cold-blooded animals including insects and molluscs although not normally affecting warm-blooded animals. However, saponins do form colloidal aqueous solutions that

can cause haemolysis of red blood cells and are therefore dangerous if injected into the human blood stream (Trease and Evans, 1993; Harborne and Baxter, 1993). The saponin glycosides were thought at first to be responsible for most of the recorded biological effects of ginseng and its preparations.

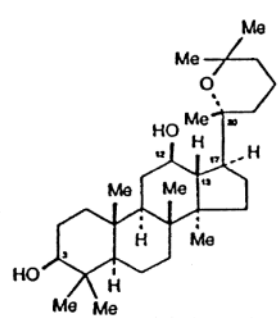
1) *Panax ginseng* C.A.Meyer

Seeking saponins in *Panax ginseng* plants, the Russian research group headed by Professor Elyakov isolated from methanolic extracts of ginseng roots a series of sugar-linked compounds which they named "panaxosides"; panaxosides A and B were reported by Elyakov *et al.* in 1962 and C, D, E and F by Elyakov *et al.* in 1964. The Russian chemists noted that on hydrolysis the saponin glycosides panaxosides A, B, and C were based on the aglycone panaxatriol and that the panaxosides D, E and F formed a separate group based on the aglycone panaxadiol. In addition Elyakov's group were able to demonstrate that the sidechains of monosaccharide molecules were different. Thus panaxoside A possessed 3 glucose units, panaxoside B 2 glucose and 1 rhamnose units, panaxoside C 3 glucose and 1 rhamnose units, panaxoside D 4 glucose units, panaxoside E 4 glucose and 1 arabinose units, and panaxoside F 6 glucose units.

At the same time, in Japan, a research group headed by Professor Shibata had isolated and described similar ginseng saponins which they preferred to name "ginsenosides" and this terminology is now universally accepted. Fujita *et al.,* (1962), studying methanolic extracts of *P. ginseng, P. japonicum* and *P. quinquefolium* roots, differentiated the saponins and their aglycones and sapogenins obtained by hydrolysis using hot methanolic hydrochloric acid. Further work (Shibata *et al.,* 1963a, 1963b) established the structure of the sapogenin panaxadiol as a dammarane-type tetracyclic triterpene.



(5-1) Dammarane



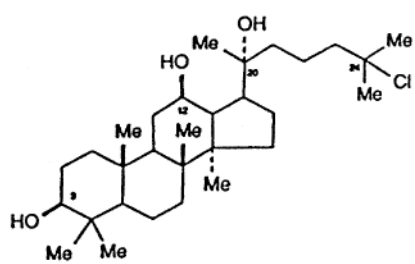
(5-2) Panaxadiol

Dammarane (5–1) is the name that was applied to the foundation triterpenoid structure of dammarenediol, a compound extracted from the resins of various tree species of *Agathis, Balanocarpus, Hopea* and *Shorea,* genera of the family

Dipterocarpaceae occurring in East Asia. East Indian dammar resin was used for technical processes such as varnishes for the preservation of oil paintings and microscope slide preparation. Shibata established the relationship of the structures of dammar resin triterpene and the ginseng triterpenes.

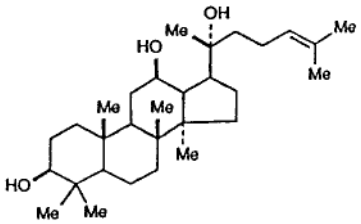
Panaxadiol (5–2), $C_{30}H_{52}O_3$, yielded the characteristic red-violet Liebermann-Burchard reaction of a triterpenoid structure and a negative tetranitromethane reaction coupled with no ultraviolet absorption peak at 210 nm indicated that it was not a normal pentacyclic oleanane structure. Further chemical reactions and ultraviolet, infrared and mass spectral observations established that panaxadiol was indeed a compound with a molecular ion peak (M^+) at *m/z* 460 confirming the formula $C_{30}H_{52}O_3$ and peaks at *m/z* 127 and *m/z* 341 were consistent with the presence of a trimethyltetrahydropyrane ring structure. In addition secondary hydroxyl groups were present at C-3 and C-12 and the trimethyltetrahydropyrane group occurred at C-20.

Shibata's team repeated Kotake's (1930) observations, obtaining panaxadiol by mineral acid hydrolysis and a chlorine-containing compound (5–3) directly by crude saponin hydrolysis with concentrated hydrochloric acid at room temperature. Dechlorination of the latter compound with potassium *tert-butoxide* produced an unsaturated compound that was converted by a mild hydrolysis process using methanolic 0.7 per cent sulphuric acid to reveal the true sapogenin which was designated as a prosapogenin (Shibata *et al.*, 1963c).

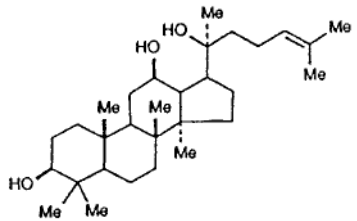


(5-3) Kotake's chlorine-containing compound

This prosapogenin was identified chemically as protopanaxadiol (Tanaka *et al.*, 1964; Shibata *et al.*, 1966)) and was shown to be an open chain compound with a free alcohol group and a terminal vinyl group; cyclisation of the C-17 side chain under strong acid hydrolysis conditions resulted in the substituted pyran ring system of panaxadiol. Further studies of the acid-catalysed isomerisation of dammarane-type triterpenes and of the mild hydrolysis of pure ginsenosides undertaken by the same team proved that the normal naturally occurring protopanaxadiol was the 20*(S)*-epimer (12β-hydroxydammarenediol II)(5–4) and not the previously assumed 20(R)-epimer (12β-hydroxydammarenediol I)(5–5) (Tanaka *et al.*, 1964, 1966, 1967, 1972).

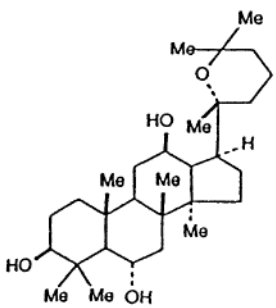


(5-4) 20(*R*)-Protopanaxadiol
(12β-Hydroxydammarenediol I)

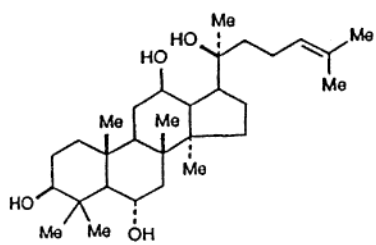


(5-5) 20(*S*)-Protopanaxadiol
(12β-Hydroxydammarenediol II)

Shibata *et al.* (1965) noted that hydrolysis of the pure ginsenoside Rg_1 using dilute mineral acid produced glucose and a crystalline compound named panaxatriol (5–6) which was shewn by spectral data analysis to be 6-α-hydroxypanaxadiol. Using methods applied to the study of panaxadiol, Shibata's team demonstrated that the true sapogenin of ginsenoside Rg_1 was 20*(S)*-protopanaxatriol (5–7) and that panaxadiol and its homologue panaxatriol, not being the true aglycones, could be regarded as artefacts.



(5-6) Panaxatriol

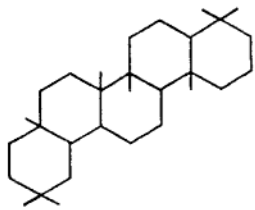


(5-7) 20(*S*)-Protopanaxatriol

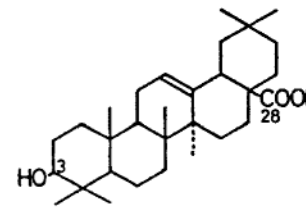
Ginsenosides based on the sapogenin (20*S*)-protopanaxadiol possess sugar moieties attached at the C-3 OH and C-20 OH positions while ginsenosides based on the sapogenin (20S)-protopanaxatriol have sugar moieties attached at C-6 OH only as in ginsenosides Rf, Rg_2 and Rh_1 or at C-6 OH and C-20 OH e.g. ginsenosides Re, Rg_1 and 20-gluco-Rf.

One further sapogenin was known to occur in some ginsenosides. As early as 1961 the German group of Hörhammer, Wagner and Loy had isolated and identified from *P. ginseng* roots a pentacyclic, oleanane-type (5–8) triterpene compound named oleanolic acid (5–9) which occurs widespread in the plant kingdom e.g. in *Olea europaea* L., the olive tree, *Thymus vulgaris* L., common

thyme, etc. Its saponin, ginsenoside-Ro, occurs commonly in ginseng species.



(5-8) Oleanane ring system



(5-9) Oleanolic acid

The dammarene saponins are hydrophilic but oleanolic acid, the aglycone of ginsenoside Ro, is lipophilic. The glycosides of ginseng are formed by the addition of sugar units to the above aglycone units, the different compounds varying according to the number, nature and occurrence of sugars such as arabinose, glucose, rhamnose and xylose. As the number of sugar units is small and variable and mixed sugars can occur the saponins are also referred to as triterpene-oligosides.

The overall yield of glycosides in *Panax ginseng* roots varies between 0.5 and 4.0 per cent and is age-dependent. Mild acid hydrolysis of the mixed ginsenosides revealed three principal structural types based on:-

a) the tetracyclic, dammarene, triterpenoid sapogenin (20-*S*)-protopanaxadiol,
b) the tetracyclic, dammarene, triterpenoid sapogenin (20-*S*)-protopanaxatriol,
c) the pentacyclic, triterpene oleanolic acid.

The international confusion concerning the nomenclature of the ginsenosides was resolved by using the designation Rx where the capital R refers to the root and the lower case x=o, a_1, a_2, b_1, b_2, c, d, e, f, g_1, g_2, g_3, h_1, h_2, etc., relating to the relative positions of the separated neutral saponin spots on thin-layer chromatograms, ginsenosides Ro and Ra being the least polar (Shibata *et al.*, 1965). In a similar manner new ginsenosides from the leaves were designated Fx, the capital F indicating "folia" meaning "leaves".

The chemical characteristics and physical properties of the commoner saponins were soon established and widely published (Table 5.1).

During the 1960's the Japanese group including Iida, Shibata and Tanaka had elucidated the characteristics and detailed chemical structures and configurations of the nine then-known ginsenosides. Using better thin layer chromatography (TLC) methods, Sanada *et al.* (1974) isolated 13 ginsenosides

Table 5.1. Physical properties of some principal ginsenosides

Ginsenosides	*Physical appearance*	*Formula*	*Melting point (°C)*	*IR (Kbr) cm^{-1}*
(20S)-Protopanaxadiol saponins				
Rb_1	White powder (EtOH-BuOH)	$C_{54}H_{92}O_{23}$	197–198	3400 (OH) 1620 (C=C)
Rb_2	White powder (EtOH-BuOH)	$C_{53}H_{90}O_{22}$	200–203	3400 (OH) 1620 (C=C)
Rb_3	White powder (iso-PrOH)	$C_{53}H_{90}O_{22}$	193–195	3420 (OH) 1620 (C=C)
Rc	White powder (EtOH-BuOH)	$C_{53}H_{90}O_{22}$	199–201	3420 (OH) 1620 (C=C)
Rd	White powder (EtOH-AcOEt)	$C_{48}H_{82}O_{18}$	206–209	3400 (OH) 1620 (C=C)
(20S)-Protopanaxtriol saponins				
Re	Colourless needles (50% MeOH)	$C_{48}H_{82}O_{18}$	201–203	3380 (OH) 1620 (C=C)
Rf	White powder (MeCOMe)	$C_{42}H_{72}O_{14}$	197–198	3380 (OH) 1620 (C=C)
20-gluco-Rf	White powder (iso-PrOH)	$C_{48}H_{82}O_{19}$	182–184	3420 (OH) 1620 (C=C)
Rg_1	White powder (MeOH-MeCOEt)	$C_{42}H_{72}O_{14}$	194–196	3400 (OH) 1620 (C=C)
Rg_2	Colourless needles (EtOH)	$C_{42}H_{72}O_{13}$	187–189	3400 (OH) 1620 (C=C)
Oleanolic acid saponin				
Ro	Colourless needles (EtOH)	$C_{48}H_{76}O_{19}$	239–241	3400 (OH) 1740 (COOR) 1728 (COOH)

from *P. ginseng* root extracts designating the compounds ginsenosides Ro, Ra, Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2, Rg_3, Rh_1 and Rh_2. They noted that the protopanaxadiol-derived Rb group (Rb_1 and Rb_2) and the protopanaxatriol-derived Rg group (Rg_1, Rg_2 and Rg_3) saponins were quantitatively the main glycosides present. In the same year Andreev and his Russian co-workers (1974) isolated 14 panaxosides from TLC plates and subsequent cooperative work has correlated the two groups of saponins. The studies of the American chemist E.J.Staba and his colleagues on the indigenous *P. quinquefolium* revealed that the saponins identified as panaquilins by earlier workers were also identical with the ginsenosides (Table 5.2) (Hou, 1978).

In the past two decades the markedly improved methods of chemical analysis, including thin layer chromatography (TLC), which permitted the early rapid identification of ginseng roots and ginseng preparations, and gas-liquid (or vapour phase) chromatography (GLC), have been largely replaced by high performance liquid chromatography (HPLC), a technique equally applicable

Table 5.2. Correlation of the nomenclature of ginsenosides, panaxosides and panaquilins

Ginsenoside	*Panaxoside*	*Panaquilin*
Ro	–	–
Ra	F	–
Rb_1, Rb_2	E	B
Rc	D	C
Rd	–	D
–	–	E_1
Rd	C	E_2
Re	B	E_3
Rf	–	–
Rg_1	A	G_1
Rg_2	–	G_2

to qualitative analysis and accurate quantitative estimation and therefore to quality control of the ginsenosides in mixtures. Nevertheless the newer planar methods such as Overpressure Layer Chromatography (OPLC), Centrifugal Layer Chromatography (CLC) and Sequential Centrifugal Layer Chromatography (SCLC) have also been used for the separation of the major ginsenosides on the laboratory scale. Multi-step procedures involving column chromatography on silica gel, the slower process of Drop Counter Current Chromatography (DCCC) and the techniques of preparative HPLC (Paik *et al.*, 1982) have been used for larger scale isolations.

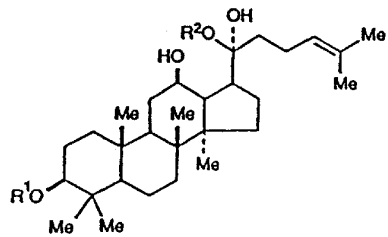
Sanada *et al.* (1974, 1978) demonstrated the simplicity and effectiveness of the separation of the 11 common ginsenosides on silica gel H thin-layer plates using the upper phase of *n*-butanol: ethyl acetate: water (4:1:5) or the lower phase of chloroform: methanol: water (65:35:10) mixtures. Their fractionation methods, involving methanol, water, ether and *n*-butanol extractions followed by silicic acid and silica gel chromatography, were summarised by Shibata *et al.* (1985).

Further glycosides were isolated from the roots and characterised including ginsenosides Ra_1 and Ra_2 (Besso *et al.*, 1982a), ginsenoside Ra_3 from both white and red ginseng (Matsuura *et al.*, 1984), the malonyl ginsenosides Rb_1, Rb_2 and Rd (Kitagawa *et al.*, 1983a, 1989; Wang *et al.*, 1993) and the minor dammarane saponins koryoginsenosides R_1 and R_2 (Kim *et al.*, 1995) (see Appendix to Chapter 5).

The ginsenosides of *P. ginseng* roots fall into at least 4 groups, protopanaxadiol type (Table 5.3), protopanaxatriol type (Table 5.4), oleanane type (Table 5.5) and miscellaneous types (Table 5.6).

Commercial ginseng roots yield about 0.05 per cent of volatile oil although fresh roots may yield up to 0.9 per cent volatile oil. The oil is responsible for the aroma of fresh ginseng but the smell is not easily detected in the powdered or dried drug after storage. The reported composition of the oil depends on the method of extraction e.g. steam distillation, ether extraction, freeze-drying and vacuum distillation, etc. Using combined gas/liquid chromatography and mass

Table 5.3. Structure of protopanaxadiol type ginsenosides

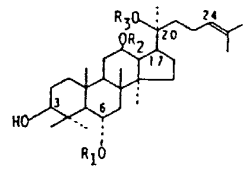


Designation		R_1	R_2
Ginsenoside	Ra_1	–Glc^2–Glc	–Glc^6–$Ara(p)^4$–Xyl
..	Ra_2	–Glc^2–Glc	–Glc^6–$Ara(f)^2$–Xyl
..	Ra_3	–Glc^2–Glc	–Glc^6–Glc^3–Xyl
..	Rb_1	–Glc^2–Glc	–Glc^6–Glc
Malonyl – ..	Rb_1	–Glc^2–Glc^6–malonyl	–Glc^6–Glc
Ginsenoside	Rb_2	–Glc^2–Glc	–Glc^6–Ara(p)
Malonyl – ..	Rb_2	–Glc^2–Glc^6–malonyl	–Glc^6–Ara(p)
Ginsenoside	Rb_3	–Glc^2–Glc	–Glc^6–Xyl
Ginsenoside	Rc	–Glc^2–Glc	–Glc^6–Ara(f)
Malonyl – ..	Rc	–Glc^2–Glc^6–malonyl	–Glc^6–Ara(f)
Ginsenoside	Rd	–Glc^2–Glc	–Glc
Malonyl – ..	Rd	–Glc^2–Glc^6–malonyl	–Glc
Ginsenoside	F_2	–Glc	–Glc
..	Rg_3	–Glc^2–Glc	–H: 20(*R*)
20(*S*)- ..	Rg_3	–Glc^2–Glc	–H: 20(*S*)
Ginsenoside	Rh_2	–Glc	–H: 20(*S*)
..	Rs_1	–Glc^2–Glc^6–acetyl	–Glc^6–Ara(p)
..	Rs_2	–Glc^2–Glc^6–acetyl	–Glc^6–Ara(f)
Notoginsenoside	R_4	–Glc^2–Glc	–Glc^6–Glc^6–Xyl
Quinquenoside	R_1	–Glc^2–Glc^6–acetyl	–Glc^6–Glc

spectrometry (GC/MS) several workers have unravelled mixtures of 50 or more components of which up to 25 percent are sesquiterpenes; principal terpene components include α-elemene, β-elemene, γ-elemene, α-aromadendrene, β-aromadendrene, alloaromadendrene, *cis*- and trans-caryophyllene, α-guaiene, β-guaiene, δ-guaiene, ε-muurolene, β- and γ-patchoulene, etc. (Sun *et al.*, 1985; Sun *et al.*, 1987). Less volatile compounds isolated from the low boiling (71–110° C.) fraction of the oil included substances such as the sesquiterpene hydrocarbon β-elemene (b.p. 73° C). Other sesquiterpenoid compounds reported include α-panasinsene, β-panasinsene, α-neoclovene and β-neoclovene and the sesquiterpene alcohols panasinsanol A and B (Iwabuchi *et al.*, 1987) and, from the rootlets, ginsenol (Iwabuchi *et al.*, 1988). Ethereal extracts of the rootlets also yielded (+)-spathulenol, (-)-4β, 10α-aromadendranediol, (-)-neointermedeol and senecrassidol (Iwabuchi *et al.*, 1990).

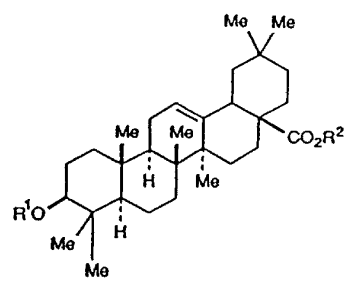
The Korean chemists investigated the occurrence of lipids in *P. ginseng* roots and Table 5.7 summarises published data.

Table 5.4. Structure of protopanaxatriol type ginsenosides



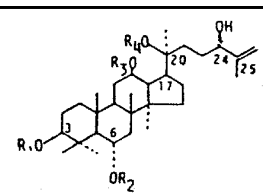
Designation		R_1	R_2	R_3
Ginsenoside	Re	–Glc^2–Rha	–H	–Glc
Ginsenoside	Rf	–Glc^2–Glc	–H	–H: 20(*S*)
20-Gluco- ..	Rf	–Glc^2–Glc	–H	–Glc
Ginsenoside	Rg_1	–Glc	–H	–Glc
..	Rg_2	–Glc^2–Rha	–H	–H: 20(*S*)
20(*R*)- ..	Rg_2	–Glc^2–Rha	–H	–H: 20(*R*)
Ginsenoside	Rh_1	–Glc	–H	–H: 20(*S*)
20(*R*)- ..	Rh_1	–Glc	–H	–H: 20(*R*)
Ginsenoside	F_1	–H	–H	–Glc
Ginsenoside	F_3	–H	–H	–Glc^6–Ara(p)
Notoginsenoside	R_1	–Glc^2–Xyl	–H	–Glc

Table 5.5. Structure of oleanane type ginsenosides



Designation	R^1	R^2
Ginsenoside Ro	–GlcA^2–Glc	–Glc

Table 5.6. Structure of miscellaneous type ginsenosides



Designation	R_1	R_2	R_3	R_4
Ginsenoside M_{7cd}	–H	–H	–H	–Glc

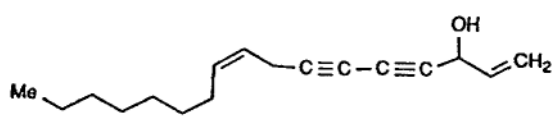
Table 5.7. The lipid content of *Panax ginseng* roots

Sample	*Total lipids per cent*	*Neutral lipids as percentage of total lipids*	*Glycolipids as percentage of total lipids*	*Phospholipids as percentage of total lipids*	*Ref.*
Fresh root	0.62	45.28	18.12	36.60	*a*
Dried root	0.89	86.48	9.20	4.32	*a*
3 yr old root	1.07–1.67	51.35–72.30	11.83–20.72	15.01–34.59	*b*
Various	1.08–2.23	64.2–73.5	15.4–17.4	10.4–19.2	*c*
Root	1.21–1.45	76.6–79.7	11.6–14.7	8.5–8.7	*d*

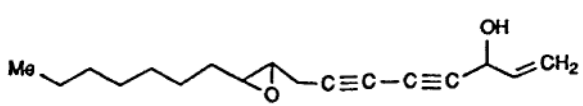
References:- *a*=Shin and Lee (1980); *b*=Sohn *et al.*, 1988); *c*=Kim *et al.* (1988); *d*=Choi and Kim (1985).

The reported data indicates that neutral lipids predominate with the glycolipids and phospholipids much more variable. The neutral lipids occurred in greatest amount in the cortex, suggesting that lipid ducts arose only in the cortex (Kim *et al.,* 1988). Shin and Lee (1980) also reported that triglycerides formed 37.6–42.5 per cent of the neutral lipid fraction and sterol esters comprised 16.5–19.6 per cent. The predominant fatty acids in order of quantitative occurrence were linoleic, palmitic, oleic and linolenic.

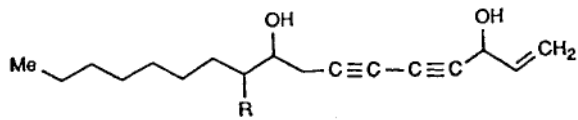
Also present in ginseng roots are the epoxides of heptadecane which are usually called polyacetylenes. The polyacetylene panaxynol (1,9-*cis*-heptadecadiene-4,6-diyn-3-ol) (5–10) was obtained as a yellow oil (b.p. 115° C) from the higher boiling (110–150° C) fraction (Takahashi *et al.,* 1966). The polyacetylenes panaxydol (9,10-epoxy-3-hydroxy-heptadeca-1-en-4,6-diyne) (5–11) (Poplawski *et al.,* 1980) and heptadeca-1-en-4,6-diyn-3,9-diol (5–12) (Dabrowski *et al.,* 1980) were subsequently isolated from an alcoholic extract of ginseng root and Kitigawa's team (1983b) extracted the related panaxytriol (hepta-1-en-4,6-diyn-3,9,10-triol) (5–13) from the ether soluble fraction of red ginseng.



(5-10) Panaxynol



(5-11) Panaxydol



(5-12) Heptadeca-1-en-4,6-diyn-3,9-diol; R = H

(5-13) Panaxytriol; R = OH

Studying extraction techniques for polyacetylene compounds in white ginseng root and using various solvents, Nho and his colleagues (1990) concluded that refluxing with methanol was the most efficient of the seven solvent systems investigated although Soxhlet extraction was almost equally effective. The solvents in order of decreasing efficiency were methanol, methylene dichloride, acetone, diethyl ether, ethyl acetate, methyl cyanide and petroleum ether. Panaxynol and panaxydol were the principal polyacetylenes present, the yield being 4.2 mg/g and 6.4 mg/g respectively. Continued interest in these compounds led to the isolation from the hexane extract of *P. ginseng* roots of a series of new polyacetylenes which were designated ginsenoynes A-E and acetylated ginsenoynes F-K (Hirakura *et al.,* 1991, 1992)(see Appendix to Chapter 5). Later Hirakura *et al.* (1994) described the isolation and characterisation of the linoleoylated polyacetylenes panaxynol linoleate, panaxydol linoleate and ginsenoyne A linoleate from the root of *P. ginseng*.

Not surprisingly ginseng roots yield about 5 per cent by weight of sugars which include the monosaccharides D-glucose, D-fructose and D-rhamnose, the disaccharides sucrose and maltose, and trisaccharides such as α-maltosyl-β-D-fructofuranoside, O-α-D-glucopyranosyl-(1→2)-O-β-D-fructofuranosyl (1→2)-β-D-fructofuranoside and O-α-D-glucopyranosyl-(1→6)-O-α-D-glucopyranosyl-(1→4)-O-α-D-glucopyranose. The principal sugar in fresh white ginseng root is sucrose, forming 92–94 per cent in 2 year old roots but decreasing in older roots (Sohn *et al.,* 1988). In red ginseng root the main sugars are sucrose and rhamnose.

Polysaccharides or glycans are usually defined as polymers of monosaccharides and their derivatives comprising ten or more units, the upper limit exceeding 1000 units. Polysaccharides are present in all parts of the ginseng plants although the roots contain greater amounts than in the rhizomes, stems and leaves in decreasing order and the main roots yield more than the lateral roots. The crude polysaccharide fraction can be obtained by extraction with boiling water with a yield of about 8–10 per cent. The roots produce mainly pectins and glucans and the leaves pectins and heteroglycans (Gao *et al.,* 1989). Pectin (some 20 per cent of the polysaccharide fraction) and the ubiquitous starch (some 80 per cent) are the major polysaccharide components found in ginseng roots. Pectic substances occur in primary cell walls and intercellular cement and are mixtures and/or chemical combinations of ara bans, galactans and methyl esters of galacturonans. Arabans possess a low molecular weight branched chain structure comprising α(1→5)- and α(1→3)-L-arabinofuranose

units. Galactans form straight chains of up to 120 units comprising β-(1→4)-D-galactopyranose units. Pectins may contain 200 or more units of α-(1→4)-D-galactopyranosyluronic acid. Ginseng pectin, which was estimated as 7.57–11.09 per cent of dried ginseng samples, comprises galactose, galacturonic acid, arabinose and rhamnose residues in the molar ratio 3.7:1.7:1.8:1 in a highly branched molecule with β-(1→3)-D-galactan as its backbone and (1→4)-galacturonic acid, (1→3)- or (1→2,4)-rhamnose and (1→5)- or (1→3,5)-arabinose residues in the side chains together with some (1→6)-linked galactose residues. The crude polysaccharides were shewn to contain ~5 per cent of proteins including aspartic acid, threonine, serine, glutamic acid, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine (Li *et al.,* 1986, 1987).

Several acidic branched chain heteroglycans with molecular weights varying from 20,000 to *ca.* 1,900,000 have been reported in roots, stems, leaves and tissue cultures of various *Panax* species. Sanchinan A, molecular weight 1,500,000, is an arabinogalactan containing about 3.27 per cent protein, from Sanchi ginseng *P. notoginseng* (Ohtani *et al.,* 1987); hetero-polysaccharide P_N from *P. ginseng* leaves, molecular weight about 1,900,000, is comprised of arabinose, galactose, glucose, rhamnose, xylose and galactosamine in the ratio 8.1:12.5:4.1:0.8:1.0:1.6 (Liu *et al,* 1988) and the ginsenans PA and PB (Takeda *et al.,* 1993) from *P. ginseng* roots have molecular masses of 160,000 and 55,000 respectively, ginsenan PA being composed of units of L-arabinose, D-galactose, L-rhamnose, D-galacturonic acid and D-glucuronic acid in molar ratios of 11:22:1:6:1 and ginsenan PB possesses hexuronic residues as methyl esters. Further analysis revealed principally α-arabino-β-3,6-galactan and rhamnogalacturonan type units. The acidic arabinogalactans ginsenans S-IA and S-IIA also from *P. ginseng* roots comprised L-arabinose, D-galactose and D-galacturonic acid in the molar ratio 8:8:1 and L-arabinose, D-galactose, D-glucose and D-galacturonic acid in the molar ratio 15:10:2:5 respectively. In these compounds the hexuronic acid residues are also methyl esters and the main spine is α-1,5-linked-L-arabino- β-3,6 branched-D-galactan (Tomoda *et al.,* 1993). The compounds are important as immunostimulatory, antitumour and anticomplementary properties have been claimed.

An interesting series of high polymer peptidoglycans, the panaxans designated A to U, were isolated and characterised by Hikino and his co-workers (1984–1986) using diluted methanol extraction followed by fractionation on a series of columns of cellulose, DEAE-cellulose, Sepharose 6B, Sephacryl-S-500 and Sephacryl-S-200. Panaxan A had a molecular weight of *ca.* 14,000 and panaxan B was estimated at *ca.* 1,800,000; these compounds comprised recurring α-(1→6)-linked D-glucopyranose units with branching at one α(1→3) position in each unit and non-reductive terminals at fixed intervals (Tomoda *et al.,* 1985). The marked hypoglycaemic activity of these compounds in normal and alloxan-induced hyperglycaemic mice suggested potential therapeutic value.

Peptides have also been reported. Using a methanol/water (1:1) extract of white ginseng and paper electrophoresis (30V/cm, AcOH/AcONa buffer, pH 5.0), Gstirner and Vogt (1966) isolated some low molecular weight peptides of

uncertain pharmacological activity. The peptides were concentrated by 2-dimensional high voltage electrophoresis on Sephadex G-50 (1000V, 8 hr) to yield four low molecular weight compounds. Subsequent hydrolysis of these isolated peptides (concentrated HCl/90(%) HCOOH (1:1), 120° C, 36 hr) and analysis by the Stein-Moore method on Amberlite IR-120 yielded the neutral amino acids alanine, glycine and serine, the acidic amino acids aspartic acid and glutamic acid and the basic amino acid arginine as common components. In addition fraction 1 contained threonine, proline (major component), leucine, isoleucine, lysine and histidine, fraction 2 threonine, valine, β-aminobutyric acid, β-aminoisobutyric acid, lysine, histidine, hydroxyproline and two unidentified compounds, fraction 3 threonine, proline (major component), methionine, leucine, alloisoleucine, isoleucine, phenylalanine, β-aminobutyric acid, tyrosine, lysine and histidine and fraction 4 an unidentified component. Tryptophan was not found in any fraction. Examining red ginseng and Korean, Canadian and American white ginsengs, Lee *et al.* (1982) noted that 15 common free amino acids occurred, the yield being lower in red ginseng than in dried white ginseng. The basic amino acid arginine comprised 68–72 per cent of the total amino acids and methionine and phenylalanine were not detected in extracts of red ginseng. Liu *et al.* (1990) examined the distribution of amino acids in *P. ginseng* roots and recorded a highest yield in the budding period (April), less in the dying-down phase in September and much less during the flowering-fruiting period in June. They also noted that the main root yielded higher levels of amino acids than the lateral roots.

Recently (1998) Chen *et al.* reported the discovery of a series of six oligopeptides related to oxidised glutathione in aqueous-methanol extracts of *P. ginseng* roots. The compounds were:—P-I=N-γ-glutamylcystinyl-bis-glycine, P-II=γ-glutamylcysteinglycine disulphide, oxidised glutathione, P-III=N,N′-bis-γ-glutamyl-cystylglycine, P-IV=γ-glutamylcysteinylglycinamide disulphide, P-V=N-γ-glutamyl-glycylcysteine disulphide and P-VI=γ-glutamylarginine. Compound P-V, a novel compound, has somnogenic properties.

Hiyama *et al.* (1978), employing HPLC analysis with a TSK gel LS160 column and water and G-3000W and G-2000W columns and H_2O/AcOH/Et_3N (100:0.3:0.3) solvent, demonstrated the presence of uridine, guanidine and adenine in white ginseng root and uracil, uridine and adenosine in lateral roots. Okuda's group found a peptide of molecular weight 1000 as well as the purine nucleoside adenosine which has hypoglycaemic activity (Hou, 1978).

Polyamines also have been reported. Putrescine was shewn to be the major polyamine in ginseng seedlings although spermidine was dominant in 2-year old plants and spermine was also present (Cho *et al.,* 1989).

Alkaloidal components were isolated in small amounts only from powdered roots. N_9-formylharman, ethyl-β-carboline-1-carboxylate and perlolyrine (Han *et al.,* 1986), the water-soluble alkaloid spinacine (4,5,6,7-tetrahydroimidazo (4,5-c)pyridine-6-carboxylic acid (Han *et al.,* 1987) and, from the ether-soluble fraction, 4-methyl-5-thiazoleethanol, norharman and harman (Park *et al.,* 1988) were recovered.

The presence of phenolic substances such as salicylic and vanillic acids was

indicated earlier and recent work involving gas-liquid chromatography (Park *et al.,* 1994) confirmed the presence of eight such aromatic acids (caffeic, cinnamic, ferulic, gentisic, p-coumaric, salicylic, syringic and vanillic acids) in white ginseng roots. Other organic acids reported included fumaric, succinic, maleic, malic, citric and tartaric and the unsaturated fatty acids oleic, linoleic and linolenic (Lee and Lee, 1961).

Trace mineral and rare elements occur in all species of ginseng. An aqueous extract contains elements such as aluminium, arsenic, boron, calcium, cobalt, copper, iron, magnesium, manganese, molybdenum, potassium, phosphorus, sodium, sulphur, vanadium and zinc as well as silicate ions (Hou, 1978). Greater amounts of trace elements were detected in leaves and stems than in roots and cultivated roots contained less trace elements than wild ginseng roots. Iron, manganese and zinc were the most important, being in highest yield in roots in about September (Zhao *et al.,* 1993), but more recent work has concentrated on the analysis for germanium and selenium in ginseng. Traces of rubidinium and strontium were also reported and cadmium and lead levels were found to be far below the toxic doses (Cai and Guo, 1992; Jiasheng *et al.,* 1994). Although trace elements are detected only in parts per million or parts per billion, they are regarded as essential for the maintenance of good health and there is much debate about their precise functions, deficiencies being related to the onset of carcinomas and endemic diseases and the breakdown of immunological defences (Lovkova *et al.,* 1996).

Other common plant constituents such as nucleosides, sterols, lipids and inorganic ions have also been recorded.

Red Ginseng

Commercially ginseng *(Panax ginseng)* is available in white and red forms. Careful chemical analysis has revealed marked differences between the two types. The airdried white ginseng as described above yielded the range of ginsenosides discussed but the heat treated red ginseng revealed some changes. Common to both varieties were the ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2, Rg_3, Rh_1 and Ro but red ginseng was found to yield greater amounts of the ginsenosides Rg_2, Rg_3 and Rh_1 Compounds apparently specific to red ginseng included 20*(R)*-ginsenoside Rg_2, 20*(S)*-ginsenoside Rg_3, 20*(R)*-ginsenoside Rh_1 and ginsenoside Rh_2 (Kitigawa *et al.,* 1983b). Subsequent work by Kasai, Matsuura and colleagues (1983, 1984) revealed the presence of additional saponins in extracts of red ginseng, including ginsenosides Ra_1, Ra_2, Ra_3, Rs_1, Rs_2, notoginsenosides R_1 and R_4 and quinquenoside R_1 (see Appendix to Chapter 5 for chemical structures). Kitigawa and his team (1987) further investigated the total saponins of white and red ginseng, employing drugs prepared from the same sample of *Panax ginseng* and a thin layer chromatography technique involving a chromatoscanner. It was concluded that the variation between the two drugs was due to heat processing changes causing a) demalonylation of the malonyl-ginsenosides occurring in the fresh root, b) elimination of the glycosyl residue at C-20 of the aglycone units in the ginsenosides, and/or c) isomerisation

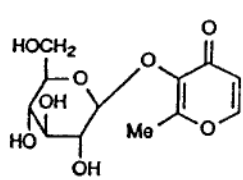
of the hydroxyl configuration at C-20 of the aglycones. Therefore heat processing changes must also occur during the preparation of commercial aqueous extracts and decoctions of ginseng and, as such preparations tend to be acid in the pH range 4.8–5.3, hydrolysis of the C-20 linkage with accompanying epimerisation at the C-20 carbon atom will take place. Resultant compounds may affect the pharmacological characteristics of the final product.

Further dammarane ginsenosides have been reported as isolated after processing from Korean red ginseng root viz. ginsenosides Rg_4 and Rg_5 (Kim *et al.,* 1996), ginsenoside Rh_4 (Baek *et al.,* 1996a), 20*(E)*-ginsenoside F_4 (Ryu *et al.,* 1996) and ginsenoside Rg_6 (Ryu *et al.,* 1997).

Employing alcian blue dye complex formation and spectrophotometry Han *et al.* (1992) confirmed that the polysaccharide yield from red ginseng after aqueous extraction was three times greater than from fresh root. The main roots yielded more polysaccharide than fine roots and the polysaccharides were located mainly in the cortex and cambium. Do *et al.* (1993) used a colorimetric method employing carbazole-sulphuric acid to measure the quantities of acidic polysaccharides in various ginsengs. They also concluded that the yield of polysaccharides from red ginseng root *(P. ginseng)* was higher than from white ginseng root, wild and cultivated American ginseng *(P. quinquefolium)* root, Sanchi ginseng *(P. notoginseng)* root and ginseng leaves.

Red ginseng was shewn to yield 724, 721 and 71 μg/g respectively of the polyacetylenes panaxynol, panaxydol and panaxytriol, estimation being performed using a capillary gas chromatographic method and a flame ionisation detector (Nho and Sohn, 1989). Another report recorded 250, 297 and 320 μg/g respectively of the same polyacetylene compounds (Matsunaga *et al.,* 1990). Using repeated column chromatography Baek *et al.* (1996b) discovered panaxynol, panaxydol, ginsenoyne A and panaxydiol chlorhydrin in red ginseng rhizomes.

Among the nonsaponin constituents of red ginseng was the food flavouring agent 3-hydroxy-2-methyl-pyran-4-one and its glucoside, which were recovered from an ethanol extract of red ginseng by ether extraction and silica gel chromatography (Wei, 1982). Such compounds may be artifacts generated during preparation of the red ginseng.



(5-14) 3-hydroxy-2-methyl-pyran-4-one 3-O-β-D-glucopyranoside

New amino acid derivatives maltulosyl-arginine and arginylfructose were isolated from Korean red ginseng and it was noted that red ginseng contained much more of the first-named compound than white ginseng, suggesting that it could be derived by the Maillard reaction of maltose with arginine under acid

conditions (*ca.* pH 3) and minimal water during the preparative heating process (Matsuura *et al.*, 1994). Further work resulted in the isolation of arginyl-fructosyl-glucose (Zheng, Y. *et al.*, 1996).

Distribution of the Main Saponins of *Panax ginseng*

The distribution of ginsenosides varies in nature and quantity throughout the plant. Typical yields of the major ginsenosides found in 4-year old South Korean ginseng plants were estimated by an HPLC-spectrophotometric method (Soldati and Sticher, 1980); results are presented in Table 5.8.

Table 5.8. Percentage yields of ginsenosides in various parts of *Panax ginseng*

Ginsenosides	Rb_1	Rb_2	*Rc*	*Rd*	*Re*	*Rf*	Rg_1	Rg_2	*Total*
Leaves	0.18	0.55	0.74	1.11	1.52	–	1.08	–	5.19
Leaf stalks	–	–	0.19	0.11	0.14	–	0.33	–	0.77
Stem	–	0.40	–	–	0.07	–	0.29	–	0.76
Main root	0.34	0.13	0.19	0.04	0.15	0.09	0.38	0.02	1.35
Lateral root	0.85	0.43	0.74	0.14	0.67	0.20	0.41	0.09	3.53
Root hairs	1.35	0.78	1.35	0.38	1.51	0.15	0.38	0.25	6.15

Saponins of *Panax ginseng* Roots

The yield of total saponins from *P. ginseng* was reported following TLC and colorimetric analysis as 1.62 per cent of which 0.71 per cent was ginsenosides Rb, 0.31 per cent ginsenosides Rg and 0.11 per cent ginsenosides Ro (Wang *et al.*, 1982). However Kitigawa *et al.* (1983b) reported 6.21 per cent total saponins from an 80 per cent methanolic extract of white ginseng cultivated in Nagano Prefecture, Honshu, Japan; the extract was partitioned using an ether-water mixture and the aqueous fraction separated and purified using a reversed phase silica gel chromatography column (Bondapak C_{18}; MeOH-H_2O). Zhang (1983) employed a colorimetric method based on the Liebermann-Burchard reaction and for dried roots reported yields of 9.74 per cent total ginsenosides in the fibrous roots, 6.41 per cent in the lateral roots and 3.31 per cent in the main root. Using an HPLC analysis method Yamaguchi *et al.* (1988) confirmed that the 6th year of root growth produced the highest total saponin yield. The decline in the 5th year was due to the rapid bulk increase of the roots but was only temporary (Table 5.9).

Table 5.9. Percentage saponin distribution in the main root of *Panax ginseng*

Main root	*1st year*	*2nd year*	*3rd year*	*4th year*	*5th year*	*6th year*
Total saponins	1.6	1.9	2.1	2.3	1.7	2.7
Diol saponins	0.44	0.35	0.36	0.58	0.33	0.65
Triol saponins	0.37	0.49	0.61	0.77	0.46	0.62
Ginsenoside Ro	0.03	0.10	0.20	0.11	0.20	0.27

Table 5.10. Percentage saponin distribution in lateral roots of *Panax ginseng*

Lateral roots	*2nd year*	*3rd year*	*4th year*	*5th year*	*6th year*
Total saponins	8.1	10	10	9.3	7.9
Diol saponins	2.0	2.8	3.1	2.7	4.2
Triol saponins	1.4	1.6	1.6	1.5	0.6
Ginsenoside Ro	0.13	0.29	0.26	0.58	0.18

The saponin yield of the smaller lateral roots was much higher and especially so in the second and third years of growth although declining in the later years (Table 5.10).

Some reported yields of individual ginsenosides in *P. ginseng* roots are summarised in Table 5.11.

Table 5.11. Reported yields of individual ginsenosides in ginseng roots

Designation		*Prosapogenin*	*Recorded yield per cent*		
			White ginseng	*Red ginseng*	
Ginsenoside	Ra_1	Protopanaxadiol	0.02	0.02	
..	Ra_2	..	0.03	0.03	
..	Ra_3	..	0.005	0.005	
..	Rb_1	..	0.47	0.37	0.39–0.49
Malonyl	Rb1	..	0.82	trace	
Ginsenoside	Rb_2	..	0.21	0.18	0.04–0.18
Malonyl	Rb_2	..	0.41	trace	
Ginsenoside	Rb_3	..	0.005	0.01	
..	Rc	..	0.15	0.13	0.48–0.88
Malonyl	Rc	..	0.30	trace	
Ginsenoside	Rd	..	0.15	0.13	
Malonyl	Rd	..	0.12	trace	
Ginsenoside	Re	Protopanaxatriol	0.15	0.20	0.29–0.73
..	Rf	..	0.05	0.05	
20-gluco	Rf	..	0.005	trace	
Ginsenoside	Rg_1	..	0.21	0.21	0.39–0.55
..	Rg_2	..	0.01	0.02	0.10–0.23
20(*R*)- ..	Rg_2	..	–	0.003	
Ginsenoside	Rg_3	Protopanaxadiol	0.003	0.014	
20(*S*)- ..	Rg_3	..	–	0.009	
Ginsenoside	Rh_1	Protopanaxatriol	0.0015	0.006	
20(*R*)- ..	Rh_1	..	–	0.007	
Ginsenoside	Rh_2	Protopanaxadiol	–	0.001	
..	Ro	Oleanane	0.02	0.04	
..	Rs_1	Protopanaxadiol	–	0.008	
..	Rs_2	..	–	0.01	
Quinquenoside	R_1	Protopanaxadiol	0.002	0.015	
Notoginsenoside	R_4	Protopanaxadiol	–	0.002	

Table 5.12. Percentage saponin distribution in *Panax ginseng* rhizomes

Rhizome	*1st year*	*2nd year*	*3rd year*	*4th year*	*5th year*	*6th year*
Total saponins	4.1	4.3	6.9	6.7	6.1	6.0
Diol saponins	0.80	0.60	1.1	1.1	0.96	1.2
Triol saponins	1.6	1.0	1.3	1.5	1.2	1.1
Ginsenoside Ro	0.25	0.59	1.1	0.95	1.2	1.1

Saponins of *Panax ginseng* Rhizomes

The rhizome or rootstock is an underground stem and for ginseng the rhizome yields most saponins in the third and subsequent years. The saponin mixture is similar to that in the roots. For example, Kuang and Xu (1982) reported the presence of nine main ginsenosides in Jilin ginseng including the ginsenosides Rb_1, Rb_2, Rc, Re, Rg_1, Rg_2 and Ro. Ginsenoside Ro, the glucuronide saponin of oleanolic acid, occurs more abundantly in the rhizome than elsewhere in the plant (Table 5.12).

Saponins of *Panax ginseng* Leaves and Aerial Stems

The leaves yielded the known diol-ginsenosides Rb_1, Rb_2, Rc and Rd and triolginsenosides Re, Rg_1 and 20(R)-Rh_2 and, in addition, three new compounds designated ginsenosides F_1, F_2 and F_3. The ginsenosides are summarised in Table 5.13. The principal saponins were ginsenosides Rd, Re and Rg_1 (Shibata *et al.*, 1985).

Subsequent work indicated that the aerial stems yielded ginsenosides Rb_2, Rd, Re, Rf, Rg_1, Rg_2 and F_1, a pattern similar to that of the leaves (Yang and Xu, 1987). Further investigations of leaves revealed the presence of 20-glucoginsenoside Rf (Cai *et al.* (1986) and confirmed the occurrence of the known ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rg_1, Rg_2, Rg_3 and Rh_1 together with the minor ginsenosides Rh_2, Rh_3 and 20*(R)*-$Rh_2$and 20*(R)*-protopanaxatriol (Chen *et al.*,

Table 5.13. Percentage saponin distribution in *Panax ginseng* leaves

Designation	*Prosapogenin*	*Recorded yield per cent*
Ginsenoside Rb_1	Protopanaxadiol	0.1
.. Rb_2	..	0.4
.. Rc	..	0.2
.. Rd	..	1.5
.. Re	Protopanaxatriol	1.5
.. Rg_1	..	1.5
20(*R*)- .. Rh_2	..	trace
.. F_1	..	0.4
.. F_2	Protopanaxadiol	0.2
.. F_3	Protopanaxatriol	0.2

1987). In 1988 Chen *et al.* isolated from stems and leaves 20*(R)*-ginsenoside Rh_2, an antitumour agent effective against the human leukaemia cell line HL-60. Zhang *et al.* (1989) added 20*(R)*-protopanaxadiol and ginsenoside Rg_4 to the growing list of minor saponins and their derivatives and later additions included 20*(R)*-dammaran-3β,6α,12β, 20,25-pentol-6-O-α-L-rhamnopyranosyl-(1→2)-O-β-D-glucopyranoside and 20*(R)*-dammaran-3β,6α,12β,20,25-pentol (Zhao *et al.,* 1990), ginsenoside F_4 (Zhang *et al.,* 1990), ginsenoside I_a (=3β,6α,12β,20*(S)*-tetrahydroxy-dammar-24(25)-ene-(20-O-β-D-glucopyranosyl)-3-O-β-D-glucopyranoside) (Don *et al.,* 1996a), notoginsenoside Fe and ginsenoside Rd_2 (Don *et al.,* 1996b) and ginsenoside F_5 (*Don et al.,* 1996c).

Chang (1998) recorded the yield of crude saponins in the leaves as *ca.* 16.5 per cent for leaves collected in July or August and lower for later collections in September. The ginsenosides Rc, Rd and Rg_1 comprised about 70 per cent of the total glycosides each month, ginsenosides Rb_1, Rb_2 and RC being minor components only. The ratio protopanaxadiol/protopanaxatriol was 1.13 for leaves collected in July but only 0.85 for leaves collected in September indicating that the diol yield was highest in July and the triol yield greatest in September.

Apart from saponins the leaves, which are used in ginseng leaf teas, have been shewn to yield ascorbic acid, glutamic and aspartic acids, leucine and trace elements such as calcium, sodium and potassium (Kwon *et al.,* 1992) and neutral and acidic polysaccharides, the former comprising arabinogalactan or (1→4)-linked glucosyl residues and the latter were pectic polysaccharides with a rhamnogalacturonan spine and neutral sidechains (Gao *et al.,* 1991). The leaves yield less polysaccharides than the roots and the roots contain mainly pectins and glucans whilst the leaves produce chiefly pectins and heteroglycans. Shin *et al.* (1997) isolated a complex pectic polysaccharide with a molecular mass of about 11,000 from the leaves and noted that it was a rhamnogalacturonan containing 15 different monosaccharides in its structure including the rare sugars 2-O-methyl-xylose, 2-O-methylfucose, apiose, 3-C-carboxy-5-deoxy-L-xylose (aceric acid), 3-deoxy-D-manno-2-octulosonic acid and 3-deoxy-D-lyxo-2-heptulosonic acid.

The leaves also contain fatty acids of which 80 per cent comprised linoleic, linolenic, palmitic and oleic acids (Park *et al.,* 1986). The volatile oil, which gives the leaf its characteristic odour, is a mixture of at least 42 compounds comprising approximately 14 per cent terpenes and sesquiterpenes, 39 per cent triglycerol lipids and 22 per cent other aromatic components (Li *et al.,* 1996a).

Saponins of *Panax ginseng* Flowers and Buds

The flower buds, which are normally removed before flowering in order to stimulate more active root growth, yielded the known saponins ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rg_1 and F_3 together with a new compound ginsenoside M_{7cd} (Yahara *et al.,* 1979). Ginsenoside Re was the major component of the saponin fraction. Recorded yields are presented in Table 5.14.

These findings were confirmed by Shao *et al.* (1987) who, using column chromatography, added the known compounds ginsenosides Rf, Rg_2 and Ro to

Table 5.14. Percentage saponin distribution in *Panax ginseng* flower buds

Designation	*Prosapogenin*	*Recorded yield per cent*
Ginsenoside Rb_1	Protopanaxadiol	0.2
.. Rb_2	..	0.2
.. Rc	..	0.2
.. Rd	..	0.5
.. Re	Protopanaxatriol	2.8
.. Rg_1	..	0.2
.. F_3	..	0.03
.. M_{7cd}	..	present

the list. Later work with cultivated Jilin (China) ginseng included the isolation of ginsenosides Ro, Rb_1, Rb_2, Rb_3, Rc, Rd and Re, 20-gluco-ginsenoside Rf and ginsenosides Rf, Rg_1 and Rg_2 (Shao *et al.,* 1989).

Jilin ginseng flower buds also yielded 0.2 per cent of volatile oil as measured by gas chromatography. The oil contained about 37 per cent sesquiterpenes together with an open chain alkane, carboxylic acids, esters and ketones (Mao *et al.,* 1989).

Saponins of *Panax ginseng* Fruits

The known saponins ginsenosides Rb_2, Rc, Rd, Re and Rg_1 were isolated from the fruits and identified by Yahara *et al.* (1976). As in the leaves, ginsenoside Re was the dominant saponin. Recorded yields are summarised in Table 5.15.

Additional minor compounds found subsequently included ginsenoside Rb_1 (Bai *et al.,* 1988) and 20*(R)*-ginsenoside Rh_2 and 20*(R)*-ginsenoside Rg_3 together with 20*(R)*-protopanaxatriol (Zhao *et al.,* 1993).

Non-saponin compounds found in the fruits included β-sitosterol and daucosterol (Zhao *et al.,* 1993). Although the total content of free and bound lipids in various parts of the plant varied from 0.91 to 3.48 per cent, the yield from seeds was about 15.08 per cent. The seed fatty acid composition was different, oleic and linoleic acids forming approximately 51.21 and 37.46 per cent respectively (Choi *et al.,* (1983). The oil from the seeds of *P. ginseng* also yielded lipids. Squalene, a biosynthetic precursor of sterols, formed 54 per cent

Table 5.15. Percentage saponin distribution in *Panax ginseng* fruits

Designation	*Prosapogenin*	*Recorded yield per cent*
Ginsenoside Rb_2	Protopanaxadiol	0.02
.. Rc	..	0.1
.. Rd	..	0.1
.. Re	Protopanaxatriol	0.6
.. Rg_1	..	0.04

of the unsaponifiable lipid fraction. Related compounds included squalene-2,3-oxide, triterpene alcohols, 4-methyl-sterols and sterols (Matsumoto *et al.,* 1986). Significantly squalene-2,3-oxide can be converted *in vitro* to 20*(S)*-dammarenediol by a microsomal fraction prepared from the hairy roots of *P. ginseng* (Kushiro *et al.,* 1997). Ginseng fruits also yield water soluble polysaccharides as a mixture of heteroglycans comprising six monosaccharides. Heteroglycan F, molecular weight *ca.* 1,900,000, yielded arabinose, galactose, glucose, rhamnose, xylose and galacturonic acid in the molar ratio of 5.9:17.4:1.0:4.3:0.3:0.7 and was based on a spine of β-(1→3)-galactose units with side chains attached at the C-4 and C-6 positions (Lui *et al.,* 1988).

(General reference sources for *P. ginseng* saponins:- Shoji, 1985 and references therein; Wei *et al.,* 1986; Thompson, 1987 and references therein; Tang and Eisenbrand (1992); for chemical nomenclature see Appendix to Chapter 5).

2) *Panax japonicus* C.A.Meyer

Japanese ginseng, known locally in Japan as *chikusetzu ninjim* or chikusetsu ginseng, is used indigenously in place of Korean ginseng as a stomachic, an antipyretic and an expectorant, usage that differs from that of the Korean drug. The Chinese Pharmacopoeia, 1985 lists the dried rootstocks (or rhizomes) of *P. japonicus* C.A.Meyer together with the dried rootstocks of *P. japonicus* C.A.Meyer var. *major* (Burk.) Wu et Feng and *P. japonicus* C.A.Meyer var. *bipinnatifidus* (Seem.) Wu and Feng.

The earliest record of its chemistry and pharmacology was published by Inoue in Japan in 1902. He isolated a saponin but no further work appeared until Muryama and Itagaki in 1923, Muryama and Tanaka in 1927, Aoyama and also Kotake in 1930 extended investigation of the crude saponin fraction but were limited by the analytical methods then available. Continuing research by Kitasota and Sone in 1932 and Kuwata and Matsukawa in 1934 revealed the presence of oleanolic acid (Hou, 1978).

Using chromatographic methods Fujita *et al.* (1962) confirmed the presence of oleanolic acid as well as arabinose, glucose and glucuronic acid. In addition they observed the presence of a small quantity of panaxadiol. Further studies undertaken by Kondo and his co-workers (1970, 1971) using improved methods produced three new saponins which they named chikusetsusaponins III, IV and V. Chikusetsusaponin III was shewn to comprise protopanaxadiol or 20-epiprotopanaxadiol combined with 2 molecules of glucose and 1 molecule of xylose, thus resembling the general structural pattern of the *P. ginseng* saponins. Chikusetsusaponin IV comprised oleanolic acid, glucose, arabinose and glucuronic acid and Chikusetsusaponin V was shewn to be identical with ginsenoside Ro. However, Chikusetsusaponin IV was found to be identical with araloside A, a saponin previously isolated from roots of *Aralia manschuria* by Kochetkov *et al.* (1962). Oleanolic acid-28-β-D-glucopyranosyl ester was isolated by Cai *et al.* (1982) and confirmed by Peng *et al.* (1987) who also found oleanolic acid-3-O-β-D-6-methylpyranoglucuronyl and in 1988

β-sitosterol-3-O-β-D-glucopyranoside. Two other oleanolic acid derived saponins, designated Pjs-2 and Pjs-4, were also isolated by Cai and Xia from rhizomes in 1984.

Known ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1 and Ro together with other chikusetsusaponins have subsequently been confirmed as common constituents in *P. japonicum* roots and rootstocks. Structures not previously tabled are presented in Tables 5.16 (protopanaxadiol type), 5.17 (protopanaxatriol type), 5.18 (oleanane type), 5.19 (octillol type) and 5.20 (miscellaneous types).

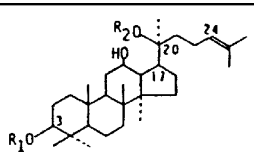
Quantitative studies of *P. japonicus* roots indicate that the oleanane ginsenoside Ro is the principal saponin present. Other glycosides occur in very small amounts, being indicated as present (Table 5.21).

The results obtained from other *P. japonicus* varieties are presented in Table 5.22 and a common pattern of oleanane saponins is apparent.

Saponins of *Panax japonicus* Rhizomes

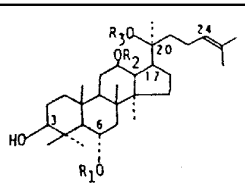
The occurrence of this species, which possesses well developed rhizomes, in both Japan and China stimulated research concerning possible phytochemical

Table 5.16. Structures of protopanaxadiol type saponins occurring in *Panax japonicus* Roots and Rootstocks



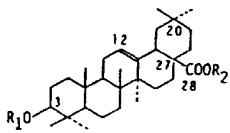
Designation	R_1	R_2
Chikusetsusaponin Ia	–Glc⁶–Xyl	–H 20(*S*)
Chikusetsusaponin III	–Glc–²Glc⁶–Xyl	–H

Table 5.17. Structures of Protopanaxatriol type saponins occurring in *Panax japonicus* roots and rootstocks



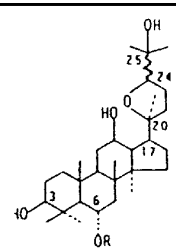
Designation	R_1	R_2	R_3
Chikusetsusaponin L_5	–H	–H	–Glc⁶–Ara(p)⁴–Xyl
Chikusetsusaponin L_{10}	–H	–Glc	–H: 20(*S*)
Notoginsenoside R_2	–Glc²–Xyl	–H	–H: 20(*S*)

Table 5.18. Structures of oleanane type saponins occurring in *Panax japonicus* roots and rootstocks



Designation	R_1	R_2
Chikusetsusaponin Ib	–GlcA–^{6}GlcA^{4}–Ara(f)	–H
Chikusetsusaponin IV	–GlcA^{4}–Ara(f)	–Glc
Chikusetsusaponin IVa	–GlcA	–Glc
Chikusetsusaponin V = ginsenoside Ro	–GlcA^{2}–Glc	–Glc
Chikusetsusaponin V methyl ester	–GlcA^{6}Me^{2}–Glc	–Glc
Oleanolic acid-3-O-β-D-6-methyl-pyranoglucuronyl	–^{6}GlcAMe	–H
Oleanolic acid-28-β-D-glucopyranosyl	–H	–Glc
Pjs-2	–GlcA^{2}–Xyl(p)	–Glc
Pjs-4	–Ara(p)	–Glc

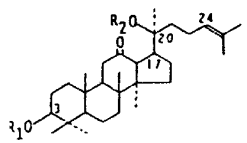
Table 5.19. Structures of ocotillol type saponins occurring in *Panax japonicus* roots and rootstocks



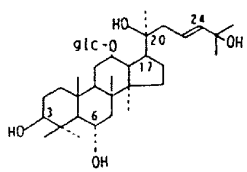
Designation	*R*	*C24*
Majonoside R_1	–Glc^{2}–Glc	(*S*)
.. R_2	–Glc^{2}–Xyl	(*S*)
Pseudoginsenoside F_{11}	–Glc^{2}–Rha	(*R*)
.. RT_2	–Glc^{2}–Xyl	(*R*)
.. RT_4	–Glc	(*S*)
.. RT_5	–Glc	(*R*)

differences Morita's group (1982) had examined rhizomes of *P. pseudoginseng* subsp. *japonicus* var. *major* (=*P. japonicus* var. *major*), a variety also known as *zu-tzing* and occurring in Yunnan, China. They reported the presence of dammarane saponins (ginsenoside Rd, 20-gluco-ginsenoside Rf and notoginsenoside R_2), oleanane saponins (chikusetsusaponin IVa and ginsenoside Ro) and (24*S*)-ocotillol saponins (majonosides R_1 and R_2). Yunnan-chikusetsu ginseng, known colloquially as *zhu-jie-shen,* also yielded mainly oleanane and

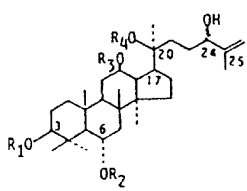
Table 5.20. Structures of miscellaneous type saponins occurring in *Panax japonicus* roots and rootstocks



Designation	R_1	R_2
Chikusetsusaponin LT_5	–Glc	–Glc^6–Glc
.. LT_8	–Glc	–Glc
.. LN_4	–Glc^6–Xyl	–Glc^6–Ara(p)



Chikusetsusaponin-L_{9a}



Designation	R_1	R_2	R_3	R_4
Chikusetsusaponin L_{9bc}	–H	–H	–Glc	–H

Table 5.21. Recorded yields of saponins in *Panax japonicus* roots

Designation	*Prosapogenin*	*Recorded yield per cent*
Chikusetsusaponin Ib	Oleanane	present
.. IV	..	0.43
.. IVa	..	present
Ginsenoside Ro	..	5.35
Chikusetsusaponin Ia	Protopanaxadiol	present
.. III	..	1.17
Ginsenoside Rg_2	Protopanaxatriol	present

protopanaxatriol saponins, the principal compounds being the oleananes ginsenoside Ro and chikusetsusaponins IV and IVa (Morita *et al.*, 1983). Investigation of rhizomes of *P. japonicus* C.A.Meyer var. *angustifolius* (Burk.) Cheng et Chu by Wang *et al.* (1985) revealed the presence of ginsenosides Rd,

Rg_1, Rh_1 and Ro, notoginsenoside R_1, chikusetsusaponins IV and IVa. zingibroside R_1, oleanolic acid-28-β-D-glucopyranoside and oleanolic acid-3-β-D-glucuronoside. Wang's team concluded that *P. japonicus, P. japonicus* var. *major* and *P. japonicus* var. *angustifolius* were chemically so similar as to be one taxa. Another variety, *P. japonicus* var. *bipinnatifidus,* was also examined by Wang *et al.* (1988a). Their results revealed the presence of the chikusetsusaponins V (ginsenoside Ro), IV and IVa, the ginsenosides Rb_1, Rd, Re, Rg_1 and Rg_2, the 24*(S)*-pseudoginsenoside F_{11} and the zingibroside R_1. The chikusetsusaponins were the dominant saponins present (Table 5.22). Wang *et al.* (1988b) then examined saponins of the rhizomes of *P. japonicus* var. *major* collected in the Qin Ling mountains of Shensi and the Hangduan mountains of Yunnan, China and observed that the dominant saponins were also oleananes (ginsenoside Ro, chikusetsu-saponin IVa, its methyl ester and oleanolic acid-28-O-β-D-glucoside) and the minor glycosides were the dammarane saponins (ginsenosides Re and Rg_2 and notoginsenoside R_2). In Japan, Morita, Tanaka and Kohda (1985) analysed the saponin composition of *P. japonicus* rhizomes known locally as *satsuma-ninjin* and gathered in the southern island of Kyushu. The dammarane saponins ginsenosides Rb_1, Rc, Re and Rg_1, notoginsenosides R_1 and R_2 and gypenoside XVII were isolated together with oleanolic acid derived chikusetsusaponin IV and ginsenoside Ro but the authors commented on the apparent absence of chikusetsusaponins Ia and III which had been found in Japanese *chikusetsu-ninjin*. From rhizomes of *daye zhuzisben* Peng *et al.* (1988) also obtained β-sitosterol-3-O-β-D-glucopyranoside. More recent work by Kanamori *et al.* (1995a, b) suggested two types of Japanese ginseng rhizome;

Table 5.22. Occurrence of individual saponins in the subterranean structures of *P. japonicus* species varieties

Designation	*Prosapogenin*	*Recorded yield per cent*		
		var. major.	*Chinese*	*var. bipinnatifid*
Ginsenoside Rb_1	Protopanaxadiol			0.122
.. Rd	..	0.67	0.04	0.085
.. Re	Protopanaxatriol		0.12	0.06
.. 20-gluco Rf	..	0.01		
.. Rg_1	..		0.15	0.085
.. Rg_2	..		0.05	0.058
.. Ro	Oleanane	0.95	3.1	2.2
Notoginsenoside R_2	Protopanaxatriol	0.03	0.02	
Pseudoginsenoside F_{11}	Ocotillol		0.24	0.016
Chikusetsusaponin IV	Oleanane		3.4	0.22
.. IVa	Oleanane	0.19	2.8	0.20
.. V	Oleanane		0.04	
methyl ester				
Majonoside R_1	Ocotillol	0.07		
Majonoside R_2	Ocotillol	0.11		
Zingibroside R_1	Oleanane			0.026

type A from Japan yielded chiefly chikusetsusaponins III, IV and IVa and ginsenoside Ro whilst type B from China contained chikusetsusaponins IV and IVa, ginsenoside Ro and pseudoginsenoside RT_1 and little dammarane saponin. The analytical range of saponins was ginsenoside Ro 5.1–14.8 per cent, chikusetsusaponin III 3.2–9.3 per cent, chikusetsusaponin IV 1.4–5.4 per cent and chikusetsusaponin IVa 0.2–0.3 per cent.

Chemical analysis of *Panax pseudoginseng*=subsp. *P. himalaicus* Hara by Kondo and Shoji (1975) and *Panax pseudoginseng* var. *major* (Burk.) Li by Morita *et al.* (1982) produced contrasting results suggesting that the saponin pattern of Himalayan ginseng is intermediate between Japanese chikusetsu ginseng and Korean ginseng but that of *P. pseudoginseng* var. *major* was closer to *P. japonicus* (Table 5.23).

Subsequent reports indicated that Himalayan ginseng grown at high altitude yielded two new saponins, the oleanane pseudoginsenosides RT_1 and RP_1 together with the known compounds chikusetsusaponins IVa and V but not IV. Interestingly, samples of *P. japonicus* and *P. pseudoginseng* subsp. *himalaicus* collected at lower altitudes all contained chikusetsusaponin IV as a major saponin but lacked pseudoginsenosides RT_1 and RP_1. Of two specimens of *P. pseudoginseng* subsp. *himalaicus* collected at high altitude one yielded the dammarane ginsenosides Rb_1, Rd, Rg_1 and F_2, and the ocotillol pseudoginsenosides F_{11}, RT_2, RT_3, RT_4 and RT_5 and the other yielded ginsenosides Rb_1, Rd, Re, Rg_1 and F_2, gypenoside XVII and pseudoginsenosides RT_3 and RT_4 (Tanaka *et al.,* 1985). A study of the saponins extracted from roots and small rhizomes of *P. pseudoginseng* Wall, subsp. *pseudoginseng* Hara grown at Nielamu, Tibet revealed the dammarane ginsenoside Rg_1 (0.02 %), the oleananes chikusetsusaponin IV (0.15 %) and pseudoginsenoside RT_1 (0.04 %) (Morita *et al.,* 1986b). A later investigation of *P. pseudoginseng* var. *elegantior* revealed the oleanane saponins chikusetsusaponin IVa and V (=ginsenoside Ro) and pseudoginsenosides RT_1 and RS_1, dammarane saponins Rb_1, Rd, Re, Rg_1

Table 5.23. Occurrence of specific saponins in *Panax pseudoginseng subsp. P.* himalaicus Hara and P. *pseudoginseng* var. P. *major* (Burk.) Li rhizomes

Designation	*Prosapogenin*	*Recorded yield per cent*	
		Himalaicum	*Major*
Ginsenoside Rb_1	Protopanaxadiol	1.05	
.. Rd	..		0.67
20-Gluco- .. Rf	Protopanaxatriol		0.01
Ginsenoside Rg_1	..		
.. Ro	Oleanane	7.25	0.95
Majonoside R_1	Ocotillol		0.07
.. R_2	..		0.11
Notoginsenoside R_2	Protopanaxatriol		0.03
Chikusetsusaponin Ia	Protopanaxadiol	present	
.. III	..	present	
.. IV	Oleanane	0.3	0.19
.. IVa	..	0.6	0.19

and Rg_2, gypenoside XVII and notoginsenosides R_1 and R_2 and ocotillol type saponins majonoside R_2 and pseudoginsenosides 24*(S)* F_{11}, F_{11} and RT_2. Morita *et al.* (1986a) concluded that this variety was therefore probably synonymous with *P. japonicus* var. *major*.

Minor saponins isolated from *P. pseudoginseng* samples included psendoginsenoside RI_2 (Bis-[oleanolic acid-3-O-β-D-glucurono-pyranosyl(2→1)-β-D-xylopyranosyl-4-O]-phthalate) and pseudogmsenoside RI_3 from subsp. *himalaicus* var. *angustifolius* (Shukla and Thakur, 1990; Shukla, Thakur and Pachaly, 1992) and chikusetsusaponin VI=20*(S)*-protopanaxadiol-3-O-β-D-glucopyranosyl(1→2)-[-βD-xylopyranosyl- (1→6)]-β-D-glucopyranosido-20-O-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside (Kohda *et al.,* 1991).

Examination of rhizomes of *P. pseudoginseng* samples collected in Chame, central Nepal, confirmed the presence of dammarane saponins Rb_1, Rb_3, Rd, Re, Rg1 and gypenoside XVII and the new saponins, 24*(S)*-pseudoginsenoside F_{11} and monoacetylginsenoside Rd (also called ginsenoside RC_1). Rhizomes gathered at Ghorapanai also yielded notoginsenoside R_1, quinquenoside R_1, majonoside R_2 and malonyl-ginsenoside Rb_1. However no specimens from either area produced oleanolic acid saponins and therefore the taxonomic status is debatable (Namba *et al.,* 1986). More work is necessary to prove species identity and the possible occurrence of chemical races within species.

The studies of Himalayan or Indian ginseng (*P. pseudoginseng* subsp. *himalaicus* and its two varieties *angustifolius* and *bipinnatifidus*) undertaken by Shukla and Thakur (1987) and Shukla (1989) revealed that on hydrolysis of the total saponins protopanaxadiol, protopanaxatriol, β-sitosterol and oleanolic acid were isolated as aglycones. Common constituents were ginsenosides Rb_1, Rb_2, Rb_3, Rc, Rd, Re, Rg_1 and Ro, pseudoginsenosides F_{11}, RP_1 and RT_1 and chikusetsusaponins IV and IVa. This pattern closely resembles that of *P. japonicus*.

Long-chain aliphatic alcohol derivatives isolated from rhizomes of *P. pseudoginseng* subsp. *himalaicus* var. *angustifolius* included tritriacontanol, 24-hydroxyhexatetra-contanoic acid, 2-methyl-hexatetracont-1-en-3,21-diol and tritriacontanyl octacosanoate (Shukla and Thakur, 1986).

Apart from saponins the rhizomes of *P. japonicus* also contain polysaccharides. Ohtani *et al.* (1989) reported the occurrence of tochibanan A, a compound of molecular mass 23,000 with a linear β-1,4-galactose spine and tochibanan B, a compound with molecular mass 40,000 comprising D-galactose (87.1 %), L-arabinose, D-glucose and D-galacturonic acid and a β-D-(1→4)-linked galactopyranosyl backbone. Side chains include galacturonic acid, galactose, arabinose and glucose (Ohtani *et al.,* 1989) The rhizomes of *P. japonicus* var. *major* yielded by a process involving saline solution extraction, dialysis, ion exchange chromatography and gel filtration two glycoproteins ZP-1 and ZP-2. The latter inhibited the growth of mycelia of the fungi *Trichoderma viride* and *Fusarium graminearum;* compound ZP-2 comprised two subunits (molecular weights 55 and 66 Kd respectively) and contained glucose, mannose, fucose, xylose, galactose, rhamnose and uronic acids together with protein rich in asparagine and glutamine (Du *et al.,* 1992).

Table 5.24. Reported yields of individual saponins in Japanese ginseng stems and leaves

Designation	*Prosapogenin*	*Recorded yield per cent*	
Ginsenoside Re	Protopanaxatriol	0.1	Hiroshima
.. F_1	..	0.01	..
.. F_3	..	0.1	..
Chikusetsusaponin L_5	..	0.7	..
.. L_{9a}		0.1	..
.. L_{9bc}		0.2	..
.. L_{10}	..	0.2	..
.. LN_4	Dammar-24-ene-3β, 20(*S*)-diol	1.4	Niigata
.. LT_5	Dammar-24-ene-3β, 20(*S*)-diol	2.0, 0.5	Tottori
.. LT_8	..	0.1	..

Saponins of *Panax pseudoginseng* subsp. *japonicus* Stems and Leaves

Yahara *et al.* (1977, 1978) noted that, although the oleanane saponins ginsenoside Ro and chikusetsusaponin IV were dominant, there were variations in the saponin pattern of wild ginseng aerial parts growing in Japanese island sites in Hiroshima (south Honshu), Niigata (northwest Honshu) and Tottori (southwest Honshu) (Table 5.24).

This generally agreed with the later observations that Japanese ginseng leaves contained high concentrations of the oleanane Ro-type ginsenosides and panaxadiol ginsenosides (Lui and Staba, 1980).

In 1984 Yang *et al.,* studying the leaf saponins of *P. japonicus* var. *major* collected in the Qin Ling mountains of China, reported nine compounds; the dominant pair were the oleananes ginsenoside Ro and chikusetsusaponin IVa and also present were the protopanaxadiol-type ginsenosides Rd, Rb_1 (trace only) and Rb_3, the protopanaxatriol-type 20-O-gluco-ginsenoside Rf and notoginsenoside R_2, and the ocotillol-type saponins majonosides R_1 and R_2. Three years later Feng *et al.* (1987) reported the presence of ginsenosides Rd (0.26%), Re (0.10%), Rg_1 (0.032%), Rg_2 (0.032%) and F_2 (0.16%) together with four new dammarene saponins designated majorosides F_1 (0.21%), F_2 (0.063%), F_3 (0.023%) and F_4 (0.063%). Two further minor saponins, majorosides F_5 and F_6 were described later by the same group (Wang *et al.,* 1989a). They also investigated the dried leaves of *P. japonicus* var. *bipinnatifidus* (Seem.) Wu and Feng and isolated the known saponins ginsenosides Rb_1, Rb_3, Rd, Re, Rg_2, F_1, F_2 and F_3, 24*(S)*-pseudoginsenoside F_{11}, majoroside F_1 and the flavone panasenoside as well as two new dammarene saponins bipinnatifidusosides F_1 and F_2 (Wang *et al.,* 1989b). Examining leaves of *P. japonicus elegantior* var. *major,* Liu *et al.* (1989) reported three dammarane saponins, ginsenosides Rd (0.5%), Rg_1 (0.016%) and Rg_2 (0.025%).

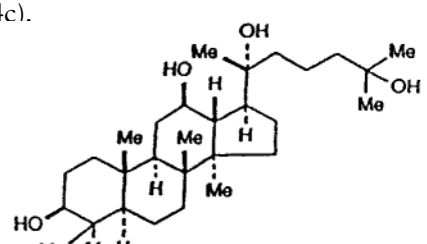
Leaves of *P. pseudoginseng* subsp. *himalaicus* also the yielded long-chain aliphatic derivatives hentriacontane, hexadecyl palmitate, dotriacontanyl palmitate, dotriacontanol, dotriacontanoic acid as well as oleanolic acid, β-sitosterol, and bis-(2-ethylheptyl)-phthalate (Shukla and Thakur, 1989).

The true status of *P. japonicus* and its subspecies and varieties needs

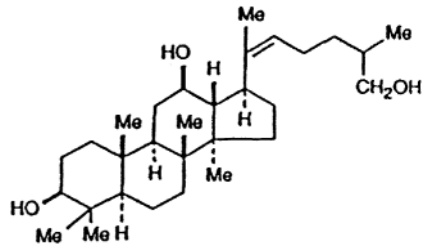
clarification. The on-going revision of the taxonomy of the *Panax* spp. may indicate that the chemical variation is due to factors such as species variation, growth location or chemical races (General reference sources for *P. japonicus* saponins:- Shoji, 1985 and references therein; Thompson, 1987 and references therein; Tang and Eisenbrand (1992) and references therein; for chemical nomenclature see Appendix to Chapter 5).

3) *Panax notoginseng* (Burk.) F.H.Chen=*P. pseudoginseng* Wallich

Sanchi or Tienchi ginseng, a species indigenous to northeastern China, was investigated for saponins by Kondo, Shoji and Tanaka (1973); they isolated 8 saponins, designating them A, B, C, D, E, F, G and H. Saponins A and D were characterised as chikusetsu saponin V (=ginsenoside Ro) and ginsenoside Rb_1 respectively. Lui and Staba (1980), comparing the common ginsengs of Canadian, American, Chinese, Japanese and Korean origin, stated that Sanchi ginseng contained the highest total ginsenoside concentration. The total yield of saponins from *P. pseudoginseng=P. notoginseng* roots as measured by TLC and colorimetry was 8.19 per cent of which 2.21 per cent comprised ginsenosides Rb and 4.30 per cent ginsenosides Rg (Wang *et al.,* 1982). Common ginsenosides occurring in this species comprised ginsenosides Rb_1, Rd, Re, 20-gluco-Rf and Rh_1 and gypenoside XVII (Besso *et al.,* 1981; Zhou *et al.,* 1981). New dammarane saponins from the Sanchi roots included notoginsenosides R_1 and R_2 and the ginsenosides Rg_2 and Rh_1 (Zhou *et al.,* 1981). Further isolations included the minor compounds notoginsennsides R_3, R_4 and R_6 (Matsuura *et al.,* 1983), notoginsenoside R_7 (panaxadiol-3-O-β-D-glucopyranoside) together with the known polyacetylene compound panaxytriol (Zhao *et al.,* 1993) and two minor epimeric dammarane saponins named notoginsenosides R_8 and R_9 were also isolated from roots by Zhao *et al.* (1996). Wei *et al.* (1985) reported two new compounds from the rootlets and recorded their names as sanchinosides B1 and B_2. The latter compound was shewn to be the known ginsenoside Rh_1 but the former was based on a new sapogenin, dammar-20(22)- ene-3β,12β,6α,25-tetrol, and sanchinoside B_1 was defined as dammar-20(22)- ene-3β,12β,25-triol-6-O-β-D-glucopyranoside. Thus, in addition to the established sapogenins 20*(S)*-panaxadiol and 20(S)-panaxatriol, *P. notoginseng* roots and rhizomes also contain the sapogenins 20(R)-protopanaxatriol, 20(R)-dammarane-3β,12β,20,25-tetrol (5–15) and dammar-20(22)-ene-3β,12β,26-triol (5–16)(Wei *et al.*, 1984c).



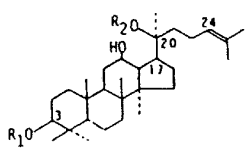
(5-15) 20(*R*)-dammarane-3β,12β,20,25-tetrol



(5-16) Dammar-20(22)-ene-3β,12β,26-triol

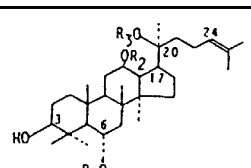
The structures of saponins not previously described as occurring in *P. notoginseng* roots are summarised in Tables 5.25 (protopanaxadiol type), 5.26 (protopanaxatriol type), 5.27 (oleanane type) and 5.28 (ocotillol type).

Table 5.25. Structures of protopanaxadiol type saponins of *Panax notoginseng* roots



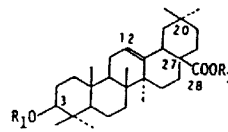
Designation	R_1	R_2
Gypenoside IX	–Glc	–Glc^6–Xyl
.. XVII	–Glc	–Glc^6–Glc
Notoginsenoside Fa	–Glc^2–Glc^2–Xyl	–Glc^6–Glc
.. Fc	–Glc^2–Glc^2–Xyl	–Glc^6–Xyl
.. Fe	–Glc	–Glc^6–Ara(*f*)
Pseudoginsenoside F_8	–Glc^{6}Ac^2–Glc	–Glc^6–Xyl

Table 5.26. Structures of protopanaxatriol type saponins of *Panax notoginseng* roots



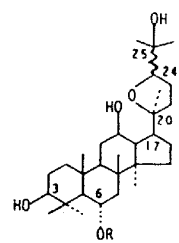
Designation	R_1	R_2	R_3
Notoginsenoside R_1	–Glc^2–Xyl	–H	–Glc
.. R_2	–Glc^2–Xyl	–H	–H: 20(*S*)
.. R_3	–Glc	–H	–Glc^6–β–Glc
.. R_6	–Glc	–H	–Glc^6–α–Glc
Pseudoginsenoside RT_3	–Xyl	–H	–Glc

Table 5.27. Structure of oleanane type saponin of *Panax notoginseng* roots



Designation	R_1	R_2
Pseudoginsenoside RT_1	–Glc^2–Xyl	–Glc

Table 5.28. Structures of ocotillol type saponins of *Panax notoginseng* roots



Designation	*R*	*C-24*
Pseudoginsenoside F_{11}	–Glc^2–Rha	(*R*)
.. RT_2	–Glc^2–Xyl	(*R*)
.. RT_4	–Glc	(*S*)
.. RT_5	–Glc	(*R*)

Quantitative analysis of the roots by Besso *et al.* (1981) and Zhou *et al.* (1981) provided the data listed in Table 5.29.

More recent work has indicated the presence of 14 known dammarane-type triterpene oligoglycosides, 9 new dammarane-type triterpene oligoglycosides named notoginsenosides A, B, C, D, E, G, H, I and J and an acetylenic fatty acid glycoside identified as notoginsenic acid β-sophoroside (Yoshikawa *et al.*, 1997). These compounds are interesting because the roots are valued in local medicine as haemostatic agents for both internal and external bleeding and as hepatoprotective agents.

Amongst other chemicals isolated from the roots of *P. notoginseng* are the peptides α-amino-β-(oxaloamino)propionic acid (Okan, 1982) and dencichin (3-[(carboxy-carbonyl)-amino]-L-alanine)(Zhao and Wang, 1986). Both compounds increase blood platelet numbers and enhance blood coagulation. *P. notoginseng* roots also yielded the polysaccharide sanchinan A (molecular weight 1.5×10^6), a compound with a β-D-(1→3)-linked galactopyranosyl backbone with branching points at C-6 to which mainly α-L-arabinofuranosyl and partly β-D-galacto-pyranosyl (1→6)-β-D-galactopyranosyl side chains are attached on average to 2 or 3 galactosyl units. Sanchinan A also incorporates a small

Table 5.29. Recorded yields of saponins of *Panax pseudoginseng* Wallich *(P. notoginseng)* roots

Designation	*Prosapogenin*	*Recorded yield per cent*
Total ginsenosides	–	8.1, 8.19
Ginsenoside Rb_1	Protopanaxadiol	1.8, 1.62, 2.21
.. Rd	..	0.2, 0.32
Ginsenoside Re	Protopanaxatriol	0.15, 0.51
20-Gluco-ginsenoside Rf	..	0.005
Ginsenoside Rg_1	..	1.9, 2.07
.. Rg_2	..	0.03
.. Rh_1	..	0.16, 0.06
Gypenoside XVII	..	0.036
Notoginsenoside R_1	..	0.16
.. R_2	..	0.04
.. R_3	..	0.007
.. R_4	..	0.028 0.002
.. R_5	..	0.02

amount of protein (*ca* 3.27%) (Ohtani *et al.*, 1987). This compound is a tissue macrophage (or reticuloendothelial) system potentiator.

Saponins of *Panax notoginseng=pseudoginseng* Corm

Matsuura *et al.* (1983) reported that the ginsenosides occurred in relatively high yields in the corms. The protopanaxadiol derived ginsenosides Rb_1, Rb_2 and Rd were found in yields of 5.2, 0.12 and 1.0 per cent respectively. The protopanaxatriol derived ginsenosides Re and Rg_1 and notoginsenoside R_1 occurred in yields of 0.63, 5.7 and 1.1 per cent respectively. As the corm is defined as a short underground shoot it can be regarded in this case as a condensed rhizome forming a very small part of the entire plant.

The reported occurrence of saponins in *P. notoginseng* rhizomes is confusing (Table 5.30). Studying the dammarane saponins, Yang *et al.* (1985) reported ginsenosides Rb_1 and Rg_1 as the most important compounds although ginsenosides Rd, Re, Rg_2 and Rh_1 and notoginsenosides R_1, R_2 and R_4 were also present. Varieties *himalaicum* and *major* would seem to be closer to *P. japonicus*.

Saponins of *Panax pseudoginseng=P. notoginseng=P. himalaicum* Stems and Leaves

After acid hydrolysis of the leaf saponins and subsequent separation by column chromatography, the sapogenins panaxadiol, panaxatriol, dammar-20(22)-en-3β,12β,26-triol, 20*(R)*-dammarane-3β,12β,20,25-tetroland an oxepane artifact derived probably by acid catalysed dehydration and rearrangement of panaxadiol were isolated (Wei *et al.*, 1984a). The leaves of Sanchi ginseng yielded

Table 5.30. Recorded yields of saponins of *Panax pseudoginseng=P. notoginseng= P. himalaicum=P. major* rhizomes

Designation	*Prosapogenin*	*Recorded yield per cent*		
		Notoginseng	*Himalaicum*	*Major*
Ginsenoside Rb_1	Protopanaxadiol	3.32	1.05	
.. Rd	..			0.67
20-Gluco- .. Rf	Protopanaxatriol			0.01
Ginsenoside Rg_1	..	4.36		
.. Ro	Oleanane		7.25	0.95
Majonoside R_1	Ocotillol			0.07
.. R_2	..			0.11
Notoginsenoside R_2	Protopanaxatriol			0.03
Chikusetsusaponin 1a	Protopanaxadiol		present	
.. III	..		present	
.. IV	Oleanane		0.3	0.19
.. IVa	..		0.6	0.19

Table 5.31. Recorded yields of saponins in *Panax pseudoginseng=P. notoginseng= P. himalaicum=P. major* stems and leaves

Designation	*Prosapogenin*	*Recorded yield per cent*	
		Notoginseng	*Himalaicum*
Ginsenoside Rb_1	Protopanaxadiol	0.03	
.. Rb_3	..	0.71	0.9
.. Rc	..	0.39	
.. Rd	..		0.1
.. Re	Protopanaxatriol		0.1
Gypenoside IX	Protopanaxadiol	0.03	
Notoginsenoside Fa	..	0.01	
.. Fc	..	0.05	
.. Fe	..	0.005	
Pseudoginsenoside F_8	Protopanaxadiol		0.1
.. F_{11}	Ocotillol		0.4

principally protopanaxadiol-derived glycosides, the dominant compounds being ginsenosides Rb_3 and Rc. Present were the ginsenosides Rb_1, Rb_3 and Rc together with gypenoside IX and the new notoginsenosides Fa, Fc and Fe (Table 5.25) (Yang *et al.*, 1983). Himalayan ginseng also yielded mainly protopanaxadiol-type saponins with ginsenoside Rb_3 being dominant; no notoginsenosides were reported (Tanaka and Yahara, 1978). The apparent dominance of panaxadiol-type saponins would seem to differentiate *P. notoginseng* leaves from the leaves of other common *Panax* spp. (Table 5.31).

The leaves also yielded the flavonoid glycoside quercetin-3-O-sophoroside (Wei and Wang, 1987).

Table 5.32. Recorded yields of saponins in *Panax pseudoginseng=P. notoginseng* flower buds

Designation	*Prosapogenin*	*Recorded yield per cent*	
Ginsenoside Rb_1	Protopanaxadiol	0.4	0.001
.. Rb_2	..	0.1	
.. Rb_3			1.2
.. Rc	..	1.0	0.42
.. Rd	..	0.1	0.067
.. F_2	..	0.1	
Gypenoside IX			0.014
Notoginsenoside Fa			0.087
.. Fc			0.015

Saponins of P. *pseudoginseng=Panax notoginseng* Flowers and Flower Buds

Taniyasu *et al.* (1982) investigated Chinese Sanchi ginseng flower buds and reported the apparent absence of protopanaxatriol-type glycosides and the dominance of ginsenoside Rc (Table 5.32).

Later the flower buds were shewn to yield ginsenosides Rb_3 and Rc, notoginsenoside Fd (gypenoside IX) and notoginsenoside Fe together with β-sitosterol and daucosterol (Zuo *et al.,* 1991). The sapogenins panaxadiol, dammar-20(22)-en-3β,12β,25-triol and 20*(R)*-dammaran-3β,20β,25-tetrol and two unnamed compounds had been isolated earlier by silica gel chromatography (Wei *et al.,* 1984b). The essential oil in the flowers comprised some 24 compounds which were principally terpenes and alkanes, dominant compounds being γ-elemene, heptocosane and pentacosane (Shuai and Li, 1986).

The pedicels or flower stalks contained ginsenosides Rb_3, Rc and Re and notoginsenosides R_1, Fd (29.6%) and Fe (Wei and Cao, 1992).

Saponins of P. *pseudoginseng=Panax notoginseng* Seeds

The seeds were analysed by Yang *et al.* (1983) who reported that the protopanaxadiol-derived ginsenosides Rb1 (0.01%), Rb_3 (1.2%), Rc (0.42%) and Rd (0.067%), gypenoside IX (0.014%) and notoginsenosides Fa (0.087%) and Fc (0.15%) occurred and again ginsenoside Rb_3 was the principal saponin present.

(General reference sources for *P. notoginseng* saponins:- Shoji, 1985 and references therein; Thompson, 1987 and references therein; Tang and Eisenbrand (1992); for chemical nomenclature see Appendix to Chapter 5).

4) *Panax quinquefolium* L.

As with the studies of Chinese and Korean ginsengs, progress in the investigation of the chemistry of American ginseng was slow. Long after Garriques' isolation of panaquilon in 1854, Wong in 1921, working in the United States, searched

unsuccessfully for alkaloids but was able to report 0.8 per cent of a pale yellow ginseng oil as well as a dark brown saponin that yielded pentose sugar and a sapogenin on hydrolysis. The oil was further investigated in 1939 by Torney and Cheng. Japanese researchers in the 1930's had shewn the presence of more saponins, less oil and proteinaceous material in the American species when compared with the Chinese and Korean species (Hou, 1978). This was confirmed by Shibata *et al.* (1965).

A detailed study of American ginseng was undertaken by Staba and his co-workers at the University of Minnesota, U.S.A. in the 1970's and 1980's. Their work embraced the distribution of phytochemicals, particularly saponin glycosides, throughout the plant and corelated the Japanese and Russian findings concerning the chemical structures of ginsenosides and panaxosides with their newly-found panaquilins from *P. quinquefolium*. The name "panaquilin" was adopted for the 11 isolated saponins in deference to Garriques original separation of a compound "panaquilon" from the plant. Fractionation into ethereal and methanol extracts revealed the occurrence of -sitosterol and stigmasterol in the ether fraction and saponins and sugars in the methanolic extract. The saponins or panaquilins were labelled A, B, C, D, E_1, E_2, E_3, G_1, G_2, (c) and (d). It was noted that panaquilins B, C, E_2, E_3 and G_2 occurred in all parts of the plant whilst panaquilins D, E_1 and G_1 were found chiefly in the subterranean parts of the plants. The leaves were found to yield panaquilins B, C, (d), E_2, E_3 and G_2. This qualitative variation was also age related, young roots presenting different saponin patterns to those found in older plants. Likewise a pattern variation occurred in the leaves at various times of the year. Subsequently, using advanced analytical methods, it was realised that these compounds were similar to or identical with known ginsenosides. Panaquilin B was a mixture of ginsenosides Rb_1 and Rb_2, panaquilin C was identical with ginsenoside Rc, panaquilins D and E_2 were ginsenoside Rd, panaquilin E_3 was ginsenoside Re and panaquilins G_1 and G_2 were ginsenosides Rg_1 and Rg_2 respectively (Hou, 1978).

Hydrolysis of the separated saponins confirmed that these compounds were also based on panaxadiol, panaxatriol or oleanolic acid. Quantitative estimations indicated about 17.3 per cent panaxadiol, 0.44 per cent panaxatriol and 0.28 per cent oleanolic acid. Thus the ratio of panaxadiol to panaxatriol was roughly 40:1 although in *P. ginseng* the ratio was approximately 1:1 and the pharmacology of the two species was therefore not identical.

Using HPLC-spectrophotometric analysis Soldati and Sticher (1980) estimated the occurrence of 1.703 per cent total ginsenosides comprising ginsenosides Rb_1 (0.263%), Rc (0.063%), Rd (0.095%), Re (1.043%) and Rg_1 (0.239%). Ginsenosides Rb_2, Rf and Rg_2 were not detected. Besso and his coworkers (1982b) reported the presence in the roots of ginsenosides Ro, Rb_1, Rb_2, Rc, Rd and Re with the additional ginsenosides Rb_3, Rg_1, Rg_2 and F_2, pseudoginsenoside F_{11} and gypenoside XVII together with a new compound, quinquenoside R_1 (=mono-O-acetyl-ginsenoside Rb_1 with the acetyl group attached at the 6-OH position of the terminal glucosyl moiety of the β-sophorosyl group). Liu *et al.* (1987) noted that the highest yield of ginsenosides occurred in the 4th and 5th years of growth and at the fruiting stage and that for 4-year old plants grown in

China the principal compounds were ginsenosides Rb_1 (0.96%), Rb_2 and Rc (0.97%), Rd and Re (2.93%) and Rg_1 (0.44%).

The work of Zheng *et al.* (1989a), comparing the roots of *P. quinquefolium* grown in China with those imported from North America, indicated a similar ginsenoside composition in all samples, the overall yield being 6.21–7.35 per cent. This finding agreed with Guo *et al.* (1991) who reported total glycoside yields of 5.80 and 7.75 per cent respectively in roots and root hairs grown in China. Ginsenosides detected by Zheng *et al.* included ginsenosides Ro, Rb_1, Rb_2, Rc, Rd, Re, Rg_1 and Rg_2. This was confirmed by Yang and his colleagues (1989) who analysed American ginseng grown in Yunnan Province, China and also noted the presence of the malonyl-ginsenosides Rb_1, Rb_2 and Rc. However they did stress that yields varied considerably dependent on the age of the plant, the time of harvesting, the commercial grade of the drug and the precise subterranean parts used. American seeds cultivated in India also produced plants yielding 9 saponins, the ginsenosides Rb being dominant. Sapogenins present after acid hydrolysis included β-sitosterol, oleanolic acid, panaxadiol, panaxatriol, dammar-20(22)-en-3β,12β,25-triol and dammaran-3β,12β,20,25-tetrol (Saxena *et al.,* 1994).

Subsequent investigations by Ko *et al.* (1995) indicated the absence of ginsenosides Ra, Rf, Rh_1 and Rh_2 from the *P. quinquefolium* samples analysed although such glycosides are present in *P. ginseng* and *P. notoginseng* roots. They reported the crude saponin yield as 7.01–7.25 per cent and that the diol/triol saponin ratio was 2.12–2.5. Analysing by colorimetry 4-year old *P. quinquefolium* roots grown in Fujian Province, China, Li *et al.* (1995) recorded a yield of 9.50 per cent total saponins and 5.054 per cent ginsenoside Rb_1.

Studying commercial samples obtained on the Taiwanese market Chuang *et al.* (1995) confirmed that the major saponins present were ginsenosides Rb_1 and Re and malonyl ginsenoside Rb_1. Also corroborating the occurrence of ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1, Li *et al.* (1996b) noted that in 4 years old roots the dominant ginsenosides were Re and Rb_1, compounds forming about 75 per cent of the total glycosides present. A French crop grown by Le Men-Olivier *et al.* (1995) also yielded ginsenosides Rb_1, Rd and Re, gypenoside XVII and pseudoginsenoside F_{11} as the main glycosides. In Canada Court *et al.* (1996) noted that the yield of ginsenosides Rb_1, malonyl Rb_1, Re and Ro increased with the age of the roots although the differences for ginsenosides Rb_2, malonyl Rb_2, Rc, malonyl Rc, Rd and Rg_1 and gypenoside XVII due to root age were small. They also agreed that ginseng harvested after 3 years growth contained less total ginsenosides than 4 year old roots.

The Chinese researchers Xie *et al.* (1996) observed that, although roots could produce 2.79 and 4.57 per cent of total ginsenosides after one and two years growth respectively under natural conditions, callus cultures and suspension cultures could yield 4.43 and 4.82 per cent total ginsenosides respectively in a much shorter time.

Yoshikawa *et al.* (1998), investigating the occurrence of bioactive saponins and glycosides in *P. quinquefolium* roots, reported the presence in the butanol-soluble fraction of 14 known dammarane-type triterpene oligoglycosides

Table 5.33. Reported yields of individual saponins in *Panax quinquefolium* roots

Designation	*Prosapogenin*	*Recorded yield per cent*
Total ginsenosides	–	5.80, 6.21–7.35, 9.50
Ginsenoside Rb_1	Protopanaxadiol	1.84, 1.57, 0.263, 0.96,
.. Rb_2	..	0.02, 0.03, 0.97
.. Rb_3	..	0.03
.. Rc	..	0.22, 0.31, 0.063, 0.97
.. Rd	..	0.77, 0.45, 0.095, 2.93
.. Re	Protopanaxatriol	0.89, 1.0, 1.043, 2.93
.. Rg_1	..	0.15, 0.239, 0.44
.. Rg_2	..	0.008, 0.03
.. Rh_1	..	0.06
.. Ro	Oleanane	0.07
.. F_2	Protopanaxadiol	0.02
Gypenoside XVII	..	0.03
Notoginsenoside R_1	Protopanaxatriol	0.16
.. R_2	..	0.04
Pseudoginsenoside F_{11}	Ocotillol	0.04
Quinquenoside R_1	..	0.01

including chikusetsusaponin IVa, pseudoginsenoside RC_1, malonyl-ginsenoside Rb_1 and the notoginsenosides A, C and K together with 5 new dammarane-type triterpene oligoglycosides named quinquenosides I-V. Four common polyacetylenic compounds and 6′-O-acetyl-ginsenoside-Rg_1 were isolated from the corresponding methanolic extract. Further study of these compounds is essential as the extracts exhibit protective activity on hepatic injury induced in mice by D-galactosamine or lipopolysaccharide. Reported yields of individual saponins in *Panax quinquefolium* roots are summarised in Table 5.33.

Amongst other chemical components occurring in the roots are palmitic acid, oleanolic acid, daucosterin, sugars (sucrose and ginseng trisaccharide), polysaccharides and amino acids. Yuan and Ouyang (1993) reported that root cell cultures and roots yielded similar saponins and polysaccharides, roots producing 8.42 per cent total saponins and 15.02 per cent polysaccharides and cell cultures 7.12 per cent total saponins and 13.93 per cent polysaccharides.

Re-evaluation of the oil content of both Chinese-grown and American-grown *P. quinquefolium* roots by Zheng *et al.* (1989b) shewed a yield of 0.04–0.097 per cent and similarity in chemical content. Sesquiterpenes were shewn to comprise about 75 per cent of the oil (Shen *et al.*, 1991). Nevertheless the composition can vary dependent on the extracting solvent e.g. 80 per cent methanol, diethylether.

As well as the common vital trace elements sodium, potassium, magnesium and calcium, *P. quinquefolium* roots contain, in order of decreasing amount, zinc (53.6 p.p.m.), barium, manganese (16.7 p.p.m.), titanium, lead (1.36 p.p.m.), chromium, cobalt, nickel and selenium (0.02 p.p.m.) (Liu *et al.*, 1987). Larger amounts of trace elements occur in the aerial parts of the plant and rhizomes and fibrous roots yield more than the roots.

Saponins of *Panax quinquefolium* Stems and Leaves

Staba's team investigated the quantitative occurrence of saponins in the aerial parts of *P. quinquefolium* (Chen *et al.*, 1981). The reported yields of the protopanaxadiol derived ginsenosides Rb_3 and Rd were 0.1 and 0.2 per cent respectively; the protopanaxatriol derived ginsenoside Re occurred at 0.1 per cent yield and the ocotillol compound pseudoginsenoside F_{11} also formed 0.1 per cent.

Ma *et al.* (1993) discovered nine saponins viz. ginsenosides Rb_2, Rb_3, Rd, Re, Rg_1, Rh_1, Rh_2, F_2 and pseudoginsenoside F_{11} in the stems and leaves of *P. quinquefolium*. Ginsenosides Rh_1, Rh_2 and F_2 had not previously been located in this species. Li and his coworkers (1996b) reported 1.33–2.64 g total ginsenosides per 100 g of dried leaves for 1 month-old leaves and 4.14–5.58 g per 100 g dried leaves for mature 4 month-old leaves. The principal ginsenosides were Rd and Re, each accounting for about 40 per cent of the total ginsenosides.

The leaves also yield polysaccharides, one of which was isolated by Miao *et al.* (1993); water-soluble polysaccharide PN had a molecular weight of 7400 and comprised main chains of β-(1→4(-linked glucose with 25 per cent of the main chains having sidechains at O-6 with a branching rate of 47.8 per cent.

The seed oil of *P. quinquefolium* contains sterols as the main unsaponifiable lipid fraction unlike *P. ginseng* seed oil which yields predominantly squalene (54%) in the unsaponifiable lipid fraction. Significantly the sterol fractions were different, *P. ginseng* yielding 28-isofucosterol (40%) and *P. quinquefolium* 24-ethyl-22E-dehydrocholesterol (66%) (Matsumoto *et al.*, 1986).

(General reference sources for *P. quinquefolium* saponins:- Shoji, 1985 and references therein; Thompson, 1987 and references therein; Tang and Eisenbrand (1992); for chemical nomenclature see Appendix to Chapter 5).

5) *Panax trifolium* L.

The roots of *P. trifolium* were shewn to yield the ginsenosides Re (0.0005%), Rf (0.0008%), Rg_1 (present), Rg_2 (0.0008%) and Ro (0.0004%). Investigating the leaves of this species Lui and Staba (1980) noted high concentrations of oleanane Ro-ginsenosides and panaxadiol ginsenosides and Lee and Der Marderosian (1988) reported the presence of ginsenosides Rb_3, Rc and Rd and notoginsenoside Fe as well as the flavonoids kaempferol-3,7-dirhamnoside and kaempferol-3-gluco-7-rhamnoside. Thus the common aglycones were (20*S*)-protopanaxadiol and kaempferol. Subsequent work revealed the presence of ginsenosides Rb_1, Rb_2, Rb_3, Rc, Rd and Ro and notoginsenoside Fe.

6) *Panax vietnamensis* Ha et Grushv.

This species entered the chemical literature when Lutomski (1992) published a review of the taxonomy, chemical composition and therapeutic action of a new species of *Panax* from Vietnam. His group had discovered seven polyacetylene compounds including two main compounds, falcarinol and heptadeca-1,8

(E)-diene-4,6-diyne-3,10-diol, and also some isomers of the latter (Lutomski and Luan, 1991). Falcarinol. a substance related to allergic contact dermatitis, had earlier been isolated from other Araliaceous species e.g. *Hedera helix* L. and related ivy species, *Schefflera arboricola* (Boll *et al.,* 1987).

Another team including Duc and Tanaka (1993–1994) investigated the occurrence of saponin glycosides isolating known dammarane glycosides including ginsenosides Rb_1, Rb_2, Rb_3, Rc, Rd, Re, 20-gluco-ginsenoside Rf, ginsenosides Rg_1, and Rh_1, 20*(R)*-ginsenoside Rh_1, pseudoginsenoside RS_1 (=monoacetyl-ginsenoside Re), gypenoside XVII, quinquenoside R_1, majonoside F_1 and notoginsenosides R_1, R_6 and F_a. The ocotillol type saponins detected were pseudoginsenoside RT_4, 24*(S)*-pseudoginsenoside F_{11} and majonosides R_1 and R_2. Oleanolic type glycosides included ginsenoside Ro and hemsloside Ma3, a glycoside previously isolated from the Cucurbitaceous plant *Hemsleya macrosperma*. The subterranean plant parts yielded mainly dammarane glycosides with a small amount of oleanolic saponins. However, the yield of majonoside R_2 the principal glycoside present, was conspicuously high being >5 per cent and comprised about 50 per cent of the total saponins.

Additional new compounds discovered were the vina-ginsenosides R_1 to R_{14}. Vina-ginsenoside R_1 was shewn to be monoacetyl-24*(S)*-pseudoginsenoside F_{11} and vina-ginsenoside R_2 was monoacetyl-majonoside R_2. Vina-ginsenoside R_3 is the first reported naturally-occurring dammarendiol and vina-ginsenosides R_5 and R_6 are ocotillol-type saponins revealing the rare -glucosyl unit (Nguyen *et al.,* 1994).

Apart from polyacetylenes and saponins these plants also yielded the sterol β-sitosteryl-3-O-β-D-glucopyranoside.

Future Propects

It is inevitable that more chemical compounds from ginseng plants will be reported and that synonymy of some compounds will be confirmed; improved isolation and analytical techniques will provide a better understanding of the readily hydrolysed sugar sidechains of the glycosides and of the structures of the complex polysaccharides. So far 6 *Panax* species have featured in current research reports and only 3 commercially useful species have been extensively investigated. The chemical and pharmacological reports do reveal differences between the species. Ko *et al.* (1995) compared these species and reported that

Table 5.34. Crude saponin content and panaxadiol/panaxatriol ratios in common ginseng roots

Species	*Crude saponin content per cent*	*Diol/triol ratio*
P. ginseng	4.81–5.24	1.27–1.45
P. quinquefolium	7.01–7.25	2.12–2.50
P. notoginseng	9.80	0.99

P. ginseng, P. quinquefolium and *P. notoginseng* yielded 12, 8 and 6 major ginsenosides respectively and *P. quinquefolium* did not reveal detectable amounts of ginsenosides Ra, Rf, Rh_1 and Rh_2. The reported crude saponin yield and related panaxadiol/panaxatriol ratio is presented in Table 5.34.

Although Japanese ginseng *P. japonicus* has recently been investigated in detail it is not apparently readily available commercially outside of Japan and possesses different pharmacological properties.

Further work on the isolated phytochemicals should permit a more precise understanding of the relationship between the common species and aid the explanation of the pharmacology of the crude extracts as well as the individual compounds.

Appendix—Chemical Nomenclature of Saponins and Some Other Chemical Substances Reported Occurring in Ginseng Species

Name	Chemical Nomenclature
Dammarane	(5α)-4,4,8,14-tetramethyl-18-norcholestane-[5*S*-[5α,8β,9α, 10β, 13β,14α,17β(*S**)]-17-(1,5-dimethylhexyl)-hexa-decahydro-4,4, 8,-10, 14-pentamethyl-14-cyclopenta [a]-phenanthrene
Aglycones	
20*(S)*-proto-panaxadiol	12β-hydroxy-dammarene-diol-11
20*(S)*-proto-panaxatriol	6α-12β-dihydroxy-dammarene-diol-11
Ocotillol	(3β,24*R*)-20,24-epoxy-dammarane-3,25-diol
Bipinnatidusosides	
-F1	dammar-25(26)-ene-3β,12β,20*(S)*,24ξ-tetraol-(20-O-β-D-glucopyranosyl)-3-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside
-F2	dammar-22(23)-ene-3β,12β,20*(S)*,24ξ-tetraol-(20-O-β-D-glucopyranosyl)-3-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside
Chikusetsusaponins	
-I	=Ginsenoside Rg_2

-Ia	β-D-glucopyranoside, (3β,12β)-12,20-dihydroxydammar-24-en-3-yl, 6-O-β-xylopyranosyl-
-Ib	3-O-α-arabino-furanosyl-(1→4)-β-D-glucopyranosiduronyloleanolic acid-6-β-D-glucopyranosyl ester
-II	β-D-glucopyranosiduronic acid, (3β)-17-carboxy-28-norolean-12-en-3-yl, 6β-D-glucopyranosyl ester
-III	20*(S)*-protopanaxadiol-3-O-β-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→6)]-O-β-D-glucopyranosyl
-IV	3-O-α-arabino-furanosyl-(1→4)-β-D-glucopyranosiduronyloleanolic acid-28-β-D-glucopyranosyl=Araloside A
-IVa	3β-O-β-D-glucopyranosiduronyl-oleanolic acid-28-β-D-gluco-pyranosyl
-IVa methyl ester	3βO-β-D-glucopyranosiduronic acid methyl ester-oleanolic acid-28-β-D-gluco-pyranosyl
-IVc	β-D-glucopyranoside, (3β,6α,12β)-20-(β-D-glucopyranosyloxy)-3,12-dihydroxydammar-24-en-6-yl 2-O-(-6-deoxy-α-L-mannopyranosyl)
-V	3β-O-β-D-glucopyranosyl-(1→2)-O-β-D-glucopyranosiduronyl-oleanolic acid-28-β-D-glucopyranosyl (=Ginsenoside Ro)
-VI	20*(S)*-protopanaxadiol-3-O-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→6)-β-D-glucopyranosido]-20-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside.
-L_5	20-O-[β-xylopyranosyl-(1→4)-α-arabinopyranosyl-(1→6)-β-glucopyranosyl]-20*(S)*-protopanaxatriol
-L_{9a}	β-D-glucopyranoside, (3β,6α,12β,23*E*)-3,6,20,25-tetrahydroxydammar-23-en-12-yl
-L10	12-O-β-glucopyranosyl-20*(S)*-protopanaxatriol
-LN4	dammar-24-ene-3β,20*(S)*-diol-12-one-3-[O-β-xylopyranosyl-(1→6)-β-glucopyranoside]-20-[O-α-arabinopyranosyl-(1→6)-β-glucopyranoside

-LT5	dammar-24-ene-3β-20*(S)*-diol-3-(O-β-glucopyranoside)-20-[O-β-glucopyranosyl(1→6)-β-glucopyranoside]
-LT8	dammar-24-ene-3β,20*(S)*-diol-12-one-3,20-di-(O-β-D-glucopyranoside)
-Pjs-2	3-O-β-D-xylopyranosyl-(1→2)-β-D-lucopyranosiduronylol eanolic acid-28-β-D-glucopyranosyl
	oleanolic acid-3-O-β-D-glucopyranosiduronic acid methyl ester
	oleanolic acid-28-β-D-glucopyranosyl ester

Ginsenosides

-A_2	β-D-glucopyranoside, (3β,6α,12β)-3,12-dihydroxydammar-24-ene-6,20-diyl bis-
-B_2	β-D-glucopyranoside, (3β,6α,12β)-20-(glucopyranoxyloxy)-3,12-dihydroxydammar-24-en-6-yl 2-O-(6-deoxy-α-Lmannopyranosyl)-
-C	β-D-glucopyranoside, (3β,6α,12β)-20-[6-O-α-L-arabinopyranosyl-β-D-glucopyranosyl)oxy]-L-12-hydroxydammar-24-en-3-yl 2-O-β-D-glucopyranosyl-
Compound or ginsenoside K	20-O-[-β-D-glucopyranosyl]-20*(S)*-protopanaxadiol
-La	β-D-glucopyranoside, (3β,12β,23*R*)-12,23-epoxydammar-24-ene-3,20-diyl bis-
-M6a.	β-D-glucopyranoside, (3β,12β)-20-(β-D-glycopyranosyloxy)-12,25-dihydroxydammar-23-en-3-yl 2-O-β-D-glucopyranosyl
-M_{6bc}	β-D-glucopyranoside, (3β,12β)-20-(β-D-glucopyranosyloxy)-12,24-dihydroxydammar-25-en-3-yl 2-O-β-D-glucopyranosyl
-M_{7cd}	20-O-β-D-glucopyranoside of dammar-25-ene-3β,6,6α,12β, 20*(S)*, 24-O-pentaol

-RA_0	20*(S)*-protopanaxadiol-3-[O-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside]-20-O-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside
-Ra_1	20*(S)*-protopanaxadiol-3-O-β-D-glucopyranosyl (1→2)-β-D-glucopyranoside-20-O-β-xylopyranosyl(1→4)-α-L-arabinopyranosyl (1→6)-β-D-glucopyranoside
-Ra_2	20*(S)*-protopanaxadiol-3-O-β-D-glucopyranosyl (1→2)-β-D-glucopyranoside-20-O-β-D-xylopyranosyl-(1→2)-β-L-arabinofuranosyl(1→6)-β-D-glucopyranoside
-Ra_3	20*(S)*-protopanaxadiol-3-O-(β-D-glucopyranosyl(l→2)-β-D-glucopyranosido)-20-O-β-D-xylopyranosyl(1→3)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside
-Rb_1	20*(S)*-protopanaxadiol-3-[O-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside]-20-O-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside
Malonyl-Rb_1	20*(S)*-protopanaxadiol-3-O-[6-O-malonyl-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-20-O-[β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl]
-Rb_2	20*(S)*-protopanaxadiol-3-[O-β-D-glucopyranosyl(1–2)-β-D-glucopyranoside]-20-[O-α-L-arabinopyranosyl(1→6)-β-D-glucopyranoside]
Malonyl-Rb_2	20*(S)*-protopanaxadiol-3-O-[6-O-malonyl-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-20-O-[α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl]
-Rb_3	20*(S)*-protopanaxadiol-3-O-β-D-glucopyranosyl (1–2)-β-D-gluco-pyranoside-20-O-β-D-xylopyranosyl-(1→6)-β-D-glucopyranosyl
-Rc	20*(S)*-protopanaxadiol-3-[O-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside]-20-O-α-L-arabinofuranosyl (1→6)-β-D-glucopyranoside
Malonyl-Rc	20-*(S)*-protopanaxadiol-3-O-[6-O-malonyl-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-20-O-[α-arabinofuranosyl-(1→6)-β-D-glucopyranosyl]
-Rd	20*(S)*-protopanaxadiol-3-[O-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside]-20-(O-β-D-glucopyranoside)

Malonyl-Rd	20*(S)*-protopanaxadiol-3-O-[6-O-malonyl-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-20-O-[β-D-glucopyranosyl]
-Rd_2	3β,12β,20*(S)*-trihydroxydammar-24(25)-ene-[20-O-α-Larabinopyranosyl(1→6)-β-D-glucopyranosyl]-3-O-β-D-glucopyranoside
-Re	20*(S)*-protopanaxatriol-6-[O-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside]-20-O--D-glucopyranoside
-Rf	20*(S)*-protopanaxatriol-6-O-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside
-Rg_1	20*(S)*-protopanaxatriol-6,20-di-O-β-D-glucoside
-Rg_1	20*(S)*-protopanaxatriol-6-O-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside
-Rg_4	3β,6α,12β-trihydroxydammar-20(22),24-diene-6-O-α-Lrhamnosyl-(1→2)-β-D-glucopyranoside
-Rg_5	20*(E)*-3β,12β-dihydroxy-dammar-20(22),24-diene-3-O-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside]
-Rg_6	β-D-glucopyranoside, (3β,6α,12β)-3,12-dihydroxydammar-20,24-dien-6-yl-2-O-(6-deoxy-α-L-mannopyranosyl)-
-Rh_1	20*(S)*-protopanaxatriol-6-O-α-D-glucopyranoside
-Rh_2	20*(S)*-protopanaxadiol-3-O-β-D-glucopyranoside
20*(R)*-Rh_2	20*(R)*-protopanaxadiol-3-O-β-D-glucopyranoside
-Rh_3	20*(Z)*-3β,12β-dihydroxy-dammar-20(22),24-diene-3-O-β-D-glucopyranoside
-Rh_4	β-D-glucopyranosyl-(3β,6α,12β,20*E*)-3,12-dihydroxydammaran-20(22),24-dien-6-yl
-Ro	=Chikusetsusaponin V
-RS_1	20*(S)*-protopanaxadiol-3-O-[6-O-acetyl-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-20-O-[α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl]

-Rs_2	20-*(S)*-protopanaxadiol-3-O-[6-O-acetyl-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-20-O-[-arabinofuranosyl-(1→6)-β-D-glucopyranosyl]
-F_1	20*(S)*-protopanaxatriol-20-O-β-D-glucopyranoside
-F_2	20*(S)*-protopanaxadiol-3,20-di-O-β-D-glucopyranoside
-F_3	20*(S)*-protopanaxatriol -20-O-[α-arabinopyranosyl-(1→6)-β-D-glucopyranosyl]
-F_4	β-D-glucopyranoside, (3β,6α,12β,20*Z*),-3,12-dihydroxydammar-20(22),24-dien-6-yl 2-O-(6-deoxy-α-L-mannopyranosyl)-
(20*E*)-F_4	β-D-glucopyranoside, (3β,6α,12β,20*E*),-3,12-dihydroxydammar-20(22),24-dien-6-yl 2-O-(6-deoxy-α-L-mannopyranosyl)
-F_5	20*(S)*-protopanaxatriol-20-O-α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside
-I(a)	3β,6α,12β-20*(S)*-tetrahydroxy-dammar-24(25)-ene-(20-O-β-D-glucopyranosyl)-3-O-β-D-glucopyranoside

Koryoginsenosides

-R_1	β-D-glucopyranoside, (E), (3β,6α,12β)-3,12-dihydroxy-6-[[6-O-(1-oxo-2-butenyl)-β-D-glucopyranosyl]oxy]-dammar24-en-20-yl
-R_2	β-D-glucopyranoside, (3β,6β,12*E*)-20-[(6-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]-12,25-dihydroxydammar-22-en-3-yl 2-O-β-D-glucopyranosyl

Majonosides

-R_1	3β,6α,12β,25-tetrahydroxy-(20*(S)*,24*(S)*)-epoxydammarane6-O-β-sophoroside
-R_2	3β,6α,12β,25-tetrahydroxy-(20*(S)*,24*(S)*)-epoxydammarane-6-O-β-xylopyranosyl(1→2)-β-glucopyranoside

Majorosides

Majoroside	cyclopenta (c) pyran-4-carboxylic acid, 1-(β-D-glucopyranosyl-oxy)-1,4α,5,6-tetrahydro-6-hydroxy-7-methyl-methyl ester, [1*(S)*-(1α,4α,6α)]-

-F_1 dammar-25(26)-ene-3β,12β,20*(S)*,24β,tetraol-(20-O-β-D-glucopyranosyl)-3-O-β-D-glucopyranoside

-F_2 dammar-25(26)-ene-3β,12β,20*(S)*,24α,tetraol-(20-O-β-D-glucopyranosyl)-3-O-β-D-glucopyranoside

-F_3 dammar-22(23)-ene-3β,12β,20*(S)*,24ξ-tetraol-(20-O-β-D-glucopyranosyl)-3-O-β-D-glucopyranoside

-F_4 dammar-23(24)-ene-3β,12β,20*(S)*,25-tetraol-(20-O-β-D-glucopyranosyl)-3-O-β-D-glucopyranoside

-F_5 dammar-22(23)-ene-3β,6α,12β,20*(S)*,24-pentaol-(20-O-β-D-glucopyranosyl)-6-O-α-L-rhamnopyranosyl-(1$\rightarrow$2)-β-D-glucopyranoside

-F_6 dammar-23(24)-ene-3β,6α,12β,20*(S)*,25-pentaol-(20-O-β-D-glucopyranosyl)-6-O-α-L-rhamnopyranosyl-(1$\rightarrow$2)-β-D-glucopyranoside

Notoginsenosides

-R_1 20*(S)*-protopanaxatriol-6-[O-β-D-xylopyranosyl-(1$\rightarrow$2)-β-D-glucopyranosyl]-20-O-β-D-glucopyranoside

-R_2 20*(S)*-protopanaxatriol-6-O-β-D-xylopyranosyl(1$\rightarrow$2)-β-D-glucopyranoside

-R_3 20*(S)*-protopanaxatriol-6-O-β-D-glucopyranosyl-20-O-β-glucopyranosyl-(1$\rightarrow$6)-β-D-glucopyranoside

-R_4 20*(S)*-protopanaxadiol-3-O[β-D-glucopyranosyl(1$\rightarrow$2)-β-glucopyranosyl]-20-O-β-D-xylopyranosyl(1$\rightarrow$6)-β-D-glucopyranosyl(1$\rightarrow$6)-β-glucopyranoside

-R_6 20*(S)*-protopanaxatriol-6-O-β-D-glucopyranosyl-20-O-α-D-glucopyranosyl(1$\rightarrow$6)-β-D-glucopyranoside

-R_7 panaxadiol-3-O-β-D-glucopyranoside

-R_8 (20*S*)-dammar-22-ene-3β-6α-12β-20-25-pentol-6-O-β-D-glucopyranoside

-R_9 (20*R*)-dammar-22-ene-3β-6α-12β-20-25-pentol-6-O-β-D-glucopyranoside

-Fa	20*(S)*-protopanaxadiol-3-O-β-xylopyranosyl(1→2)-β-glucopyranosyl-(1→2)-β-glucopyranoside-20-O-β-glucopyranosyl(1→6)-β-glucopyranoside
-Fc	20*(S)*-protopanaxatriol-3-O-β-xylopyranosyl(1→2)-β-glucopyranosvl-(1→2)-β-glucopyranoside-20-O-β-xylopyranosyl-(1→6)-β-glucopyranoside
-Fd	β-D-glucopyranoside, (3β,12β-3-(β-D-glucopyranosyloxy)-12-hydroxydammar-24-en-20-yl 6-O-β-D-xylopyranosyl-
-Fe	20*(S)*-protopanaxadiol-3-O-β-glucopyranosyl-20-O-β-D-arabinofuranosyl-(1→6)-β-D-glucopyranoside

Pseudoginsenosides

-F_8	mono-acetyl-3,20-di-O-glycosyl-20*(S)*-protopanaxadiol
-F_{11}	6-O-α-rhamnopyranosyl(1→ 2)-β-glucopyranoside of 3β,6α, 12β,25-tetrahydroxy-(20*S*,24*R*)-epoxydammarane
-RC_1	β-D-glucopyranoside, (3β,12β)-20-(β-D-glucopyranosyloxy)-12-hydroxydammar-24-en-3-yl 2-O-(6-O-acetyl-β-D-glucopyranosyl)
-RI_2	bis-[oleanolic acid-3-O-β-D-glucurono-pyranosyl(2→1)-β-D-xylopyranosyl-4-O]-phthalate
-RP_1	β-D-glucopyranosiduronic acid, (3β)-17-carboxy-28-norolean-12-en-3-yl 2-O-β-D-xylopyranosyl-
-RT_1	β-D-glucopyranosiduronic acid, (3β)-28-(β-D-glucopyranosyl-oxy-28-oxoolean-12-en-3-yl 2-O-β-D-xylopyranosyl
-RT_2	β-D-glucopyranoside, (3β,6α,12β,24*(R)*)-20,24-epoxy-3,12,25-trihydroxy-dammaran-6-yl
-RT_3	β-D-glucopyranoside, (3β,6α,12β)-3,12-dihydroxy-6-(-D-xylopyranosyloxy)-dammar-24-en-20-yl
-RT_4	6-O-β-D-glucopyranoside, (3β,6α,12β,24*(S)*)-20,24-epoxy3,12,25-trihydroxy-dammaran-6-yl
-RT_5	6-O-β-D-glucopyranoside, (3β,6α,12β,24*(R)*)-20,24-epoxy3,12,25-trihydroxy-dammaran-6-yl

Quinquenosides

-R_1	mono-O-acetyl-ginsenoside-Rb_1
-I	3-O-[6-O-*(E)*-2-butenoyl-β-D-glucopyranosyl(1←2)-β-D-glucopyranosyl]-20-(β-D-glucopyranosyl)20*(S)*-protopanaxadiol
-II	3-O-[6-O-(E)-2-octenoyl-β-D-glucopyranosyl(l←2)]-20-O-(β-D-glucopyranosyl(1←6)-β-D-glucopyranosyl)-20*(S)*-protopanaxadiol
-III	3-O-[β-D-glucopyranosyl(l←2)-6-O-acetyl-β-D-glucopyranosyl]-20-O-(β-D-glucopyranosyl-20*(S)*-protopanaxadiol
-IV	3-O-[β-D-glucopyranosyl(l←2)-β-D-glucopyranosyl]-20-O-β-D-glucopyranosyl(←6)-3β,7β,20*(S)*-trihydroxydammar-5,24-diene
-V	3-O-[-D-glucopyranosyl(1←2)-β-D-glucopyranosyl]-20-O-[α-D-glucopyranosyl(1←4)-β-D-glucopyranosyl(l←6)-β-D-glucopyranosyl]-20*(S)*-protopanaxadiol

Sanchinosides

-B_1	dammar-20(22)-ene-3β,12β,25-triol-6-O-β-D-glucopyranoside
-B_2	β-D-glucopyranoside, (3β,6α,12β)-3,12,20-trihydroxydammar-24-en-6-yl
-C_1	β-D-glucopyranoside, (3β,6α,12β)-3,12-dihydroxydammar-24-ene-6,20-diyl bis-
-E_1	β-D-glucopyranoside, (3β,12β)-20-[(6-O-β-D-glucopyranosyl)-oxy]-12-hydroxydammar-24-en-3 -yl 2-O-β-D-glucopyranosyl-

Zingiberosides

A_1	β-D-glucopyranoside, (3β, 25*S*)-spirost-5-en-3yl 2-O-(6-deoxy-α-L-mannopyranosyl)-
A_2	β-D-glucopyranoside,(3β, 24*R*, 25*R*)-24-hydroxy-spirost-5-en3-yl 2-O-6-(6-deoxy-β-L-mannopyranosyl)-

A_3	β-D-glucopyranoside, (3β, 25*S*)-spirost-5-en-3-yl O-6-deoxy-α-L-mannopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→4)]-
-R_1	3-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosiduronyl-oleanolic acid

Other Compounds

Amino Acid Derivatives

Arginylfructosyl-glucose	1-(arginine-N$^{\alpha}$-yl)-1-deoxy-4-O-(α-D-glucopyranosyl)-D-fructose
Dencichin	3-[(carboxy-carbonyl)amino]-L-alanine

Flavone Glycoside

Panasenoside	4H-1-benzopyran-4-one, 3-[2-O-β-D-glucopyranosyl-β-D-galactopyranosyl)oxy]-5,7-dihydroxy-2-(4-hydroxyphenyl)-

Polyacetylenes

Ginsenoyne A	8-[3-(6-heptenyl)-oxiranyl]-1-octene-4,6-diyn-3-ol
Ginsenoyne B	10-chloro-1,16-heptadecadiene-4,6-diyne-3,9-diol
Ginsenoyne C	1,16-heptadecadiene-4,6-diyne-3,9,10-triol
Ginsenoyne D	8-(3-heptyloxiranyl)-4,6-octadiyn-3-ol
Ginsenoyne E	(2R-cis)-8-(3-heptyloxiranyl)-1-octene-4,6-diyn-3-one
Ginsenoyne F	8-[3-(6-heptenyl)-oxiranyl]-1-octene-4,6-diyn-3-ol, acetate
Ginsenoyne G	8-(3-heptyloxiranyl)-4,6-octadiyn-3-ol, acetate
Ginsenoyne H	8-[3-(6-heptenyloxiranyl)-4,6-octadiyn-3-ol, acetate
Ginsenoyne I	8-(3-heptyloxiranyl)-[2*R*-[2α(3*R*,4*E*), 3α]]-1,4-octadien-6-yn-3-ol
Ginsenoyne J	[*S-(E,Z)*]-1,4,9-heptadecatrien-6-yn-4,6-diyn-3-ol
Ginsenoyne K	10-hydroperoxy-1,8-heptadecadiene-4,6-diyn-3-ol
Panaxydol	3-hydroxy-9*S*,10*R*-epoxyheptadeca-1-ene-4,6-diyne

Acetylpanaxydol	3-acetyloxy-9*S*, 10*R*-expoxyheptadeca-1-ene-4, 6-diyne
Panaxydol chlorhydin	10-chloro-3,9-dihydroxyheptadeca-1-ene-4,6-diyne
Panaxyne	tetradeca-13-ene-1,3-diyne-6,7-diol
Panaxyne-epoxide	tetradeca-13-ene-1,3-diyne-6,7-diol
Panaxytriol	heptadeca-1-ene-4,6-diyne-3,9-diol-10-triol
10-Acetyl-panaxytriol	heptadeca-1-ene-4,6-diyne-3,9-diol-10-acetate
Falcarinol= Panaxynol	heptadeca-1,9-diene-4,6-diyne-3-ol
Panaxacol	heptadeca-3-oxo-4,6-diyne-9,10-diol

Sesquiterpeniod Compunds

Ginsenol	(1*S*-(1α,3α*β*,4α,7α*β*))-octahydro-2,2,4,7α-tetramethyl-1,4-ethano-3αH-inden-3α-ol
Panasinsanol A	cyclo[c] inden-8-ol, decahydro-2,2,4α,8-tetramethyl-, [2αS-(2aα, 4α*β*,8α,8a*R**)]-
Panasinsanol B	cyclo[c] inden-8-ol, decahydro-2,2,4α,8-tetramethyl-, [2αS-(2aα, 4α*β*,8,8a*R**)]-

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6. THE PHARMACOLOGY AND THERAPEUTICS OF GINSENG

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During the past 50 years a vast amount of literature of widely varying quality has accumulated concerning the pharmacology of ginseng and its preparations and by 1990, despite the scepticism of many orthodox Western physicians and pharmacologists, ginseng had already attracted considerable interest and an ever increasing degree of respectability as a herbal medicine worthy of further study and of discussion at medical and scientific meetings worldwide.

Initial studies concentrated on the proof and explanation of traditional use in eastern Asia and it was assumed that the main active compounds were the saponin glycosides, the ginsenosides. As chemical separation of pure ginsenosides improved and samples of individual compounds became available, the biochemical mechanisms underlying the pharmacological effects were explored with varying degrees of success. More recent studies, which are reviewed in this chapter, do suggest that it is the combinations of ginsenosides and other compounds that may play the important pharmacological role.

EARLY USE AS A GENERAL TONIC

The earliest Chinese texts praised the virtues of ginseng stating that if it was taken constantly it was a tonic to the five viscera (liver, lungs, heart, spleen and kidneys), quieting the spirits, establishing the soul, allaying fear, expelling evil effluvia, opening up the heart and brightening the eyes, benefiting the understanding and invigorating the body and prolonging life (Hou, 1978).

Several more recent workers have shewn that ginseng extracts from stems or leaves given orally or intraperitoneally to young rodents produce body weight increase, increase in protein and ribonucleic acid (RNA) in muscle and liver cells, and no increase of weight in the prostate glands or seminal vesicles (Wang *et al.,* 1982). Increased synthesis of protein, deoxyribonucleic acid (DNA) and RNA in the bone marrow and protein and RNA in the kidneys was also found and ginseng stem and leaf given orally accelerated the growth of young pigs. It was noted that the saponins of the aerial parts of *P. ginseng* were apparently greater stimulators of protein and nucleic acid synthesis than the root saponins (Wen *et al.,* 1982). Such observations underline the importance of working with standardised preparations or pure compounds. It was concluded that the mechanism of growth promotion of ginseng stem and leaf saponins was different

to that of androgenic steroids, being direct action on the syntheses of RNA and protein (Wang *et al.,* 1982).

Ginseng root extract was shewn to stimulate the incorporation of labelled precursors into rat kidney nuclear RNA in a dose dependent manner, maximal effect for incorporation being produced within 8 hrs and and for sequential cytoplasmic RNA synthesis within 10 hrs. The incorporation of leucine into rat renal protein was also increased within 12 hrs of intraperitoneal administration (Nagasawa *et al.,* 1977). Ginseng was shewn to stimulate protein synthesis in human fibroblasts, confirming that constituents of ginseng extract are able to act directly on human cells with resultant accumulation of protein. Ginseng extracts were also capable of inhibiting proteolysis of long-lived proteins in human diploid fibroblasts (Lu and Dice, 1985).

The incorporation of [^{3}H]-uridine into liver and kidney RNA and [^{3}H]-leucine into protein was increased in mice receiving 25 mg/kg total leaf and stem saponins orally daily for 7 days although [^{3}H]-thymidine incorporation was unaltered. In reserpinized animals ginsenosides had no effect on the formation of RNA and protein in liver and kidney (Zhang *et al.,* 1985). Synthesis of DNA in liver and kidney was also unchanged although DNA increased in bone marrow cells (Wen *et al.,* 1982). Bone marrow mitosis was enhanced with resultant increase in the numbers of totally nucleated cells in the bone marrow and of reticulocytes in the peripheral blood (Yamamoto *et al.,* 1977). In further work individual pure ginsenosides Rb_2, Rc, Re and Rg_1 were administered to rats intraperitoneally at a dose of 5–10 mg/kg with consequent increase in DNA, RNA, protein and formation of lipids in bone marrow cells. Ginsenosides Rb_2, Rc and Rg_1 caused decreased *c*AMP (cyclic adenosine monophosphate) and increased *c*GMP (cyclic guanosine monophosphate) in the bone marrow 20 min after injection (Yamamoto *et al.,* 1978). Such evidence supports the early view of ginseng as an effective tonic.

GINSENG AND THE QUALITY OF LIFE

Much attention has been directed to the quality of life in stressful societies, communities in which the social pressures, especially in cities, are markedly increased by the accelerated rate of human activities such as advanced high technology processes in the factory or office, rapid transport in crowded areas or on congested high-speed motorways, strained or overpowering interpersonal business relationships in work, entertainment and sport and even family relationships. Unable to slow down or opt out sufferers turn to drug treatments to give a reasonable quality of life and to offset such strains, which are sometimes identified as functional fatigue.

Ginseng has been used as an agent to counter the subtle changes in health (fatigue, lack of energy, anxiety, restlessness, depression, etc.) and improve the quality of life of humans and several authors, using controlled clinical trials, have demonstrated the efficacy of standardised extracts of ginseng in combination with minerals (Tesch *et al.,* 1987; Dörling and Kirchdorfer, 1989; Pieralisi *et al.,* 1991). Such combination therapy has produced improved alertness, better physical activity and a feeling of well-being in both middle-aged persons and

the elderly. Nevertheless there has been criticism of the methodology used in many natural product trials, particularly with respect to poorly documented methods, uncontrolled routines, unsatisfactory choice of participating individuals and the short duration of the trials.

The Swedish group of Wiklund *et al.* (1994) devised two self-administered questionnaires, the Psychological General Well-Being Index and the Sleep Dysfunction Scale, to assess the quality of life in 205 participants taking Gericomplex capsules (=Geriatric Pharmaton, Pharmaton S.A.), capsules containing 40 mg standardised Ginseng Extract G115 and added vitamins, minerals and trace elements, and 185 persons taking identical-looking placebo capsules. Participating volunteers in the test group were aged 43.6±8.4 years and in the placebo group 41.8±8.9 and all worked for a high-technology organisation. Participants took one capsule after breakfast and one after lunch under supervision and the trial lasted 12 weeks. Assessments under 15 headings measured for all participants before and after treatment indicated that the administration of the combined therapy to an apparently healthy population of working people produced significant advantages over placebo treatment in terms of self-assessed feelings of vitality, alertness, relaxation and appetite. Not surprisingly the beneficial results were more pronounced in those whose initial Quality of Life assessment was poorest.

A subsequent trial conducted by the London-based group of Ussher *et al.* (1995) involved 95 British middle managers in an eight week double-blind placebo controlled study. Results again shewed that dietary supplements including ginseng were most effective for those on a relatively poor diet and revealed the need for further studies on different age groups and on the contributions of ginseng extract on the one hand and vitamins and minerals on the other.

Older people with age-associated memory impairment but not medical or neurological disorders producing cognitive deterioration were tested for psychological well-being and perceived quality of life by the Italian team of Neri *et al.* (1995). Sixty persons (18 male and 42 female, mean age *ca.* 61 years (minimum 51, maximum 65 years)) were divided equally into drug-treated and placebo-treated groups. The trial commenced with a 15 day run-in period and then a double-blind treatment period of 9 months. Participants were given two capsules daily, one after breakfast and the other after lunch. The drug-treated group received Gegorvic Pharmaton=Geriatric Pharmaton capsules and the placebo-treated group identical capsules containing an innocuous substitute. Using rating scales indicating Life Satisfaction in the Elderly and Symptom Rating and the Randt Memory Test, data was gathered at the beginning of the run-in period, at the baseline commencement of treatment and then at the end of 3, 6 and 9 months. Statistical analysis revealed that only in the treated group were memory index scores positively and significantly correlated with the quality of life and psychological well-being, indicating the selective action of the ginseng containing drug combination.

Le Gal *et al.* (1996) developed a method for evaluating complaints reported by patients suffering from functional fatigue. Patients chose the five titles best describing their problems from a list of 20 prepared titles. After treatment with

Pharmaton capsules (standardised ginseng extract G115 formulated with 9 vitamins and 8 minerals) fatigue scores were calculated on the basis of the patients' assessments of their problems. Such scores were calculated at the start of the trial, after 3 weeks and after 6 weeks. In a multicentre, comparative, double blind clinical trial involving 232 patients (117 in the Pharmaton group and 115 in the placebo group) Pharmaton capsules were shewn to be effective in countering functional fatigue, shewing superiority over placebo treatment whilst being equally tolerable.

A retrospective cohort study by Lillo (1998) in Spain embracing the period 1980–1993 involved 1,800 mainly elderly subjects (age range 15–95 years) who, apart from 93 persons, were receiving medical treatment for pathological conditions with drugs such as cerebral vasodilators, cardiac vasodilators, antiarrhythmics, car-diotonics, antihypertensives and nootropics. In addition to their specific prescribed medication other than tonics or vitamins patients received daily Ginseng G115 capsules with added vitamins, minerals and trace elements. Careful monitoring of all patients in the out-patient clinic was maintained for periods of 3 to 21 months (average 10±3.3 months). Data collated included age, sex, life-style, height, body weight, nutrition state, appetite, duration of treatment, health condition and previous and actual pathology. Further data included clinical parameters such as blood pressure, heart rate, electrocardiogram, Karnofsky's index and mental state (SCAG, the Sandoz Clinical Assessment Scale in Geriatrics based on 17 items). At the end of the study the investigator assessed the overall effectiveness of the treatment and concluded that of the 1800 patients 1257 shewed very good response, 426 demonstrated a good response, 96 a moderate response and only 21 patients were considered to offer a poor response. In addition it was reported that just 17 patients reported moderate tolerance to the treatment but it was not necessary to suspend treatment. Improved appetite was reported by 1298 patients although there was minimal deviation in body weight.

Also published in 1998 was a controlled, randomised double-blind study in parallel groups involving 72 senile patients (mean age±standard deviation=67.5± 4.5 years) with compensated non-insulin dependent diabetes and mild cognitive disorders (Della Marchina and Renzi, 1998). After a 15-day run-in period during which all patients received placebo capsules, the participants were divided into two groups, one receiving placebo and the other standardised Ginseng G115 extract with added vitamins and minerals. All patients received one capsule twice daily for 9 months and were assessed with appropriate psychometric and biochemical tests at regular intervals. At the end of the treatment period the improvement of drug treated patients when compared with placebo treated subjects was significant in the areas of memory, Symptoms Rating Test, Randt Memory Test, Global Deterioration Test And Life Satisfaction in the Elderly Scale.

That ginseng is involved in the improvement in the quality of life is clear and this agrees with the ancient use of ginseng as a nutritive and restorative tonic strengthening the debilitated body and encouraging recovery from illness. Nevertheless experiments employing ginseng and vitamin supplements do not

indicate the true effect of ginseng extract. Therefore a Mexican research team (Caso Marasco *et al.*, 1996) compared treatment with Pharmaton capsules with added vitamins and trace minerals and treatment with identical capsules without the Ginseng G115 extract. The randomised, double-blind clinical study was completed by 501 patients in the age range of 18 to 65 years. Of these 338 were treated with the ginseng combination and 163 with the multivitamins alone. Patients made 4 monthly visits to participating physicians and data accumulated included physical measurements e.g. weight, blood pressure and heart rate, and questionnaire details concerning the quality of life under 11 headings and assessed on a 6-point scale. Although 625 persons enrolled for the trial, 124 (44 in the ginseng group and 80 in the multivitamins group) were excluded due to non-compliance or voluntary withdrawal. A few patients withdrew because of side effects. It was concluded that the superiority of the Pharmaton capsule treatment over the multivitamin treatment was due to the action of ginseng which produced a significantly improved quality of life. Multivitamin treatment caused an obvious increase in body weight but ginseng treatment had no such effect; this was probably due to the action of ginseng saponins on carbohydrate and lipid metabolism. In addition multivitamin treatment was accompanied by a rise in diastolic blood pressure, a phenomenon not observed with ginseng and suggesting the adaptogen property of ginseng against stress effects. One patient who was taking the ginseng capsules was proved to have the symptoms of brucellosis or undulant fever. The disappearance of the disease state without interference with the trial indicated a probable immunomodulatory function of ginseng. The value of ginseng therapy for high physical and mental stress states and for improvement of the quality of life was again clearly established.

ABSORPTION, DISTRIBUTION, EXCRETION AND METABOLISM OF GINSENG SAPONINS

Ginsenosides are large molecules which are apparently hydrolysed in the acid medium of the stomach, rapidly absorbed into the blood stream and excreted shortly afterwards yet their effects linger for a long period. Unravelling this problem has proved difficult as suitable sensitive radioimmunoassay methods are only slowly being developed. Nevertheless data is steadily accumulating.

Administering radioactively labelled ginseng saponins to rats orally, Joo *et al.* (1982) observed that total recovery of the radioactivity was only about 30 per cent and they concluded that the saponins had bound with macromolecular and membrane structures in forms which were not readily extractable. The saponins were widely distributed in the body tissues and especially in liver, kidney, blood serum, stomach and gastrointestinal tract.

Early study of the pharmacokinetics of ginseng saponins revealed that little of the important protopanaxadiol-derived compound ginsenoside Rb_1 is absorbed from the upper digestive tract after oral administration (100 mg/kg) in rats. Intravenous injection (5 mg/kg) in rats resulted in the serum level declining slowly and biexponentially with a half-life in the β-phase of about $14\frac{1}{2}$ hours. Ginsenoside Rb1 persists for a long time in the serum and tissues, persistence being due to the

high activity of plasma protein binding, but eventually it is slowly excreted in significant amounts into the urine, although not apparently into the bile. The unabsorbed ginsenoside Rb_1 was rapidly decomposed in the digestive tract and/or metabolised chiefly in the large intestine (Odani *et al.,* 1983a).

The other important saponin, ginsenoside Rg_1, derived from protopanaxatriol, was absorbed rapidly from the upper parts of the digestive tract (up to one fifth of the dose taken orally) and the serum level of ginsenoside Rg_1 attained a peak in 30 min and tissue maximum levels were reached in about 1.5 h. Ginsenoside Rg_1 was not apparent in the brain tissues of the rat and was not significantly metabolised in the liver. The decomposition and/or metabolism occurred mainly in the rat stomach and large intestine and excretion of ginsenoside Rg_1 was via rat urine and bile in the ratio 2:5 (Odani *et al.,* 1983b). Employing ^{3}H-labelled ginsenoside Rg_1 and single intravenous or oral doses in mice, Huo *et al.* (1986) reported that the descending order of tissue distribution was kidneys, adrenal gland, liver, lung, spleen, pancreas, heart, testes and brain. After oral ingestion, the absorption of ginsenoside Rg_1 was 49 per cent and *in vitro* the drug protein binding in the plasma, liver, testes and brain was 24, 48, 22 and 8 per cent respectively. About 17.5 per cent only of orally-administered ginsenoside Rg_1 remained unchanged, the rest being metabolised. Strömbon and Sandberg (1985), also using mice, reported that about 30 per cent of orally administered tritium-labelled ginsenoside Rg_1 was absorbed within one hour and confirmed the distribution in body organs, adding that the concentration in muscle and endocrine organs was very low and that intact ginsenoside Rg_1 was excreted in small amounts in mouse urine and faeces but the concentration of accompanying metabolites was high.

Experimenting with the ginsenosides Rb_1, Re and Rg_1 given orally to mice, Han and his colleagues (1986) observed that the gastrointestinal uptake of ginsenosides varied from about 10–50 per cent; administration of higher doses than normal resulted in lower absorption uptake. The major component excreted in the urine was intact ginsenoside but in 26 hours only 1–2 per cent of the ingested dose was excreted. At the subcellular level tritium-labelled protopanaxadiol-derived ginsenoside Rb_1 was not detected in the mitochondrial (or cell powerhouse) fraction but was demonstrated to possess strong binding affinity with high molecular weight membrane fractions of organ homogenates and with serum proteins. However, the protopanaxatriol-derived ginsenosides Re and Rg_1 showed very weak binding to such fractions.

Although little is known of the pharmacokinetics of other ginsenosides, Sawchuck *et al.* (1980) suggested that the protopanaxadiol-derived ginsenosides such as ginsenosides Rb_2 and Rd possessed plasma protein binding of >99 per cent and long elimination half lives on intravenous injection (*ca.* 445 min) while the protopanaxatriol-derived ginsenosides such as ginsenosides Re and Rg_1 demonstrated lower protein binding (45.5 per cent and 33.2 per cent respectively) and much shorter elimination half lives (49.8 and 82.6 min respectively).

The mechanism of degradation of the protopanaxatriol-derived ginsenoside Rg_2 was investigated by Chen (1987), who reported that on incubation with rat gastric juice at 37° C three metabolites were formed, 25-hydroxy-20*(S)*-ginsenoside Rg_2, 20*(R)*-ginsenoside Rg_2 and 25-hydroxy-20(R)-ginsenoside Rg_2. Further

breakdown occurred when these compounds were incubated in rat intestinal fluid. In a series of papers the Japanese group of Karikura *et al.* (1991) carefully studied the degradation, distribution and metabolites of ginsenosides both *in vivo* and *in vitro* using chromatographic methods, ^{1}H- and ^{13}C-nuclear magnetic resonance spectroscopy and fast atom bombardment mass spectrometry. Orally administered ginsenosides were rapidly partially hydrolysed in the gastric acid medium and then underwent further hydrolysis in the intestinal tract and colon. In addition hydroperoxidation of ginsenosides Rb was detected in the rat stomach, the major hydroperoxide being the 25-hydroperoxy-23-ene derivative of ginsenoside Rb_1. Significantly the pattern of hydrolysis of the 20*(S)*-protopanaxatriol-derived ginsenoside Rg_1 in the rat stomach was different from that of the 20*(S)*-protopanaxadiol-derived ginsenosides Rb_1 and Rb_2 in the rat colon. Hydrolytic degradation differs for the various ginsenosides and is dependent on the protopanaxadiol or protopanaxatriol nucleus and also on the side-chain substituent sugars at C-3 and especially at C-20, the possible sugars being glucose, arabinose, rhamnose and xylose. Chromatographic analysis of rat large intestine contents revealed the presence of the hydrolytic and oxidative products gypenoside XVII, ginsenoside Rd, ginsenoside F_2, compound K (20-O-[β-D-gluco-pyranosyl]-20*(S)*-protopanaxadiol) and 25-hydroperoxy-23-ene-ginsenoside Rb_1. In *in vitro* experiments using the crude enzyme hesperidinase ginsenoside Rb_1 degraded to gypenoside XVII, ginsenoside F_2 and compound K, and ginsenoside Rb_2 yielded 3-O-β-D-glucopyranosyl-20-[α-L-arabino-pyranosyl(1→6)-β-D-glucopyranosyl]-20*(S)*-protopanaxadiol, ginsenoside F_2 and compound K; thus hydrolysis by the β-glucosidase present in the rat intestine was different from that with crude hesperidinase. It was also noted that tetracycline-resistant bacteria decomposed ginsenosides Rb_1 and Rb_2 to their respective prosapogenins but not ginsenoside Rd and the respective hydroperoxides. Ginsenoside Rd and the hydroperoxides of ginsenosides Rb_1 and Rb_2 were produced by the action of enteric enzymes.

To further clarify the degradation pattern, the important ginsenosides Rb_1 and Rg_1 were cultured anaerobically with fresh human faeces and it was confirmed that they were metabolised by successive hydrolyses, ginsenoside Rb_1 rapidly within 8 hours and ginsenoside Rg_1 slowly within 48 hours. The proposed pathways by successive removal of glucose units were ginsenoside Rb_1→ginsenoside Rd→ ginsenoside F_2→compound K→20(S)-protopanaxadiol and ginsenoside Rg_1→ ginsenoside Rh_1→20(S)-protopanaxatriol (Kanoaka *et al.*, 1994).

Hasegawa *et al.*, 1996) administered *P. ginseng* extract orally to human subjects and to specific pathogen-free rats. The main metabolites of the panaxadiol ginsenosides Rb_1, Rb_2 and Rc and the panaxatriol ginsenosides Re and Rg_1, identified after anaerobic incubation with faecal flora, were 20-O-β-D-glucopyranosyl-20*(S)*-protopanaxadiol **(I)**, 20-O-[α-L-arabinopyranosyl(1→6)-β-glucopyranosyl]-20(S)-protopanaxadiol **(II)**, 20-O-[α-L-arabinofuranosyl(1→6)-β-glucopyranosyl]-20(S)-protopanaxadiol **(III)** and 20*(S)*-protopanaxatriol. The hydrolytic degradation rate and mode would be affected by the fermentation media. Significantly these 4 main metabolites and 20*(S)*-protopanaxatriol were found in the urine (2.2–96 μg/ml) and blood (0.3–5.1 μg/ml) of human subjects and also in the urine and blood of rats.

Further work by Hasegawa *et al.* (1997) shewed that *Prevotella oris* bacterial strains in humans possessed the potential to convert ginsenosides Rb_1 and Rd to metabolite (**I**), ginsenoside Rb_2 to metabolite (**II**) and ginsenoside Rc to metabolite (**III**). The protopanaxatriol-derived ginsenosides Re and Rg_1 were unaffected. In the trial 79 per cent of 58 human subjects aged between 1 and 64 years yielded faecal specimens capable of such conversions and it was considered reasonable to involve intestinal *P. oris* in the conversion of protopanaxadiol saponins to metabolites (**I-III**). It was also speculated that metabolites (**I-III**) were the most likely forms in which such saponins were absorbed from the intestines because only the final metabolite (**I**) was detected in the blood stream at 1.0–7.3 μg/ml after oral administration of ginsenoside Rb_1 (125 mg/kg) in mice, no intact ginsenoside Rb_1 or intermediate derivatives being present. The ginseng metabolites (**I**), 20-O-[α-D-arabinopyranosyl(l→6)-β-D-gluco-pyranosyl]-20*(S)*-protopanaxadiol (**IV**) and 20-O-[α-D-arabinofuranosyl(1→6)-β-D-glucopyranosyl]-20*(S)*-protopanaxadiol (**V**), produced by human intestinal bacteria, have been tested *in* vitro for antigenotoxicity versus benzo[α]pyrene-induced mutagenicity in *Salmonella typhimurium* TA98 and TA100 and clastogenicity in Chinese hamster lung fibroblast cells (Lee *et al.*, 1998). The mutagenicity of benzo[α]pyrene was inhibited by metabolites **I, IV** and **V** in a dose-dependent manner and metabolites **I** and **V** reduced the frequency of benzo[]pyrene-induced chromosome aberrations. Some such metabolites of the ginseng saponins have been patented as immunopotentiators with inhibitory actions on the vascularisation of tumours and the extravasation of cancer cells (see Chapter 9).

The progress of pharmacokinetic studies on the many constituent chemicals in ginseng is still hampered by lack of reliable, sensitive radioimmunoassay techniques. Although little published work based on human subjects is apparently available, reports concerning rats, mice, rabbits and mini-pigs are in the literature and the mini-pig is considered similar to man in its metabolism of ginsenosides. Thus Jenny and Soldati (1985) determined the half-life of ginsenoside Rb_1 in the β-phase in the mini-pig as 16 hours but for ginsenoside Rg_1 the half-life was only 27 min. More research is necessary to provide similar data on the metabolism of the large molecules of other ginsenosides, polysaccharides, polyacetylenes, etc. and on the occurrence, identity, fate and functional significance of the ginseng metabolites.

STRESS AND ADAPTOGENIC ACTIVITY

Probably the most important use of ginseng and its saponins is as anti-stress agents. Stress, a normal feature of life, can readily be reproduced experimentally and is apparent in all impaired and injured animals. Stress manifests in many forms e.g. reaction to external conditions such as the "fight or flight" phenomenon, heat, cold, noise, starvation, physical restraint, etc., reaction to psychiatric states e.g. fear, anxiety, emotional strains, psychosomatic diseases, etc., and reaction to disease, bacterial and viral infection, physical injury, wounds, surgical operations, chemical agents, pollutants, etc. In particular, the pace of modern society places individuals in abnormal situations where stress is often

markedly increased e.g. overworking under harsh managerial pressure, excessive worry, fear situations, etc. As early as 1936 Hans Selye had stated that there was a general adaptation syndrome revealed in rats that had suffered severe trauma from agents such as cold, surgical injuries and poisoning and that the adaptation was independent of the nature of the damaging agent. The syndrome developed in several steps including:-

a) increase in the size of the adrenal glands;
b) decrease in the size of the thymus gland, the spleen, the lymph glands and the liver;
c) breakdown of adipose tissue;
d) formation of acute erosions in the digestive tract, etc.

After the initial general alarm reaction, the body commences adaptation to the new situation. Therefore there is a continuing search for agents that ameliorate stress syndromes by encouraging or accelerating such adaptation or normalisation.

The eminent Russian pharmacologists I.I.Brekhman and I.V.Dardymov, working in the Biologically Active Substances Division of the Siberian Branch of the USSR Academy of Sciences in Vladivostok in the 1950's and 1960's, carefully studied the ability of ginseng (*Panax ginseng*), Siberian ginseng (*Eleutherococcus* spp) and related Araliaceous drugs to increase the nonspecific resistance of the host to several types of stresses. This normalising phenomenon presented by ginseng was referred to as "adaptogenic activity", a controversial concept. In effect there was a higher state of defence preparation and the adaptogen, which was also known as a non-specific immunomodulator or neuroimmunological regulator, was defined as being:-

a) innocuous, causing minimal disorder in the physiological functions of the organism,
b) non-specific in action i.e. it should increase resistance to adverse influences of a wide range of factors of physical, chemical and biological nature, and
c) normalising, in that it acts irrespective of the direction of the preceding pathological changes (Brekhman and Dardymov, 1969a).

Although the adaptogen proposition was not readily accepted by many orthodox allopathic-trained pharmacologists, evidence steadily accumulated.

THE MECHANISM OF STRESS REACTION

As one of the main uses of ginseng is as an antistress agent, it is necessary to consider the mechanism of stress reaction. In the living body such reaction is controlled by

1) the hypothalamus, which is embedded in the lower or "visceral" part of the brain and has an important automatic regulatory role concerning the internal environment of the body e.g. water balance, food intake, mammalian body temperature, hormone release via the pituitary gland, as well as responding to signals from the conscious or thinking areas of the brain,

2) the pituitary gland which controls the other endocrine or ductless glands and is a pea-shaped endocrine structure also in the brain and connected to the hypothalamus by the infundibulum, and
3) the adrenal or suprarenal glands which are located one above each kidney and yield the steroid hormones and catecholamines.

The hypothalamus receives signals from higher centres of the brain indicating a stress situation; such stimulation causes release of chemical messengers called peptide neurohormones into the blood stream prompting the activation of the pituitary gland with the release of signals to the adrenal glands via the adrenocorticotrophin hormone (ACTH). Responding to ACTH stimulation the adrenal cortex, the outer area of the adrenal gland, secretes corticosteroids (glucocorticoids e.g. hydrocortisone or cortisol) which rapidly mobilise the body's carbohydrate, protein and fat reserves. In man hydrocortisone forms 95 per cent of the total glucosteroids formed in the adrenal cortex. Production and distribution of glucosteroid stress hormones is increased under stress although limited in the body at rest even when ginseng is administered. Ginseng encourages adrenal gland response in stress situations and also facilitates a rapid shutdown when the stress is removed. If the stress is prolonged the adrenal glands conserve their reserves and do not release so much hormone i.e. there is a greater sensitivity to stress. Significantly ginseng saponins, despite their triterpenoid structure which closely resembles steroid structure, have little effect on normal organisms although effective in stressed, injured or impaired animals by stimulation of the pituitary-adrenocortical system.

TEMPERATURE STRESS REACTION

Ginseng and its extracts can prevent body temperature variations in animals subjected to the stress of cold and heat exposure, although not apparently affecting normal subjects at room temperature. This was realised in 1958 when the Chinese physiologists Sung and Chi published experimental results indicating that powdered *P. ginseng* root contained active principles with a protective property against temperature stresses. Using male white rats fed either on a normal diet or a normal diet with 5 per cent added ginseng powder, they were able to demonstrate that after 3 weeks the ginseng-fed group were more able to withstand heat stresses of 5–6 minutes at 78–90° C or 60 minutes at -2° C. In an alternative experiment one group of rats received 2.4 ml of 50 per cent ginseng extract one hour before the temperature stress whilst the other (control) group received water only. Ginseng treated rats, on being returned to normal conditions after hot chamber treatment, recovered almost immediately but the control rats similarly treated recovered slowly over 20–60 minutes, either crouching almost stationary or convulsing during the normalisation. The rats were sacrificed after the temperature stress experiments. Analysis of the adrenal glands shewed that the vitamin C content was depleted after hot or cold temperature stress but such depletion was reduced in ginseng treated rats. Thus Sung and Chi had confirmed that ginseng was capable of increasing the nonspecific resistance of animals to temperature stress situations (Hou, 1978).

The increased tolerance to cold as manifested by prolonged survival times was also produced in adrenalectomised mice using alcoholic extracts of ginseng or hydrocortisone (Kim, 1963). In the following year Tsung, Cheng and Tang (1964) subjected 92 animals (white mice) to survival tests either in a hot chamber at 45–47° C or a cold chamber at -2° C; the experiments were terminated when half of the animals had died. The test animals received intraperitoneal injections of 10 ml/kg 50 per cent aqueous extract of ginseng and control animals were similarly given an equal volume of normal saline solution. A comparable alternative experiment involved animals from which the adrenal glands had been surgically removed prior to the experiment. These experiments convincingly confirmed that ginseng extract treatment enabled the test animals to tolerate high and low temperature stresses but the antistress ability was abolished if the adrenal glands were removed. Therefore Tsung *et al.* concluded that the pituitary gland-adrenal gland system must be implicated in the mechanism of antistress activity.

Using 240 mice, C.C.Kim (1964) observed that the level of total serum protein fell in mice acclimatised to cold conditions although it increased markedly in mice exposed to the cold after treatment with ginseng injections. Exposure to cold also led to a lower serum haemoglobin level and to lower red blood cell counts but ginseng was able to reverse such tendencies, thus countering the cold stress. White blood cell counts too were lower in unprotected experimental animals. Again ginseng had proved effective versus temperature stress.

During the next decade many reports confirmed the effectiveness of ginseng against temperature stress (Hou, 1978) and it was clearly shewn that ginseng countered the fall in body temperature induced by stress (Bao *et al.,* 1984b). Cheng *et al.* (1986b), also employing mice as test animals and temperature stress conditions at 45° C, observed that the saponins of Chinese red ginseng root and Chinese ginseng stem and leaf inhibited increase in rectal temperature at the alertness stage but, surprisingly, the median lethal time was prolonged by Chinese *P. ginseng* root and stem but not by *P. quinquefolium* saponins. This pharmacological variation was probably due to the interspecies variation in saponin composition. In a similar series of experiments involving the saponins of *P. ginseng, P. notoginseng* and *P. quinquefolium,* Yan *et al.* (1987) were able to show that the total saponins of all three species would oppose the reduction of the vitamin C content in stressed rat adrenal gland although only the total saponins of the first two species could reduce the ACTH-induced decrease in adrenal vitamin C and the atrophy of the spleen and thymus gland, confirming the apparent lack of antistress action of *P. quinquefolium* saponins.

Continuing the study of temperature stress Cheng *et al.* (1986a), using rats, a temperature of 45° C for 20 minutes and ginseng root saponins, observed a fall of rectal temperature from 40.3° to 39.0° C. Ginseng also reversed the decrease in acetylcholine levels in the brain and markedly increased the plasma corticosterone levels but there was no effect on amino acid levels (gamma-amino-butyric (GABA), glutamic and aspartic acids) in the brain. For mice at 20° C, it was noted that ginseng root saponins (70mg/kg) reduced brain levels of GABA but not glutamate or aspartate. Investigating cold temperature stress the same

group (Cheng *et al.,* 1987) observed that for mice kept at -2° C for an hour oral ginseng root saponins (50 or 100 mg/kg) reduced the rate of fall in body temperature. For mice at -4° C brain noradrenaline, 5-hydroxytryptamine (5-HT) and 5-hydroxy-indole-acetic acid (5-HIAA) levels decreased but increased after administration of ginseng root saponins (100 mg/kg). Dopamine levels were unchanged by cold temperature stress. Rats also experienced falls in rectal temperature and plasma corticosterone when confined at -2° C; after administration of ginseng total saponins (70 mg/kg) the rectal temperature remained unchanged but the brain acetylcholine and plasma corticosterone had increased; brain GABA, glutamate and aspartate was unchanged. Yuan *et al.* (1989) also recorded that heat increased rectal temperature and serum corticosterone levels in mice (45° C for 15 min) but brain 5-HT and noradrenaline decreased and dopamine remained unchanged. Ginseng root saponins (200 mg/kg by intraperitoneal injection) inhibited the increase in serum corticosterone and the decrease of brain 5-HT and noradrenaline in heat-stressed mice but the dopamine level was unchanged. It was noted that ginseng root saponins produced reduction of body temperature at normal room temperature conditions and inhibited the rise of body temperature under heat environmental conditions. Reserpine, a tranquillising indole alkaloid, neutralised the temperature lowering action of ginseng root saponins at room temperature and at the higher temperatures of heat-stress conditions.

Investigating the mechanism of the protective effects of ginseng root saponins on immunity in heat-stressed mice, Wu *et al.* (1993) observed that the percentage of lymphocytes and T-lymphocytes in the white blood cells was reduced in a heat environment of 45° C for 15 minutes but the serum corticosterone was increased. If ginseng root saponins (50 or 100 mg/kg^{-1}) were administered intraperitoneally 15 minutes before the heat stress, the suppression of the blood T-lymphocytes was reversed although the increase in serum corticosterone was unaffected. Ginseng root saponins at 50mg/kg^{-1} also inhibited the reduction of peripheral lymphocytes.

Experimenting with a combined ginseng and multivitamin-mineral preparation and using a cold-hypoxia-restraint animal model, Ratan Kumar *et al.* (1996) studied cold tolerance and recovery from acute hypothermia with the aim of unravelling the respective roles of ginseng root extract and the vitamin-mineral combination. Male albino Wistar rats were treated by gastric cannula with single doses of *P. ginseng* root extract (0.1–2.0 μg/g) or multivitamin-mineral preparation (1.0–20.0 μg/g) or ginseng-multivitamin-mineral preparation (1.1–22.0 μg/g) before transfer to a decompression chamber (5° C, 428 mmHg); the rats were removed when their colonic temperature reached 23° C and were restrained in a recovery chamber at 32±1° C until the colonic temperature returned to 37° C. Adaptogenic activity was calculated in terms of the time for the colonic temperature to fall from normal to 23° C and then to recover to 37° C. Single or multiple doses of the three preparations were assessed for periods of 5 or 30 days. It was concluded that the adaptogenic effect was roughly equal to the sum of the effects of the two components, the ginseng extract inducing the resistance to cooling and the multi-vitamin-mineral combination prompting the more rapid recovery from acute hypothermia.

PHYSICAL STRESS REACTION

In earlier times and particularly in indigenous Chinese medicine ginseng root was employed as a remedy for premature weakness or exhaustion induced by physical or mental stress. In our own time improved physical and mental activity has frequently been reported and confirmed as the most important effect of ginseng (Sonnenborn, 1987). The period of active work is extended and the onset of fatigue correspondingly delayed. Fatigue and weariness occurs after prolonged activity, manifesting itself as irritability, failing concentration, reduced ability to rationalise and consequently inefficient working. Certainly repeated laboratory experiments had confirmed improved running ability, increased climbing performance and prolonged swimming endurance of test animals (Hou, 1978; Fulder, 1993).

Other types of physical stress such as immobilisation and positive radial acceleration stresses can similarly be effectively countered. Under such conditions ginsenosides restrict any major changes in the weights of the adrenals, thymus, spleen and thyroid as compared with control animals and modify blood sugar and liver glycogen changes.

Positive gravity effects induced by radial acceleration generate considerable stress reaction. Kim and Koh (1964a), using 840 mice, found that a 2 mg/kg body weight/day injection of an ethanol extract of ginseng root increased tolerance to such stress. Mice that had been acclimatised to radial stress had decreased levels of total serum protein and ginseng increased the levels and prevented decrease in the albumin/globulin ratio at 3G and 5G forces.

From the time of the earliest swimming tests undertaken with mice by Brekhman and his Russian colleagues in 1957 there has been positive evidence of improved performance after oral administration or injection of ginseng extracts. Significantly the antifatigue action was more pronounced after a course of ginseng injections for a longer period. Ten years later trials were conducted during a period of 60 days with test swims every 5 days and the results were again positive. For the first 10 days on which the mice swam no ginseng was given but from the 11th to 40th days on alternate days half the mice received 0.1 ml per 20 g body weight per mouse of a 10 per cent aqueous liquid extract of ginseng root. The control group of mice received a corresponding injection of a 2 per cent solution of ethanol in water. Swimming commenced 20 minutes after an injection. At the end of the trial it was reported that the average swimming time of the control group was 47–61 minutes but for the test group it was 96–117 minutes, an increase of almost 100 per cent. In addition 80 per cent of the control mice died as a result of complete physical exhaustion but only 40 per cent of the ginseng-treated animals succumbed (Brehkman, 1967).

Further work by the Brekhman group proved that extracts from wild and cultivated ginseng roots caused virtually the same responses in the swimming and rope-climbing trials. Testing individual *Panax* saponins, it was concluded that the potencies varied in the range from 100 to 1000 times greater than for crude root extract. The panaxosides A (=ginsenoside Rg_1) and C (=ginsenoside Rd), both protopanaxatriol derivatives, were more potent than the panaxosides D (=ginsenoside Rc), E (=ginsenoside $Rb_{1,2}$) and F (=ginsenoside Ra) which are

all protopanaxadiol derivatives. The panaxosides (=ginsenosides) were more potent than the aglycones panaxatriol and panaxadiol and potency was apparently related to the sugar content of the molecule. Thus panaxoside C (=ginsenoside Rd) with four sugar substituents is roughly twice as potent as panaxoside A (=ginsenoside Rg_1) which possesses only three sugar units (Brekhman and Dardymov, 1969b).

In the early 1960's Kitagawa and Iwaki confirmed and expanded the swimming studies by using four different ginseng extracts viz. ether extract, alcohol extract, aqueous extract without prior extraction with an organic solvent and aqueous extract after extraction with ether, at dose levels of 200 mg/kg body weight (ether extract) or 100 mg/kg (other extracts). The mice were organised in groups of five and swam to exhaustion in water at 32° C. The recorded swimming times for control group mice varied in the range 34–194 seconds; mice treated with ginseng extract three days before swimming yielded times of 55–123 seconds (ether extract), 52–164 seconds (alcohol extract) and 80–198 seconds (total aqueous extract) and mice treated with ginseng extract one hour before swimming registered times of 74–280 seconds (ether extract), 131–577 (alcohol extract) and 104–242 seconds (total aqueous extract). Treated animals therefore shewed an improved performance in the range 60–200 per cent better than the control mice and the most effective treatment was one hour before exercise (Hou, 1978).

Using 450 mice, the Swiss laboratories of Pharmaton Ltd. undertook large scale testing of their carefully prepared and standardised Ginseng Extract G115 (standardised mixture of the ginsenosides Rb_1, Rb_2, Rb_3, Rc, Rd, Re, Rf, 20-gluco-Rf, Rg_1, Rg_2 and Rh_1 in a constant ratio marketed by Pharmaton S.A., Lugano, Switzerland). Two dose levels of Ginseng Extract G115 were employed, 3 mg/kg mouse body weight/day (=0.06 mg/20 g) and 30 mg/kg mouse body weight/day (=0.6 mg/20 g), the former approximating to the clinical dose and the latter to ten times the clinical dose. Swimming performances in a water bath at 18° C were assessed after G115 administration for periods of 14, 21 or 28 days. During the test each mouse swam to exhaustion before being allowed to dry out in a warm air stream; after one hour of rest the swim was repeated and the recorded times for the two swims were averaged. The results revealed that mice treated with 0.06 mg/20 g/day for 14 days slightly improved their performance, but those receiving 0.6 mg/20 g/day improved their performances some 12–20 per cent when compared with control mice. For the 21 days test, those receiving 0.06 mg/20 g/day improved some 19–22 per cent as compared with the control group and those given 0.6 mg/20 g/day improved 20–27 per cent. The final group after 28 days yielded even better results, the 0.06 mg/20 g/day group improving by about 52 per cent as compared with control mice at the first test and, less well, by 38 per cent at the second test; with the higher dose (0.6 mg/20g/day) the first test improvement was about 48 per cent and the second test yielded about 38 per cent improvement (Rückert, 1974). These results were convincing yet the Pharmaton group continued their work with 1000 mice and produced confirmatory evidence which also proved that better swimming performances were obtained if the ginseng extract was ingested over a longer period of time. Extending this work to human subjects and using Geriatric Pharmaton G115 (a standardised combination of

ginseng G115, dimethylaminoethanol bitartrate, vitamins, mineral salts, trace elements and lipotropic substances produced by Pharmaton S.A., Lugano, Switzerland), enhanced performances were reported for athletes, middle-aged men and the elderly (Rückert, 1975).

In Japan further proof was obtained using six techniques viz. exploratory movement, hole cross, rotating rod, sliding angle, spring balance and rectal temperature tests. Mice were exhausted by four hours of oscillation movements. Aqueous extracts of ginseng or its saponins were injected peritoneally on cessation of the exercise. The aqueous extract of ginseng root significantly accelerated the recovery of exploratory movement and raised the rectal temperature, and ginsenoside Rg_1 and the lipophilic fraction of ginseng extract produced the most marked antifatigue effect in every test applied (Saito *et al.,* 1974).

This anti-stress action in swimming and forced exercise endurance tests has been repeatedly confirmed in more recent publications (Banerjee and Iquierdo, 1982; Saito and Bao, 1984a; Luo *et al.,* 1993; Grandhi *et al.,* 1994). Nevertheless Han *et al.* (1985) had carefully considered Brekhman's swimming test experiments and came to the conclusion that pure ginsenosides did not yield the same results as impure ginseng preparations combining ginsenosides and antioxidants such as phenolic acids and maltol.

Using mice running to exhaustion in a treadmill, Filaretov *et al.* (1988) reported an increased working capacity of 132 per cent after a single dose of ginseng and 179 per cent after 7-day treatment. They concluded that there were two stages of adaptation controlled by the pituitary-adrenocortical system, a normal level and a higher state of excitation as the work load increased. Another explanation presented for this obvious delay in the onset of fatigue and weariness, and tested in rats and in human clinical studies, is the more economical release of body energy due to the more efficient use of glycogen and high energy phosphate during physical activity. Thus there is a decrease in muscle adenosine triphosphate (ATP), in glycogen, a large glucose polymer, and in creatine phosphate and a smaller accumulation of the muscle lactic and pyruvic acids than is normally encountered during the hard exercise that causes fatigue. The glycogen reserve was gradually used up probably at the expense of a higher lipid and fatty acid oxidation with reduced lactate and pyruvate levels in blood and a resultant raising of the aerobic/anaerobic threshold to a higher production level (Sonnenborn, 1987).

Such observations suggest that ginseng extracts contain active principles that are capable of influencing pathways of substrate metabolism during sustained physical work. Possible explanations include the effect of the activation of the lactate-dehydrogenase enzyme in the liver, and muscle oxidation of fatty acids rather than glucose. The latter is possible as stress releases adrenocorticotrophin (ACTH) and thence hydrocortisone (cortisol) which stimulates the concentration of free fatty acids in the plasma thus permitting their utilisation as a source of energy. Hydrocortisone also moderately promotes oxidation of fatty acids in the cells, possibly as a consequence of reduced availability of glycolytic products after sustained work. The significantly raised oxygen capacity of the heart with an accompanying positive effect on the coronary reserve indicates the definite contribution of ginseng administration. Alternatively, it is possible that the

antioxidant effects of phenolic substances such as salicylic and vanillic acids and so far unisolated phenolic compounds occurring in impure ginseng extracts are significant. Such compounds may remove substances from the unwanted sludges produced by abnormal oxidation at cell level.

Dose levels were considered in a study by Forgo and Kirchdorfer (1982) involving 30 established male athletes. Measuring parameters including maximum oxygen absorption, heart rate during physical effort and blood lactate levels they concluded that there was no significant difference between a commercial G115 ginseng extract containing a standardised 4 per cent of ginsenosides and a specially prepared standardised extract containing 7 per cent ginsenosides. Therefore large doses were not justified and most workers have used 2×100 mg capsules of ginseng extract per day for human subjects, the most satisfactory results being obtained with the standardised product.

Nevertheless Murano and Lo Russo (1984) in a 60-day clinical trial employed 40/80 mg ginseng extract G115 taken orally daily and 65 athletes (professional and amateur of both sexes and aged 18–70 years). The professional sportspeople shewed significantly improved mental parameters as compared to baseline but little change for physical parameters. The amateur athletes, presumably less fit, revealed both physical and mental improvements.

In Italy Pieralisi *et al.* (1991) also studied the effects of a standardised ginseng extract formulation on physical performances during exercise. A double-blind, randomized, crossover study involved 50 healthy, male, sports teachers aged 21–47 years. Daily for 6 weeks the participants ingested either two placebo capsules or two capsules containing ginseng extract, dimethylaminoethanol bitartrate, vitamins, minerals and trace elements. After six weeks the treatments were reversed for a further six weeks, and finally a single-blind placebo washout period of one week was undertaken. Employing an exercise test regime with increasing work loads on a treadmill, it was found that the total work load and maximal oxygen consumption during exercise were significantly greater after taking ginseng capsules ($p<0.0001$) than after placebo ingestion. It was also noted that at the same work load, oxygen consumption, plasma lactate levels, ventilation, carbon dioxide production, and heart rate were significantly lower after ginseng administration than after placebo treatment. Pieralisi and his colleagues stated that the standardised ginseng preparation increased work capacity by improving muscular oxygen utilization and that the effects were more pronounced in the 23 participants with a maximal oxygen consumption below 60 ml/kg/min before the treatment than in sportsmen with levels of 60 ml/kg/min or higher. The authors stressed that the ginseng preparation employed was a combination of Ginseng Extract G115 with dimethylaminoethanol bitartrate, vitamins, minerals and trace elements and therefore the important constituents of ginseng probably played the vital role. As the effects on the choline-acetylcholine complex of dimethyl-aminoethanol bitartrate administration are somewhat ambiguous at the dose-level employed, further work using ginseng extract alone is desirable. The results obtained confirmed the earlier work of Forgo and Kirchdorfer (1981).

This apparent improvement of physical performance by increasing body resistance to stress and fatigue was also tested in normal healthy human subjects

by Engels *et al.* (1996) using a randomised, double-blind, placebo-controlled programme. The test group of normal, healthy females was given 200 mg per person per day of a concentrated extract of *P. pseudoginseng* in addition to their normal supplement-free diet. Before and after the 8-week trial all participants performed a graded maximal cycle ergometry test to exhaustion and answered a standard habitual physical activity questionnaire. It was concluded that in normal healthy persons ginseng supplementation had no effect on maximal work output, resting, exercise and recovery oxygen uptake, ventilation, heart rate and blood lactic acid. Also, as habitual physical activity scores of all the participants, irrespective of whether they were ginseng or placebo takers, were almost identical before and after the 8-week trial period ($p>0.05$), it was suggested that chronic dietary supplementation with ginseng did not produce in normal healthy persons an enhancement of work performance or change in energy metabolism or improvement of recovery response from maximal physical output.

A Canadian group (Morris *et al.,* 1996) also employing a cycle ergometer concluded that there was no ergogenic effect upon their 8 subjects (aged 27.2± 4.8 years) after ginseng ingestion (8 or 16 mg/kg body weight daily for 1 week). Although the rate of perceived exhaustion was significantly greater and the time to exhaustion was significantly shorter during the control ride than for the placebo and ginseng trial rides they concluded a negative result and unfortunately did not continue their trial over a longer period of time. Such results are not surprising in view of the findings of Forgo and Schimert (1985); using a 60-day treatment comprising ginseng extract G115, vitamins, minerals and trace elements they noted that professional sportsmen who would be at peak fitness shewed improved memory and attention reaction only whereas the less fit amateur players shewed both physical and mental improvement. In a subsequent paper Tesch *et al.* (1987), who studied a group of healthy, middle-aged men for two months in a placebo controlled trial, recorded the cardiocirculatory, metabolic and haematological characteristics during submaximal effort. They concluded that treatment with standardised ginseng G115 with added vitamins, minerals and trace elements (Gericomplex, Pharmaton S.A.) over a period yielded significantly lower heart rates and blood lactate levels at the same work load than in corresponding placebo trials although there was only a non-significant increase in the duration of exertion.

A slightly different approach was used by the Israeli team of Gross *et al.* (1995) who, in a 12-week report of an on-going study, investigated the effects of standardised ginseng extract G115 on pulmonary functions, oxygenation and general functions including walking capacity. The trial was limited to 15 severely ill patients (11 male, 4 female) with a mean age of 67±12 years. All the patients had severe chronic respiratory diseases and most were oxygen dependent at times. Initial medical histories, blood pressure and heart rate were recorded; then pulmonary functions, respiratory muscle strength and endurance were measured before the administration of 100 mg capsules of ginseng G115 twice a day for 12 weeks. Patients were reassessed every 6 weeks; spirometric data, strength and endurance of the respiratory system measured with a maximum pressure measuring device and 6 min walking distances were recorded and oxygen and carbon dioxide levels were obtained by arterial blood gas analysis. Collated

results reveal that the daily ginseng G115 treatment improved pulmonary functions and oxygen capacity in patients with severe chronic pulmonary diseases. After 6 weeks treatment there was significant improvement of pulmonary function and a concomitant improvement in 6 min walking distance and this was maintained and slightly improved for the further 6 weeks, mean values being 600±93 m at the outset, increasing to 854±101 m after 6 weeks and to 1123±119 m after 12 weeks. Forced vital capacity, which normally decreases with age, was much increased from 32.1 per cent to 67.3 per cent ($p<0.05$) after 6 weeks and 72.8 per cent ($p<0.01$) after 12 weeks. The maximum ventilation volume, an index of ventilatory endurance, increased by 11 per cent ($p<0.01$) after 6 weeks and by an additional 7.3 per cent ($p<0.01$) after 12 weeks treatment. These increases indicated a significant improvement in respiratory muscle strength. The forced expiratory flow rates FEF_{50} and FEF_{75} which were initially very poor at 15.9 and 23.3 per cent respectively, increased to 22.6 per cent ($p<0.05$) and 28.7 per cent ($p<0.02$) respectively after 6 weeks and then to 27.7 per cent ($p<0.01$) and 30.1 per cent ($p<0.05$) respectively after 12 weeks, indicating improved effort independent airflow in the small airways. In addition there was a significant increase in oxygenation, PaO_2 changing from 47.0±4 torr to 65.3±3 torr ($p<0.05$) after 6 weeks and 69.3±4 torr ($p<0.01$) after 12 weeks. As in the earlier work of Forgo's team, improvements in oxygen absorption and therefore aerobic metabolism are related to significantly reduced lactate levels and thus improved physical performance.

The results of recent work in Canada (Wang and Lee, 1998) using root saponins from *P. quinquefolium* in 4 day treatment of untrained rats at a dose level of 10–20 mg/kg daily indicated that prolonged aerobic endurance resulted at about 70 per cent VO_2 max. Ginseng saponins increased the plasma free fatty acid level when compared against saline controls as well as maintaining the plasma glucose level during exercise. Glycogen levels in liver and skeletal muscles of exhaustively exercised animals were slightly higher in treated animals. Wang and Lee also concluded that ginseng functioned by altering the fuel homeostasis during prolonged exercise probably by raising free fatty acid utilisation in preference over glucose for cellular energy demands thus enhancing exercise endurance. They also emphasised that the ginsenosides Rb_1 and Rg_1 were necessary for enhanced aerobic exercise performance.

As the Chinese and Vietnamese soldiers used ginseng in wars of the 20th century and Russian astronauts and athletes also improved their performance with ginseng or eleutherococcus, it is obviously desirable that further and more prolonged careful work is undertaken with precisely standardised ginseng preparations including isolated ginsenosides and other ginseng phytopharmaceuticals and with healthy and less healthy human volunteers exhibiting a wider age range.

STRESS DUE TO DISEASE STATES AND TOXIC SUBSTANCES

Disease states, surgical operations and ingestion of toxic substances are also stress situations. During the past 25 years several workers have described the protective action of ginseng extracts against toxic agents such as tetrachloromethane (=carbon

tetrachloride), lead tetraethyl, nitrogen mustard gas, poisonous alkaloids and alcohol, against anti-cancer drugs such as 5-fluoracil and mitomycin C, in preventing galactosamine-induced liver cell damage, and against radiation sickness in test animals (Court, 1986) and many reports confirmed that ginseng is a useful agent for combatting the effects of disease states and toxic substances (Fulder, 1993). Ginseng preparations have been shewn to improve liver function.

Post-operative stress in women who had undergone hysterectomy and climacteric post-menopausal stress in other women was investigated in Vienna by Reinold (1990). Typical symptoms are vasomotor conditions such as sweating, hot flushes and skin eruptions, neurovegetative conditions such as headache, dizziness, palpitations, parathesia and weakness and psychosomatic aspects such as fatigue, insomnia, reduced performance, anxiety, etc.. The combined effect is a reduced quality of life. Not all the effects are due to hormone deficiency and therefore a ginseng formulation was examined as an alternative to a lengthy hormone replacement therapy. Forty-nine women comprising 33 post-hysterectomy convalescent patients and 16 post-menopausal cases were treated with the standardised ginseng extract G115, 100 mg being taken orally every 12 hours for 12 weeks. The effect of the treatment was assessed every 2 weeks during the period of 12 weeks using the gradings:- very good, good, moderate and not detectable, and tolerance was graded:- very good, good and poor. In addition laboratory tests (e.g. haemoglobin content, erythrocyte count, glucose level, serum glutamate-oxaloacetate transferase (SGOT), serum glutamate-pyruvate transaminase (SGPT), sodium, potassium and chloride levels, etc.) were undertaken after 8 and 12 weeks; these tests shewed no significant variations at any time. Appropriate gynaecological tests (e.g. mammary gland assessment, speculum examination, cytological smears, etc.) and common symptoms, body weight and general well-being were checked. The tolerance and efficacy of the treatment was generally reported as "very good", the success rate being better in the post-operative cases. No oestrogenic side effects were encountered.

The debilitating effect of multiple stress situations in everyday life can produce a similar asthenic state. The Argentinian group headed by Rosenfeld (1989) investigated the treatment of this condition with ginseng in a similar manner i.e. 100 mg capsules of standardised ginseng extract G115 every 12 hours for 8 weeks. For two weeks before ginseng treatment the patients received placebo capsules. Fifty patients in the age range 24–66 years who had shewn no improvement during the placebo treatment and exhibited varying degrees of psychophysical asthenia, depression and neurological disorders were assessed using three methods. Firstly, the Toulouse Test was employed to measure concentration and attentiveness; results shewed a statistically highly significant improvement ($p<0.01$). Secondly, the Wechsler-Bellevue Test based on the Bellevue Intelligence Scale devised by Wechsler (1930) was used to assess intellectual and cognitive functions and produce the total intelligence quotient (total IQ), verbal IQ and performance IQ. At the end of 8 weeks treatment there was significant increase in intellectual and cognitive functions in every heading assessed with the exception of vocabulary and, in particular, highly significant improvements ($p<0.01$) of information, understanding, analogy creation, visual intake, observation, practical judgement and attentiveness; IQ values were likewise improved. Thirdly and finally, the SGAG scale was used

to measure general psychophysical criteria such as mental clarity, short-term memory, mood, loss of drive, neglect of personal care, unsociable behaviour, fatigue, loss of appetite, etc. After treatment the individual criteria were significantly improved and the global evaluation score showed highly significant improvement. Efficacy was rated as 88 per cent good and 12 per cent moderate by self assessment but 96 per cent effective and 4 per cent moderate by physicians' assessment. There were no obvious side effects.

Baek *et al.* (1995) studied the use of ginseng to counter cadmium accumulation in the liver. Detoxication effects of ginseng usually increased proportionately to the increase in cadmium concentration and it was concluded that administration of ginseng extract with cadmium chloride increased metallothionein concentration with decreased toxicity of cadmium in the liver.

In 1982 Song *et al.* had observed that the serum level of glutamate-pyruvate transaminase (SGPT) was markedly raised in mice suffering tetrachloromethane-induced liver damage. Treatment with a total saponin preparation prepared from *P. notoginseng* roots (80 mg/kg subcutaneously) markedly reduced this level. At the same time incorporation of [^{3}H]-thymidine into liver DNA and [^{3}H]-leucine into liver and serum protein increased significantly, suggesting that ginseng saponins could stimulate damaged liver regeneration. Three years later it was reported that the ginsenosides Rb_1 and Rg_1 completely suppressed tetrachloromethane toxicity in rat hepatocytes at 10 g/mL (Nakagawa *et al.*, 1985). Wee *et al.* (1996), studying tetrachloromethane intoxicated rats, noted similarly that Korean red ginseng prevented necrosis of the hepatocytes and reduced the change of fats. As the serum demonstrated increased ability to suppress oxygen radicals, it was concluded that red ginseng increased the antioxidative potential of the body against tetrachloromethane poisoning and thus inhibited hepatocyte necrosis. Using a total red ginseng saponin mixture standardised in terms of ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1, Jeong *et al.* (1997) noted that the raised levels of serum glutamate-oxaloacetate transaminase (SGOT) and SGPT caused by tetrachloromethane was partially countered by the saponin mixture. Histological examination revealed that liver vacuolisation and lymphoid cell aggregation induced by tetrachloromethane in male Sprague-Dawley rats were definitely reversed by premedication with red ginseng saponins. Although the results of this work did shew partial recovery from the hepatotoxicity induced by tetrachloromethane, recovery which was ascribed to the saponins administered, it is probable that other phytochemicals present also contributed to the protective effect. Significantly Kim and Kim (1996), studying thioacetamide-induced hepatotoxicity in rats, reported markedly increased serum aspartate amino-transferase, alanine transferase, 5'-nucleotidase and bilirubin levels and increased liver Ca^{++} with decreased protein content which was protected against by *P. ginseng* ethanol extract and by silymarin (a benzopyranone derivative) but not by ginseng saponin. In 1997 Nanba and Kadota patented the isolation of ginsenosides Re and Rg_1 from P. *notoginseng* and liver protecting agents containing these glycosides. They stated that in mice these saponins protected the liver from D-galactosamine-induced damage (see Chapter 9).

Poisoning with tetrachloromethane produces raised serum lipid peroxide levels

and marked loss of cytochrome P450. The ginseng polyacetylenes panaxydol, panaxynol and panaxytriol inhibited liver lipid peroxidation and prevented leakage of lactate dehydrogenase (LDH) to the blood serum and panaxynol reduced the serum lipid peroxide levels induced by tetrachloromethane. Thus polyacetylenes protected against tetrachloromethane poisoning by inhibiting lipid peroxidation both *in vivo* and *in vitro* possibly by direct action on the liver microsomes (Kim *et al.,* 1989c).

Benzene poisoning induces aplastic anaemia; Li *et al.* (1994) noted that within four weeks of benzene contamination ginseng treated rats shewed improved bone and blood marrow pictures (increased erythrocyte, leucocyte and haemoglobin counts) and also diminished production of lactic acid in erythrocytes and raised the oxygen utilisation of hexose sugars (mannose>glucose>fructose>galactose).

Another potential group of phytochemical components with probable hepatic protective properties comprise the ginseng polysaccharides. Tetrachloromethane damaged primary cultured rat hepatocytes were treated with ginseng polysaccharide fraction (0.1, 0.3 and 1.0 mg/mL^{-1}) resulting in a marked inhibition of the release of lactate dehydrogenase (LDH) and glutamic oxaloacetic transaminase (GOT) into the culture medium in a dose-dependent manner. Oral administration of the polysaccharide fraction to tetrachloromethane intoxicated rats (100 or 200 mg/kg) prevented decrease in body weight and increase of the ratio of liver to body weight. The elevation of GOT, glutamate-pyruvate transaminase and phosphatase (ALP) activity in serum, characteristic of tetrachloromethane hepatotoxicity, was suppressed by administration of the polysaccharide fraction. Malonyldialdehyde levels, which were raised in the serum and liver tissue of intoxicated rats, were lower in polysaccharide-treated animals. Such results indicate that the ginseng polysaccharides may be the important antihepatotoxic agents (Kim, 1995).

Treatment with anticancer chemotherapeutic agents such as cyclophosphamide, phenylhydrazine and ^{60}Co cobalt radiation damages the red and white blood cells. However, haematopoietic activity promoted by ginseng saponins has been shewn to effect recovery of erythroid burst-forming and erythroid colony-forming precursor cells in mice *in vivo* and *in vitro*. In particular an ethyl acetate soluble fraction containing ginsenosides Rg_2 and Rh_1 was implicated (Park *et al.,* 1989; Huang *et al.,* 1990). The immunosuppression caused by cyclophosphamide was opposed by Chinese and Korean red ginseng total saponins, the former being more potent than the latter. In mice the saponins antagonised the cyclophosphamide-induced suppression of phagocytosis by the mobile and attached macrophages, retarded the hypersensitivity reaction, reversed the decrease in white blood cells and opposed the suppression of haemopoiesis (Yuan *et al.,* 1992). Total saponins of *P. quinquefolium* were shewn to partially restore the activity of cyclophosphamide-depressed bone marrow stem cell proliferation and splenocyte proliferation in mice and to enhance the interleukin-IL-3 and -IL-6-like substances production from the splenocytes. Similarly total saponins of *P. ginseng* markedly induced colony-stimulating factor and the glycoproteins interleukin-IL-2 and -IL-6 in splenic cells (Wang *et al.,* 1996). Such observations indicate that the *P. ginseng* and *P. quinquefolium* saponins may oppose the depressant effects of

cyclophosphamide and other chemotherapeutic agents on the bone marrow stem cell proliferation by control of haemopoietic growth factor production in the splenocytes (Zhang *et al.,* 1992a). Ginsenosides Rb_2, Rc and Rg_1 were subsequently confirmed as stimulants of DNA synthesis in bone marrow cells possibly by the involvement of cyclic nucleotides. Therefore preparations of red ginseng or other ginsengs may be useful in the prevention and treatment of haematopoietic damage induced by chemical agents (Yamamoto *et al.,* 1996).

Irradiation with γ-rays in the treatment of cancers or ultraviolet ray exposure may result in cell damage and it has been suggested that ginseng pretreatment will reduce radiation sickness. In order to assess the effectiveness of ginseng root powder against radiation damage Chang and his co-workers in 1980 (Sonnenborn, 1987) conducted a placebo-controlled trial involving 50 cervix cancer patients. There were only slight pathological changes in the blood picture although the thrombocyte count shewed a statistically positive increase; leucocyte and erythrocyte counts and the haemoglobin value indicated no clear distinction between ginseng and placebo. A number of reports have concentrated on the protective effect of ginseng proteins and polypeptides isolated by PAGE (polyacrylamide gel electrophoresis) and HPLC (Kim, 1990). Such ginseng protein fractions comprised polypeptides with molecular weights in the range 21,000 to 100,000 and reportedly increased the DNA repair capacity. That pretreatment with ginseng aqueous extract was effective versus radiation damage in mouse lymphoctes in culture was demonstrated by Kim *et al.* (1996a). Ginseng aqueous extract was added to the culture 48 hr before irradiation (100 Gy γ-rays) and within 3.6 hr DNA double strand break repair was in progress. Further work (Kim *et al.,* 1996b) on the radioprotective effect of ginseng pretreatment on murine hair follicles in mid growth cycle after 3 days of 3 Gy irradiation compared with controls suggests that an aqueous fraction extracted from *P. ginseng* roots may also reduce the body surface cell damage due to γ-irradiation.

Ginseng polysaccharides also protected against X-ray irradiation in mice. Tian *et al.* (1992a) noted that pretreatment with ginseng polysaccharides prior to X-ray irradiation (3.0 Gy) caused in bone marrow cells a reduction in the rate of chromosome aberration and aberrated cells as compared with untreated irradiated animals. Counts of nucleated cells in the spleen, the concanavolin A-proliferative reaction of the splenocytes, glycoprotein IL-2 production by the splenocytes and the natural killer activity were higher in the X-ray (2.0 Gy) irradiated group that had been treated with ginseng polysaccharide than in control animals. The effect was maintained for 3 days after irradiation (Tian *et al.,* 1992b). Treatment with ginseng polysaccharides also reduced dose dependently (125–500 mg/kg/day) the free radical content in irradiated animals (3.0 Gy) as compared with untreated but irradiated control animals. Effects lasted for 3 hours after irradiation (Tian *et al.,* 1995).

The accumulating reports suggest that the radioprotection effects of ginseng may act through the enhancement of the DNA repair capacity of treated cells but the exact mechanism is, as yet, uncertain.

Electroshock is a specific form of stress that can be measured effectively in test animals and Banerjee and Iquierdo (1982), using Swiss albino mice, administered 180–200 ng/kg ginseng orally in drinking water for 16–18 days

before testing or 250 ng/kg by intraperitoneal injection 30–60 minutes before testing and discovered that ginseng gave good protection against electroshock stress when compared with control animals receiving water only.

It is clear that further study is needed to satisfactorily explain the body defence stimulating action of ginseng. Ginsenosides, polyacetylenes, polypeptides and polysaccharides probably all contribute to the overall protective phenomenon.

GINSENG AND ANTI-ULCER ACTIVITY

It has long been known that gastric ulcers are frequently a reaction to stress. As early as 1827 the American Indian doctor John Williams in the book *"The American Indian Doctor: Dr. John Williams' Last Legacy, A Useful Family Herbal"* praised ginseng roots as an excellent medicine to alleviate "inward hurts and ulcers" when used in combination with other herbal drugs such as comfrey, spikenard, elecampane, camomile, angelica and fir boughs in a dilute alcoholic vehicle (Harriman, 1973). Tang and Craze in their book *"Chinese Herbal Medicine"* (1995) also refer to the use of ginseng to treat peptic ulcer due to stagnating stomach qi (or ch'i), weakness of spleen or excess heat and advise avoidance of alcohol, coffee and tea.

Cheng *et al.* (1985) noted that ginseng pectin polysaccharide and dextrin could reduce the amount of gastric acid and the activity of the enzyme pepsin. Ginseng pectin inhibited histamine-induced secretion of gastric acid and was effective versus indomethacin-induced and pyloric ligation-induced ulcers. In the same year Zhang and Hu (1985) reported the effectiveness of ginseng flower saponins on gastric ulcers induced by ligation of the pylorus or aspirin or reserpine. Later work by Sun *et al.* (1992a, 1992b) involving *P. ginseng* root and leaf polysaccharides indicated that the weakly acid polysaccharide fraction GL-4 from the leaves inhibited gastric ulcers caused in rats by water immersion stress, indomethacin and pyloric ligation. The most effective polysaccharide, which was capable of preventing ulcerogenesis in a dose dependent manner in mice, had an average relative mass of 16,000 daltons and was a pectic polysaccharide based mainly on galactose and galacturonic acid with small amounts of rhamnose, arabinose, mannose, glucose and glucuronic acid. Kujohara *et al.* (1994) indicated that the polysaccharide GL-BIII from the leaves comprised terminal, 4-substituted and 3,4-disubstituted galacturonic acid units together with 4-substituted glucuronic acid units. Further analysis (methylation and gas chromatography—mass spectrometry) revealed a galacturonic acid-(1→4)-rhamnose unit in addition to longer acidic units consisting of 2-substituted rhamnose and 4-substituted galacturonic acid.

Further research should reveal more precise structures and differentiate saponin glycoside and polysaccharide actions on ulcerative conditions.

GINSENG, MEMORY AND INTELLECTUAL SKILLS

Experiments involving human intellectual skills such as telegraphy and proof reading and animal experiments involving negotiation of a spiral maze confirmed

improved mental activity and therefore ginseng pretreatment was suggested for tasks requiring speed, accuracy and stamina. Ginseng administered orally was successfully used to counter the decline in learning ability that is normally produced under physiological stress (Bao *et al.,* 1984a,c). In a 12-week double-blind, placebo-controlled trial involving 16 fit male volunteers (aged 20–24 years) and an oral dosage of 100mg of Ginseng G115 twice a day, D'Angelo *et al.* (1986) noted statistically significant improvements in attention, information-processing, reaction times and well-being and, in particular, in mental arithmetic.

More recent studies by Petkov and Mosharrof (1987) have offered a more detailed explanation of this improvement of learning, memory and physical capability induced by administration of standardised Ginseng G115 extract. Learning and relearning can be considered in terms of memory. Ginseng was considered particularly useful if the breakdown of mental activity was due either to senescence or to individual specificity. Age is a very important factor especially as one can by 75 years of age lose 25% of the memory capacity held at 20 years of age. Fortunately such loss does vary considerably from person to person. Slowing down of the cerebral processes is accompanied by a decrease in the deposition of biogenic amines and acetylcholine, the compounds essential for nerve ending transmission, and is manifested more obviously by lack of attention, decrease in concentration and lapses in memory.

Memory is associated with the hippocampus, an elongated structure composed of a modified form of cerebral cortex forming ridges on the floor of each lateral ventricle of the brain. The hippocampus is regarded as the brain's critical decision-making neuronal mechanism, determining the importance and type of incoming sensory signals. It has been suggested that the hippocampus acts as the encoding centre for conversion of short term memory into long term memory and facilitates and controls the long term memory storage of data i.e. knowledge.

Benishin *et al.* (1991) noted that, in rats, ginsenoside Rb_1 obtained from *P. quinquefolium* roots was able to partially prevent the memory deficits caused by the cholinergic agent hyoscine (=scopolamine). Although the ginsenoside Rb_1 had no apparent effect on acetylcholinesterase activity, it facilitated the release of acetylcholine from hippocampal slices and thus the uptake of choline into the nerve endings without alteration in calcium influx. Therefore they concluded that the ability of ginsenoside Rb1 to reduce or prevent memory deficits was probably related to facilitation of acetylcholine metabolism in the central nervous system. Other workers confirmed such observations. Thus Ni *et al.* (1993) used a T-maze delayed alternation task technique and rats whose spatial memories had been disrupted by intraperitoneal injection of hyoscine (0.025–0.1 mg/kg). The hyoscine effect was dose-dependent but the hyoscine (0.1 mg/kg) action could be reversed by physostigmine (0.4 mg/kg) and also by orally administered ginseng extract (0.5–4.0 g/kg dried root). Ginseng extract given orally 60 min before testing improved the maze solving problem in a dose-dependent fashion, opposing the memory deficit induced by hyoscine. Oral ginseng given for 7 days in drinking water (2.0 and 4.0 g/kg/day) also produced dose-dependent reversal of the hyoscine-induced performance disruption and improved spatial working memory. The Japanese team of Yamaguchi *et al.* (1995) similarly recorded the

impaired performance of rats in a radial-arm maze, the apparent deterioration of initially correct performances being induced also by hyoscine treatment. A single intraperitoneal injection of ginsenoside Rg_1 but not ginsenosides Rb_1 or Rd, prevented the impairment of performance but it was noted that the inhibition of the reduction in initially correct responses was associated with a bell-shaped dose-response curve for ginsenoside Rg_1. As ginsenoside Rg_1 was unable to counter the spatial learning deficits prompted by a lesion in the medial septum, it was suggested that the cholinergic neurons in the medial septum were involved in the correction of the impaired performance. Later the same group (Yamaguchi *et al.*, 1997) observed that, in young adult rats suffering hyoscine-induced cognitive impairment, the choline acetyltransferase activity increased in the medial septum but not in the diagonal band, caudate and hippocampus 30 min. after injection of the protopanaxatriol ginsenosides Rg_1 or Re. Significantly the protopanaxadiol ginsenosides Rb_1 and Rd had no effect on choline acetyltransferase activity. Aged rats performed a smaller number of initially correct responses when introduced into the radial arm maze and this was related to a lower level of choline acetyltransferase activity in the medial septum although not in the diagonal band. However in aged rats repeated intraperitoneal injections of ginsenoside Rg_1 caused an increase in the number of initially correct responses and an increase in choline acetyltransferase activity in the medial septum although not in the diagonal band. It was therefore suggested that the protopanaxatriol type ginsenosides Rg_1 and Re ameliorated the cognitive deficit in aged rats through an increase in choline acetyltransferase activity in the medial septum.

Although brain function is still poorly understood it seems clear that the chemistry of the cholinergic system in the brain reticular formation and hippocampus is critical. In living systems free radicals, strongly active, highly reactive substances, such as singlet oxygen, superoxide anion and hydroxy radical are formed at tissue level in parallel with oxygen consumption for cell respiration and such radicals can affect targetted cells. Naturally occurring endogenous protective systems normally neutralise free radicals but if such systems are inadequate or the production of free radicals is excessive, the brain becomes disturbed. Free radicals are normally produced in small amounts only and are mopped up endogenously by scavenger enzymes e.g. superoxidodismutase, catalase, peroxidase, tocopherols, ascorbic acid, etc. In the event of scavenger enzymes and antioxidants failing to neutralise the free radicals, reaction with unsaturated fatty acids in the various biomembranes yields lipid peroxides. Such peroxides are normally reduced to less reactive hydroxy acids although some may break down to yield malondialdehyde. It remains to be seen whether the naturally occurring antioxidants found in ginseng extracts are major factors in the improvement of memory and intellectual skills experienced by many ginseng users.

GINSENG AND SLEEP

Many ginseng users claim improved sleep patterns as an advantage gained by regular ingestion of the roots. That *Panax ginseng* extract given orally in drinking water modulated sleep in unrestrained rats was demonstrated by Rhee *et al.*

(1990) who observed that the amount of wakefulness was significantly decreased during a 12 hr period of light whilst the amount of slow wave sleep was increased. Sleep was apparently unaffected during the dark period. The same group (Lee *et al.,* 1990a) observed that the amount of slow wave sleep and wakefulness fluctuated significantly during 48 hours food deprivation and also during the following recovery periods. However, employing age-matched male rats chronically treated with ginseng extract via drinking water, the fluctuation was markedly reduced. Therefore it was suggested that the beneficial effect of ginseng might be related, in part, to improvement of sleep caused by a stabilising effect on sleep-waking disturbances.

Investigating a ginseng fraction rich in protopanaxadiol type saponins with rats as experimental animals, Shimizu *et al.* (1991) reported a tranquillising effect with acutely increased diurnal slow-wave sleep and chronically decreased nocturnal locomotor activity. The Tokyo group also studied the effects of an enzyme-treated ginseng extract (Shimizu *et al.,* 1992). The cyclomaltodextrin glucanotransferase-treated *Panax ginseng* extract lacked bitter taste and its anti-fatigue properties were tested using exercise-loaded sleep-deprived male rats which had undertaken forced locomotion on a treadmill for three hours prior to the dark period. Without treatment the tired rats demonstrated a significant increase in slow wave sleep and correspondingly less wakefulness during the 12 hour dark period. Forty mg of the enzyme-treated ginseng given orally shortly before and after the treadmill exercise prevented the fatigue-prompted increase of slow-wave sleep and significantly returned the amount of nocturnal wakefulness to normal. Thus the enzyme-treated ginseng extract exerted an anti-fatigue effect in the presence of physical stress.

GINSENG AND THE AGEING PROCESS

As people's lives become longer, particularly in the more civilised societies which have adequate medical and preventative services, ageing presents many problems. As we age we become less physically fit, we show obvious changes such as whitening or loss of hair, wrinkled skin which recovers more slowly from the pinch test, weakened hearing and vision, general slowing down of physical activities and we become more liable to suffer from various illnesses. Less obvious is the deterioration of the body organs prompting glandular disorders, reduction of hormone output leading to sexual impotency, gradual mental deterioration and breakdown of the immune system. Any life style, drug or medicine that can delay or slow this inevitable decline and improve the quality of life is therefore important. The ancient Chinese were convinced that ginseng was the tonic that fulfilled this role. After all, it is an adaptogen coping with stress, it is a metabolism regulator for proteins, carbohydrates and lipids including cholesterol, a regulator that also stimulates production of bone marrow DNA, protein and blood cells, and it is a controller that ensures the harmony of visceral action and stimulates immune function.

Interest was focused on ginseng and the elderly when reports of trials in Eastern Europe and Italy were published in the 1970's. One German group

conducted a trial involving 540 patients divided into groups, one group receiving ginseng extract, the second group ginseng extract with added vitamins and the final group a placebo. The results of a wide range of tests indicated improvements in psychological performance, mental and psychological coordination and mood as well as better control of blood pressure and blood sugar. Similar results were obtained by the Italian team who, in 1972, stressed the value of ginseng in improving the lives of old people suffering incoordination, reduced mobility, fatigue, failing concentration and memory and disturbed psychological behaviour (Fulder, 1993).

In 1979 Quiroga and Imbriano reported that cerebrovascular deficits in 36 per cent of a group of 134 aged patients were corrected very favourably and in 54 per cent favourably by treatment with ginseng extract G115 at 100–200 mg per day for 90 days. Improvement of the circulatory insufficiency was assessed as 60 per cent. In a subsequent report of a double-blind trial involving 45 patients (Quiroga, 1982) the effect of the ginseng treatment was compared with use of 1.5 mg/day of the ergot alkaloid-derived vasodilator co-dergocrine (Hydergine). For ginseng an improvement quotient of 34 per cent was stated and for hydergine 58 per cent with the placebo shewing only 1 per cent. Ginseng was, however, less likely to cause adverse reactions. Confirming such beneficial use of ginseng, Ragusin *et al.* in 1980, in a clinical trial, administered 40–80 mg/day of ginseng extract G115 or a similar placebo to 98 geriatric patients. Parameters recorded included effects on tension, depression, sleep, memory deficit, muscle fatigue, work capacity and appetite. The test group of patients shewed significant improvement when compared with the placebo group (Owen, 1994).

Neuronal energy requirements remain high throughout life, the most efficient source of brain energy being aerobic glycolysis during which adenosine triphosphate (ATP) is continuously synthesised. The ageing process alters cerebral metabolism with lowered glucose and oxygen consumption producing reduced ATP synthesis in persons demonstrating senile cerebral insufficiency (Sebban, 1982). Studying the effect of ginseng extract G115 on rabbit brain both *in vitro* and *in vivo,* Samira *et al.* (1985) concluded that there was a significant increase of glucose uptake with a corresponding decrease of lactate, pyruvate and lactate/pyruvate ratio, indicating aerobic rather than the less economical anaerobic pathway. Thus G115 can be considered as a metabolic stimulant for brain tissue at doses equivalent to the recommended single human therapeutic dose, suggesting that altered neuronal metabolism rather than impaired cerebral blood flow is the problem in old age. Li *et al.* (1997), using fluorescence spectrometry and the dye 1,6-diphenyl-1,3,5-hexatriene, noted that the neuronal membrane fluidity decreased with age and was marked in old age. Ginsenoside Rg1 (10–40 mg/kg^{-1}) significantly increased the fluidity of ageing cortical cells and this may, in part, account for the anti-ageing effect of ginseng. There was thus general agreement with the observations of Zhang *et al.* (1995) that ginsenoside Re also enhanced the membrane fluidity of both juvenile and mature human red blood cells and thus promoted protective and anti-ageing effects on cell membranes.

Today gerontologists believe that malondialdehyde binds nonspecifically with enzyme biomacromolecules to yield lipofuscin, a pigment accumulated in living

cells as part of the ageing process. Diseases of old age may be related to the membrane damage caused by free radical chain reactions and the protein binding of malondialdehyde. Therefore naturally occurring antioxidants able to reduce the lipid peroxide content of the cells are regarded as anti-ageing compounds It is also suggested that internally in the nervous system ginseng acts in the presence of tocopherol against the free radicals by interruption of their formation.

Choi and Oh (1984) noted that the diol- and triol-type ginsenosides inhibited lipoperoxide formation in both *in vitro* and *in vivo* experiments and that the anti-oxidation effects of the triol-saponins were greater than the diol-saponin effects *in vitro*. However in *in vivo* experiments diol-type, triol-type and total saponins behaved similarly. Irrespective of the route of administration (oral or intraperitoneal) the greatest activity was in the liver with less in the kidneys and blood stream and the enzymes superoxide dismutase and peroxidase were inhibited. Oral rather than interperitoneal red ginseng extract was found to be more effective as a lipid peroxidation inhibitor than white ginseng extract; in *in vitro* experiments it was shewn to be more effective in the enhancement of liver superoxide dismutase, in electron donation and in the prevention of pyrogallol auto-oxidation. White ginseng extract was reported as more effective in preventing liver peroxide formation; liver peroxidase was enhanced 5.4–9.4 per cent for both red and white ginseng extracts. The butanol fraction of the ginseng extract contains the saponins and is the most effective as an anti-ageing medication. Further work confirmed that in rats the saponin hydrolysates prosapogenin, panaxatriol and panaxadiol inhibited lipid peroxide formation and superoxide dismutase and peroxidase activities *in vivo* and *in vitro* and contributed to the anti-ageing effect. The hydrolysates inhibited superoxide dismutase and peroxidase activities more efficiently than red ginseng extract (Choi and Oh, 1985). It was also reported that ginseng leaf saponins administered orally at 100 mg/kg daily to young rats (3–6 months old) for 3 months stimulated growth but for older rats (*ca* 18 months) there was a tonic effect.

Study of the ultrastructure of the myocardium for degenerative signs such as accumulation of lipofuscin and indistinct appearance of the crustae and outer membrane of the mitochondria and collagenous fibres and fibroblasts revealed that ginseng treatment produced decreased degeneration (Wang *et al.,* 1986). Aerobic cells are usually protected from free radical damage by enzymic antioxidants such as catalase, glutathione peroxidase, glutathione S-transferase, glutathione reductase and superoxide dismutases, enzymes that scavenge available free radicals. Non-protein antioxidants also present include albumin, ceruloplasmin and non-protein-bound sulphydryls including glutathione. Superoxide generation increases with age whilst copper and zinc superoxide dismutase and catalase enzymes decrease with advancing age. Ginsenoside Rb_2 treatment was shewn in senescence-accelerated mice to significantly increase the antioxidative enzymes copper and zinc superoxide dismutase and also manganese superoxide dismutase in the liver. In addition ginsenoside Rb_2 stimulated increase of antioxidative catalase activity as well as marked elevation of the antioxidant serum albumin and non-protein bound SH levels in the liver. A further benefit of ginsenoside Rb_2 treatment was the significant decrease in

hepatic malonyldialdehyde levels. Thus the anti-ageing effect can be related to an increase in antioxidants giving protection against reactive oxygen species (Chung *et al.*, 1994). The anti-superoxidation activities of several ginsenosides have been investigated *in vitro* using rat liver homogenates with added hydrogen peroxide or Fe^{2+} ions to generate the superoxide anions and hydroxyl radicals. Dong *et al.* (1996) concluded that total ginseng saponins and ginsenoside Rc offered considerable antioxidant protection although the total saponins and ginsenosides Rb_1, Rc and Rd but not Rb_2 suppressed the peroxidation induced by hydrogen peroxide. Discussing the probable mechanism these workers noted that ginseng and its glycosides did not react directly with oxygen-containing radicals or with their inducers (hydrogen peroxide and Fe^{2+} ions). Therefore the anti-superoxidation action is by activation of the endogenous free radical scavenging system. They also observed that the saccharides linked at C-20 in the protopanaxadiol nucleus played an important role in protection against lipid peroxidation. Recent work by Boulianne's team in Toronto (1998) has demonstrated that the life-span of fruit flies can be increased by about 40 per cent by inducing the development of the human enzyme superoxide dismutase (SOD) in their motor neurones. SOD inactivates and therefore scavenges the highly reactive oxygen radicals which are regarded as the cause of the cellular damage related to ageing effects. In fruit flies it is suggested that the weak link in the defences against free radicals occurs in the motor neurons and this may be also true for humans. As ginseng has a stimulating effect on SOD production in man, this may in part explain the anti-ageing properties of ginseng. Recently Kim *et al.* (1998c), studying glutamate-induced neurodegeneration in cultured rat cortical cells, confirmed that pretreatment with ginsenosides Rb_1 and Rg_3 inhibited the overproduction of nitric oxide characteristic of glutamate neurotoxicity as well as inhibiting the formation of malondialdehyde resulting from lipid peroxidation. Such neuroprotective activity would in part account for the potential value of ginseng in the treatment of senile dementia.

An alternative study by Zhang *et al.* (1992) investigated the rate of unscheduled DNA synthesis in cultured human lung fibroblasts through many generations. DNA-damage was induced with mitomycin-C and studied using a ^{14}C- and ^{3}H-thymidine double labelling technique. Unscheduled DNA synthesis was found to decrease during ageing, being significantly greater in the 28th generation than at the 40th generation. It was noted that mitomycin-C (1–10 ng/mL) induced both DNA-damage and repair in the 27th generation but damage only in the 40th generation. Ginseng saponins from root, stem and leaf, and fruit were found to antagonise the increase in unscheduled DNA repair induced by mitomycin-C in young cells but such saponins increased the unscheduled DNA synthesis in aged cells. Therefore it was concluded that ginseng saponins prompted antimutagenic and antiageing effects through the regulation of DNA repair.

Ginsenoside Rg_1 reputedly possesses both antiageing and nootropic functions, that is, the ability to retard ageing and to improve the cognitive state of the mind and particularly the memory. Liu and Zhang (1996) used Northern and Western blot analyses to estimate the levels of *c-fos m*RNA and *fos* protein in the hippocampus of both young and aged rats with or without ginsenoside Rg_1

treatment. The expression of the *c-fos* gene and protein was found to be reduced in aged rats. Treatment with ginsenoside Rg_1 however caused a dose-dependent increase in both aged and young rats and there was also an increase in hippocampal cAMP. It was concluded that changes at the genomic and protein levels caused by the increase in cAMP offered a possible explanation of the mechanism of action of ginsenoside Rg_1 on the ageing process and memory deterioration.

Immunoregulatory function in all animals, including man, declines with increasing age. In aged cells blood cell membrane permeability increases, membrane fluidity decreases and lymphocyte production in response to mitogenic stimulation falls with a decrease of glycoprotein interleukin IL-2 production. Ginsenoside Rg_1 was shewn to be capable of enhancing the proliferation of lymphocytes and the generation of interleukin IL-2 in old rats, with resultant increase of interleukin IL-2 ribonucleic acid and IL-2 protein contents. However, this phenomenon was not observed in young rats, suggesting that ginsenoside Rg_1 is an immunoregulator rather than an immunopotentiator (Liu and Zhang, 1995). Further studies involving incubation of lymphocytes isolated from healthy aged humans (Liu *et al.,* 1996) confirmed that ginsenoside Rg_1 significantly increased lymphocyte subtypes, lymphocyte phenotype expression and protein tyrosine kinase activity in the elderly.

There is little doubt that the anti-ageing effect of ginseng is related to the free-radical scavenging activity of the saponins. However, in addition to the inhibition of lipid peroxide formation in the tissues and the elevation of blood and brain superoxide dismutase activity, there is also evidence of the reduction of lipofuscin in brain neurons and in liver as a result of treatment with ginseng stem and leaf saponins (Wu *et al.,* 1992) and also improvement of DNA repair in the ageing cells. Not surprisingly a number of patents have been registered for geriatric tonics, for ageing, for cerebral vascular disease in the aged, for senile dementia and for Alzheimer's disease (see Chapter 9).

GINSENG AND ALCOHOL

Alcohol is foreign to the human system and is normally destroyed in the liver by oxidation yielding acetaldehyde which is in turn destroyed by aldehyde dehydrogenase. Ginseng saponins significantly increased the rate of oxidation of ethanol in alcohol-fed rats (Joo *et al.,* 1982). The liver decontaminates the body, converting chemical waste products to both useful and harmlessly useless products. In response to stress hormones the liver accelerates the conversion of waste and the generation of new protein enzymes and ginseng is known to facilitate such functioning of the liver (Fulder, 1980). Studying the effects of red ginseng extract and vitamins on alcohol-intoxicated mice, Saito *et al.* (1984b) stated that tocopherol inhibited alcoholic excitation and red ginseng extract and pantethine prevented memory failure in intoxicated mice. Using healthy human volunteers, Lee *et al.* (1987) demonstrated that in 10 out of 14 cases ginseng extract (3g/65 g body weight) accelerated blood-alcohol clearance by 32–51 per cent.

Hepatic cell damage due to alcohol can be considerable, mitochondria being swollen and the rough endoplasmic reticulum dilated. With ginseng saponin

treatment the hepatocytes were not so damaged (Joo, 1992). The acetaldehyde level in the liver and the serum of rats treated with ethanol was much higher than for non-treated animals but only slightly higher for the ethanol and ginseng group. Careful analysis of individual lipids e.g. phospholipids, cholesterol, fatty acid and triglycerides revealed a decrease in phospholipid biosynthesis and an increase in fatty acid and triglycerides as a result of ethanol feeding; these effects were significantly countered by co-feeding ginseng saponins. The saponins apparently stimulate the microsomal ethanol-oxidising system and the aldehyde dehydrogenase enzyme action and therefore there is faster removal of acetaldehyde with rapid shunting of excess hydrogen into lipid biosynthesis (Kwak and Joo, 1988). In rats it has been shewn that plasma levels are about 20 per cent lower when ethanol is administered orally with aqueous red ginseng extract than when ethanol is given alone. However orally administered ginseng has no effect on the plasma levels produced by intraperitoneal injection of ethanol (Lee *et al.,* 1993). These observations support the earlier recorded use of ginseng to promote faster disposal and elimination of alcohol from the blood after drinking (Fulder, 1980) and justify the potential value of the patents listed in Chapter 9 as treatments for alcohol-induced problems. Further work is needed concerning the value of standardised ginseng in the treatment and recovery of human subjects from alcoholism and its associated problems e.g. memory loss and nervous reactions.

GINSENG AND MORPHINE AND RELATED OPIOIDS

The relationship between morphine and associated opioids and ginseng has proved both remarkable and unexpected. Opioids are widely used as legitimate, effective analgesics, especially in terminal afflictions such as cancers although continued use of morphine-type drugs is rapidly accompanied by the development of tolerance, the craving for ever-increasing doses, and by psychic and physical dependence. Seeking effective antagonists of the narcotic and addictive effects of opium and morphine, H.S.Kim and his colleagues in a long series of studies from 1985 onwards have demonstrated the effectiveness of ginseng versus morphine tolerance. The protopanaxadiol-type ginsenosides Rb_1 and Rb_2 and the protopanaxatriol-type ginsenosides Re and Rg_1 inhibited the development of morphine-induced tolerance in mice and ginsenosides Rb_1 and Rg_1 also had an inhibitory effect on naloxone-induced withdrawal jumping response. In addition the ginsenosides inhibited body weight loss in physically dependent mice undergoing multiple injections of morphine (Kim *et al.,* 1989a). Naloxone ((-)-N-allyl-7,8-dihydro-14-hydroxy-nor-morphinone hydrochloride), an opioid receptor antagonist, partially blocked the analgesic effect of a large dose of morphine and also completely inhibited the development of an acute type tolerance. Ginseng total saponin extract, on the other hand, did not oppose the analgesic effect of a large dose of morphine although partially inhibiting the development of both acute and delayed-type tolerance. Therefore it was concluded that the partial inhibition of the development of acute and delayed-type tolerance by ginseng total saponins was not mediated by the opioid receptors (Kim *et al.,* 1989b).

The metabolism of morphine in the liver includes the partial conversion of morphine to the ketone morphinone, a reaction prompted by the enzyme morphine-6-dehydrogenase. Morphinone is considered nine times as toxic as morphine but demonstrates only half the analgesic effect. On conversion to morphinone-protein-sulphydryl conjugate by covalent bonding between morphinone and the sulphydryl groups, the opiate receptors become blocked irreversibly with diminishing analgesic action, so encouraging more and higher dosages of morphine to achieve the same analgesic effect. The subsequent development of tolerance and addiction can be detoxified by reaction with liver glutathione. Ginseng functions by blocking morphine-6-dehydrogenase and also by maintaining, or even increasing, the hepatic glutathione level so inhibiting reduction of non-protein sulphydryl levels. This has been proved *in vitro* using the standardised extract G115 and the protopanaxatriol-type ginsenosides, especially ginsenoside Rg_1 (Kim and Jeong, 1994). Ginseng leaf saponins were also shewn to antagonise the analgesic action of morphine and to inhibit the development of morphine induced tolerance and physical dependence and also to inhibit the reduction in liver glutathione induced by repeated morphine injections (Kim *et al.,* 1989e).

The effect of ginseng on the nociceptors, nerve ending sensory receptors detecting and transmitting the pain normally caused by chemical or physical damage to the tissues, is analgesic. In rats, ginseng at 200 mg/kg produced analgesia and hypothermia which was not reversed by naltrexone (17-(cyclopropylmethyl)-4,5α-epoxy3,14-dihydroxy-morphinan-6-one), a narcotic antagonist used to treat opioid dependency orally. Morphine at 8 mg/kg also produced analgesia and hypothermia but the analgesic response to morphine was antagonised by 25 mg/kg and 50 mg/kg doses of ginseng extract although not by 12.5, 100 and 200 mg/kg doses. The hypothermic effect induced by morphine was opposed by ginseng extract in doses of 12.5 to 500 mg/kg. The cataleptic (trance-like) effect of morphine in 50 mg/kg doses was antagonised by 25 mg/kg ginseng extract. From such results Ramarao and Bhargava (1990) concluded that the analgesia and hypothermia induced by ginseng extract was via a non-opiate mechanism and that ginseng opposed the acute pharmacological effects of morphine. Subsequent work (Bhargava and Ramarao, 1991) confirmed the inhibitory effect of ginseng extract in appropriate doses on the occurrence of tolerance to the pharmacological actions of morphine. The analgesic and hypothermic effects of red ginseng extract occur at relatively high doses and are not mediated via the endogenous opiates or opiate mediators as the effects are not antagonised by naltrexone. Ginseng produces partial analgesia in disorders of pain sensation, and hyperaesthesia (morbid sensitivity of the nerves) produced in animal pain models is not caused by chemical sympathectomy. Although the effect may be the result of depression of both dosal horn neurons in the spinal cord and the nociceptors sensitised by continuous impulse discharges at nerve injury sites, a non-opioid mechanism is probable (Kim and Kim, 1995). Current research based on the formalin test (injection of the hind paws of mice with 1 per cent formalin solution) suggests that the antinociceptive action of the ginsenosides (administered intrathecally) was due to blocking of peptide

SP-induced nociceptive information to post-synaptic site(s) at the spinal level. Normal reaction to formalin injection is biting or licking of the affected area but ginsenoside pretreatment inhibited the biting/licking reponse in a dose-dependent manner. The peptide compound substance P (SP), a probable neurotransmitter conveying information from pain receptors to the central nervous system, also prompted pain behaviour including licking, scratching and biting of the hind portion of the body. Co-administration of substance P (SP) with ginsenosides inhibited the SP-induced pain response. Therefore it was concluded that the ginsenosides were indeed probable nociceptive pain signal blockers (Yoon *et al.*, 1998).

Apart from the inhibitory effect on morphine tolerance and psychic dependence by ginseng extracts and particularly by the extract G115, there is also a marked preventative effect on addiction withdrawal symptoms. This has been demonstrated in morphine-dependent animal experiments but the precise mechanism of action is, as yet, not understood. Certainly the withdrawal symptoms are accompanied by an increase in the dopamine and cyclic adenosine monophosphate (*c*AMP) levels, a reduction in the acetylcholine level in the brain and decrease in serotonin (5-hydroxytryptamine) release from the brain stem. Dopamine (hydroxytyramine) is a normally inhibitory neurotransmitter produced in neurones in the substantia nigra of the basal ganglia of the brain stem and is involved in motor control; *c*AMP is an intracellular hormonal mediator arising within the cells and in its short life controls reactions such as promotion of enzyme activity, alteration of cell permeability, prompting of muscle contraction and relaxation, inciting synthesis of specific intracellular proteins, initiation of secretion, etc.; acetylcholine is another neurotransmitter occurring in the brain, spinal cord, nervous system ganglia, at the terminals of the motor neurones controlling skeletal muscle fibres and in the postganglionic fibres of the parasympathetic nervous system, and serotonin is a further neurotransmitter that is produced by nuclei originating in the median raphe of the brain stem and projecting into the dorsal horns of the spinal cord, the hypothalamus and other parts of the brain. Serotonin inhibits pain pathways in the spinal cord and is involved in control of mood, prolactin secretion, sleep and circadian rhythms.

More recent work by Kim *et al.* (1995) confirmed the potential value of ginseng total saponins at 200 mg/kg intraperitoneally in rodents in the prevention and therapy of the adverse reactions of morphine, ginseng total saponins being able to inhibit the development of sensitivity or reverse tolerance to the ambulatory-accelerating effect of morphine and to prevent the development of dopamine supersensitivity caused by chronic administration of morphine (10 mg/kg per day for 7 days). Similarly, in mice, ginseng total saponins blocked the development of reverse tolerance to the ambulation-accelerating effect induced by the CNS stimulant methamphetamine (2 mg/kg, subcutaneously) and also prevented the development of dopamine receptor supersensitivity induced by the chronic administration of methamphetamine. As ginseng saponins oppose the development of reverse tolerance to both morphine and methamphetamine as well as inhibiting dopamine supersensitivity, it was suggested that reverse tolerance may be related to enhanced dopamine receptor supersensitivity. Oh *et al.* (1997), observing that

ginseng pretreatment reduced the magnitude of methamphetamine-induced dopamine (3,4-dihydroxy-phenylethylamine), 3,4-dihydroxy-phenylacetic acid and homovanillic acid depletions, suggested that ginseng total saponins could partially prevent methamphetamine-induced striatal dopaminergic depletions.

Because the ginseng (*P. ginseng* root) total saponins inhibit the hyperactivity and conditioned place-preference response induced by psychostimulants and opiates, it has been concluded that the mechanism is direct or indirect modulation of dopaminergic activity. As the protopanaxadiol-derived ginsenoside Rb_1 and the protopanaxatriol-derived ginsenoside Rg_1 are the major *P. ginseng* root saponins, Kim *et al.* (1998a) investigated their effects on morphine-induced hyperactivity and place-preference. Both ginsenosides inhibited morphine-induced hyperactivity but not apomorphine-induced climbing behaviour, thus agreeing with the hypothesis that ginsenosides modulate catecholaminergic activity preferentially at pre-synaptic sites. Morphine-induced conditioned place-preference for greater time in the dark compartment, however, was inhibited only by the protopanaxatriol-derived ginsenoside Rg_1 with resulting test animal preference for more time in the white compartment. At low doses ginsenosides Rb_1 and Rg_1 were equally effective as inhibitors of catecholamine secretion at the pre-synaptic site but at higher doses ginsenoside Rg_1 was the more effective inhibitor. This could explain why morphine-induced conditioned place-preference was inhibited by ginsenoside Rg_1 only. Such results suggest that ginsenoside Rg_1 has potential as an agent for the prevention and treatment of the adverse effects of morphine type drugs.

Researchers at the Medical and Pharmaceutical University at Toyama, Honshu, Japan investigated the effect of majonoside R_2, the principal saponin from *P. vietnamensis,* Vietnamese ginseng, on morphine-induced antinociception. Majonoside R_2, *P. vietnamensis* extract and *P. vietnamensis* total saponins inhibited or attenuated the μ-opioid agonist morphine-induced antinociception as judged by the tail-pinch and hot-plate tests in mice and also reversed the tail-flick latency increased by conditioned fear stress in rats. Repeated administration of *P. vietnamensis* saponin or majonoside R_2 suppressed the development of morphine tolerance as judged by the tail-pinch test (Huong *et al.*, 1996, 1997a).

Clinical trials are necessary to clarify not only the potential use of ginsengs in the treatment of morphine tolerance in man but also the possible value in the prevention of adverse reactions of methamphetamine and cocaine.

GINSENG AND THE CENTRAL NERVOUS SYSTEM

There are many contradictory accounts of the effects of ginseng, its extracts and its individual isolated ginsenosides on the central nervous system (CNS). Early work recorded that the stimulant effect of ginseng diminished the depressant action of hypnotic drugs such as chloral hydrate and barbiturates. Unlike amphetamine and related anorexies, ginseng can in small doses produce the CNS stimulant effect with no interference with normal sleep. Larger doses decreased motor activity yielding a general sedative effect.

Such puzzling results stimulated Professor Takaji's Japanese group to carefully study the actions of the individual glycosides as well as ginseng extracts and

saponin fractions. One fraction, containing the diol glycosides ginsenosides Rb_1 and Rc, yielded sedative, tranquillising, analgesic and muscle-relaxing properties; a second fraction, containing the triol glycosides ginsenosides Rg_1, Rg_2 and Rg_3, shewed both stimulant and depressant activities in addition to muscarinic and histaminic actions. The last named two fractions possessed a depressant action characterised by decreased spontaneous movement, lowered body temperature, diminished alertness and relaxed muscle tone. From the accumulated results it can be concluded that *S*-protopanaxatriol series ginsenosides of the Rg group are principally stimulants, whilst the compounds of the *S*-protopanaxadiol series are sedatives, and the main saponin glycosides of *P. ginseng* roots are the triol ginsenoside Rg and the diol ginsenoside Rb groups. All the glycosides investigated (ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2, Rg_3) demonstrated antifatigue activity and increasing walking activity but were moderate depressants of the electroencephalogram (EEG) pattern and of the activity of cats; ginsenosides Rb_2, Re and Rg_1 were the most potent. It was suggested that the stimulant-depressant combination exercised a regulatory function on the CNS and should have potential value in the treatment of emotional disorders (Hou, 1978).

The conclusions drawn were supported by the subsequent studies of Yoshimura *et al.* (1988a) which revealed that resident mice given ginseng saponins (25, 50 and 100 mg/kg by intraperitoneal injection) demonstrated dose-dependent suppression of aggression as assessed by offensive sideways posture and biting behaviour although the combative behaviour was not changed when the intruding mice were treated with crude saponins. Ginsenoside Rb_1 (2.5, 5 or 10 mg/kg by intraperitoneal injection) also suppressed the combative actions of the resident mice although ginsenoside Rg_1 given similarly had no effect. When ginsenosides Rb_1 and Rg_1 were administered separately to intruder mice, the agonistic behaviour of the resident mice did not change. Although in locomotor activity tests ginseng usually does not depress motility (Banerjee and Iquierdo, 1982), it was noted that the highest dose of ginseng crude saponins did reduce locomotion frequency. In further work (Yoshimura *et al.,* 1988b) ginseng crude saponins (50 mg/kg) and the diol ginsenoside Rb_1 (2.5 mg/kg) administered chronically or acutely by intraperitoneal injection (50 mg/kg ginseng crude saponins or 2.5 mg/kg ginsenoside Rb_1) suppressed maternal aggression in isolated post-partum mice in a dose-dependent manner without causing impairment of motor function. The triol ginsenoside Rg_1, however, also at a dose of 2.5 mg/kg tended to increase maternal aggression. It was concluded that both the crude ginseng saponins and ginsenoside Rb_1 apparently possessed a psychotropic action on combative behaviour.

GINSENG AS A BIOLOGICAL RESPONSE MODIFIER

Understanding the actions of ginseng has, until now, always been complicated by the mixed nature of the chemical compositions of the ginseng products used. Therefore it has not yet been possible to clearly define the biological response modifier. Ginseng preparations have been shewn to improve liver function as well as immune function and it is therefore probable that the protective action

is related to an immunomodulatory effect. Pretreatment with ginseng extracts was found to prolong the survival times of test animals suffering experimental trypanosomiasis, to prevent the development of fevers induced by typhoid and paratyphoid vaccines in rabbits, and to retard the development of diseased (leukopenic) white blood cells in bacillary dysentery. Ginseng treatment stimulated the production of specific antibodies in guinea pigs immunised with influenza vaccine and in rats immunised with diphtheria toxoid and was more effective at lower doses. In mice, ginseng extracts offered protection against experimental viral infection (Semliki forest virus) and increased the antiviral resistance developed by an interferon inducer of fungal origin viz. 6-MFA. Ginseng extracts were shewn *in vitro* to be effective inducers of interferon production in human peripheral white blood cells and to increase the natural killer and antibody dependent cytotoxic activity; thus immunomodulatory activity has been confirmed (Sonnenborn, 1987).

Knowledge of the body's defence mechanisms is a prerequisite for understanding of ginseng's immunomodulatory properties. Phagocytes, white blood cells that engulf and destroy harmful particulate matter, micro-organisms such as bacteria, and waste matter, may be predominantly sessile macrophages (monocytes, histiocytes, reticulocytes, etc.) or mobile macrophages (polymorphonuclear neutrophils which form some 62 per cent of the white blood cells). They form a first line of defence giving nonspecific resistance or innate immunity. The second line of defence is usually called acquired or adaptive immunity and comprises two groups a) the development of circulating globulin molecules known as antibodies which attack invading agents and b) the formation of the lymphocytes (some 30 per cent of the white blood cells). Lymphocytes include the B-lymphocytes derived from the foetal liver and the T- lymphocytes arising from the stem cells of the thymus gland. The B-lymphocytes are involved in the antibody-mediated immune response system producing humoral antibodies while the T-cells control the cell-bound defence mechanism. T-cells include the important T4 cells with helper and inducer actions and T8 cells with suppressor and killer actions. Contact between an antigen and a helper T4 cell prompts the circulating B-cells to multiply and differentiate into plasma cells which develop and release antibodies and memory cells essential for sustained immunity.

The phagocytic activity of the guinea pig tissue macrophage system (frequently but incorrectly called the reticuloendothelial system) was increased after intragastric infusion of total ginsenosides from *P. ginseng* stems and leaves for 3 days at a daily dose of 400 mg/kg (Cui *et al.,* 1982). Nie *et al.* (1989) also noted that ginsenosides effectively increased the number of macrophages as well as stimulating the activities of the enzymes acid phosphatase, cytochrome oxidase and succinate dehydrogenase. There was no apparent linear parallel dose/effect relationship, low doses producing a stimulating effect but large doses had little effect. Intraperitoneal injection of ginsenosides in mice also increased the serum levels of specific antibodies and of the common antibodies IgA, IgG and IgM (Cui *et al.,* 1982). Jie *et al.* (1984) treated mice orally for 5–6 days with 10, 50 or 250 mg/kg of an aqueous total ginsenoside extract. In response to a primary or secondary challenge with sheep red cells there was enhanced production of

antibodies in a dose dependent manner. At the highest dose level, the primary IgM antibody response was increased by 50 per cent and the secondary IgG and IgM antibody responses by 50 per cent and 199 per cent respectively. Natural killer cell activity was also increased markedly.The effect of ginsenosides on natural killer cell activity and its correlation with the pituitary-adrenal axis system were studied using a mouse surgical stress model (Lui and Yang, 1991). Ginsenoside was found to antagonise the reduction in killer cell activity and to reduce the plasma ACTH and cortisone increase induced by stress. Regulation of the natural killer cells was thus possibly via the pituitary-adrenal axis in surgical stress. Such immunostimulating effects observed *in vivo* mirrored the *in vitro* stimulation of interferon production.

The action of crude extract, total saponins and especially the principal saponin, majonoside R2, from Vietnamese ginseng was shewn by the results of bactericidal and carbon clearance tests to enhance phagocytic activity in mice both *in vivo* and *in vitro* (Huong *et al.,* 1997b). Test animals were protected from the toxic action of *Escherichia coli* ATCC 25922 and there was a significant increase in the phagocytic index.

The effects of long term oral administration of ginseng *(P. ginseng)* extract to mice was investigated by Kim *et al.* (1997). They were particularly interested in the serum protein profile and the occurrence of immunoglobulin (Ig) isotypes. Healthy female mice received either 30 mg/kg/day or 150 mg/kg/day of ginseng extract orally. Serum protein electrophoretograms shewed that the levels of γ-globulin decreased dose-dependently to 82 per cent and 56 per cent of control values at 30 and 150 mg/kg/day respectively. The levels of total protein, albumin, α_2- and β-globulin fractions and the ratio of albumin to globulin did not vary significantly but the α_1-globulin level increased by 24 per cent with both dose regimens. Of the Ig isotypes (including IgA, IgG_1, IgG_{2a}, IgG_{2b}, IgG_3 and IgM), serum IgG_1 was dose-dependently reduced to 68 per cent of the control values with the dose regimen of 150 mg/kg/day. There was no marked change of other Ig isotypes. The IgG_1 isotype is seldom cytotoxic and can therefore function as a blocking antibody; its selective reduction by ginseng extract without changes in the cytotoxic antibodies such as Ig_{2a} may aid the prevention and inhibition of cancers.

Immunomodulatory actions have been investigated in double blind trials employing aqueous ginseng extract, the carefully standardised preparation G115 (Pharmaton SA), and placebo. In an eight week trial 60 healthy volunteers aged between 18 and 50 years were divided into three groups (Scaglione *et al.,* 1990). One group was given capsules containing 100 mg of an aqueous extract of ginseng root, a second group received similar capsules containing 100 mg of the standardised ginseng extract G115 and the final group received similar capsules containing lactose and caramel. The capsules were ingested orally, one capsule every 12 hours. Volunteers were tested at the commencement of the trial, after 4 weeks treatment and finally after 8 weeks treatment. Tests or measurements applied in triplicate to venous blood samples were 1) chemotaxis of circulating polymorphonuclear leukocytes (a measure of the specific attraction of leucocytes etc. by substances dissolved in the medium less the spontaneous migration), 2)

phagocytosis (the destroying activity of phagocytes which being electronegative attract the electropositive substances such as dead tissues, foreign particulate matter, etc. as well as rough surfaced particulate matter; the result, the phagocytic index, is expressed as the ratio of phagocyted microorganisms to the total number of polymorphonuclear leukocytes), 3) the phagocytosis index, the ratio of phagocyting polymorphonuclear leukocytes to the total number of polymorphonuclear leukocytes, 4) intracellular killing expressed as a percentage of killed microorganisms, 5) the total lymphocytes, 6) the T-helper (T4) subset, 7) suppressor cells (T8) subset, 8) the T4/T8 ratio, 9) blastogenesis of circulating lymphocytes assessed after mitogen stimulation (concanavalin A, pokeweed and lipopolysaccharide) and ^{3}H-thymidine labelling, and 10) the natural killer cell activity assessed by ^{51}Cr release in tumoural K562 target cells. The results obtained indicated that the ginseng extracts did stimulate an immune response in man. There was a significant increase in chemotaxis for both extracts after 4 weeks ($p<0.05$) and a more significant increase after 8 weeks ($p<0.001$) although no such increase was observed in the placebo group. Both the phagocytic index and the phagocytic fraction shewed similar trends, rising significantly after 4 weeks ($p<0.001$) for the G115 extract and maintaining high levels until the 8th week; results for the unstandardised extract were less significant, attaining a reasonable level ($p<0.05$) only after 8 weeks. Intracellular killing with both ginseng preparations also rose markedly ($p<0.01$ after 4 weeks, $p<0.001$ after 8 weeks); the placebo treatment also increased intracellular killing by the end of the trial ($p<0.05$).

Scaglioni and his colleagues correlated the total T-lymphocytes with the helper T4 and suppressor T8 cells. They noted that the total number of lymphocytes was elevated for the ginseng-treated volunteers from the fourth week onwards and significantly by the 8th week ($p<0.001$). For the T4 helper cells it was clear that the G115 extract produced a significant increase after 4 weeks ($p<0.05$) and this was maintained for the rest of the trial ($p<0.001$). The aqueous extract group yielded a lesser increase, only significant after 8 weeks ($p<0.05$) and the placebo group showed no change. The T8 cell count revealed no significant increase over the trial period for all three groups. The T4/T8 ratio showed little change although the G115 group produced a significant result after 4 weeks and this was maintained up to the 8th week ($p<0.05$). These results indicate that the standardised G115 preparation induced an onset of immune response earlier than the aqueous extract did.

Testing with mitogens (substances stimulating cell division) viz. concanavalin A, pokeweed mitogen and lipopolysaccharide, enabled assessment of the mitogen-induced blastogenesis or generation of circulating lymphocytes by measurement of radioactivity counts per minute of incorporated ^{3}H-thymidine. Although the placebo group remained unaffected and the response to the first two mitogens only caused a significant increase ($p<0.05$) after 8 weeks treatment for both the ginseng aqueous extract and the G115 extract, lipopolysaccharide caused a very significant increase ($p<0.001$) only for G115 extract.

Scaglioni's group also investigated the activity of natural killer cells using K562 target cells as foreign tumour cells and measurement of the ^{51}Cr isotope

released on their destruction. The results showed that only extract G115 produced a statistically significant enhancement of activity after 4 weeks ($p < 0.05$) and more so after 8 weeks ($p < 0.001$).

The results of these *in vivo* experiments confirm the cited earlier *in vitro* work of Singh *et al.* and Jie *et al.* in 1984 indicating that ginseng extracts can stimulate immune reactions and that the standardised G115 extract can positively influence a higher number of subsets in the immune system. Another interesting observation was reported by Mizuno *et al.* (1994); they observed that a hot water extract prepared from wild ginseng and given orally to mice shewed mitogenic activity to lymphocytes but extract of cultured ginseng did not. Polysaccharides may be involved and lymphocyte stimulation by lipopolysaccharide requires further investigation.

A further study by Scaglioni *et al.* (1994) concerned 40 volunteers who were smokers (20 cigarettes per day) and suffered from chronic bronchitis. The participants were divided into two groups; the test group received 100 mg ginseng extract G115 at 12 hour intervals and the placebo group were given similar capsules containing 100 mg lactose and caramel. Parameters were determined on macrophages from bronchoalveolar lavage at the start and at the 4th and 8th weeks. Alveolar macrophages were separated from the lavage fluid and immediately assayed. Phagocytosis frequency (phagocyting alveolar macrophages), phagocytosis index (percentage of C. *albicans* phagocyted after 20 min incubation at 37° C) and intracellular killing power towards the yeast-like organism *Candida albicans* (percentage of yeast cells killed and stained with methylene blue (0.01 per cent in distilled water)) were estimated. Results revealed that the extract G115 could improve the immune response of alveolar macrophages in human subjects and significantly so by the 8th week of treatment ($p < 0.001$ vs the respective controls). The ability of extract G115 to restore and increase the activity of alveolar macrophages renders such treatment useful in treating chronic bronchitis and related respiratory disorders. Such observations were supplemented by the work of Rimar *et al.* (1996) who had perfused rabbit lungs with artificially digested extract G115. Using extract G115 in undigested, gastric digested and intestinal digested forms, they were able to demonstrate that the pulmonary vasoconstriction induced by compound U46619 and the free radical injury caused by electrolysis could be countered by all three preparations. Acetylcholine induced vasodilation following injury could also be maintained using all three preparations. Therefore artificially digested and normal oral G115 extracts have potential as pulmonary vasodilators protecting against free radical injury.

Another research group (Song *et al.*, 1997) investigated the value of ginseng treatment for patients with cystic fibrosis. The main pathogen affecting such patients is *Pseudomonas aeruginosa* which causes chronic lung infection, pneumonia with resultant progressive pulmonary insufficiency. Rats were injected subcutaneously with an aqueous ginseng extract (25 mg/kg) for 10 days and control animals received cortisone (25 mg/kg) and saline (0.9 per cent, 1 ml/kg). Two weeks after the challenge with *P. aeruginosa,* the ginseng-treated animals demonstrated a significant improvement in bacterial clearance from the lungs, less severe lung pathology, a lower incidence of lung abscesses and fewer mast

cell numbers in the lung foci. In addition, lower total immunoglobulin G (IgG) levels and higher IgG_{2a} levels were detected in serum against *P. aeruginosa* sonicate and there was a shift from an acute to a chronic type of lung inflammation as compared with the cortisone-treated and saline-treated control groups. Ginseng treatment of this pneumonia in rats promotes a cellular response that suggests potential value in the treatment of chronic *P. aeruginosa* lung infection in human cystic fibrosis patients.

Increased immune response should augment the efficacy of vaccination against the common cold and/or influenza syndrome. Therefore Scaglioni *et al.* (1996) devised a double-blind, placebo-controlled, randomized, multicentre study; 227 suitable volunteers were grouped into 114 participants receiving Ginsana G115 standardised extract (100 mg orally every 12 hours) and 113 taking similar placebo capsules (also every 12 hours). Vaccination with anti-influenza polyvalent vaccine 0.5 ml was effected 4 weeks after commencement of the trial and the capsules were taken for 12 weeks from the start of the investigation. Data regarding safety parameters (24 laboratory tests e.g. sedimentation rate, haemoglobin, albumin, glucose, creatinine, etc.) were collected at the start and finish of the trial. There was no statistically significant variation between the initial and final results for the Ginsana G115 or placebo groups. At the three medical centres natural killer cell activity and antibody titre was assessed at 0, 4, 8 and 12 weeks; concomitant diseases were checked at 2, 4, 8 and 12 weeks and adverse events at 2, 4, 6, 8, 10 and 12 weeks. Only 9 patients (8 taking ginseng G115 and 1 placebo) reported minor side reactions e.g. nausea, insomnia and epigastralgia. The results of the trial clearly demonstrated that the standardised ginseng extract G115 improved the immune response *in vivo* in human subjects thus protecting against common cold and influenza. Natural killer activity and antibody titre were significantly higher ($p<0.001$) after 8 and 12 weeks. Therefore significantly fewer volunteers in the G115 treatment group succumbed to these complaints.

Using *Echinacea purpurea* rhizomes and *Panax ginseng* roots See *et al.* (1997) reported that extracts of both plants enhanced the cellular immune function of peripheral blood mononuclear cells from normal individuals and from patients with depressed cellular immunity due to chronic fatigue syndrome or acquired immunodeficiency. Natural killer cell function was enhanced against K562 cells and antibody-dependent cellular cytotoxicity was improved versus human herpes virus 6 infected H9 cells.

Other workers investigated the immunological effects of the ginseng heteropolysaccharides, compounds with molecular weights in the range 20,000 to 180,000 and comprising structural units such as arabinose, fucose, galactose, glucose, rhamnose, xylose and galacturonic acid and obtained from various *Panax* species and from the waste after such species are propagated by tissue culture. For example, sanchinan A, isolated from *P. notoginseng* roots, had a molecular weight of 1,500,000 and proved to be an arabinogalactan with remarkable tissue macrophage system stimulating effects (Ohtani *et al.,* 1987).

Working with mice, Wang *et al.* (1981) recorded the effects of gastric infusion of a ginseng polysaccharide preparation at doses of 50–400 mg/kg body weight per day for 3–7 days. The effects were 1) stimulation of the phagocytic function

of the tissue macrophage or reticuloendothelial system, 2) an increase in the serum specific antibodies and IgG antibodies, and 3) an increase in the relative percentages of B-lymphocyte cells. They also noted that a gastric infusion of 50mg of the ginseng polysaccharide preparation/kg/day in guinea pigs increased the serum complement level. Their conclusions indicated that ginseng polysaccharides apparently enhanced immune function.

Ginseng polysaccharide administered orally, intraperitoneally or subcutaneously to mice at doses of 100/200 mg/kg for 5–8 days countered the immunodeficiency induced by cyclophosphamide and also normalised suppressed macrophage phagocytosis and haemolysin formation and the delayed hypersensitivity reaction (Wang *et al.,* 1985; Yuan *et al.,* 1986). Similar conclusions were drawn by Kim *et al.* (1991) who observed that a ginseng polysaccharide fraction inhibited the decreases in the ratio of spleen weight to body weight, the white blood cell count and plaque-forming cell count induced by cyclophosphamide and also increased these factors in normal mice. In normal animals the ginseng saponin fraction increased the haemoglobin level and plaque-forming cell count in the spleen. The polyacetylene panaxytriol (20 mg/kg) prevented decreases in the white blood cell count caused by cyclophosphamide although neither the saponin fraction nor panaxytriol had any effect on the plaque-forming cell count and the antibody titres in cyclophosphamide treated mice. Such observations indicate that it is the polysaccharide fraction that probably reduces the immunotoxicity of cyclophosphamide and may have potential as a stimulator of immune functions in man.

Some polysaccharides comprising arabinose, galactose, glucose, mannose, xylose and uronic acid demonstrated varying degrees of anticomplementary action mediated by classical and alternative pathways whilst still retaining immunostimulating effects such as enhanced immune-complex binding to macrophages (Sun *et al.,* 1994; Gao *et al.,* 1996). Kim *et al.* (1998b) also reported strong anticomplementary activity initiated by the total saponins and the major saponins and discussed structure-activity relationships.

Studying immune deficiency in guinea pigs with a decomplementary and hypophagocytic state induced by cobra anticomplementary factor, Zhuang *et al.* (1996) administered ginseng polysaccharides (20 mg/kg intraperitoneally twice daily for 6 days) and discovered that the normal serum complement level was not influenced although there was recovery from the low complement level and reduced phagocytic rate caused by the cobra anticomplementary factor. Examination of isolated neutrophils by electron microscopy revealed that the polysaccharides reduced the number of neutrophilic granules which had increased following cobra anticomplementary factor treatment.

Patents for potential immunostimulatory pharmaceutical products based on ginseng polysaccharides and polypeptides are listed in Chapter 9.

GINSENG AND TUMOUR GROWTH

Cancers are a scourge of modern society. Cancers attack the fundamental life processes of the cells, normally altering the total genetic complement or genome

of the cell with mutation of one or more genes or fracture of a segment or segments of the DNA strand or loss of segments of the chromosomes. Mutation may occur by chance but other factors can initiate cancer development e.g. specific chemicals known as carcinogens, ionizing radiations, physical irritation, hereditary pre-disposition and certain viruses. The resultant cancer cell overgrows, that is, it is not constrained in its growth by naturally-occurring chemical limiters called chalones. In addition cancer cells tend not to adhere to one another and can therefore stray freely around the body via the blood and lymph systems and can set up new cancerous centres or metastases which are commonly known as secondaries. There is accumulating evidence that malignant transformation of a cell, be it blood, lung or brain, is due to the change of particular genes and emphasises the need for the development of highly sequence-selective DNA-interacting drugs. Protein kinase C (PKC), a Ca^{++}/phospholipid-activated protein kinase, is a cell biopolymer controlling many cell functions by selective protein threonine/serine phosphorylation in the presence of adenosine triphosphate. When PKC is out of control there is a series of signals leading to the uncontrolled cell proliferation typical of cancer.

The cancerous condition develops frequently in patients manifesting reduced resistance as in old age and such cancers can be combatted by strengthening the general body resistance whilst simultaneously treating the cancer with suitable drug therapy or appropriate radiation treatment. Therefore adaptogens such as ginseng or eleutherococcus should function well by stress resistance and antitoxic effect. Ginseng enhances the formation of antibodies and immune functions in cancer patients and in microbe-infected experimental laboratory animals, possibly by elevation of the *c*AMP (*cyclic* adenosine monophosphate) levels. Both antitumoral activity and stimulation of the immune function of cancer patients when ginseng was administered have been observed in experimental animals and in human subjects. The reduced susceptibility of the host to bacterial, viral or tumour attack or infection has been referred to as "non-specific immunostimulation", "para-immunity" or "biological response modification (BRM)" (Sonnenborn, 1987). A wide range of disparate plant and pharmaceutical agents has been suggested as inducers including 2-mercapto-ethanol derivatives, synthetic compounds such as cimetidine and levamisole, some peptides from human casein hydrolysate, β-1,3-glucans, extracts of the thymus gland, plant extracts from families such as Loranthaceae (e.g. *Viscum album* L., Mistletoe), Echinaceae (e.g. *Echinacea* spp., Coneflowers), Compositae (e.g. *Silybum marianum* (L.) Gaertn., Milk Thistle), Araliaceae (e.g. *Eleutherococcus senticosus* Maxim., Siberian ginseng; *Panax* spp., ginsengs), etc., etc.

Mitchell (1985) stated that a substance considered as a Biological Response Modifier should fulfil one or more of the criteria:-

1) a compound or preparation reacting to the tumour by either raising the count or activity of the effector cells or stimulating production of the mediator (e.g. interferons, a class of proteins inhibiting the growth and multiplication of viruses in cells, and lymphokinins associated with the T lymphocytes of the thymus gland);

2) a substance that functions as an inhibitor of the immune system suppression system and thus stimulates the individual body defence mechanism indirectly;
3) a compound that elevates or stimulates the immune defence system and therefore functions as a positive inducer or mediator;
4) a substance increasing tolerance to cytotoxic antitumour therapy by encouraging leucocyte proliferation in the bone marrow;
5) a compound modifying the surface area of the tumour cells so that the effectiveness of cytotoxic medicaments is increased or the capacity for metastase formation is reduced;
6) a substance inducing cell transformation that will arrest, cancel, reverse or discriminate against the "primitive tumour cell".

Considering these stated criteria it is clear that ginseng has potential as a supporting agent in classical cancer therapy. The investigation of ginsengs as potential anticancer agents was undertaken by Russian pharmacologists in the 1960's and 1970's. Under experimental conditions preparations of ginseng, particularly ethereal and alcoholic extracts, and isolated ginsenosides were shewn to inhibit urethane-induced adenomas of the lung, 6-methyl-thiouracil-induced tumour of the thyroid gland and indole-induced myeloid leukaemia in laboratory animals. It was realised that ginseng could decrease the transplantability and size of tumerous foci when cells of Ehrlich's ascitic tumour were introduced intravenously into mice. The formation of spontaneous tumours of mammary gland and spontaneous leukaemia in mice could also be reduced although leukaemia-L1210 tumours were unaffected (Hou, 1978). Investigating the inhibitory effect of the phytoadaptogenic drugs bioginseng, *Eleutherococcus senticosus* and *Rhamnus carthamoides* on the growth of tumours induced by N-nitrosoethylurea on the nervous systems of rats, Bespalov *et al.* (1992) observed that all three drugs prolonged life and reduced the size and frequency of the tumours and that ginseng possessed the greatest anticarcinogenic activity and *Eleutherococcus* the least.

Therefore ginseng was apparently potentially useful in the attack on neoplasms or tumours such as adenomas (benign glandlike tumours), carcinomas (malignant cancers of the lining tissues of the skin and internal organs), leukaemias (malignant disorders of the white blood cells), melanomas (pigmented tumours which may become malignant due to the overgrowth of melanin-producing cells in the basal layers of the skin) and sarcomas (malignant tumours of the connective tissues of bone, muscle or tendon).

Despite the promising observations of the early workers, some pharmacologists questioned the methodology employed and doubted the validity of the results obtained. Nevertheless, as cancers tend to develop in persons with lowered resistance, any preparation or substance with the ability to bolster the immuno-defence system should retard cancer initiation. Better designed experiments shewed that ginseng saponins increased phagocytosis in the tissue macrophage (reticuloendothelial) systems of normal and tumour-infected mice and stimulated cellular and humoral immune function but, as yet, no satisfactory explanation of this immunomodulatory action has been presented.

Early work shewed that light petroleum and ethyl acetate fractions of ginseng root extract effectively inhibited the *in vitro* growth of mouse leukaemia L5178Y and mouse sarcoma S180 cells in a dose dependent manner. The toxic effect on the cancer cells was correlated with an inhibitory action on macromolecule biosynthesis, the light petroleum fraction in particular inhibiting protein synthesis whilst the ethyl acetate fraction inhibited certain RNA species (Yun *et al.,* 1980).

Ginsengs have been proposed as oral antitumour agents, the ginsenosides Rb_1 and Rg_1 being specifically implicated. Activity was claimed against a range of tumours in human subjects as well as in laboratory animals; for example, treatment of mice bearing the sarcoma S180 with 50 mg/kg of ginsenoside Rg1 for 7 days yielded a 52 per cent tumour inhibition (Arichi *et al.,* 1982). The subcellular organelles of Morris hepatoma cells grown in a culture medium containing ginsenosides were observed to be well developed with a well-organised distribution when compared with untreated control cells. Thus ginsenosides could reverse the transformation of Morris hepatoma cells (Odashima *et al.,* 1979). Following on this work it was noted that ginsenoside Rh_2 was incorporated into the cell membranes of B16 melanoma skin cancer cells and erythrocytes and the fluidity of the cell membranes was modified as determined by polarisation changes of the cells labelled by 1,6-diphenyl-1,3,5-hexatriene. It was suggested that this phenomenon was related to the phenotypic reverse transformation of the cancer cells (Ohta *et al.,* 1985). The effects of ginsenosides Rh_1 and Rh_2 were then studied employing mouse melanoma B16 cells in culture. In a concentration dependent manner ginsenoside Rh_2 inhibited the growth of the B16 cells at 5–15 μM concentration, initiating morphological changes and stimulating melanin synthesis at high cellular densities. On removal of ginsenoside Rh_2 after 2–6 days, the cellular growth rate recovered slightly although incompletely during the next four day period of experiment. Although 20(S)- and 20(R)-ginsenosides Rh_1 did not inhibit growth of B16 cells, they, like ginsenoside Rg_3, did stimulate melanin synthesis in a concentration dependent fashion (Tahara *et al.,* 1985). Ginsenoside Rh_1 did not inhibit growth even at a concentration exceeding 100 μM but it did stimulate expression of the melanotic phenotype. Significantly ginsenosides Rh_1 and Rh_2 vary only in the possession of a glucose unit at C-6 and C-3 respectively but differ markedly in their effects on B16 melanoma cells; this may be related to the occurrence of ginsenoside Rh_2 in the lipid fraction of the B16 melanoma cell membrane whilst ginsenoside Rh_1 was not so detected (Odashima *et al.,* 1985). Ginsenoside Rh_2 was also found to cause flattening of cells cultured in a collagen gel with resultant development of non-overlapping monolayers as well as markedly increased adhesiveness of cell to cell and cell to substrate, factors which tend to reduce the spread of cancers. Ginsenoside Rh_1 did not produce such effects (Ohta *et al,* 1987). Chen *et al.* (1988) isolated the related compound 20*(R)*-ginsenoside Rh_2 from the stems and leaves of *P. ginseng* and reported that at 2 g/mL concentration the growth of human leukaemia cell line HL-60 was inhibited. Subsequent work confirmed that ginsenoside Rh_2 has strong affinity for the lipid layer and is quickly absorbed into the cell membrane lipid bilayer altering the layer and thus affecting certain functional molecules on the cell surface. Therefore glycosidase glucosyl transferase, receptor protein and adhesion protein may be

affected by ginsenoside Rh_2, changing signals or their transmission to the nucleus. Consequently the expression of certain genes, such as c-myc oncogene, changes although ginsenoside Rh_2 does not act directly on the expression of c-myc oncogene which is probably regulated at the level of protein synthesis and/or protein stability and c-myc proteins express at every phase in the cell cycle of the cancer cell lines examined. Certainly ginsenoside Rh_2-treated cancer cells expressed a phenotype closer to that of their normal counterparts (Ohta *et al.,* 1990). Ginsenoside Rh_2 has also been shewn to retard the growth cycle of S180 sarcoma tumour cells from the S period to the G_2 period in mice; ginsenoside Rh1 did not demonstrate the same effect (Ma *et al.,* 1991).

Kikuchi and his colleagues (1991) studied the *in vitro* and *in vivo* effects of the diol-type ginsenoside Rh_2 on human ovarian tumour growth using a cell line derived from the ascites (i.e. free fluid in the peritoneal cavity) of a patient with serous cystadeno-carcinoma of the ovary. The tumour growth was inhibited dose-dependently in the range 10–100 μM by ginsenoside Rh_2 and the DNA, RNA and protein syntheses of the tumour cells were likewise inhibited dose-dependently at above 15 μM of ginsenoside Rh_2. Experiments on nude mice with transplanted human ovarian tumour cells demonstrated that combination therapy with cisplatin (a platinum-containing cytotoxic drug) and 10 μM ginsenoside Rh_2 resulted in significant inhibition of tumour growth 31 days after inoculation and produced increased survival times when compared with untreated animals and those treated with either cisplatin or ginsenoside Rh_2 alone. The effective synergistic combination yielded no adverse effects, thus suggesting clinical potential. A similar synergistic inhibition of the growth of cancer cells had been demonstrated *in vitro* earlier (Hwang *et al.,* 1989) using a ginseng and vitamin C combination against cultured mouse leukaemia cells (L1210 and P388) and human rectal and colonic cancer cells.

Ginsenoside Rg_3, also a diol-type saponin, was shewn to inhibit neoplasm metastasis and tumour cell infiltration in mice (Kitigawa *et al.,* 1993) and inhibition of lung tumour metastasis also in mice was demonstrated using ginsenoside Rb_2 and 20*(R)*- and 20*(S)*-ginsenosides Rg_3 extracted from red ginseng (Mochizuki *et al.,* 1995). These workers concluded that the mechanism of action was probably related to variations in cell adhesion and tumour cell invasion and antiangiogenetic activity. One year later Shinkai *et al.* (1996), using a cell monolayer invasion model, confirmed that ginsenoside Rg_3 was a potent inhibitor of cell invasion by rat ascites hepatoma cells, melanoma cells, small lung carcinoma cells and human pancreatic adenocarcinoma cells. The structurally analogous ginsenoside Rb_2, 20(R)-ginsenoside Rg_2 and 20*(S)*-ginsenoside Rg_3 demonstrated little inhibitory action and ginsenosides Rb_1, Rc, Re, Rh_1, 20(R)-Rh_1 and Rh_2 were ineffectual. Ginsenoside Rg_3 was also shewn to be an effective inhibitor of experimental pulmonary metastasis induced by highly metastatic mouse melanoma B16FE7 cells. Ginsenoside Rg_3 was subsequently demonstrated as an inhibitor of intestinal adenocarcinomas. Iishi *et al.* (1997) used bombesin (gastrin releasing peptide) which significantly enhanced the occurrence of intestinal tumours and cancerous metastases in the peritoneum after 45 weeks. Rats were treated with subcutaneous injections of the carcinogenic azoxymethane (7.4 mg/kg body weight weekly) for 10 weeks, then subcutaneous injections of bombesin (40 μg/kg body weight on

alternate days) and finally from week 20 subcutaneous injections of bombesin and ginsenoside Rg_3 (2.5–5.0 mg/kg body weight) on alternate days until termination of the trial (week 45). Although ginsenoside Rg_3 had little or no effect on the bombesin enhancement of intestinal tumours as it did not affect tumour growth or vascularity, it did significantly decrease cancer metastasis and thus the spread of cancer via peritoneal fluid.

Total ginsenosides (500 mg/day subcutaneously for 8 days) administered to mice implanted with S180 cancer cells reduced the rate of tumour bearing and the weight of individual tumours. Increase of natural killer cells, interferon and interleukin-2 produced by spleen cells suggests that ginsenosides play a positive role in the functioning of the natural killer cell-interferon-interleukin-2 regulatory network (Yu and Yang, 1987).

Investigating intermediate degradation products, prosapogenins and sapogenins prepared from Korean *P. ginseng* red ginseng saponins, Back *et al.* (1995) noted that cytotoxic activity varied against various cancer cell lines. Stereoisomerism did not appear to be significant although activity was inversely proportional to the number of sugar units linked to the sapogenin. Diol-type saponins, sapogenins and prosapogenins demonstrated higher cytotoxicity than the corresponding triol-types. In the same year Im *et al.* (1995) tested ginseng leaf saponins hydrolysed under alkaline conditions. The products obtained included monogluco-ginsenoside Rh_1, monogluco-ginsenoside Rh_2 and compound K (20-O-[β-D-glucopyranosyl]-20(S)-protopanaxadiol) and a mixture of the three demonstrated antitumour activity against human cancer cells.

Multidrug resistance is a problem in cancer chemotherapy. However Park *et al.* (1995c) reported the value of 20-(S)-ginsenoside Rg_3, a red ginseng saponin, as an agent with a potent inhibitory effect on multidrug resistance in the treatment of human fibrocarcinoma resistant to the periwinkle alkaloid vincristine. Therefore ginseng can be used as a supporting treatment in chemotherapy strategies or radiotherapy.

Ginseng total saponins were successfully employed in the prevention of bone marrow haemopoietic stem cell destruction by harringtonine during the treatment of maurine L1210 leukaemia (Fan and Han, 1979). Aqueous extracts of ginseng are known to counter the side effects of the anticancer agents 5-fluorouracil and mitomycin C, decreasing the leucocyte count and reducing urine flow, renal plasma flow, glomerular filtration rate and urinary excretion of sodium (Kim and Kim, 1982), although Zhou and Han (1983) warned that the cytosine arabinoside-induced damage to bone marrow haematopoietic precursor cells in mice was increased by pretreatment with ginseng total saponins, orally or intraperitoneally. Therefore care is needed.

You *et al.* (1995) reported that intrahepatic sarcoma-180 tumour cells could be treated successfully in mice using the combination of radiotherapy and oral *Panax ginseng* extract. Oral administration of *P. ginseng* root extract lengthened the survival time of tumour-bearing mice by 15.4 per cent and radiation treatment extended lifespan by 16.9 per cent but the combination of both treatments was much more effective increasing life by 82.9 per cent. Histopathological investigations revealed that the radiation treatment destroyed the cancer cells and

also liver cells but the ginseng extract stimulated the recovery of the liver cells without infiltration of tumour cells. This agrees with the subsequent observations of Pande *et al.* (1998) who also noted that ginseng extract was non-toxic in mice at dose levels up to 1200 mg/kg. At 10–20 mg/kg doses the survival time of irradiated Swiss albino mice was considerably enhanced compared with untreated animals. In addition, radiation-induced damage to germ cells and loss of body weight were also reduced in pretreated mice. The radioprotective effect was attributed to increased levels of glutathione in the liver.

The chemically interesting polyacetylenic compounds isolated from both red and white ginseng roots proved to be equally fascinating pharmacologically. Kim *et al.* (1988b) noted that the compounds panaxydol (3-hydroxy-9*S*,10R-epoxyheptadeca-1-ene-4,6-diyne) and panaxynol (heptadeca-1,9-diene-4,6-diyne-3-ol) caused concentration-dependent haemolysis but panaxytriol (heptadeca-1-ene-4,6-diyne-3,9,10-triol) had no such effect. With liposomes comprising phosphatidyl-choline and phosphatidic acid, all three polyacetylenes suppressed osmotic behaviour to the same extent. However, with liposomes of phosphatidyl-choline, phosphatidic acid and cholesterol, panaxytriol produced the least suppression. Significantly it was observed that panaxydol caused non-specific injury to L-1210 leukaemia cells by disrupting the cell membrane, nuclear envelope and mitochondria and it was concluded that the cytotoxicity of the polyacetylene compounds was due to the damaging effect on the cell membranes. Therefore panaxytriol, causing little damage to the cell membranes, was the least effective; this was due to its polarity and its minimal effect on the cholesterol of the lipid bilayers.

Polyacetylenic alcohols isolated from *P. ginseng* and *P. pseudoginseng* have been shewn to suppress tumour activity *in vitro* (Saita *et al.,* 1994). Panaxynol, panaxydol and panaxytriol were studied with respect to *in vitro* cell growth. Water solubility varied but α-cyclodextrin complexes enabled solutions of active compounds to be formulated. The inhibitory effect of these compounds was much stronger against malignant cells than against normal cells: Inhibition was cytotoxic at high concentrations and cytostatic at low concentrations. Continuous contact between the compounds and the target cells is not apparently necessary and the mode of action is more concentration dependent than time dependent (Matsunaga *et al.,* 1990). The action of panaxytriol was tumour-dependent and against B-16 melanoma the growth inhibition was definitely dose-dependent rather than time-dependent, panaxytriol being effective in mice at 40 mg/kg intramuscularly (Katano *et al.,* 1990). Matsunaga *et al.* (1994) noted the antiproliferative activity of panaxytriol against several types of tumour cells and observed that panaxytriol and mitomycin C are synergistic against the human gastric carcinoma cell line (MK-1) or, if used singly, additive. The synergism was considered probably due to an acceleration of the mitomycin C effect on cellular accumulation prompted by the panaxytriol and also enhancement of mitomycin C accumulation in MK-1 cells caused by the decreased fluidity of the cell membranes in the presence of panaxytriol. Panaxynol, panaxydol and panaxytriol were also shewn to be cholesteryl ester transfer protein inhibitors in human subjects (Kwon *et al.,* 1996).

Many polyacetylenic compounds have now been isolated from various *Panax* species and from tissue and callus cultures. Most are C14 and C17 compounds and the key structural unit appears to be hept-1-ene-4,6-diyne-3-ol. Thus panaxyne from *P. ginseng* roots is less effective against leukaemia L1210 cells because its structure is tetradeca-13-ene-1,3-diyne-6,7-diol and therefore lacks the key group (Kim *et al.,* 1989d).

More research concerning the action and therapeutic application of polyacetylene compounds which have been isolated from both whole plant and callus extracts of various *Panax* species should concentrate on neoplasm inhibitors, leukaemia L1210 and sarcoma inhibitors and the use of 5-lipoxygenase inhibitors as allergy inhibitors.

Although saponins and polyacetylenes have been given anti-tumour roles in the overall action of ginseng, there is a further chemical group, the polysaccharides, which have also been cited as tumour inhibitors. Hatono *et al.* (1986) patented a purified polysaccharide preparation from the roots of *P. notoginseng;* the molecular weight was >100,000 and the product contained arabinose, galactose and glucose in the proportions 0.5:5.0:94.5. It was claimed as a tumor killing factor for use in cancer therapy. Other patented examples are cited in Chapter 9.

Explanation of the mechanism of action of such compounds on cancers has prompted many suggestions. The oral ingestion of ginseng polysaccharides prolonged the survival time of mice inoculated with S180 or Ehrlich tumour cells but did not affect Ehrlich cells *in vitro*. In tumour-bearing mice immunised with red sheep cells, oral administration of *P. ginseng* polysaccharides (400–800 μg/kg/day for 10 days) stimulated the production of plaque-forming cells, rosette-forming cells and antibodies by the spleen. In normal mice such immunological changes were not apparent. This suggests that the anti-tumour action of ginseng polysaccharides is linked to immunostimulation in the host animal (Qian *et al.,* 1987).

Also, the ascites free fluid in the peritoneal cavity of sarcoma-180-bearing mice or hepatoma-bearing humans contains a lipolytic or fat-splitting factor, toxohormone-L. Lee *et al.* (1990b) discovered that an acidic polysaccharide from Korean red ginseng with a pectin-like α-1,4-polygalacturonan backbone and some acetoxyl groups significantly inhibited toxohormone-L-induced lipolysis at concentrations of 10 μ/ml. It was also noted that the inhibitory effect of the main root of red ginseng was 2.3 times greater than that of white ginseng. The anticancer action is probably also related to hormone balance. It was reported (Zhu *et al.,* 1991) that ginseng polysaccharides produced in mice splenocytes dose-dependent increases in the glycoproteins interleukin-2 and interferon and also in the natural killer cells. In animals bearing B16 melanoma tumours the levels of such compounds were lowered but could be reinstated by administration of ginseng polysaccharides (200, 100, 50 mg/kg/14 consecutive days intraperitoneally). This also supports the view that ginseng's anti-tumour action is related to its immunomodulatory action.

Lee *et al.* (1997) studied the purified lectin-free ginseng acidic polysaccharide ginsan, a compound with a molecular weight of about 150,000, isolated from *P. ginseng*. Ginsan stimulated the proliferation of B-cells and T-cells and the cytotoxicity of spleen cells to a wide range of tumour cells *in vitro*. The ginsan-

activated killer cells were produced in the presence of adherent macrophages and CD^{4+} cells; ginsan also activated macrophages to generate reactive nitrogenous intermediates and become tumouricidal. In addition, ginsan was effective *in vivo* versus B16 melanoma cell lines and in the benzo(a)pyrene-induced autochthonous lung tumour model. As ginsan appears to be relatively non-toxic having been injected at 1 g/kg body weight in mice without deaths, it is possibly a potential non-toxic antineoplastic immunostimulator activating several effector arms of the immune system. Subsequent work (Kim *et al.,* 1998d) confirmed the inhibition of benzo(a)pyrene-induced autochthonous lung tumours in mice. Spleen cells became cytotoxic to a wide range of tumour cells after 5 days of culture with ginsan polysaccharide in a non-major histocompatability restricted manner. Seeking an explanation of the antineoplastic action it was reported that ginsan could generate lymphokine-activated killer (LAK) cells from both natural killer (NK) cells and T-cells through endogenously produced multiple cytokines; ginsan in association with rIL-2 synergistically developed LAK cells (2–15 fold). Ginsan was also shewn to inhibit pulmonary metastasis of B16-F10 melanoma cells and to intensify the suppression of lung colonies by rIL-2. Such properties suggest an immunopreventive and immunotherapeutic role for ginsan-type polysaccharides.

Inhibition of protein kinase C has been related to the suppression of tumours and Park *et al.* (1994a) reported that methanol and acetone protein extracts of ginseng as well as the ginsenosides Rb_1, Rg_1 and Rh_2 inhibited protein kinase C. The methanol extract contained mainly glycopeptides with molecular weights below 18 Kd_a whilst the acetone fraction yielded mainly 18 Kd_a polypeptides.

As mentioned earlier, a Korean team (Kim *et al.,* 1997) studied the long term effect of oral administration of *P. ginseng* extract recording the serum protein profile and immunoglobulin (Ig) isotype occurrence. Study of the occurrence of Ig isotypes including IgA, IgG_1, IgG_{2a}, IgG_{2b}, IgG_3 and IgM revealed a dose dependent decrease of serum IgG_1 to 68 per cent of the control values when ginseng extract at 150 mg/kg daily was administered. Because the other Ig isotypes were not significantly affected it was suggested that, as the IgG_1 isotype is rarely cytotoxic and can therefore act as a blocking antibody, the partial removal of the IgG_1 isotype from the serum by ginseng action could permit more cytotoxic antibodies such as IgG_{2a} to act thus preventing or inhibiting cancer growth.

A recent report by the Chinese group Chen *et al.* (1998) also stresses the efficacy of ginseng extracts as potent anti-tumour agents improving the cell immune system. Effective against croton oil-induced skin papillomas in mice at doses of 50–400 mg/kg, ginseng extracts can reduce the number and incidence and prolong the latent period of occurrence of such tumours; similarly the growth of transplantable mouse sarcoma S180 and melanoma B16 are inhibited. Weaker extracts (0.1 and 0.25 mg/ml) were shewn to possess antioxidant properties inhibiting Fe^{2+}/cysteine-induced lipid peroxidation. Such results were obtained with extracts of red ginseng containing mixtures of ginsenosides, polyacetylenes, polysaccharides, etc. and laboratory animals were employed. Nevertheless the problem with ginseng is the lack of carefully designed clinical trials or clinical reports concerning successful prevention or treatment of cancers in man.

In the Korea Cancer Centre Hospital and General Hospital, Yun and Choi

(1995) undertook a comparative study of 1,987 pairs of patients. A "pair" consisted of one patient diagnosed with cancer and one without cancer and the object of the study was to compare the differences in each pair. The members of the "pair" were selected on the basis of sex, age and date of admission to the same hospital. Trained interviewers collated information on each patient's age, sex, marital status, history of ginseng use, socio-demographic status, education, lifelong occupational history, smoking habits and alcohol consumption. In addition data was collected concerning age of first use of ginseng, type of ginseng used, frequency of use and duration of treatments. After assessment of the results it was concluded that regular ingestion of *Panax ginseng* could reduce the risk of cancer by 50 per cent. For all types of cancer and all forms of ginseng the incidence of tumours decreased steadily with increasing duration of ginseng consumption. Patients who had taken ginseng for one year had 36 per cent less cancer incidence than non-users and those who had ingested ginseng for 5 or more years had 69 per cent less cancer incidence. It was also noted that those who had used ginseng treatment less than 50 times experienced a 45 per cent reduction but those who had used it more than 500 times had a 72 per cent reduction. The results indicated that ginseng was a more effective protection against cancers of the larynx, oesophagus, ovaries, pancreas and stomach but had no significant effect on bladder, breast, cervix and thyroid cancers. Further controlled trials are obviously necessary.

Some authors have stressed the antimutagenic properties of ginseng preparations using the mutagen mitomycin C as control. Ginsenosides from *P. ginseng* stems and leaves administered intraperitoneally or orally prevented the induction of micronuclei in murine bone marrow cells. When ginsenosides and mitomycin C were administered simultaneously the occurrence of micronuclei was much reduced. Such reduction was considered to be antimutagenic (Lu *et al.,* 1991). The Russian group of Umnova *et al.* (1991) prepared bioginseng from ginseng callus cells by alcoholic extraction and lyophilisation and, using Chinese hamster cells, demonstrated that bioginseng could reduce the rate of spontaneous sister chromatid exchanges and the occurrence of mitomycin-C induced chromosome aberrations. Bioginseng also protected Ehrlich tumour cells against the mutagenic action of nitrosomethylurea. In a further communication these workers (Salikhova *et al.,* 1994) confirmed the antimutagenic effect of bioginseng against nitrosomethylurea and cyclophosphamide treatment and suggested that the sister-chromatid exchange decrease was related to increased DNA repair induced by bioginseng. Zhu *et al.* (1994) also observed that the frequency of chromosome aberration induced by mitomycin-C in mice was significantly reduced *in vivo* in the presence of ginseng stem and leaf saponins and the best protection of genetic materials was obtained by treatment prior to mutagen application. Further work involving human subjects is essential.

GINSENG AND THE CARDIOVASCULAR SYSTEM

Ginseng has, as an adaptogen, been credited with the ability to normalise both high and low blood pressure conditions. Therefore it would appear to be potentially useful in the treatment of hypertension, a condition of high blood

pressure predisposing to strokes and heart attacks and associated with old age. Certainly it had been valued for the treatment of impaired circulation and the sedative effect lowering the blood pressure was well-known. Early workers in the 1920's and 1930's using rats, dogs and rabbits as test animals established that the effects were dose dependent, smaller doses causing an increase and larger doses a lasting decrease in blood pressure (Hou, 1978).

Lee *et al.* (1981) examined the effects of ethereal, ethanolic and aqueous *P. ginseng* extracts on cardiovascular function in dogs after intravenous injection (40 mg/kg). The ether extract caused significant decrease of heart rate and central venous pressure, the ethanol extract significant decrease of heart rate and mean arterial pressure and the aqueous extract significant decrease of cardiac output, stroke volume and central venous pressure but the total peripheral resistance was markedly increased.

Using intravenous injections of total ginsenosides in dogs, Chen *et al.* (1982) confirmed that the peak value of left ventricular pressure and the arterial systolic pressure were rapidly decreased. Heart rate and renal arterial blood flow decreased although renal vasoresistance was significantly increased. This vasoconstrictory effect of ginsenosides was not blocked by α-adrenoreceptor blocking or serotonin receptor blocking agents. Other workers reported that the cardiovascular responses to the stem and leaf saponins were similar to those observed with the root saponins (Pan *et al.,* 1985). Pan and Li (1991) also noted that, in mice, ginseng flower saponins as well as root saponins at appropriate oral dosage could raise the level of myocardial cyclic adenosine monophosphate (*c*AMP), an intracellular hormonal mediator. Flower saponins also affected myocardial cyclic guanosine monophosphate (*c*GMP) at suitable dose levels, the ratio *c*AMP/*c*GMP in mice increasing progressively at dosages of 50, 25 and 12.5 mg/kg.

Several Chinese workers have indicated that various ginseng saponins can reduce the size of myocardial infarction, the area of dead tissue developed after coronary occlusion has obstructed the blood flow to the cardiac muscles or myocardium. Experimentally this can be achieved by ligation of the left descending coronary artery (*ca* 40 min) and subsequent reperfusion (*ca* 120 min). In various animals (dogs, guinea pigs, rats and mice) Chen *et al.* (1981) observed that ginsenosides increased myocardial tolerance to hypoxia (oxygen deficiency), a decrease in myocardial oxygen consumption apparently occurring during hypoxia. The survival times for mice given ginseng extract intraperitoneally and subjected to hypoxia were prolonged (Lu *et al.,* 1987). Ginsenosides have also been reported by other workers to protect mice against metabolic disturbances and myocardial damage associated with severe anoxia and anoxaemia (lack of oxygen in the tissues) (Yunxiang and Xiu, 1987). Myocardial necrosis can be also induced with isoproterenol (isoprenaline) and changes in the electrocardiogram and serum creatine phosphokinase, lactic dehydrogenase and γ-glutamyl-transferase levels can be normalised with ginsenosides from ginseng root, stem, leaf and fruit. Such actions are comparable to those of propranalol, a heart sympathetic stimulation inhibitor used in the treatment of cardiac arrhythmias associated with heart disease (Chen *et al.,* 1986).

P. notoginseng saponins (50 mg/L and 100 mg/L) slowed the breakdown of adenosine triphosphate (ATP) in cultured chick embryo neurons after 2 hours of hypoxia and stimulated the restoration of ATP during 30 min reoxygenation. The saponins (100 mg/L), whether administered at the commencement of hypoxia or after induction of hypoxia, also reduced the release of creatine kinase (Jiang *et al.*, 1995). Ginsenosides improved the survival rate of cultured rat hippocampal neurons under anoxic conditions, reducing the efflux of K^+ and lactate dehydrogenase (Wang *et al.*, 1995). Similar conditions probably apply to myocardial neurons. Chan *et al.* (1997) confirmed the myocardial protective effect in the rat heart of the naturally occurring triacylglycerol trilinolein isolated from ginseng. Pretreatment of isolated cardiomyocytes with trilinolein at the low concentration of 10^{-9} M reduced the $^{45}Ca^{2+}$ influx caused by hypoxia/normoxia by 34 per cent. When the isolated perfused rat heart was subjected to 1 hr global hypoxaemia without reperfusion it was observed that pretreatment with 10^{-7} M trilinolein for 15 min reduced by 37 per cent the size of the infarct or dead tissue area caused by stagnation of the blood circulation. Determination of superoxide dismutase-mRNA by Northern blot analysis in the *in vivo* heart that had undergone 30 min ischaemia (hypoxic reduction of blood supply) followed by 10 min reperfusion indicated that pretreatment with 10^{-7} M trilinolein resulted in a synergistic action with antioxidant systems preventing a rise in superoxide dismutase-mRNA. It can therefore be concluded that ginsengs operate by inhibition of $^{45}Ca^{2+}$ influx, by restoration of high energy phosphates during reoxygenation and by improvement of antioxidant activity.

The individual saponin glycosides were shewn to act in different ways. Ginsenosides Rg, Rg_1 and total flower saponins were recorded as cardiac performance improvers whilst ginsenosides-Rb and total leaf saponins had the opposite effect. Negative chronotropic effects (retardation of the rapidity of the periodically-recurring phenomena e.g. heart beat) and negative inotropic effects (not modifying the force or speed of contraction of the cardiac muscle) *in vitro* have been demonstrated for ginseng saponins; the mechanism of action resembles that of verapimil (5-[N-(3,4-dimethoxyphenethyl)-methylamino]-2-(3,4-dimethoxyphenyl)-2-iso-propyl-valeronitrile hydrochloride), a drug reducing the work load on the heart by reducing the oxygen requirements of the myocardium and decreasing peripheral resistance and used for angina pectoris treatment (Wu and Chen, 1988). However *in vitro* experiments had also indicated an increase in coronary blood flow together with a positive inotropic effect (Lei *et al.*, 1986).

Ginseng saponins Rc and Rd provided some antiarrhythymic action against aconitine- and barium chloride-induced arrhythmias in rats and adrenaline-induced arrhythmias in rabbits. The mode of action resembled that of amiodarone and prompted prolonged RR, PR and QT_C intervals on the electrocardiogram (Li and Zhang, 1988).

P. notoginseng saponins (12.5 and 25 μg/mL) and ginsenosides Rb_1 (10 μg/mL) and Rg_1 (10 μg/mL) decreased cardiac creatine phosphokinase release, reduced myocardial Ca^{++} accumulation, reduced malondialdehyde production and blocked reduction of superoxide dismutase activity in isolated rat hearts with global ischemia and reperfusion. Therefore *P. notoginseng* saponins and the ginsenosides Rb_1

and Rg_1 tend to prevent cardiac ischemia by inhibition of lipid peroxidation (Li *et al.*, 1990). Panaxadiol and panaxatriol saponins were shewn to dose-dependently decrease the action potentials of normal cultured myocardial cells suggesting selective Ca^{++} channel blockade and to protect against free radical oxidative damage induced by xanthine and xanthine oxidase (Zhong *et al.*, 1991). Ginsenosides of group Rb but not Rg possess Ca^{++} antagonist activity and a protective effect on experimental myocardial infarction in rabbits. Arrhythmias induced by reperfusion were prevented by panaxatriol pretreatment at 5 or 50 μg/mL. Panaxatriol saponins inhibited the release of creatine phosphokinase and lactate dehydrogenase as well as malondialdehyde production from ischaemic reperfused hearts at 50 μg/mL. There was also action on superoxide dismutase at 5 and 50 μg/mL, action probably due to inhibition of free radical accumulation and lipid peroxidation (Li *et al.*, 1992a). Total *P. quinquefolium* saponins (90–180 mg/kg i.p.) administered to rats having the myocardium damaged by injury to the left anterior descending coronary artery were also shewn to protect the myocardium with an anti-ischaemic action probably related to a decrease in free fatty acid levels and an elevation of lactate dehydrogenase activity. The total saponins may also have produced a Ca^{++} channel blocking effect (Jin and Lu, 1992).

Investigating the effect of 11 ginsenosides on the action potentials induced by microelectrodes on cultured cardiomyocytes Jiang *et al.* (1993) noted that ginsenosides reduced the amplitude, ginsenosides Rg_2, Rg_1 and Re in decreasing order being the most effective and similar in action to the Ca^{++} channel blocker nimodipine. Ginsenosides Rd, Rf and Ro had no effect on action potential and the protopanaxatriol-derived ginsenosides Re, Rg_1, Rg_2 and Rh_1 were more potent than protopanaxadiol-derived ginsenosides Rb_1, Rb_2, Rb_3 and Rc. Therefore the Ca^{++} channel blocking action of the triols is greater than that of the diols. Significantly ginsenoside Re was patented in 1995 as a treatment for cardiac arrhythmia (see Chapter 9).

Further work on the protective action on myocardial ischaemia and reperfusion injury of saponins from *P. notoginseng* and *P. japonicus* and of isolated gypenosides indicated that all possessed some effectiveness, the first two named acting by prevention of Ca^{++} overload and the gypenosides acting by anti-lipid peroxidation (Ha *et al.*, 1994). It is clear that some ginseng constituents are antioxidants restricting lipid peroxidation, others are less effective or ineffective and some inconsistencies are undoubtedly due to the variations in qualitative and quantitative chemical composition of the plant materials or saponin mixtures used. Hence the importance of standardised experimental materials.

GINSENG AND ATHEROSCLEROSIS

Atherosclerosis involves the irregular deposition of lipids such as cholesterol and triglycerides in yellowish plaques of atheroma in the subintimal layers of the inner walls of arteries and arterioles. Calcification may also occur with consequent hardening of the arteries. Atherosclerosis most commonly affects coronary arteries, cerebral arteries and peripheral arteries of the lower limbs. As a progressive condition it is particularly associatedm with old age.

Atheromatous plaques and related scarring cause narrowing of the arteries and ultimate occlusion of these vessels with ischaemia or reduction of blood supply to the structures supplied by such arteries and resultant infarction due to dead tissue formed by the lack of blood supply. According to the area involved atherosclerosis can lead to hypertension, angina pectoris, myocardial infarction, arrhythmias, paralysis, gangrene of the extremities and cerebral insufficiences leading to confusion, amnesia, personality changes or strokes.

Ginseng was suggested as a medicament for the control of cholesterol levels and for the treatment of anaemia. Experimental evidence does shew that ginseng is capable of increasing the red blood cell count, of promoting serum protein synthesis and of stimulating RNA formation in the liver and DNA synthesis in bone marrow. Cholesterol, a steroid alcohol, occurs naturally in the body and is found particularly in the bile and gall bladder and in the lipoproteins of the blood plasma. Although endogenous cholesterol can be formed in all cells of the body, blood cholesterol is usually produced in the liver, the body organ that controls the normal cholesterol level in the blood. High levels of cholesterol can occur in insulin and thyroid hormone deficient subjects or in persons consuming a high fat, high cholesterol containing diet. Dietary cholesterol, also called exogenous cholesterol, derives from foods such as milk, cream, butter, cheese, eggs, beef dripping, offal and other meats. Ginsenosides stimulate cholesterol synthesis in the liver and its conversion to other steroids as well as probable excretion in bile and faeces. At the same time, total serum cholesterol concentration falls by about 25 per cent due to reduced absorption from the gastrointestinal tract and increased rate of cholesterol metabolism in the body (Karzel, 1974).

Compounds responsible for the lowering of total serum cholesterol and low density lipoprotein (mainly cholesterol) levels occur particularly in petroleum ether extracts of ginseng root although also in aqueous extracts, lowering cholesterol and triglyceride levels in the blood, and lipogenic and cholesterol enzyme (cholesterol-7α-hydroxylase and β-hydroxy-β-methylglutaryl-CoA) activities in the liver (Qureshi *et al.,* 1983). Moon *et al.* (1984) studied such effects during a 4-week trial of red ginseng crude saponins administered to rats fed on a diet of 2 per cent cholesterol and 10 per cent olive oil. Oral saponin treatment (150 mg/kg/day) did not affect the high density cholesterol level although the plasma total cholesterol level was lowered and the triglyceride levels markedly raised. Other workers have implicated ginsenosides Rb_2 and Rc taken orally and it was noted that ginsenoside Rb_2 treatment of hyperlipemic animals could result in elevated high density lipoprotein levels. High density lipoproteins comprise about 50 per cent protein with smaller concentrations of lipids and are deposited in adipose tissues. Ginsenoside Rb_2 stimulated the lipolytic activity of the lipoprotein lipase causing a concomitant decrease in triglyceride levels and very low density lipoprotein-triglyceride levels in serum (Yokozawa *et al.,* 1985a). Prednisone acetate can be employed to induce increased levels of total lipids, triglycerides and total cholesterol as well as a decrease in serum cortisol. In rabbits it has been shown that *P. ginseng* stem and leaf total saponins given orally (60 mg/kg^{-1}/day) markedly inhibited such induced changes. The total leaf saponins

comprised ginsenosides Rb_2, Rc, Rd, Re, Rg_1, Rg_2, 20*(R)*-Rg_2, Rh_1, F_2 and F_3, ginsenoside Re being the most important (Dou *et al.*, 1997). Using Hep G2 liver cells cultured in a cholesterol-rich medium, Park *et al.* (1995b) concluded that the serum cholesterol-lowering effects of ginseng components such as total saponins, ginsenosides Rb_1 and Rb_2 and the non-saponifiable fraction of the ether extract could be partially attributed to increased hepatocellular acyl CoA: cholesterol acyl transferase activity. Further studies are needed using standardised preparations.

Several open clinical studies have indicated the potential value of ginseng in the prophylaxis of atherosclerotic or arteriosclerotic conditions although no large scale placebo controlled double blind studies have as yet been reported (Sonnenborn, 1987). Joo *et al.* (1982) noted that ginseng saponins stimulated phospholipid (very low density lipoprotein) biosynthesis and also that in cholesterol-fed rabbits the ginseng saponins reduced the penetration of cholesterol into the aortic tissue. Therefore a potential preventative action against atheroma formation was indicated. Using standardised ginseng extract G115 (Pharmaton S.A., Lugano, Switzerland) and rhesus monkeys *(Macaca muletta),* Dixit *et al.* (1991) confirmed that lowering of serum triglycerides and cholesterol occurred in hyperlipidemic animals and a 34–72 per cent reduction was reported. The high density lipoprotein-cholesterol/ total cholesterol ratio was increased and the reduction in low density lipoprotein and very low density lipoproteins again prompted the suggestion that ginseng G115 preparations might have beneficial effects as antiatherogenic agents. Saponins from Jilin ginseng rhizome, from ginseng stems and leaves and from *P. quinquefolium* white ginseng were found, in rats, to inhibit the formation of thrombi, decrease osmotic pressure, decrease volume swelling of erythrocytes and increase the fluidity of erythrocytic membranes, factors of value in countering atherosclerosis and ageing (Yang *et al.*, 1992).

Ginseng saponins have also been incorporated in formulations and patented preparations (see Chapter 9) intended to reduce blood clotting in thrombosis cases. Ginseng has antiplatelet action. The blood platelets or thrombocytes are formed by fragmentation of megakaryocytes, a large type of white blood cell formed in the bone marrow. The platelets, round or oval discs about 2 microns in diameter, pass into the blood circulation and play an important role in the blood clotting mechanism. Normal blood contains 200,000 to 400,000 platelets per cubic millimetre and the platelets function to activate the blood clotting mechanism and to plug damage to the blood vessels. When in contact with damaged vascular surfaces e.g. collagen fibres in the vascular wall or damaged endothelial cells, the platelets swell, adopt irregular shapes with projecting processes, and become sticky, adhering to the collagen fibres. Secretion of adenosine diphosphate (ADP) and enzymes leads to the formation of thromboxane A in the plasma and the combination of ADP and thromboxane A activates more platelets to become adhesive and to accumulate and coalesce to form platelet plugs that can effectively block small rents and tears in vessel walls. Greater vascular damage requires the larger structure of the thrombus or clot. As the platelet plug blocks the smaller tears, vasoconstrictor substances are released constricting the blood vessel and initiating clot formation. The

prothrombin activator secreted catalytically converts prothrombin to thrombin which enzymatically converts the soluble plasma protein fibrinogen to insoluble fibrin threads that make a meshwork trapping blood cells, platelets and plasma so forming the blocking blood clot.

Total saponins from *P. quinquefolium* roots were shewn to significantly decrease platelet aggregation rates and to increase superoxide dismutase activity in hyperlipidaemic rats (Li *et al.,* 1996). Protopanaxatriol-derived saponins were isolated from Sanchi ginseng *(P. notoginseng)* and included ginsenosides Re, 20-gluco-Rf, Rg_1, Rg_2, Rh_1 and F_1 and notoginsenosides R_1, R_2, R_3 and R_6. Such triol saponins (1–4 mg/mL) inhibited ADP, collagen and arachidonic acid induced rabbit platelet aggregation *in vitro*. In addition, *in vivo* in rats the protopanaxatriol-derived saponins (75–300 mg/Kg intraduodenally) dose dependently inhibited platelet aggregation, platelet thromboxane A2 release and experimental thrombosis. Thus the anti-thrombotic mechanism was concluded to be mediated by inhibition of platelet aggregation and thromboxane A2 release (Su *et al.,* 1996). Such results indicated potential value in the prevention and treatment of atherosclerosis.

It had already been recorded that the polyacetylenes panaxydol and panaxynol caused concentration-dependent haemolysis (Kim *et al.,* 1988b) and therefore the effect on blood platelets was investigated. Ginseng was known to have an effect on platelets and panaxynol, obtained from the ethereal fraction of ginseng root extract, was considered the most potent antiplatelet factor in ginseng and its action was mainly due to the inhibition of thromboxane formation. Panaxynol caused a marked reduction of the platelet aggregation induced by collagen, arachidonic acid, ADP and thrombin. Ginsenosides from the butanol fraction of ginseng root extract had no such effect although ginsenoside Ro did inhibit adenosine triphosphate release by washed rabbit platelets. The aggregation ability of platelets after panaxydol treatment was not readily regained. In human blood plasma panaxydol prevented secondary aggregation and completely obstructed the epinephrine and ADP-induced adenosine triphosphate release from platelets. Panaxynol, but not the ginsenosides, inhibited thromboxane B_2 formation by platelets although panaxynol and ginsenoside-Rb_2 inhibited increase of intracellular Ca^{+2} caused by collagen (Teng *et al.,* 1989).

A later report suggested that panaxynol from the ether-soluble fraction and the ginsenosides Ro, Rg_1 and Rg_2 from the butanol-soluble fraction were the main antiplatelet components. Panaxynol inhibited the platelet aggregation, the adenosine triphosphate release reaction and thromboxane formation in rabbit platelets but the ginsenosides Ro, Rg_1 and Rg_2 suppressed the release reaction only (Kuo *et al.,* 1990). It has also been suggested that as ginsenoside Rg_1 has potent antiaggregation activity *in vitro* and *in vivo* it may be useful for the prevention and treatment of thrombotic cardiovascular disorders (Yamomoto *et al.,* 1988).

Investigation of the effects of ginseng saponins on blood coagulation was commenced by Matsuda *et al.* (1985). 20 *(S)*-ginsenoside Rg_3 and 20*(R)*-ginsenoside Rg_3 inhibited the platelet aggregation induced by collagen and ADP and 20 *(S)*-ginsenoside Rg_3, 20*(S)*-ginsenoside Rh1 and 20*(R)*-ginsenoside Rh_1 inhibited thrombin-induced conversion of fibrinogen to fibrin. Extending this work further (Matsuda *et al.,* 1986), the action of some ginsenosides on blood coagulation and

fibrinolytic mechanisms *in vitro* were compared with the standard anticoagulants aspirin, heparin and dextran sulphate. Ginsenoside Rg_2 demonstrated marked inhibitory action on the platelet aggregation caused by collagen, endotoxin and arachidonic acid when compared against aspirin as standard at 1.0 mM concentration. Ginsenoside Ro reduced the conversion of fibrinogen to fibrin caused by thrombin at a concentration of 0.1–1.0 mM. On the basis of the action of the enzyme urokinase on plasminogen-containing fibrin plates, it was thought that the ginsenosides Rb_1, Rb_2, Rc, Re, Rg_1, Rg_2 and Ro might promote the action of that enzyme in the fibrinolytic system. Intraperitoneal injection of panaxadiol (200 mg/kg) in rats reduced the viscosity of whole blood and plasma and in rabbits (50 or 70 mg/kg intravenously) inhibited both platelet aggregation and blood coagulation (Xu *et al.*, 1988). Other workers stated that only ginsenoside Rg_1 had potent antiaggregation activity *in vitro* when tested against human platelets stimulated with the aggregating agents collagen and arachidonic acid and suggested that the mechanism of action was by impairment of the thromboxane-A_2-mediated pathway at post-receptor sites. A dose of 50 mg of ginsenoside Rg_1 given orally significantly reduced platelet aggregation (Yamamoto *et al.*, 1988). Also working with human platelets, Kimura *et al.* (1988) reported that ginsenoside Rg1 alone inhibited adrenalin and thrombin-induced platelet aggregation and the release of 5-hydroxytryptamine in a concentration-dependent manner in the range 5–500 μg/mL. Adrenalin and thrombin induce elevation of the calcium Ca^{++} level in the second phase of clotting but ginsenoside Rg_1 reduced such cytosolic free Ca^{++} levels and, in turn, inhibited 5-hydroxytryptamine release and platelet aggregation. Hence the repeated suggestions that ginsenoside Rg_1 may be potentially useful in the treatment of atherosclerosis and thrombotic conditions. More recently Park *et al.* (1994b) concluded that panaxadiol and panaxatriol did not inhibit Ca^{++} influx in adrenaline-stimulated human platelets although inhibiting the formation of thromboxane-A_2 and thus platelet aggregation, probably by blocking the conversion of arachidonic acids to thromboxane-A_2.

Another suggestion was presented by Shi *et al.* (1990) who noted that, in comparison with control animals, aortic atherosclerotic plaque was reduced in rabbits treated orally with *P. notoginseng* total saponins (100 mg/kg/day) for 8 weeks. They observed an increase in prostacyclin (prostaglandin PGI_2) in the carotid artery and decreased thromboxane A_2 in blood platelets and suggested that the anti-atherosclerotic action of the total saponins might be due to correction of imbalance between prostacyclin and thromboxane-A_2. Similar results were reported by Terano *et al.* (1994) who had administered ginsenoside Rc to arteriosclerosis and thrombosis patients for one week. Increase in prostacyclin levels in the urine occurred although the thromboxane A_2 level was unchanged. Rat cell culture experiments confirmed that ginsenoside Rc enhanced the expression of the cyclooxygenase gene in the ribonucleic acid (RNA) in the blood vessel walls, promoting prostacyclin formation. In turn the prostacyclin inhibited vascular smooth muscle cell generation and platelet aggregation as well as exhibiting vasodilatory action, factors reducing thrombosis.

The Korean group of Park *et al.* (1995a) studied the non-saponin, lipophilic fraction extracted from red ginseng roots and noted that it inhibited in a

dose-dependent manner the aggregation of human blood platelets induced by thrombin (0.1 units/ml). It also inhibited the Ca^{++} influx into the platelets, markedly inhibited thromboxane-A_2 formation and caused a rise in cyclic guanosine monophosphate (*c*GMP) concentration. Therefore it was concluded that regulation of the levels of *c*GMP and thromboxane-A_2 was the probable mechanism of inhibition of platelet aggregation due to thrombin. Further studies (Park *et al.*, 1996) using a lipophilic fraction in corn oil prepared from *P. ginseng* roots as a food supplement indicated that the lipophilic fraction increased *c*GMP directly and cyclic adenosine monophosphate (*c*AMP) indirectly and thus inhibited thrombin- or collagen-induced platelet aggregation by increasing the thrombin time and the activated partial thromboplastin time for conversion of fibrinogen to the fibrin threads that trap platelets, blood cells and plasma to form clots. Therefore dietary lipophilic fraction produces an antithrombotic effect *in vivo*.

The value of *P. notoginseng* saponins in the treatment of cerebral ischaemia was demonstrated *in vivo* and *in vitro* in rats. The saponins were administered at a dosage of 200 mg/kg intraperitoneally for 1–3 days and were shewn *in vivo* to significantly inhibit abnormal increases of platelet aggregation and platelet adhesiveness in rats subjected to permanent occlusion of the middle cerebral artery. Platelet aggregation induced by ADP *in vitro* was also inhibited by *P. notoginseng* saponins. Therefore it was suggested that the increased fluidity of the platelets with resultant reduced platelet adhesiveness and reduced platelet aggregation accounted, at least in part, for the anti-cerebral ischaemia action of ginseng (Ma and Xiao, 1998).

Sanchi ginseng *(P. notoginseng)* differs from other common ginsengs in its indigenous usage as a haemostatic agent. Such action has been ascribed to α-amino-β-(oxaloamino)propionic acid, a compound also increasing the blood platelet count and obtained from Sanchi roots (Okan, 1982)) and similarly ascribed to L-dencichin (3-[(carboxy-carbonyl)amino]-L-alanine) (Zhao and Wang, 1986). A freeze-dried powder prepared from an ethanolic extract of roasted *P. notoginseng* roots tended to shorten whole-blood coagulation times and plasma recalcification times one hour after oral administration of 500–2000 mg/kg to rats but did not alter thrombin times (Goto *et al.*, 1987).

GINSENG AND DIABETES

The value of ginseng preparations in the treatment of diabetes mellitus is debatable. Diabetes, diabetes mellitus or sugar diabetes, is a common condition occurring worldwide and amongst all classes of people but more frequently amongst the poor and the aged in modern industrialised communities. In such societies it is rated as the third most common cause of death after cancers and cardiovascular conditions. The disease is characterised by impaired carbohydrate metabolism caused by inadequate production in the pancreatic islets of Langerhans of the hormone insulin, a small protein molecule (molecular weight 5808) comprising two amino acid chains connected to each other by disulphide linkages. In the absence of insulin in the blood stream the blood sugar level rises abnormally (hyperglycaemia) and sugar passes readily into the urine with

resultant glucosuria or polyuria. Such rapid liquid excretion leads in turn to the characteristic thirst of diabetics. Lack of insulin may also be due to autoimmune damage to the islets of Langerhans. In diabetic patients protein and fat metabolism is enhanced with the breakdown of tissue proteins, lipids and fatty acids and the resultant occurrence of nitrogenous and ketone compounds in urine. Overweight persons are more prone to diabetes and have a shorter life expectancy. Complications of untreated diabetes include cataracts and blindness, ketoacidosis (high urinary ketone levels), gangrene of the feet, heart disorders, aetherosclerosis and renal failure.

The causes of diabetes are multifactorial depending on hereditary traits, age, pregnancy, obesity, stress, drug-related factors (corticosteroids and some diuretics), hormonal imbalances, some infections and stress. The common treatment for diabetes is the subcutaneous injection of insulin preparations. Insulin is not considered effective orally and as injection is inconvenient and unpleasant any alternative is worthy of investigation. Ginseng has been suggested as such an alternative because the early Chinese repeatedly recorded its use for the relief of diabetic symptoms.

In the 1950's Chinese researchers confirmed that ginseng extracts and, in particular, red ginseng extracts reduced blood sugar levels and urine acetate in alloxan-induced hyperglycaemia in mice, rats and dogs. Although ginseng demonstrated a hypoglycaemic action it could not adequately correct the metabolic malfunction in alloxan-diabetic dogs and therefore it was concluded that ginseng was no substitute for insulin, not even in combination with a controlled diet (Hou, 1978).

The antihyperglycaemic action of ginseng and its extracts was truly established but some later publications claimed that ginseng and its extracts could also effect an increase in blood glucose level accompanied by an increase in muscle and liver glycogen and a decrease in inorganic phosphates. An alternative view was that ginseng and its extracts turned the metabolic flow towards lipogenesis by conversion of sugars and consequently the sugar level fell in liver, kidneys, muscles and blood. Such observations were confirmed and indicated a ginseng component capable of lowering the blood glucose level and stimulating insulin release in diabetic animals (Hou, 1978). Martinez and Staba (1984) reported that plasma glucose levels in resting rats were reduced by orally administered saponin extracts of Canadian white, American red, Sanchi, Korean red and Shui-Chi ginsengs. Guodong and Zhongqi (1987), using isolated rat pancreatic cells *in vitro,* demonstrated that ginsenosides promoted insulin release which was independent of extracellular calcium and utilised a different mechanism to that of glucose. Another report indicated that *in vivo* in rats a ginseng extract increased the number of insulin receptors in bone marrow and reduced the number of glucocorticoid receptors in rat brain homogenate (Yushu and Yuzhen, 1988). Other workers investigated the effect of ginsenoside Rg_1 on insulin binding in mouse liver and brain membranes. Administered at a dose of 10 mg/kg daily ginsenoside Rg_1 significantly increased ^{125}I-labelled insulin binding in both liver and brain, the increase being related to an increase in the number of insulin receptors rather than to a change in receptor affinity (Chilyan *et al.,* 1991). It

was thought that all of these suggestions contributed to the antidiabetic action of ginseng as the diabetogenic action of adrenal corticoids has been established and the number of insulin receptors usually decreases with ageing.

In the mid-1980's the interest of the Japanese team of Kawashima, Oura and Yokazawa turned to the antidiabetic potential of ginsenoside Rb_2. Their work shewed that in rats with streptozotocin-induced diabetes ginsenoside Rb_2, 10 mg in 0.5 mL saline per day administered by intraperitoneal injection, caused a moderate reduction of the blood glucose level, lowered the serum lipid level, especially the very-low-density lipoprotein, and reduced serum triglyceride, nonesterified fatty acids and total cholesterol. Ginsenoside Rb_2 also reduced the 3-hydroxybutyrate and acetoacetate levels thereby indicating an improvement of diabetic keto-acidosis. In addition there was a rise in glucokinase activity in the liver and a decrease of glucose-6-phosphatase activity. Hepatic lactate levels were unchanged or slightly decreased. Body weight increased although the test animals ate less food than the corresponding control animals. Nevertheless ginsenoside Rb_2 improved diabetic symptoms such as over-eating, polyuria and glycosuria (Yokozawa *et al.*, 1985b). Not surprisingly, in 1986 a Japanese patent was secured for ginsenoside Rb_2 as an effective antidiabetic and in 1994 another Japanese patent was obtained for blood sugar lowering diabetic foods containing ginseng extract and vitamins (see Chapter 9).

Further work demonstrated a marked decrease in the blood urea nitrogen level in the streptozotocin-induced diabetic animals treated with ginsenoside Rb_2 and this was accompanied by increased total protein and amino acids such as lysine, glycine, glutamic acid, arginine, etc., in serum but there were no significant changes in serum albumin. In the liver the urea content was also reduced with a corresponding increase of ribonucleic acid. The increase in ribosomes was mainly due to membrane-bound ribosomes. Ginsenoside Rb_2 normalised the liver concentrations of glutamic acid, phenylalanine and tyrosine. Thus ginsenoside Rb_2 suppressed total urinary nitrogen excretion, increasing nitrogen retention in the body and improving the overall nitrogen balance (Yokozawa *et al.*, 1989). Administration of ginsenoside Rb_2 (10 mg/day) for 3 days produced no significant increase in serum protein and albumin levels but at 6 days there was a significant increase. Using radioactively labelled [^{14}C]-leucine it was proved that activated protein biosynthesis was occurring after 3 days although obvious increases appeared at 6 days (Yokozawa *et al.*, 1990). However it was noted that after 6 days treatment with ginsenoside Rb_2 there was no change in the blood insulin level. Therefore the ginsenoside Rb_2-induced lowering of blood sugar level and the improvements in sugar and lipid metabolism in rats with streptozotocin-induced diabetes is not associated with an increase in insulin. Two years later Joo *et al.* (1992) analysed the livers of rats suffering from streptozotocin-induced diabetes and observed the decreased enzyme activities of glucose-6-phosphatase, acetyl-coA-carboxylase and 6-phosphogluconate dehydrogenase. *In vivo* treatment of diabetic rats with ginseng saponins increased the activity of these enzymes although other hepatic enzymes such as pyruvate kinase, malic enzyme and glycogen phosphorylase were unaffected. In addition, ginseng saponins exerted a hypoglycaemic effect and

insulin biosynthesis in the liver was apparently enhanced. Wang *et al.* (1993) reported that ginseng stem and leaf total saponins (150 mg/kg/day) given orally to diabetic rats for 20 weeks decreased blood sugar levels and lipid peroxidation as well as increasing the depressed superoxide dismutase activity. It was therefore suggested that ginsenosides, like soyasaponins, protected diabetic rats from free radical injuries to some extent, thereby improving quality of life. Later work by Ohnishi *et al.* (1996), employing orally administered aqueous extracts of *P. ginseng* roots in mice, indicated that blood glucose levels in normal and epinephrine-induced hyperglycaemic mice were reduced significantly after 4 hours. Analysis of the livers of normal and hyperglycaemic mice and comparison with controls revealed a significant increase in facilitative glucose transporter isoform 2, the liver type glucose transporter protein GLUT2. Therefore it was suggested that this increase in GLUT2 protein accounted in part for the hypoglycaemic action of ginseng.

Enzyme studies involving rats had suggested that the hypoglycaemic action of ginsenoside Rb was probably due to changes in glucokinase and glucose-6-phosphatase activity but subsequent discovery in *P. ginseng* roots of high polymer peptidoglycans, glycans and other peptides, compounds with hypoglycaemic activity, and the publication of results suggesting that chemically pure ginsenosides do not produce an insulin-mimetic effect *in vivo,* ginsenosides Rb_1 and Rg_1 being found to decrease islet insulin concentration to an undetectable level, has stimulated further research seeking the real hypoglycaemic agent (Waki *et al.,* 1982). Peptidoglycans A-E isolated from several species (*P. japonicus, P. quinquefolium, P. pseudoginseng, P. notoginseng, P. bipinnatifidus* and *P. transitorius*) have been shewn to have hypoglycaemic properties in mice and were patented in Japan in 1985 (see Chapter 9). Glycans, quinquefolans A-C, were isolated from aqueous extracts of *P. quinquefolium* roots and, on intraperitoneal injection into normal and alloxan-induced hyperglycaemic diabetic mice, reduced blood glucose levels (Oshima *et al.,* 1987). Zhang *et al.* (1988) suggested that a peptide isolated from ginseng possessing an amino acid sequence of Glutamic acid-Threonine-Valine-Glutamic acid-Isoleucine-Isoleucine-Aspartic acid-Serine-Glutamic acid-Glycine-Glycine-Glycine-Aspartic acid-Alanine was insulin-like.

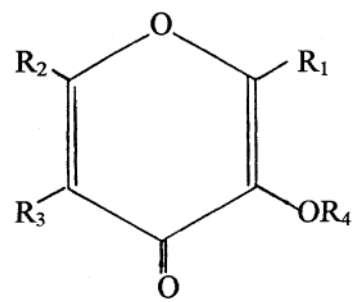
Further compounds were sought by many scientists. One suggestion was the nonsaponin component DPG-3–2 isolated from *P. ginseng* roots. DPG-3–2 had been shewn to stimulate insulin biosynthesis in pancreatic preparations from various hypoglycaemic test animals but not from normoglycaemic animals (Waki *et al.,* 1982). In 1984 Hikino and his team reported the isolation of a series of 21 high polymer peptidoglycans from various *Panax* spp. Panaxans A-E were obtained from the polysaccharide fraction and further work produced panaxans F-U (Konno *et al.,* 1984, 1985). Panaxans A-E from Korean or Chinese roots were reported to possess greater haemolytic activity than panaxans Q-U from Japanese ginseng. All of these compounds shewed dose-dependent hypoglycaemic activity in normal and alloxan-induced diabetic mice if given by intraperitoneal injection although they were ineffective if given orally as the high polymer glycans are unlikely to be absorbed from the gastrointestinal tract. It was also stressed

that the differential effects in young and elderly animals required further study.

Nevertheless Hikino and Konno (1990) patented the isolation and characterisation of a group of polysaccharides named Karusans A-E from ginseng roots; these compounds were considered potentially useful as hypoglycaemic agents (see Chapter 9).

Seeking insulin-like substances in Korean red ginseng roots, Takaku *et al.* (1990) isolated adenosine (adenine riboside) and pyroglutamic acid, compounds inhibiting epinephrine-induced lipolysis yet stimulating insulin-mediated lipogenesis from glucose. Pyroglutamic acid, an amino acid described as a selective modulator, exhibited selective modulation towards the opposite metabolic pathways in rat adipocytes, inhibiting lipolysis yet stimulating lipogenesis.

Another group of compounds investigated was the γ-pyrones, compounds of the formula:



(6-1)

where R_1, R_2, R3=H, alkyl, alkoxyalkyl, haloalkyl, alkenyl, oxo or oxy-substituted alkyl and R_4=H, $C_{2\text{-}6}$ acyl (Yoon and Kawamura, 1994). In particular ginseng roots yield maltol (3-hydroxy-2 methyl-4-pyrone), a compound which, given to mice at a dose-level of 5 mg every other day for 2–20 weeks, produces autoimmune diabetes by the 35th week.

Using Korean ginseng extract and adult streptozotocin-diabetic albino Wistar rats, Hassan *et al.* (1994) administered 25 mg/kg or 100 mg/kg ginseng extract with or without 15 mg/kg of glipizide (sulphonyl urea). Hypoglycaemia, hypocholesterolemia and hypotriglyceridemia were observed in all treated animals and there was an increase in serum phospholipids and, in 80 per cent of treated animals, an increase in potassium ion concentration (hyperkalemia) and magnesium and zinc levels. Serum calcium levels fell in 60 per cent of treated animals. There was little or no hepatotoxicity but the alkaline phosphatase level was slightly increased.. This work shewed that chronic treatment with ginseng extract alone at a lower dose regimen produced more significant effects on the studied parameters after 4 weeks and was preferred to combined therapy.

Clinical trials on human diabetic patients have been few. El-Nasr *et al.* (1982) conducted a placebo-controlled crossover study of diabetics treated with 80 mg of standardised ginseng extract G115 for 3 weeks in each month for 3 consecutive months. They reported that post-prandial blood glucose levels and diabetic neuropathy improved significantly during the trial. Sotaneimi *et al.* (1995) investigated the effect of ginseng treatment on newly diagnosed

non-insulin-dependent diabetes mellitus patients using a double-blind placebo-controlled trial involving 36 human subjects. The treatment was either 100 or 200 mg doses of ginseng or placebo given daily for 8 weeks. Ginseng treatment elevated mood, improved psychophysical performance and reduced fasting blood glucose and body weight. The 200 mg ginseng dose improved glycated haemoglobin, serum aminoterminalpropeptide and physical activity. Placebo treatment reduced body weight and changed the serum lipid profile but did not alter the fasting blood glucose level and it was concluded that ginseng could be a useful adjunct in the management of non-insulin-dependent sugar diabetes.

Therefore there is still no real evidence that ginseng can substitute for insulin, but it may improve the lifestyle of diabetics by off-setting side effects such as general malaise, fatigue, impotence, thirst and anaemia and may even help to reduce the insulin dosage required.

Further work on individual saponin glycosides, polyacetylenic compounds, polypeptides and polysaccharide complexes in the laboratory to prove the mechanism of action and, in medical practice, to prove therapeutic reliability is still required.

GINSENG AND APHRODISIAC ACTIVITY

The effect of ginseng on sexual activity has intrigued the general public and many research workers. Bao *et al.* (1984b) observed that there was no disturbance of the ovarian cycle in stressed female mice if ginseng was administered. Bao *et al.* (1984c) also reported that ginseng extracts given to male mice prevented stress-induced decrease of sexual activity. Similarly Lian and Zhang (1998) also noted that repeated daily hanging stress reduced sexual activity in male mice as assessed by licking, mounting and mating activity. The plasma testosterone level was reduced but treatment with ginsenoside Rb_1 (2.5, 5 or 10 mg/kg, i/p) before each stress event countered the repeated stress-induced sexual deficiency and raised the plasma testosterone level. It was concluded that ginsenoside Rb_1 was capable of maintaining the normal plasma testosterone level.

The reported occurrence of oestrone, oestradiol and oestriol in liposoluble fractions of ginseng extracts, based on TLC results, was not confirmed. Some workers likened tail erection produced in test animals to aphrodisiac agent effect. Other workers suggested that increased deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and protein formation due to ginsenoside administration offered an explanation. The hormone-like phenomenon was also related to the superficial structural resemblance of steroid hormones and digitalis cardioactive glycosides to the ginsenosides, but closer investigation of the spatial conformations of such compounds clearly shows that the ginsenosides are quite different and would not interfere with the steroid hormone receptors and, under physiological conditions in human subjects, the ginsenosides do not interfere with the progesterone receptors. Thus, despite the many reports of the use of ginseng in the treatment of impotence and penile dysfunction, no simple explanation has, as yet, emerged concerning an action probably involving mixed metabolic processes, mixed ginsenosides and possibly some so far unidentified related

compounds. Therefore the reputed sex hormone-like activity of ginseng has not, as yet, been adequately explained. It could be argued that improved general health and quality of life as a result of ginseng treatment would be accompanied by improved normal activities such as sexual relationships.

GINSENG IN COSMETIC PREPARATIONS

Ginseng saponins are structurally near chemical relatives of the antiinflammatory compounds such as steroids and glycyrrhetinic acid from liquorice (*Glycyrrhiza glabra* L.). It is thus not surprising that antiinflammatory properties have been reported for ginseng extracts and ginseng has been suggested as an ingredient of cosmetic creams with non-allergenic, cutaneous bioactivity for the treatment of wrinkles and eczema. The many recent patent applications involving cosmetic creams, anti-wrinkle creams, acne applications and hair growth preparations with supporting claims of efficacy suggest probable effectiveness (*cf* Chapter 9). Curri *et al.* (1986) had reported that ginseng extract applied topically as a phospholipid liposomal formulation produced a favourable effect on skin ageing, moisturisation of the stratum corneum and improved skin elasticity. Saponins of ginseng, the ginsenosides Rb_1, Rb_2, Rc, Re and Rg_1, as well as 70 per cent methanolic extract of *P. ginseng,* increased the production of glycosaminoglycans by cultured human skin fibroblasts; ginsenoside Rb_2 was particularly effective but the non-saponin fractions had no action (Tanaka *et al.,* 1991). Resultant skin improvement suggested a cosmetic application. Another dermal application of ginseng involved the treatment of the viral conditions herpes labialis (cold sores) and herpes simplex. Ginsenosides of the leaves and stems of *P. ginseng,* particularly ginsenosides Rb and especially ginsenoside Rb_2, were shewn to suppress replication of viruses such as herpes viruses HSV-I and HSV-II, adenovirus-III, etc. Creams containing ginsenosides were tested *in vitro* and *in vivo* on herpes labialis induced by HSV-I; the success rate was 87.1 per cent (Li *et al.,* 1992b). Korean red ginseng was formulated as a vasodilator in a cosmetic preparation described as a non-invasive novel method for lip augmentation capable of increasing lip size for up to 4 hours (see Frome, B.M. in Chapter 9).

Ginseng has also been formulated with many other plant materials and some chemical compounds in cosmetic products but there is still need for reliable, unbiased tests proving efficacy. Otherwise many products will continue to be regarded as doubtful placebos.

CONCLUSIONS

The above survey of the pharmacology and therapeutic potential of ginsengs indicates that ginseng can strengthen the debilitated body, stimulating recuperation and improving the quality of life. Ginseng has been shewn as an agent capable of improving memory and intellectual skills at all ages and it is also certain that ginseng is of value in the countering of stresses due to temperature variation, physical strain, disease states and toxic substances. Less clear is the anti-ulcer effect as ginsengs are usually used in combination with other plants containing substances such as mucilages, pectin polysaccharides and dextrins.

There is evidence of the protective effects of ginseng in old age. Protection from neural degeneration, preservation of antioxidant levels and inhibition of malondialdehyde formation collectively retard the inexorable advance of age related deterioration. Therefore ginseng has potential in geriatric tonics and medicines for the treatment of conditions such as normal ageing, cerebral vascular disease in the aged, senile dementia and Alzheimer's disease.

Other potential applications of ginseng in anticancer treatment, as liver protective agents, in alcohol intoxication therapy, for morphine, cocaine and amphetamine withdrawal problems, in topical preparations for skin affections such as acne and eczema and in cosmetics still require careful clinical trials to demonstrate indisputably that standardised ginseng phytochemicals or formulations are really effective in human subjects.

At the practical level, it has been suggested that young and healthy persons should take ginseng in short courses of 2–3 weeks with a two week interval between consecutive courses. The recommended daily dose is 0.5–1.0 g of powdered root or 200 mg of ginseng extract daily divided into two doses, one in the morning two hours before food and one in the evening at least two hours after food. It has also been recommended that ginseng should not be taken continuously for periods exceeding three months and others suggest occasional use in treatments comprising a one month course followed by a two months interval before further treatment. Ginseng treatment can be continuous in the aged and the chronically sick. Concurrent use of stimulants such as coffee is not encouraged.

SUMMARY

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22. Ginseng and Cosmetics

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7. THE SIDE EFFECTS OF GINSENG ADMINISTRATION

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Much publicised adverse reactions to synthetic medicines created a climate of mistrust amongst the vulnerable sick and such hostile reaction to allopathic medicines has contributed to the oft quoted idea that if the medicine comes from natural sources it must be safe. Nevertheless some writers have referred to the potential hazards of ginseng use. Long term usage in particular might involve hypotension, hypertension and steroid type poisoning, yet very few reliable reports of adverse reactions to ginseng use are available despite the worldwide consumption of ginseng.

Palmer *et al.* (1978) referred to a single case of a 70 year old woman who, after 3 weeks ingestion of ginseng powder, presented with tender, swollen breasts with diffuse nodularity despite an improved feeling of well-being; the symptoms subsided on discontinuance of the ginseng treatment. In explanation they suggested a mild hormonal action. In the same year Koriech (1978) had reported five cases involving female patients demonstrating enlargement of the nipples accompanied by increased libido.

Siegel (1979) also suggested avoidance of large doses of ginseng because of a possible corticosteroid effect although he noted few serious side effects in a study of 133 patients. In a 2-year study of participants using ginseng roots, capsules, tablets, teas, extracts, cigarettes, chewing gum and candies, mainly orally, the reported side reactions included morning diarrhoea (47), skin eruptions (33), sleeplessness (26), nervousness (25), hypertension (22), euphoria (18) and oedema (14). His work has been criticised because it did not form a true "double blind" trial and his patients' other life-style activities, e.g. ingestion of large quantities of caffeinated beverages, may have contributed to the resultant side effects. He stated that long term abuse might be associated with hypertension prompted by the dammarenetriol glycosides or hypotension due to the dammarenediol glycosides.

Punnonen and Lukola (1980), citing a single case, also drew attention to the oestrogen-like effect of ginseng and Greenspan (1983) connected ginseng ingestion and vaginal bleeding. Another report described a single case of postmenopausal vaginal bleeding alleged to be associated with the facial use of a topical ginseng cream and related to an oestrogen-like action (Hopkins *et al.*, 1988).

Nevertheless Hess *et al.* (1982), studying two generations of Sprague-Dawley rats and employing the standardised Korean ginseng root extract G115, could find no treatment-related effects on lactation, reproduction, body weights, food

consumption, haematology, clinical chemistry, gross and histo-pathology, etc. during weekly tests and later, gross autopsies.

In Switzerland the Pharmaton group subjected their standardised product Ginsana G115 to extensive toxicity tests, teratogenicity tests, carcinogenicity tests and tests designed to reveal any other abnormal pathology. The accumulated results demonstrated the safety and reliability of their standardised products. Beagle dogs treated with subchronic doses of 1.5, 5 or 15 mg/kg per day for 90 days demonstrated no treatment-related effects concerning body weight gain, food consumption, ophthalmology, haemotology, clinical chemistry or gross and histopathological findings (Hess *et al.,* 1983).

Hou (1978) summarised the then published observations on toxic doses. In 1926 Yonekawa had reported the lethal dose of the then isolated saponin glycoside ginsenin as 2–3 g/kg in mice. Almost forty years later Kitagawa and Iwaki (1963) stated that the lethal dose of ethereal extract of ginseng was 5g/kg in mice. Brekhman (1969) reported that for mice the LD_{50} value (dosage lethal to 50 per cent of the animals in a test) was 10–30 g/kg of *Panax ginseng* whole root and for the pure ginseng saponins, the panaxosides, given orally 1.4 g/kg. Thus he concluded that, for mice, the toxicity of the isolated total saponins was 10–20 times less than for the pure saponins. Another worker, Kaku (1975), recorded the LD_{50} dosage given intraperitoneally in mice as 1.25 g/kg.

More recent work in Korea has suggested that the average LD_{50} dose of purified ginseng saponins in male mice is 270 mg/kg intravenously, 342 mg/kg intraperitoneally, 505 mg/kg intramuscularly, 950 mg/kg subcutaneously and >5000 mg/kg orally (Rhee *et al.,* 1982). Despite such findings Barna (1985) commented on the dearth of well-controlled and quantitative human experiments although our Western medical literature contained a growing number of adverse reports referring to mastalgia, hypertension, morning diarrhoea, skin eruptions, sleeplessness, nervousness, oedema, depression and amenorrhoea. Such reactions may have been related to the high doses of ginseng consumed by users who received little guidance from labels on ginseng packaging, ginseng being regarded in America in 1985 as a food supplement.

Antibiotic drugs are widely used in western medicine and, not surprisingly, Kim and his colleagues (1987) investigated possible *in vitro* reactions between common antibiotics and ginseng. Using the Checkerboard Method they were able to demonstrate that there was no reaction between kanamycin, oxytetracycline and chloramphenicol respectively and ginseng saponin versus *Pseudomonas aeruginosa* and between ampicillin and chloramphenicol respectively and ginseng saponin versus *Mycobacterium smegmatis*. There was some synergistic action between ampicillin, kanamycin, oxytetracycline and chloramphenicol respectively and ginseng saponin versus *Bacillus subtilis* and ampicillin and cephalexin respectively with ginseng versus *Staphylococcus aureus*. No antagonism between the antibiotics and ginseng was discovered.

In 1995 Ryu and Chien reported from Taiwan of a severe headache reaction to the ingestion of a large quantity of ethanol-extracted ginseng. Cerebral angiograms revealed a "beading" appearance in the anterior and posterior cerebral and superior cerebellar arteries, consistent with cerebral arteritis in the

28-year old subject. They concluded a causal relationship between ginseng ingestion and cerebral arteritis. In the same year, Chan (1995) stressed the danger of widespread usage of Chinese herbal medicines containing contaminating poisonous substances and particularly anticholinergic alkaloids such as the atropine-like compounds in *Datura metel* and *Mandragora officinarum* L., the latter being a possible adulterant of ginseng.

In the following year a Canadian physician reported a ginseng-digoxin interaction (McRae, 1996). A 74-year old male patient, who had been maintained on digoxin for 10 years and normally presented with digoxin serum levels between 0.9 and 2.2 nmol/l, was routinely examined and surprisingly an elevated digoxin serum level of 5.2 nmol/l was recorded. Decreasing the patient's digoxin dose and ultimately discontinuing it after 10 days had little effect on the serum levels which remained high for a further 14 days. The patient showed no obvious symptoms but the riddle was solved when the patient admitted taking ginseng. It was then found that ginseng would cause the apparent elevated digoxin estimation. The author suggested that ginseng components which are chemically related to the cardiac glycosides may have been converted in vivo to digoxin. However, as the patient was asymptomatic and the ginseng was in fact the Siberian ginseng *Eleutherococcus senticosus,* it seems more plausible that the serum assay method employed produced a false-positive result which was too high (*Can. Med. Ass. J.,* (1996). Therefore much care is needed in selecting suitable biochemical methods of analysis and it is important to know the precise identity of any other medication, allopathic or herbal, taken concurrently by the patient.

The latest report of probable drug interaction concerned warfarin and ginseng. A 47-year old American, who had received a mechanical heart valve implanted in the aortic position, was treated with warfarin coumarin-type anticoagulant therapy for 5 years in order to prevent possible embolisms. In addition he had taken as required the antianginal and antihypertensive diltiazem hydrochloride for 7 years, the involuntary muscle relaxant nitroglycerin for 5 years and the diuretic salsalate for 3 years. The International Normalised Ratio (INR) had varied between 3.0 and 4.0 for 9 months prior to the ingestion of Ginsana ginseng capsules. To improve his "energy level" the patient took one capsule three times a day. Four weeks prior to the ginseng his INR was 3.1 yet within two weeks of ginseng ingestion it had fallen to 1.5 but within two weeks of the cessation of the ginseng treatment the INR had returned to 3.3, indicating a probable warfarin-ginseng incompatability (Janetzky and Morreale, 1997).

During a clinical trial involving 382 patients ingesting capsules of a ginseng-vitamin combination one patient reported a cutaneous reaction and another complained of headache, general discomfort and nausea symptoms; neither was serious and the symptoms rapidly disappeared on cessation of treatment. Two patients reported increased libido as a side effect (Caso Marasco *et al.,* 1996). Another recent trial involving 227 volunteers only recorded 8 minor adverse reactions, 4 being insomnia, one nausea with vomiting, one nausea with epigastralgia, one epigastralgia and one anxiety and, as before, the

symptoms rapidly subsided on cessation of treatment (Scaglione *et al.,* 1996). One volunteer reported insomnia after ingesting placebo capsules.

Reporting a retrospective cohort study involving 1800 patients during the period 1980–1993, Lillo (1998) commented on the small number of adverse reactions. Only 17 adverse events were recorded, comprising epigastric disorders (12 cases), hypertension (1 case), muscular pain (1 case) and erythema (3 cases). No suspension of treatment or prescribed counter measures were required. In addition it was noted that there were no clinically significant variations in body weight or systolic and diastolic blood pressures and it was concluded that ginseng was a valuable and safe preparation.

Similarly Della Marchina and Renzi (1998), conducting a randomised double-blind trial on 72 senile patients with compensated non-insulin dependent diabetes and mild cognitive disorders, noted that Ginseng Extract G115 was well tolerated as only 4 cases were recorded with adverse events of short duration (nausea and gastric upset) related to the treatment. Such events did not require specific treatment.

Considering the amount of ginseng consumed worldwide, it is clear that very few cases of drug interaction have been substantiated and ginseng, especially if administered as a carefully standardised product, must remain a substance characterised by low toxicity, fairly large oral dosage, slow action on ingestion and steady development of improved natural resistance and recuperative power.

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8. THE QUALITY CONTROL OF GINSENG

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Plant drugs usually present difficult standardisation problems. The chemical constituents are frequently present as a collection of closely related compounds, each compound possessing its own specific chemical features and pharmacological actions. In addition, plant material is variable according to species variation, time of collection, growth conditions, storage conditions, etc. Nevertheless plant derived drugs should in general conform to the current conventional standards for medicinal products. Therefore such drugs should be:-

a) reliable, being of consistent nature, substance and quality,
b) safe, causing no untoward effects at normal recommended dose levels or during prolonged use, and
c) efficacious, the consumer not being misled by false or unsubstantiated extravagant claims.

Pharmacopoeial monographs are not universally available for ginseng. Pharmacopoeias from Austria, China, Czechoslovakia, France, Germany, Japan, Russia and Switzerland, the Martindale Pharmacopoeia in Britain and the unofficial British Herbal Pharmacopoeia include information on *Panax ginseng* C.A.Meyer. The Chinese Pharmacopoeia also includes Radix notoginseng from *P. notoginseng* (Burkhill) Hoo and Tseng, Rhizoma Panacis from P. *japonicus* and Rhizoma Panacis Majoris from *P. japonicus* var. *major* and *P. japonicus* var. *bipinnatifidus*. The Japanese Pharmacopoeia includes Red Ginseng, the dried and steamed root of *P. ginseng*.

The labelling of crude drugs of natural origin is often misleading as a name such as ginseng can be easily misinterpreted especially when applied to wrong species. In the United States of America canaigre, the roots of *Rumex hymenosepalus* Torrey, family Polygonaceae, have been marketed under the common names "Wild red American ginseng" and "Wild red desert ginseng". The species, which is not related botanically to true ginseng, grows wild in the dry regions of Texas and Mexico and does not yield ginsenoside saponins and therefore cannot possess the pharmacological properties of true ginseng. It does contain tannins and anthraquinones and its astringent properties are exploited in indigenous medicine in the treatment of diarrhoea, skin complaints, and sore throats. The U.S. Herb Trade Association in 1978 introduced a policy of clearly indicating that this plant is not a ginseng at all. Being rich in tannins it is however a potential carcinogenic agent (Tyler *et al.,* 1988).

A species more closely related to Panax ginseng is *Acanthopanax senticosus* (Rupr. et Maxim.) Harms=*Eleutherococcus senticosus* Maxim. Occurring as a tall shrub indigenous to eastern Siberia, the Shansi and Hopei provinces of China and Korea, it is known as Eleuthero or Siberian ginseng and also belongs to the family Araliaceae. A further complication is the pharmacological action of the constituents, which, although not identical with the ginsenosides, do produce stimulant, tonic and antistress activities similar to ginseng. Therefore, as with *R. hymenosepalus,* standardisation is essential and precise, distinct labelling imperative.

Apart from correct identification of the macroscopical and/or microscopical characteristics of the plant material by traditional pharmacognostical methods, there is need to carefully store and preserve ginseng preparations whether crude plant materials or extracts or other pharmaceutical preparations or products such as cosmetics, confectionary and cigarettes containing ginseng as an additive.

The stability of the major constituents in well-stored ginseng was confirmed by Shibata (1994) who investigated 1200 year old samples of ginseng stored since 756 A.D. in the Imperial Treasure House of Shosoin in Nara, Japan and was able to show that the roots retained their characteristic active principles, the ginsenosides. Notwithstanding such evidence changes in physical and chemical properties of ginseng are likely to occur during collection, preparation for the commercial market and subsequent storage before sale.

A prerequisite of successful storage of ginseng roots or powder is careful drying and maintenance of a dry atmosphere. During storage at high relative humidity the levels of saponins in white ginseng, particularly of ginsenosides Rb1, Rc, Re and Rg, were reduced significantly. Storage at relative humidities between 75 and 96 per cent produced an initial increase in glucose and fructose content followed by gradual decrease indicating hydrolysis of the glycosides. At relative humidities <67 per cent the glucose/fructose levels increased progressively but the sucrose content fell especially at relative humidities >75 per cent (Noh *et al.,* 1983). Stoffert (1997), investigating the harvesting and marketing of *P. quinquefolium* in Canada, observed that fresh roots could be stored for 4–6 weeks without loss of quality and advised a drying temperature of 38° C because at below 30° C mildew was likely to develop and above 40° C caramelisation would occur.

Studying changes in moisture and ginsenoside contents during storage, Lui and Li (1995) noted that red ginseng and sundried ginseng contained more moisture than artificially dried root and reported that after 1 year the ginsenoside content was reduced by 25.18 per cent for red ginseng and 32.85 per cent for sundried ginseng although lyophilised ginseng with a much lower moisture content shewed little change. The variability of the commercial drug was investigated because an earlier communication (Choi *et al.,* 1985) had indicated that after 3 years storage the total saponins of red ginseng had decreased by 12 per cent whilst those of airdried white ginseng had reduced by 27 per cent. They also stressed the variability of the panaxatriol/panaxadiol ratio on storage and the concomitant variation in biochemical and pharmacological effects.

An alternative method of preservation is freeze-drying the product. Using a

colorimetric method for analysis of 7 products, Tai (1982) noted that freeze-dried ginseng retained a higher total ginsenoside content. This was confirmed by Zhang (1983) who, using a colorimetric method based on the Liebermann-Burchard reaction, concluded that the total ginsenoside content was highest in freeze-dried roots, less in sun-dried roots and least in boiled roots. Xu *et al.* (1986) also confirmed that freeze-dried roots were richer in saponins than naturally dried roots and that steam-dried roots yielded the least amount of saponins but more of the ginsenosides Rh_1 and Rh_2 (0.485 and 0.0274 per cent respectively). Zhang (1983) also noted that the highest yield of total ginsenosides occurred in fibrous roots (9.74 per cent), less in lateral roots (6.41 per cent) and least in main root (3.31 per cent). Comparing fresh ginseng stored at 4° under 87–92 per cent relative humidity for 10 weeks with a corresponding freeze-dried sample Jang and Shim (1994) concluded that there were no significant changes between the samples although about 9 per cent shrinkage had occurred, pH was slightly elevated and the reducing sugar content increased during storage. Longer term storage was investigated by Yu and Hou (1996) who stored ginseng samples at -15° C with appropriate humidity for more than 5 years and reported that worm infestation, fungal growth, discolouration and cracking did not occur and flavours were well preserved.

Preservation of crude drugs during storage can also be enhanced by treatment of the plant material with ethylene oxide gas or by γ-ray irradiation. Both procedures efficiently destroy microorganisms such as moulds. Cho *et al.* (1994) demonstrated that irradiated ginseng powders (5–10 kGy) could be stored for 7 months at 30° C in different relative humidities without microorganism contamination. Although ginseng saponins were stable to sterilisation, sulphur-containing amino acids, reducing sugars and acidity changed considerably during ethylene oxide treatment. Later Kwon *et al.* (1990) confirmed that ^{60}Co irradiation did not affect the saponins, especially ginsenoside Rg_1, and that the free carbohydrates underwent little change. They, like other workers, concluded that it was safe to irradiate ginseng at 10 kGy in order to extend the shelf life of ginseng preparations but stated that at dose levels exceeding 10 kGy there was a significant decrease in sulphur-containing amino acids and tyrosine. Subsequently they investigated the effects of ethylene oxide fumigation and γ-irradiation at 5 kGy on the chemical constituents of exportable ginseng leaf tea; the ginsenosides and ginseng fatty acids remained stable under both treatments but glucose, histidine and lysine, whilst virtually unchanged by γ-irradiation, decreased considerably under ethylene oxide sterilisation (Kwon *et al.,* 1992). Han *et al.* (1995a) confirmed that ginsenosides and polyacetylenes in ginseng products changed little after 10 kGy irradiation although polyacetylenes and phenolic acids varied from fresh ginseng powder. Han's group considered that γ-irradiated ginseng was more stable than ethylene oxide-treated material although both samples yielded similar antioxidant activity (Han *et al.,* 1995b). Examining γ-irradiated *P. quinquefolium* samples, Chen *et al.* (1995) observed that unstable free radicals indicating permanent radiation damage were formed when the radiation dose exceeded 12 kGy. More recent studies (Byun *et al.,* 1997) confirmed that γ-irradiation of Korean red ginseng powder

Figure 10. Mature washed and cleaned white *Panax ginseng* roots.

at 7.5 or 10 kGy produced no significant changes in composition, pH, browning pigment, hydrogen donating activity, fatty acids, minerals and saponins although glucose, sucrose and maltose content was significantly increased ($P<0.05$). Yoshikawa *et al.* (1993) reported that a far-infrared method using an oven temperature of 45° C effectively dried ginseng root without reduction in ginsenoside and malonyl-ginsenoside content as estimated by high performance liquid chromatography (HPLC). Compared with normal air-drying and hot air drying techniques the far-infrared method was much faster.

In ideal circumstances ginseng extracts are prepared from well-washed, clean, dry roots (Fig. 10) which have been checked carefully by botanical, microbiological and chemical methods for identity and the absence of of pesticides, aflatoxins, heavy metals, etc., and assayed for the presence of individual ginsenosides. The roots are comminuted before extraction with an alcohol/water solvent at room temperature. After separation of the liquid extract by filtration and pressing the resultant marc, the bulked liquids are concentrated to a viscous liquid which is sterilised by irradiation prior to spray-drying to yield a dry extract in powder form. This resultant product can be assayed for individual ginsenoside content and batches mixed to yield a consistent commodity that can be incorporated in pharmaceutical preparations e.g. liquid medicines such as elixirs, capsules, tablets, etc., before terminal assay. Thus products such as Pharmaton's G115 set a high pharmaceutical standard. Unfortunately not all commercially available preparations emulate these standards.

Ginseng extracts and liquid pharmaceutical preparations have been tested for stability. Ginsenosides are normally labile under acidic conditions and common acid hydrolysis is accompanied by side reactions such as epimerization, hydroxylation and cyclisation of side chains of the sapogenins. For example, the C-20 glycosyl linkage of ginsenosides is readily hydrolysed on heating with acetic acid to yield an equilibrated mixture of 20*(S)*- and 20*(R)*- epimers. Also demalonylation of malonyl ginsenosides, elimination of the C-20 glycosyl residues and isomerisation of resultant C-20 hydroxyl group configurations can occur during steaming and heating preparation processes. The pharmacological activities of the changed compounds due to poor formulation, unsatisfactory processing or bad storage may be very different to those of the original material.

Measuring moisture, protein, fat, ash, fibre, total sugars and saponins, Cho *et al.* (1981) reported on the variability of the commercial solid extracts, one of which contained 46.5 per cent moisture. The total saponin content of other extracts varied; the two best extracts yielded 16.16 and 13.12 per cent respectively but the remaining extracts yielded less than 9.0 per cent. Choi *et al.* (1981) observed changes in concentrated red ginseng extract manufactured from ginseng tails by aqueous extraction and subsequent concentration under reduced pressure. Initially during storage at 60–100° C the colour darkened quickly and the glucose, fructose and sucrose levels decreased. HPLC analysis shewed a change in the saponin pattern and particularly so in the protopanaxatriol group. Peak heights for ginsenosides Re and Rg_1 decreased and peak heights for ginsenosides Rg_2 and Rh increased. Refluxing white and red ginsengs in water for 6 hrs Kitigawa *et al.* (1987) observed the degradation of the ginsenosides. On the one hand the

bisdesmoside group (ginsenosides Rb_1, Rc, Rd, Rf, Rg_1 and Ro) decreased quantitatively and, in particular, ginsenosides Rb_1 and Rg_1 were reduced by about half. On the other hand the monodesmoside group (ginsenosides Rg_3 and Rh_1) increased sixfold. A few years later Lee *et al.* (1994), studying the storage of aqueous solutions of red ginseng saponins and particularly ginsenoside Rb_1, concluded that the best pH range for stable liquid preparations was 6–8 and that the shelf life was about 570 days. It was also noted that the injection excipients mannitol and benzyl alcohol had no effect on the degradation of ginsenoside Rb1. The accumulated evidence underlines the importance of good storage, good analysis and quality control.

A modern formulation and presentation was described by the Russian group of Shygarova *et al.* (1997). Tablets of ginseng extract were coated with a shellac substrate in a fluidizing bed of methyl cellulose and Polysorbate 80, the coating providing constituent stability and tablet strength.

The introduction of adsorption column chromatography by the chemist M.Tswett in 1903 for the separation of chlorophyll pigments adsorbed on powdered calcium carbonate in a vertical glass column by elution with alcohol, enabled the separation of relatively large amounts of plant constituents but the method was very slow. Separation of amino acids by partition paper chromatography as developed by the team of Consden, Gordon, Martin and Synge in England in 1944 proved useful, especially in medical analysis. Paper chromatography was soon applied to other types of compounds including glycosides but was limited as loads were small and large sheets of wet paper were difficult to handle. The subsequent development of thin layer chromatography (TLC), a flat bed technique involving the passage of a suitable solvent (the mobile phase) across a uniform layer of a finely divided insoluble adsorbent (the stationary phase), combined the separating properties of the column adsorbent with the visual convenience of paper chromatography. This method was relatively quick, easy to perform and simple to interpret and in 1957 was successfully applied to the analysis of drugs by the German pharmacognosist Egon Stahl. Although initially applied to alkaloids, naturally occurring nitrogenous compounds, TLC was soon applied to glycosides and, inevitably, to the saponin glycosides of ginseng.

TLC proved to be a rapid quality control method that established identity by the occurrence of characteristic ginsenoside spots that reacted predictably with specific chromogenic reagents; in addition adulteration or substitution could be readily detected by the appearance of atypical spots. Commonly the saponins and sapogenins are extracted in alcoholic solvents e.g. methanol, ethanol, butanol, etc., the solvents are concentrated and the resultant test solution applied to chromatographic plates precoated with silica gel or silica gel GF254 and calcium sulphate or CM-cellulose, and developed with solvent mixtures such as chloroform: methanol: water (63:35:10) or (70:15:8), 1,2-dichloroethanol: butanol: methanol: water (30:40:15:25), chloroform: methanol: ethyl acetate: water (20:20:40:10), butanol: chloroform: methanol: water (40:20:15:10), etc. Identification of elixirs and capsules prepared from ginseng root powder and extract using TLC separation of panaxadiol and panaxatriol was described by Betz *et al.* (1979).

The location of specific spots on chromatographic plates can be established by application of spray reagents such as ferric chloride: sulphuric acid: acetic acid mixture and anisaldehyde: sulphuric acid: acetic acid reagent followed by heating at 105° C; this method was used to differentiate red and white Korean ginseng and Siberian ginseng (Jolliffe and Din, 1983). A further development was high performance thin layer chromatography (HPTLC), a fingerprint identification technique which was recommended by Peishan and Yuzhan (1987) for the differentiation of white and red *P. ginseng, P. quinquefolium* (American) and *P. notoginseng* (Sanchi) in commercial samples of ginseng by a peak grouping method. In order to reduce background spots and tailing it was recommended that the test samples were subjected to a preliminary adsorption cleanup on a small alumina column prior to recovery in butanol. Development with a chloroform: ethyl acetate: methanol: water (15:40:22:10 by volume) solvent system on silicagel was claimed to produce better resolution, greater spot capacity and improved reproducibility as compared with established systems. Fluorescence scanning after visualisation using a 5 per cent sulphuric acid-ethanol dipping agent yielded a ninefold enhanced sensitivity. Dallenbach-Toelke *et al.* (1987) discussed the theoretical aspects of optimising the mobile phase for the eight most important ginsenosides, noting that a small change in selectivity had a marked effect on resolution and that overpressured TLC could be used with HPTLC plates.

Quantitation after TLC separation has prompted the development of many methods involving fluorimetry, colorimetry, densitometry and elution techniques.

Figure 11. High Performance Liquid Chromatography apparatus with supporting equipment.

Colorimetric methods are not specific and frequently afford higher than normal results. TLC-fluorimetric methods are also limited in application because it is difficult to differentiate the ginsenosides Rb_1/Rb_2, Re/Rf and Rg_1/Rg_2.

The most important developments in the field of chromatographic analysis of ginseng compounds during the past twenty years are based on High Pressure or High Performance Liquid Chromatography (HPLC) and Reverse Phase High Pressure Liquid Chromatography (RP-HPLC) (Fig. 11). The system is fundamentally liquid column Chromatography using relatively narrow bored columns (*ca* 5 mm diameter) operating at temperatures from room temperature up to about 200° C and pressures up to about 200 atmospheres (20,000 kPa). A precolumn may be used to provide a preliminary clean-up of the test solution before separation in the main column. Flow rates of the mobile phase are usually in the range 1–10 ml/min depending on the diameter of the column used and on the pressure applied. Modern apparatus can be used for all types of liquid Chromatography including adsorption, partition using bonded liquid phases, reversed phase, gel filtration, ion exchange and affinity methods. Suitable apparatus will also provide for programmed graded elution using two or more solvents. Amongst the many recommended systems are an octadecylsilylated (ODS) porous glass column at room temperature with a methyl cyanide: 50mM potassium dihydrogen phosphate mobile phase (Takai *et al.,* 1989), an ODS column with an acetonitrile: water: potassium hydrogen phosphate mobile phase (Kanazawa *et al.,* 1992), an ODS chemically modified porous glass column with acetonitrile: water: ammonium acetate mobile phase (Gu *et al.,* 1994), an amino column with acetonitrile and aqueous 2-tert-butylanthraquinone solution as mobile phase (Park *et al.,* 1995) and a LiChrosorb NH_2 amino-bonded column and an acetonitrile: water: 2-propanol gradient system (Park *et al.,* 1996). Modified porous glass-ODS columns were however preferred to silica ODS columns, having a better optimum pore size through a narrower distribution range and better capacity for ginsenoside separation despite smaller capacity factors for the common ginsenosides (Kanazawa *et al.,* 1993).

Although many HPLC systems have been recommended for the separation of ginsenosides, the general concensus would suggest preparatory use of a cleanup column (e.g. Sep-Pak C18 cartridge) which reduces the front peaks caused by impurities in the methanol extracts of samples and yields a clearer background and smoother baseline in the final HPLC (Lang *et al.,* 1993). An alternative procedure involves extracting the powdered crude drug several times in 70 per cent aqueous methanol at room temperature for 30 minutes each time. The bulked extracts are evaporated to dryness under reduced pressure and the residue dissolved in water before loading on to a Sep-Pak C18 cartridge. The cartridge is washed with water and then 20 per cent methanol before elution of the ginsenosides in methanol and HPLC on a 4 mm ×250 mm ODS porous glass column at room temperature using methyl cyanide-50 mM potassium hydrogen phosphate (K_2HPO_4)(25.5:74.5) as the mobile phase and a flow rate of 2.0 mL/min. (Takai *et al.,* 1989). Direct ultraviolet absorbance detection can be undertaken without preliminary derivative formation at wavelengths in the range 198–204 nm. Samukawa *et al.* (1995a) also used a C18 column with a

methyl cyanide-water gradient system to provide an improved method of simultaneous separation and estimation of 22 major and minor neutral and acidic ginsenosides. The method has commercial potential for routine analysis of red and white ginseng products. Court *et al.* (1996), analysing *P. quinquefolium* samples, employed a phosphate buffer-acetonitrile gradient system with a C_{18} reversed phase column. The ginsenosides were extracted in methanol in an ultrasonic bath before direct liquid chromatographic separation and determination of the neutral triterpene saponins (ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rg_1 and Ro, gypenoside XVII and pseudoginsenoside-F_{11}). Acidic ginsenosides (malonyl-ginsenosides Rb_1, Rb_2, Rc and Rd) were determined indirectly after hydrolysis with aqueous potassium hydroxide.

Other suggestions have included photoreduction fluorescence detection after conversion of the ginsenosides to highly fluorescent dihydroxy anthracene derivatives (Park *et al.,* 1995a), multichannel photodiode array ultraviolet absorbance (Meier and Sticher, 1986, Van Breemen *et al.,* 1995), thermospray HPLC/mass spectrometry (Park *et al.,* 1995b) and evaporative light scattering detection (Park *et al.,* 1996). The thermospray LC/MS method is claimed to be 10–200 times better than the more conventional HPLC/UV method. An advantage of photodiode array ultraviolet absorbance method is coupling to online electrospray mass spectrometry which permits rapid species differentiation. Reported detection limits for the methods vary from 25 to 250 ng and resolution times are in the range 20–45 minutes. A recently published reversed-phase HPLC method designed for the identification and quantification of ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1 claims good resolution and assay within 15 minutes (Gómez-Serranillos *et al.,* 1997).

The major advantages of HPLC methods are speed, effective separation producing characteristic fingerprint patterns, quantitative as well as qualitative application, preparative possibilities and reproducibility. As the pharmacology of the individual constituents is so variable and their occurrence in the crude drug and in extracts and formulations derived therefrom is also so variable, it is essential that reliable, standardised medicinal products are available on the market and HPLC, being so easily applied to pharmaceutical formulations, provides the desired analytical answer. For example, the application of TLC and HPLC has clearly differentiated American ginseng *(P. quinquefolium)* from Chinese ginseng *(P. ginseng)* because the 24*(R)*-pseudoginsenoside F_{11} spot appears above the ginsenoside Rg_1 spot only in *P. quinquefolium* extracts whilst *P. ginseng* extracts yielded a ginsenoside Rf spot below the Rg1 spot (Dou *et al,* 1998).

The availability of simple, rapid and relatively cheap methods for establishing the identity of ginseng crude drugs and pharmaceutical products during the past 30 years would suggest the universal application of quality control standards, yet evidence has accumulated concerning unreliable products on the commercial market. Siegel (1977), drawing attention to mislabelled herbs on the American market, recorded adulteration and substitution of ginseng and especially ground ginseng, substitutes including *Mandragora officinarum* L. containing hyoscine (scopolamine), *Rauvolfia serpentina* Benth. yielding

reserpine and *Cola* spp. containing caffeine and other additions were procaine, nicotinic acid and vitamin C. Over 20 years later attention was still being drawn to the potential danger of anticholinergic drug adulteration in imported Chinese herbal medicines, adulterants including *Datura metel* flowers and *Mandragora officinarum* roots from Solanaceous species (Chan, 1995). Such adulteration could have been easily and cheaply confirmed by simple TLC methods.

Corrigan (1981), analysing 35 commercial ginseng preparations available in the British Isles, commented on the great variability in quality. His work agreed with the findings of other workers in Europe (Soldati and Sticher, 1980) and in America (Liberti and Der Marderosian, 1978). Problems such as incorrect species, mixed species, underweight products, failure to pass tablet disintegration tests, absence of ginsenosides and erroneous and inadequate labelling were encountered. Nevertheless good products were and are available.

Many workers have commented on the variability of the starting materials. The main root of 4–6 year old *P. ginseng* may yield 1.5 per cent of total glycosides, side roots may yield 3–4 per cent and the finest roots may yield as much as 9 per cent. Sprecher (1987) commented that the man-like roots were in fact less rich in the valued ginsenosides than the lateral root system yet indigenous medicine preferred the main root. Within the root the ginsenosides occur mainly in the periderm and outer cortex rather than in the bulkier central phloem and xylem (Tani *et al.,* 1981) although for *P. quinquefolium* Smith *et al.* (1996) noted that the ginsenosides were distributed in the periderm and cortex and in the xylem and pith of roots and rhizomes. Therefore very careful mixing of powdered ginseng is essential prior to filling non-assayed capsules with measured amounts of powdered root, but the resultant product will still be chemically and therefore pharmacologically variable. Ginseng extracts also present problems as, for example, in Korea where white ginseng and ginseng lateral roots are both used for extract production (Cho *et al.,* 1981). In Switzerland also, Soldati and Sticher (1980), using HPLC methods, demonstrated that, even with carefully standardised extraction methods and estimation of the total ginsenosides, the resultant products were still variable. For 3 different batches of carefully prepared standardised ginseng extract containing 4 per cent total ginsenosides and for 4 different lots of soft gelatin capsules their findings shewed considerable variations (Table 8.1)

Other workers (Sollorz, 1985; Meier *et al.,* 1985) have stressed the variability of the proportions of the ginsenosides Rb_1 and Rg_1 in commercially available

Table 8.1. Variable ginsenoside content of "Standardised" ginseng

Saponin glycoside	*Percentage content*	
	Ginseng extract	*Ginseng capsules*
Ginsenoside-Rb_1	0.976–1.338	1.820–2.148
Ginsenoside-Rb_2	0.425–0.567	0.708–0.780
Ginsenoside-Rc	0.603–0.714	0.952–1.110
Ginsenoside-Rd	0.229–0.286	0.342–0.382
Ginsenoside-Re	0.352–0.586	0.910–1.196
Ginsenoside-Rf	0.238–0.285	0.422–0.626
Ginsenoside-Rg_1	0.548–0.946	1.308–1.660
Ginsenoside-Rg_2	0.065–0.142	0.090–0.272

Table 8.2. The variable ginsenoside content in red ginseng samples

Ginsenoside-Rb	0.231–0.586	Ginsenoside-Re	0.029–0.408
.. -Rb_2	0.097–0.311	.. -Rg	0.240–0.643
.. -Rc	0.150–0.331	.. -Rg_2	0.120–0.271
.. -Rd	0.082–0.274	.. -Ro	0.120–0.276

products recording ratios of 6:1, 2:1, 1:1 and 1:2. This is significant because such ginsenosides are to some extent pharmacologically antagonistic.

Li *et al.* (1986), using high performance TLC (HPTLC) on silicagel plates, developing with chloroform: methanol: water (65:35:10) or n-butanol: ethyl acetate: water (4:1:1), spraying with 10 per cent sulphuric acid solution, heating the sprayed plates at 110° for 7 minutes and measuring by light scanning at 520 nm, noted the variability of Chinese, Japanese and Korean red ginseng; the total saponins were estimated as 2.411–5.688 per cent. Thus the quantitative range of individual ginsenosides in "red ginseng" products requires standardisation (Table 8.2).

Although ginsenoside estimation has dominated quality control reports, Kitagawa and his colleagues (1987), comparing fresh ginseng and white and red ginseng prepared from the same roots, stated that fresh and red ginseng contained glycerogalactolipids and steryl glucoside fatty esters and white ginseng yielded the latter in small quantities only but there was also variation in the polyacetylenic compounds present, the fresh and white ginsengs producing panaxynol and panaxydol whereas red ginseng provided in addition heptadec-1-ene-4,6-diyne-3,9-diol and panaxytriol. As the heat treatment of red ginseng also caused demalonylation of malonyl ginsenosides, elimination of glycosyl residues at C-20 of ginsenosides and isomerisation of the hydroxyl configuration at C-20, careful analysis is necessary.

Such results underline the need for satisfactory analytical standards if ginseng is to be accepted in medical practice. Sound, published research, sponsored by ethical pharmaceutical companies such as Pharmaton (Boehringer-Ingelheim) has ensured the availability of efficient standardisation techniques employing advanced analytical methods. For example, Dr. Soldati of Pharmaton and Professor Sticher of Zürich (1980), noting the variability of ginsenoside content throughout the ginseng plant and the great variations in commercial ginseng products, have clearly shown the application of HPLC techniques to the successive stages of manufacturing viz. i) control of initial raw material, ii) control of the extraction processes, iii) controlled mixing of standardised extracts and iv) control of the final galenical preparation whatever its form. Consequently good botanical material can be extracted, formulated and standardised using sound pharmaceutical practices and the resultant medicines are stable, standardised and chemically and pharmacologically reliable. Thus the widely used standardised ginseng extract G115 (Pharmaton SA) contains 4 per cent total ginsenosides adjusted to yield 6 principal and 2 secondary ginsenosides within defined limits. Not surprisingly this product has been successfully used in many clinical trials.

Despite such advances, more than a decade later (1998) there is still no universal legal requirement that standardised ginseng only should be sold and

several researchers have reported the continuing sale of ginseng devoid of ginsenosides or of variable composition (1.9–9 per cent total ginsenosides). No less than 50 products from 11 countries were analysed and 6 of the products sold in Sweden, the United Kingdom and the United States contained no specific identifiable ginsenoside and one product, which had caused an unsuspecting athlete to fail a dope test, was found to contain a large amount of ephedrine (Cui *et al.,* 1994). In the following year Ma *et al.* (1995), employing HPLC, also reported a survey of more than 60 commercial ginseng and ginseng tissue culture products and again stressed the wide variability of commercial products. Many commercial products comprise powdered ginseng root mixed with appropriate excipients and compressed into tablets. Samukawa *et al.* (1995b), undertaking careful HPLC analysis of roots, observed that the mixture of panaxadiol and panaxatriol glycosides gradually increased in quantity in successive years of growth and the proportion of the oleanane ginsenoside Ro increased significantly in the sixth year of growth. Therefore they stressed the importance of estimating this glycoside during analysis.

In Taiwan Chuang and his colleagues (1995) investigated 37 commercial samples of Ginseng Radix prepared from 3 species, *P. ginseng, P. notoginseng* and *P. quinquefolium*. The samples were gathered from the Taiwanese herbal markets and analysed for their ginsenoside and malonylginsenoside contents. Results shewed that red and white Ginseng Radix derived from *P. ginseng* yielded less total saponins which were mainly ginsenosides Rb_1 and Rg_1; Ginseng Radix from *P. notoginseng* yielded more total saponins and these were mainly ginsenosides Rb_1, Rd and Rg_1 and Ginseng Radix from *P. quinquefolium* similarly yielded more total saponins but these were chiefly Rb_1, Re and malonylginsenoside Rb_1. Thus there was great variability in the commercial material and therefore in its pharmacological effects.

More recently Cui (1995) and Cui *et al.* (1996), using a GC-GCMS (gas chromatography-gas chromatography mass spectrometry) technique, examined 20 ginseng extract preparations and 17 different commercial ginseng preparations available on the Swedish market. They noted that some liquid preparations and red ginseng samples produced artefacts derived by epimerisation and hydration and that individual capsules varied between 2.1 and 13.3 mg of total ginsenosides and that red ginseng preparations and liquid ginseng preparations yielded, after release of the sugar moieties from the ginsenosides, significant amounts of the 20-epimers of 20-*(S)*-protopanaxadiol and 20-*(S)*-protopanaxatriol together with the corresponding 24,25 hydrated compounds. They also reported that liquid preparations contained, in addition to the naturally occurring ginsenosides above and their artificial derivatives, two epimeric pairs of prosapogenins corresponding to ginsenosides Rg_3 and 20-*(S)*-Rg_3 and Rh_1 and 20-*(R)*-Rh_1. Such findings suggest that conversions such as hydrolysis, epimerisation and hydration of aglycone side chains can and do occur during the processing of ginseng and especially under weakly acidic conditions (pH 3.0–3.5) in weak alcohol (*ca* 9–10 per cent) at room temperature. Therefore careful and detailed analysis is imperative and effective standards must be set if products yielding reliable dose-response activities are to be developed and marketed safely.

An interesting technique for the rapid identification of the country of origin of ginseng roots was published by Kwon *et al.* (1996). Based on the infrared reflectance spectra and using InfraAlyzers 500 and 400 and InfraAlyzer Data Analysis Software (IDAS), the method offered rapid sample information, automatic sample group separation for discriminatory analysis and fast print-outs of reports. Tested on 1500 samples of roots from China and Korea, the technique offered 95 per cent accuracy in proving countries of origin, period of cultivation, crude saponin content and moisture content.

Ginseng products may be contaminated by pesticide residues. Examining many samples of ginseng teas and herbal products and their hot water extracts, Yasuda *et al.* (1986) recorded 0.732 p.p.m. of the organochlorine pesticide benzene hexachloride in dried ginseng, 0.241 p.p.m. in ginseng powder and 0.133–1.13 p.p.m. in ginseng extracts. Dieldrin 0.05 p.p.m. was detected in ginseng extracts and heptachlor epoxide was found in ginseng extracts (0.05–0.35 p.p.m.) and dried ginseng (0.24 p.p.m.). Significantly up to 25 per cent of the benzene hexachloride could pass into the hot water extracts and thus into final products. Such observations stress the importance of testing for pesticide contamination and ensuring that the products obey local legal requirements e.g. in the United States limits on diazinon (*Fed. Regist.,* 1986), iprodione (*Fed. Regist.,* 1987) and chlorothalonil (*Fed. Regist.,* 1997). Many papers have been published describing methods such as high performance capillary column gas chromatography and reverse phase HPLC applied to the analysis of organochlorine and organophosphorus insecticides and various fungicides encountered in ginseng samples.

As the pharmacology of the chemical constituents individually and in different combinations is so variable and their occurrence in the crude drug and in extracts and formulations derived therefrom is also so variable, it is essential that reliable, standardised medicinal products are available on the market and effective storage requirements and sell-by dates should be stipulated.

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9. PATENTS

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The wide range of uses of ginseng has resulted in many patent applications during the past two decades. Those referred to in American Chemical Abstracts *(Chem. Abs.)* particularly during the past decade are summarised.

Patents have been registered concerning the production of natural ginseng plants using modern horticultural methods for growth and preservation of the plants.

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High saponin containing plants. Yokoyama, M. and Yanagi, M., Jpn. Kokai Tokkyo Koho **JP 62,111,696;** 22.05.1987; *Chem. Abs.,* **107,** P131214.

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Ginseng-based biologically active food additive. Skoblick, T.I., Yudina, T.I., Ustinova, T.A., Dedyaeva, E.M., Khaitov, R.M., Oradovskaya, I.V., Fadeeva, I.D., Lokhova, F.S. and Zajtsev, A.N., **Russ. RU 2,057,463;** 10.04.1996; *Chem. Abs.,* **126,** P18018.

Panax extract in the manufacture of health vinegar. Takahashi, K., Kanetani, T. and Nishijima, A., **Jpn. Kokai Tokkyo Koho JP 08,256,754;** 08.10.1996; *Chem. Abs.,* **126,** P30662.

Ginseng has also been employed in animal products.

Vitamin and ginseng enriched chicken feeds. Ogawa, H., **Jpn. Kokai Tokkyo Koho JP 82 26,549;** 12.02.1982; *Chem. Abs.,* **96,** P198450.

Vitamin-enriched fish feed. Ogawa, H., **Jpn. Kokai Tokkyo Koho JP 82 26,550;** 12.02.1982; *Chem. Abs.,* **96,** P198451.

Pet food containing oleic acid, silicic acid and therapeutic plant extracts. Kanamaru, M. **Jpn. Kokai Tokkyo Koho JP 08,140,587;** 04.06.1996; *Chem. Abs.,* **125,** P166270.

Health pet food. Kanamaru, M., **Jpn. Kokai Tokkyo Koho JP 08,191,668;** 30.07.1996; *Chem. Abs.,* **125,** P194148.

Other formulations have offered cosmetic applications such as treatment of hair loss, hair growth, skin protection, massage facilitation and wrinkle prevention, transient lip augmentation preparations where ginseng is employed as a vasodilator, deodorant sprays, etc.

Cosmetics containing ginseng polysaccharides. Hikino, H. and Hayashi, T., **Jpn. Kokai Tokkyo Koho JP 61,115,013;** 02.06.1986; *Chem. Abs.,* **105,** P120516.

Skin conditioners containing alicyclic compounds and blood-circulation promotors (including ginseng). Ogawa, T., Mujamoto, T., Sada, M., Abe, T. and Nishijima, Y., **Jpn. Kokai Tokkyo Koho JP 61 56,114;** 20.03.1986; *Chem. Abs.,* **105,** P66253.

Cosmetics for preventing skin discolouration. Juchi, S., **Jpn. Kokai Tokkyo Koho JP 61,122,209;** 10.06.1986; *Chem. Abs.,* **105,** P120522.

Skin lightening cosmetics containing plant extracts and L-ascorbic acid or other substances (including ginseng). Shinho, T, Suzuki, J. and Masuda, M., **Jpn. Kokai Tokkyo Koho JP 08 92,056;** 09.04.1996; *Chem. Abs.,* **125,** P41456.

Cosmetics containing diisopropylamine dichloroacetate and plant extracts. Hasunuma, K., **Jpn. Kokai Tokkyo Koho JP 62,148,414;** 02.07.1987; *Chem. Abs.,* **107,** P183354.

Cosmetics for the treatment of alopecia containing testosterone 5a-reductase inhibitors from plant extracts. Tsuboi, M., Ando, Y. and Matsui, K., **Jpn. Kokai Tokkyo Koho JP 62,116,520;** 28.05.1987; *Chem. Abs.,* **108,** P26820.

Benzyl-β-primeveroside and sustained-release fragrance compositions containing the glycoside (from *Panax ginseng*). Saito, J., Konno, C. and Hikino, H., **Jpn. Kokai Tokkyo Koho JP 02,196,795;** 03.08.1990; *Chem. Abs.,* **113,** P 217822.

Body weight-controlling, blood lipid-reducing creams, ointments or membrane-forming preparations. Si, S., Song, S. and Li, H., **Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1,062,082;** 24.06.1992; *Chem. Abs.,* **117,** P220132.

Astringent cosmetics containing plant extracts and amino acids. Mizuno, Y. and Ito, K., **Jpn. Kokai Tokkyo Koho JP 01 47,708;** 22.02.1989; *Chem. Abs.,* **111,** P102551.

Cosmetics containing ginseng extracts and cholesterol derivatives. Abe, T. and Kondo M., **Jpn. Kokai Tokkyo Koho JP 05 51,314;** 02.03.1993; *Chem. Abs.,* **118,** P260711.

Non-invasive novel method for lip augmentation. Frome, B.M., **U.S. US 5,571,794;** 05.11.1996; *Chem. Abs.,* **125,** P339066.

Therapeutic massage topical formulations (cations, anions and ginseng extract). Ishino, M., Ikegami, Y., Yokoyama, Y. and Mizuno, H. **Jpn. Kokai Tokkyo Koho JP 05,194,245;** 03.08.1993; *Chem. Abs.* **119,** P234049.

Creams or pastes for the whole body or facial massage. Takano, H., **Jpn. Kokai Tokkyo Koho JP 07,157,410;** 20.06.1995; *Chem. Abs.,* **123,** P152905.

Skin tonic cream containing hypocrellin (and *Panax notoginseng*). Lui, S., **Faming Zhuanli Shenqing Gongkai Shuomingshu CN 85,103,693;** 19.11.1986 ; *Chem. Abs.,* **108,** P81819.

Skin preparations containing ceramides, glucosylceramides and/or galactosyl-ceramides and extract of *Swertia japonica* and/or *Panax ginseng* for prevention of ageing. Miyamoto, T. and Uchida, R., B; **Jpn. Kokai Tokkyo Koho JP 01 22,811;** 25.01.1989; *Chem. Abs.,* **111,** P83901.

Topical and cosmetic preparations containing saikosaponins for activation of skin cell proliferation. Nishima, T. and Iwamoto, A., **Jpn. Kokai Tokkyo Koho JP 05 262,635;** 12.10.1993; *Chem. Abs.,* **120,** P173475.

Cosmetics containing γ-amino-β-hydroxybutyric acid and plant extracts for prevention of skin-aging. Hasanuma, K. Jpn. **Kokai Tokkyo Koho JP 07 76,513;** 20.03.1995; *Chem. Abs.,* **122,** P322227.

Skin cosmetics containing terrapin oil and other ingredients (including ginseng). Sawada, K., **Jpn. Kokai Tokkyo Koho JP 07,157,421;** 20.06.1995; *Chem. Abs.,* **123,** P152600.

Topical preparations containing erythritol and medicinal plant extracts for rough skin. Shimada, T. and Yamada, H., **Jpn. Kokai Tokkyo Koho JP 07,215,838;** 15.08.1995; *Chem. Abs.,* **123,** P296251.

Skin-care preparations containing caffeine, seaweed products and ginseng extracts. Saito, M., Sumida, Y, Oota, K. and Murakami, M., **Jpn. Kokai Tokkyo Koho JP 08,104,618;** 23.04.1996; *Chem. Abs.,* **125,** P67214.

Skin care products containing caffeine, seaweed products and ginseng extracts. Saito, M., Sumida, Y, Oota, K. and Murakami, M., **Jpn. Kokai Tokkyo Koho JP 08,104,618;** 23.04.1996; *Chem. Abs.,* **125,** P67214.

Use of ginsenoside Ro or a plant extract containing same for promotion of collagen synthesis for pharmaceuticals and cosmetics. Meybeck, A., Bonte, F., Dumas, M. and Chaudagne, C., **PCT Int. Appl. WO 95 25,524;** 28.09.1995; *Chem. Abs.,* **123,** P350231.

Skin lightening cosmetics containing culture products of *Fomes japonicus* and their extracts and ginseng extracts, sodium chondroitin sulfate, and hyaluronic acid. Naeshiro, H., Hashimoto, A. and Ando, H., **Jpn. Kokai Tokkyo Koho JP 04 09,316;** 14.01.1992; *Chem. Abs.,* **116,** P158607.

Cosmetics containing hydroxy-carboxylic acids and plant extracts. Dampierou, C., **Fr. Demande FR 2,736,263;** 10.01.1997; *Chem. Abs.,* **126,** P255278.

Saponin ethers with quaternary ammonium compounds for hair and skin care products. Young, D.K. and Byung, J.H., **Fr. Demande FR 2,648,138;** 14.12.1990; *Chem. Abs.,* **115,** P56953.

Flavors for pseudoginseng containing tooth pastes. Li, J., **Faming Zhuanli Shenqing Gongkai Shuomingshu CN 85,106,825;** 25.03.1987; *Chem. Abs.,* **108,** P156284.

Toothpaste containing medical plant extracts and vitamins for controlling gray hair. Zhang, S., **Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1,039,535;** 14.02.1990; *Chem. Abs.,* **113,** P217826.

Vitamin-enriched denture adhesive cream. Runkel, A., **Ger. Offen. DE 4,437,824;** 27.04.1995; *Chem. Abs.,* **123,** P40996.

Antiinflammatory and antimicrobial mouthwashes for controlling oral diseases (containing *Panax pseudoginseng).* Wu, W., **Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1,099,981;** 15.03.1995; *Chem. Abs.,* **123,** P152889.

Ginseng saponins as stimulators of hair growth. Osaka Yakuhin Kenkyusho KK, **Jpn. Kokai Tokkyo Koho JP 60 38,314;** 27.02.1985; *Chem. Abs.,* 103, P16852.

Hair growth promoting agents. Yoshino, T., **Jpn. Kokai Tokkyo Koho JP 61 69,712;** 10.04.1986; *Chem. Abs.,* **105,** P29797.

Hair tonics containing alicyclic compounds and blood-circulation promotors (including ginseng). Mujamoto, T., Abe, T. and Nishijima, Y., **Jpn. Kokai Tokkyo Koho JP 61 56,118;** 20.03.1986; *Chem. Abs.,* **105,** P66247.

Hair tonics containing *cis*-6, *cis*-9, *cis*-12 -octadecatrienolic acid and blood circulation accelerators. Mujamoto, T. and Motoi, T., **Jpn. Kokai Tokkyo Koho JP 62,132,809;** 16.06.1987; *Chem. Abs.,* **107,** P161378.

Hair tonics containing cyproterone acetate and blood circulation accelerators. Mujamoto, T. and Maeno, K., **Jpn. Kokai Tokkyo Koho JP 62,103,005;** 13.05.1987; *Chem. Abs.,* **107,** P183324.

Hair tonics containing spironolactone and blood circulation accelerators. Mujamoto, T. and Maeno, K., **Jpn. Kokai Tokkyo Koho JP 62,103,006;** 13.05.1987; *Chem. Abs.,* **107,** P183325.

Composition for treatment of the hair against seborrhoea, pruritis, pityriasis and hair loss. Abramowicz Frajdenrajch, S., **Fr. Demande FR 2,588,756;** 24.04.1987; *Chem. Abs.,* **107,** P242431.

Hair growth-promoting cosmetics containing γ-amino-β-hydroxybutyric acid (and ginseng extract). Hasunuma, K., **Jpn. Kokai Tokkyo Koho JP 62,255,409;** 07.11.1987; *Chem. Abs.,* **108,** P209987.

Hair growth stimulating preparations containing medicinal plant extracts (including ginseng). Huang, M., Hang, W. and Zhong, Q. **Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1,043,624;** 11.07.1990; *Chem. Abs.,* **114,** P171037.

Hair growth stimulating preparations containing *c*AMP, pyruvic acid, fermentation metabolites, and natural products (including ginseng). Minabe, T., Fujii, K., Kanatani, A. and Tanici, A., **Jpn. Kokai Tokkyo Koho JP 03,167,113;** 19.07.1991; *Chem. Abs.,* **115,** P239325.

Hair preparations containing succinic acid salts and ginseng extracts. Abe, T. and Myamoto, T., **Jpn. Kokai Tokkyo Koho JP 04,338,319;** 25.11.1992; *Chem. Abs.,* **118,** P131751.

Hair bleaches containing per sulfate and powdered ginseng root. Hartmann, P., **Ger. Offen. DE 4,126,429;** 11.02.1993; *Chem. Abs.,* **118,** P131758.

Hair growth stimulants containing dimethylsilicones (including ginseng extract). Hirano, A., Awamura, K. and Nogawa, Y., **Jpn. Kokai Tokkyo Koho JP 05 97,631;** 20.04.1993; *Chem. Abs.,* **119,** P55711.

Hair tonics containing hyaluronic acid and plant extracts. Myamoto, T., **Jpn. Kokai Tokkyo Koho JP 06 09,349;** 18.01.1994; *Chem. Abs.,* **120,** P226533.

Hair preparations containing *Aconitum* extracts in combination with hair growth stimulants (including ginseng). Minamino, H. and Iwamoto, Y., **Jpn. Kokai Tokkyo Koho JP 05 286,837;** 02.11.1993; *Chem. Abs.,* **120,** P86079.

Hair tonics containing *Artemisia capillaris* extracts (and *Panax schinseng* extracts and tested on male subjects). Tanaka, K. and Myamoto, T., **Jpn. Kokai Tokkyo Koho JP 06 145,027;** 24.05.1994; *Chem. Abs.,* **121,** P91314.

Hair tonics containing natural ingredients. Utsuki, Y., **Jpn. Kokai Tokkyo Koho JP 06,271,429;** 27.09.1994; *Chem. Abs.,* **122,** P63986.

Hair tonics containing bovine bone marrow extract, *Swertia japonica* extract and *Panax ginseng* extract. Myamoto, T., Hashimoto, M. and Aoyanagi, S., **Jpn. Kokai Tokkyo Koho JP 07,291,838;** *Chem. Abs.,* **124,** P66203.

Hair growth-stimulating cosmetics containing bisabolol hydrogenation product. Nakagawa, N. and Myamoto, T., **Jpn. Kokai Tokkyo Koho JP 08 48,616;** 20.02.1996; *Chem. Abs.,* **124,** P324973.

Hair growth-promoting aerosol containing plant extracts. Yasuda, M., Tsunakawa, M., Matsuo, K., Aoshima, K., Ohta, M. and Kinno, S., **Eur. Pat. Appl. EP 640,333;** 01.03.1995; *Chem. Abs.,* **122,** P248015.

Hair dye containing plant pigments and ginsenosides. Hartmann, P., **Ger. Offen. DE 4,402,203;** 27.07.1995; *Chem. Abs.,* **123,** P179087.

Cleansing agents containing nonionic surfactants for scalp and hair (and including ginseng extract). Hashimoto, K., **Jpn. Kokai Tokkyo Koho JP 07,112,925;** 02.05.1995; *Chem. Abs.,* **123,** P122728.

Hair tonics and growth stimulants containing kinetins and other ingredients. Yokoyama, T. **Jpn. Kokai Tokkyo Koho JP 08,109,115;** 30.04.1996; *Chem. Abs.,* **125,** P67164.

Hair preparations containing capronium chloride. Amanya, T. and Yamamoto, Z., **Jpn. Kokai Tokkyo Koho JP 08,127,518;** 21.05.1996; *Chem. Abs.,* **125,** P95541.

Cosmetics containing germanium compounds extracted from plants. Kawai, R, **Jpn. Kokai Tokkyo Koho JP 62,111,908;** 22.05.1987; *Chem. Abs.,* **107,** P140906.

Extraction of saponins from the fresh leaf and stem of ginseng for manufacturing cosmetics. Liu, X., Li, Q., *et al.,* **Faming Zhuanli Shenqing Gongkei Shuomingshu CN 1,054,535;** 18.09.1991; *Chem. Abs.,* **116,** P136035.

Cosmetic or dermatologic composition containing at least one saponin of the ginsenoside type, and its application particularly to hair care. Maybeck, A., Bonte, F. and Dumas, M., **PCT Int. Appl. WO 94 06,402;** 31.03.1994; *Chem. Abs.,* **121,** P17710.

Antipollution cosmetic composition. Mausner, J., **U.S. US 5,571,503;** 05.11.1996; *Chem. Abs.,* **126,** P22787.

Deodorant spray for body odour and bad breath. Ashitagawa, T., Hoshi, K. and Mizushima, Y., **Jpn. Kokai Tokkyo Koho JP 08,191,880;** 30.07.1996; *Chem. Abs.,* **125,** P230223.

Cosmetic composition for protection against atmospheric pollutants. Courtin, O., **Fr. Demande FR 2,688,137;** 10.9.93; *Chem.Abs.,* **120,** P200170.

The surface tension-reducing properties of the saponins have been exploited in the formulation of bath preparations and water cleansing agents.

Ginseng extract containing vitamin B_1 derivatives for bath preparations. Oshio, H., Aki, N., Noda, E. and Okada, A., **Jpn. Kokai Tokkyo Koho JP 05 09,110;** 19.01.1993; *Chem. Abs.,* **118,** P175530.

Bath preparations containing granular carbohydrates (including ginseng extract). Shiraishi, T., **Jpn. Kokai Tokkyo Koho JP 05,301,815;** 16.11.1993; *Chem. Abs.,* **120,** P61952.

Bath preparations containing gas (and *Panax notoginseng).* Shiraishi, T., **Jpn. Kokai Tokkyo Koho JP 05 331,042;** 14.12.1993; *Chem. Abs.,* **121,** P91316.

Bath preparations containing oils and saponins (including ginseng extract). Muramatsu, T. and Suzuki, S., **Jpn. Kokai Tokkyo Koho JP 05,331,044;** 14.12.1993; *Chem. Abs.,* **121,** P91318.

Shampoo and bath preparations. Huang, Y., **Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1,095,920;** 07.12.1994; *Chem. Abs.,* **122,** P322192.

Bath preparations containing ascorbic acid and other ingredients. Ikuta, T. and Uryu, T., **Jpn. Kokai Tokkyo Koho JP 08,198,743;** 06.08.1996; *Chem. Abs.,* **125,** P256785.

Toilet Soap. Yushchenko, V.A., Mel'nik, E.A., Drashchinskaya, V.A., Tysyachnaya, L.G., Medyanik, I.A., Kuznetsov, B.E., Tsesarskaya, V.I., Aleksandrova, I.V., Danilina, A.N. and Anisimov, O.L., **U.S.S.R. SU 1,046,280;** 07.10.1983; *Chem. Abs.,* **100,** P70382.

Transparent soaps containing medicine. Li, S., Wu, F. and Wu, D., **Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1,087,944;** 15.06.1994; *Chem. Abs.,* **123,** P116282.

Ginseng soaps. Lui, W., **Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1,088,977;** 06.07.1994; *Chem. Abs.,* **123,** P173582.

Fragrance-free deodorants from ginseng extract. Sawaguchi, M., Moroishi, Y., Asano, T., Noda, K. and Tomita, T., **Jpn. Kokai Tokkyo Koho JP 62,246,369;** 27.10.1987; *Chem. Abs.* **108,** P43199.

In addition ginseng has been used in a number of miscellaneous applications. Chinese herbal medicine compound liquid disinfectant (including ginsenosides).

Wang, C., **Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1,102,055;** 03.05.1955; *Chem. Abs.,* **123,** P308661.

Excess sludge treatment. Taniguchi, T., **Jpn. Kokai Tokkyo Koho JP 63,242,400;** 07.10.1988; *Chem. Abs.,* **110,** P179003.

Agents for bulking prevention in treatment of organic waste water with activated sludge. Taniguchi, T., **Jpn. Kokai Tokkyo Koho JP 63,242,398;** 07.10.1988; *Chem. Abs.,* **110,** P178975.

Combustion improvers for petroleum-derived fuels. Honma, F., **Jpn. Kokai**

Tokkyo Koho JP 63,112,690; 17.05.1988; *Chem. Abs.,* **109,** P76416.

Preparation of combustion catalysts for gasoline and diesel oil. Jin, G., Hu, J. and Pan, T., **Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1,091,461;** 31.08.1994; *Chem. Abs.,* **123,** P291531.

Oil combustion aid Jie-You-Ling and its preparation. Yang, Z. and Huang, J., **Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1,099,407;** 01.03.1955; *Chem. Abs.,* **123,** P318591.

10. OTHER GINSENGS

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Apart from other species of *Panax* that sometimes enter the commercial market, there are several plants from completely different sources that are sometimes known as "ginsengs".

The best known is undoubtedly *Eleuthero,* the misnamed Siberian ginseng, which comprises the roots and rhizomes of the erect Araliaceous shrub *Eleutherococcus senticosus* (Rupr. & Maxim.) Maximowicz (=*Acanthopanax senticosus*). The plant grows freely and abundantly in eastern Siberia, the northern Chinese regions of Shan-si and Hop-ei, and in Korea. The plant is known in northeast China as ciwuija (pronounced "su wah ja"). A shrub growing to about 2–3 m in height, it is characterised by downward-pointing, spiky thorns, a feature giving rise to its common names of devil's bush and touch-me-not and to its specific name *senticosus* (Latin for prickly or spiky). It is also known colloquially as wild pepper. The aerial stems and branches are covered with a light grey or greyish-brown bark. Being Araliaceous, the leaves of this species do resemble ginseng, being palmately divided and long stalked. The leaves bear tiny serrations and prickles on their margins. The flowers, which bloom in July, are small, the male being violet and the female yellow, and are arranged in umbellate formations. The glossy, oval, berry-like fruits turn black as ripening takes place in September. The slender, cylindrical, branched rhizomes are loosely called roots and are pale yellowish brown in colour with a yellowish brown cortex and a white central pith. The rhizome is soft when fresh and hardens on drying; it has a strong, agreeable odour and a spicy and not unpleasant taste.

Because *Eleutherococcus* has been much prized for its anti-stress action, there has been considerable research into its chemical composition. Like ginseng it yields several glycosides, the main types being lignans, phenylpropanoids and coumarins rather than the dammarane saponins of true ginseng. The initial glycosidal compounds were called eleutherosides, eleutheroside A being β-sitosterol glucoside (daucosterol), eleutheroside B was identified as the monoglucoside of syringin (sinapic alcohol), eleutheroside B_1 was the 7-O-glucoside of isofraxidin (=6,8-dimethoxycoumarin), eleutheroside B_4 was isolated as sesamin, eleutheroside C was methyl-α-D-galactoside and eleutherosides D and E are closely related syringaresinol-di-O-β-D-glucoside isomers; eleutheroside F was undefined. The phenylpropanoids comprised caffeic acid and its ester and coniferyl aldehyde. The terpenoid oleanolic acid has also been recorded (Newall *et al.,* 1996). Polysaccharides are present including glycans referred to as eleutherans A-F based on galactose, glucose, maltose and sucrose and

contributing to the immunostimulant effect of the drug (Hikino *et al.,* 1986). The roots yield about 0.8 per cent of volatile oil.

Much of the literature available has been published in Russian, often with brief English summaries. Some English literature is available although most textbooks only offer sketchy outlines of the characteristics of the plants. In many ways the pharmacology of eleutherococcus resembles that of *Panax ginseng* and related species.

Eleutherococcus is usually considered more effective as an anti-stress agent than ginseng. Although it has no apparent history of folklore usage in Russia and does not appear in the early pharmacopoeias, in modern "traditional" Chinese medicine it is valued as an enhancer of stamina thus countering fatigue, an improver of physical performance during exercise and an agent encouraging recovery after physical effort. Its tonic action also boosts the immune system. The stimulant and tonic effects are reputedly stronger and longer acting than ginseng. The commercial product is normally powdered root diluted with a suitable innocuous agent before packing in capsules or tabletting although dried aqueous extracts are also used at a dose level of 200–1600 mg. As with ginseng, the commercial product may well be unstandardised. It is also relatively expensive and, like ginseng, subject to abuse syndrome.

Eleutherococcus has similar immunomodulatory characteristics to *P. ginseng*. Thus increased resistance to induced infection can be demonstrated e.g. for listeriosis in rodents ingesting prophylactic doses although parallel therapeutic administration yielded reduced resistance. Also improved specific antiviral immunity in rodents and man (influenza and coryza), anti-toxic substance protection (cardiac glycosides in frog, diethylglycolic acid in mouse and alloxan in rat, anti-cancer drugs in man) and protection against the toxic effects of X-ray radiation have been reported. The high molecular weight polysaccharide compounds have been implicated as the agents lessening thioacetamide, phytohaemagglutinin and X-radiation toxicity. Placebo controlled, double blind clinical trials involving human subjects have indicated the positive effects of an ethanolic extract of eleutherocoocus in healthy patients. The increased total lymphocyte count and particularly the increased T-lymphocyte cell count was prompted by a daily regimen of 30–40 mL of ethanolic eleutherococcus extract containing 0.2 per cent w/v eleutheroside B. As granulocyte and monocyte levels were not significantly changed, it was obvious that the lymphocytes were specifically targetted (Newall *et al.,* 1996).

The anti-stress action and adaptogenic responses of eleutherococcus preparations are as controversial as for *P. ginseng* preparations. Nevertheless there is a considerable literature available, especially from Russian sources, confirming the value of eleutherococcus in man and laboratory animals for the improvement of stamina and physical work, mental activities such as proof reading, telegraphy and maze tests, resistance to high and low temperatures, resistance to radial acceleration and motion sickness, adaptation to hypoxia, in the reduction of the days lost due to sickness, and for effective athletic performances. Trials involved large numbers of healthy animal and human subjects with a wide range of ages. Similar trials on unhealthy human subjects revealed particular value in atherosclerosis (except in patients with high blood

pressure), acute pyelonephritis, normalisation of hypertensive and hypotensive states, acute craniocerebral trauma, certain neuroses, rheumatic heart disease (due to anti-aggregatory action on blood cells), chronic bronchitis and for children in abating forms of pulmonary tuberculosis (Farnsworth *et al.,* 1985). In 1995 Zhekalov reported a trial involving the adaptation of 205 males aged 18–19 years to conditions in the Mongolian mountain-desert region. Monitoring parameters such as mental and physical work capacity, changes in dynamic and isothermal regimes, and systolic and arterial pressure, Zhekalov noted that preparations of *E. senticosus* and of *Rhododendron adamsii* Rehd. prompted more rapid normalization to the new external conditions. As with *P. ginseng* extracts, published reports concerning improvement of overall work output are confusing. In both cases there is a claim of more economical use of glycogen and high energy phosphates and more efficient metabolism of lactic and pyruvic acids under stress conditions. Precise control of the adaptogenic phenomenon is not understood but is believed to involve energy, nucleic acid and protein regulation in the tissues (Farnsworth *et al.,* 1985).

Recent Japanese work indicated a protective action of eleutherococcus n-butanol extract against cold water stress-induced gastric ulcers in rats. Pre-administration of the extract orally for 2 weeks at 500 mg/kg/day was most effective, inhibiting ulcer formation by 61.1 per cent compared with a control group treated with distilled water. It was suggested that principally (51.3 per cent) syringaresinol-di-O-β-D-glucoside and to a lesser extent (21.4 per cent) chlorogenic acid were responsible (Fujikawa *et al.,* 1996). Clinical application has yet to be proved.

In animal studies the cardiovascular actions of eleutherococcus have been compared with aspirin, the anti-aggregatory action being assigned to the 3,4-dihydroxybenzoic acid found in the root. The activity of this compound versus collagen and adenosine diphosphate-induced platelet aggregation resembled that of aspirin but it was less effective than aspirin versus arachidonic acid-induced platelet aggregation. Anti-inflammatory and anti-oedema actions in mice have also been reported (Farnsworth *et al.,* 1985; Yun-Choi *et al.,* 1987).

Studies on the central nervous system reactions revealed stimulant effects in rabbits but sedative effects in rats and mice (Farnsworth *et al.,* 1985).

Like *P. ginseng* preparations, both hypoglycaemic and hyperglycaemic properties have been reported as the result of animal experimentation involving rabbits and mice. Hypoglycaemia was apparent after intraperitoneal injection of aqueous eleutherococcus extracts and was, as with *P. ginseng,* attributed to polysaccharide compounds absorbed intraperitoneally but only slowly orally. The polysaccharides were eleutherans A-G. The results obtained with human subjects are unconvincing, both positive and negative observations being recorded, suggesting that normalisation tends to occur but no positive beneficial effect was recorded for diabetes mellitus patients (Farnsworth *et al.,* 1985).

Eleutherococcus, like true ginseng, has a reputation as a sexual tonic. Gonadotrophic activity in immature male mice, oestrogenic activity in immature female rats and anabolic build-up in immature rats have been described. In addition, eleutherococcus was claimed to enhance the reproductive capacity of

bulls and cows with no adverse effects on measurable blood parameters such as haemoglobin, total plasma protein, albumin and globulin, and protein coefficient (Farnsworth *et al.,* 1985).

Further pharmacological actions described for eleutherococcus include increased liver regeneration in partially hepatectomised mice, increased catecholamine accumulations in brain, adrenal glands and urine, some effect on induced hypothermia in rabbit, rat and mouse (Farnsworth *et al.,* 1985) and *in vitro* inhibition of hexobarbitone metabolism (66 per cent) (Medon *et al.,* 1984). The anti-tumour action of the total polysaccharide fraction on mouse sarcoma S180 and human chronic myelogenous leukaemia K562 cells was described by Tong *et al.* (1994).

Eleutherococcus has been widely used and especially so in Russia yet relatively few cases of side effects have been recorded. Potential side effects include alterations in heart rhythm, insomnia, irritability, melancholy and anxiety in hypochondriacs ingesting higher doses of extract and some hypersensitivity reactions. The drug is generally regarded as non-toxic because trials involving many species of laboratory animal and humans have yielded no toxic effects. A chronic toxicity study in which rats were fed 5 mL/kg of an ethanolic extract for 320 days revealed no toxic or lethal reactions. Likewise teratogenicity studies in laboratory animals yielded no abnormalities (Farnsworth *et al.,* 1985). Nevertheless a more recent report refers to the sudden rise in the serum digoxin level of a 74-year old man but without toxic effects. The cause was simultaneous ingestion of Siberian ginseng and the level returned to an acceptable level on cessation of Siberian ginseng treatment but returned when ginseng was resumed several months later. The exact cause is so far unknown but the result could be due to an unexplained false serum assay result (McRae, 1996). Therefore care is needed when the Siberian ginseng is taken in association with other medicines.

The recommended dose of powdered dried eleutherococcus root for healthy persons is 0.6–3.0 g daily for up to 4 weeks. For healthy individuals Russian workers suggested a recommended dose of 2–16 mL of an ethanolic extract (1:5) up to three times a day for up to 60 consecutive days. The regimen for unhealthy persons was 0.5–6.0 mL for up to three times a day for up to 35 days. In all cases it was considered good practice to follow the dosing periods with drug-free periods of 2–3 weeks (Farnsworth *et al.,* 1985).

Eleutherococcus, like Asiatic ginseng, is an adaptogenic plant with several pharmacological actions. Its application in medicine is, as yet, restricted by lack of effective standardisation and therefore lack of consistent effect.

Brazilian ginseng is the name applied incorrectly to the roots of *Pfaffia paniculata* (Martius) Kuntze and *P. iresinoides,* plants belonging to the family Amaranthaceae. Saponin glycosides, estimated at ~4 per cent, are derived from the hexacyclic norterpene pfaffic acid and possess cytotoxic activity (De Oliviera *et al.,* 1980; Nishimoto *et al.,* 1988). Known colloquially by the Portuguese name "paratudo", meaning "for all" or "for everything", Brazilian ginseng is considered a restorative particularly in convalescence, during the menopause and for disturbed emotional states. It is not advised during pregnancy. The recommended daily dose is 1 g usually in 500 mg capsules taken orally before breakfast. The chemistry

and medicinal usage of *P. paniculata* was reviewed by De Oliviera in 1986. Occupational asthma induced by powdered *P. paniculata* inhalation whilst processing "Brazilian ginseng" capsules was reported by Subiza *et al.* (1991). They noted that powdered Korean ginseng, tested in a similar manner, did not cause any such reactivity. Antiinflammatory activity of *P. paniculata* and *P. stenophylla* (Sprengel) Stuchl. has also been described (Mazzanti *et al.*, 1993).

"Wild red American ginseng" or "Wild red desert ginseng" are misleading trivial names applied to the roots of *Rumex hymenosepalus* Torrey, a plant belonging to the family Polygonaceae and more correctly known as canaigre. It is indigenous to the arid areas of Mexico and Texas. As this plant is not Araliaceous it is not surprising that it does not yield dammarane-type saponin glycosides. Chemical investigations have shewn that the roots yield about 30–45 per cent tannins accompanied by anthraquinones. The indigenous uses of canaigre root by the Mexican Indians relate solely to the astringent properties of the plant. Therefore topical preparations have been developed for the treatment of skin irritations and internal medicines are used for sore throats and to treat diarrhoeal conditions. There are no reports of canaigre being used in stress conditions or as an adaptogen or immune system tonic. The American Herb Trade Association decided in 1978 to discourage the use of the name ginseng for this plant; it was also noted that the high tannin content was potentially carcinogenic.

There are other medicinal plants in Chinese medicine which have a similar action to ginseng although ginseng is the most valued and the most expensive. Such plants are available on the herbal market and are derived from varying plant families but share the general name "shen". Thus ginseng is known as "jen-shen" or alternatively "huang-shen", the yellow-shen.

"Sha-shen" or "Pai-shen", white-shen, comprises the wild and cultivated roots of *Adenophora verticillata* of the plant family Campanulaceae; a close relative, *A. tetraphylla* or Southern Sand Root or "Nan sha shen", is mentioned in many current books on Chinese medicine. Such plants are used for their general tonic and restorative actions and also for lung disorders. In traditional Chinese therapy sha-shen was employed for lung complaints with burning and jen-shen (ginseng) for lung complaints with cooling, it being claimed that sha-shen was a tonic for the yin whilst jenshen was a tonic for the yang of the five viscera.. The roots yield milky white juices and are whiter in appearance than ginseng roots. Sha-shen roots taste bitter and cool.

"Hsuan-shen" or "Hai-shen", black-shen, is derived from the roots of *Scrophularia oldhami* Oliv. and is a close relative of the common figwort *S. nodosa* L. ("Xuan-shen") of the family Scrophulariaceae. The plant emerges in March, produces a stem resembling that of ginseng but the leaves are opposite, long and serrated resembling wild sesame, bears white or greenish-blue flowers in August and finally yields black seeds. The plant attains a height of 1.2–1.5 m. The root tastes bitter and cool with a fishy odour. Like ginseng it has general tonic and restorative actions but is also used as a diuretic and in the treatment of fevers. The epithet "black" refers to the darkness of the stem, roots and seeds and, by colour association reminiscent of the Doctrine of Signatures, black-shen is used for kidney conditions.

"Tan-shen", "Dan-shen" or "Ch'ih-shen", red-shen or scarlet root, is the Labiateous plant *Salvia miltiorrhiza* Bunge. Emerging in February it reaches a height of only about 0.3 m. Its opposite leaves resemble those of its close relative *Mentha x piperita* L. (peppermint). Purple flowers appear in April followed by red fruits. In traditional medicine red-shen is used as a tonic stimulating the blood circulation and encouraging the formation of blood cells and is also administered generally for blood disorders.

"K'u-shen", bitter-shen, is the roots of the Leguminous species *Sophora angusifolia* or *S. flavescens,* common in central China and Manchuria. The plant is characterised by typical Leguminous yellowish-white flowers and long, narrow pods. The elongated, yellowish root is very bitter. In traditional medicine it is employed as a bitter tonic for many disorders such as fevers, dysentery, scrofulous swellings and jaundice.

"Tzu-shen", "Quan-shen" or "Mou-meng", the purple-shen, is known in Europe as bistort or English serpentary and comprises the roots of the shade-loving *Polygonum bistorta* L., family Polygonaceae. The purple-white flowers appear in May and the fruit yields black seeds. The roots are purplish-black with a bitter, astringent taste due to tannins. Hence the traditional use as a treatment for dysentery, haemorrhages, amenorrhoea and fevers and as a general tonic. By colour association the plant roots were also used for kidney and blood conditions.

"Tai-tzu-shen" has been used as a ginseng substitute because of its tonic, restorative action and comprises the roots of the Caryophyllaceous species *Pseudostellaria rhaphanorrhiza* Pax. or *P. heterophylla* (lesser ginseng root).

"Tang-shen" is a name applied to several varieties of plants but principally to the roots of *Codonopsis tangshen* Oliv., a Campanulaceous perennial herb growing in northwest China and known as bastard ginseng.. The synonymous *C. pilosa* or *Campanumaea pilosula* root is known as "Dang-shen" or "Relative Root". Tangshen grows to a height of 1 m with long oval leaves. The yellowish-brown root attains a length of 25 cm and is deeply wrinkled; the central pith is pale-coloured and the root has a brittle fracture. The taste is sweet and malty. The medicinal applications resemble true ginseng but the therapeutic effect is weaker; in particular it is valued as an energy provider, for building immunity and improving resistance, in recuperation from surgical trauma and as a prophylactic for some forms of heart disease. In traditional Chinese medicine it is regarded as a substitute for true ginseng when prices are high or supplies scarce (Hou, 1978).

A recent publication bears the title *Withania somnifera:* the Indian ginseng, Ashwagandha (Singh and Kumar, 1998). *Withania somniferum* Dunal is a Solanaceous plant found in southern Europe, India and Africa. It has a reputation in Indian indigenous medicine as a sedative, hypnotic and antiseptic agent. Extracts of the plant demonstrate antimitotic activity. Typically the plant yields tropane alkaloids and steroidal lactones named withanolides but no ginsenosides.

The occurrence of these substitutes, adulterants and misnamed plants on the commercial market confirms the need for adequate quality control. There has been little scientific investigation of many of the alternative plants and detailed data is needed concerning chemical composition, pharmacological action and possible therapeutic application.

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