Natural Toxins and Chemopreventives in Plants

Roger A. Coulombe, Jr.

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Introduction

Our food contains, in addition to the many well-known major (protein, fat, carbohydrate, and fiber) and minor (vitamins, minerals, and nonessential compounds) nutrients, thousands of naturally present toxic plant compounds. Some are known or strongly suspected to cause cancer in laboratory animals and, thus, may be potentially carcinogenic in people. Many of these compounds are commonly termed "nature’s pesticides" because they are often toxic to predators, such as insects and animals, thereby conferring a competitive advantage to the plant that produces them. Other natural toxins in plants have no known role. Although these chemicals are in every meal we eat, they have received little attention compared to that given to minute residues of synthetic chemicals such as PCBs and pesticides. Our food contains significantly greater amounts of natural plant toxins and carcinogens than the synthetic kind, and our bodies aren’t able to distinguish between the two. Still, while popular notion remains that "natural is good," it is clear that natural toxins pose a far greater health risk than that posed by synthetic chemicals in our foods.

Fortunately, our food also contains natural chemicals that can counteract the adverse effects of many natural and synthetic toxins. While much more work on these "antitoxins" or "chemopreventives" is needed, the data thus far are very encouraging that some plant foods can actually reduce the incidence of certain types of cancer. Hundreds of animal and epidemiological studies have identified several foods or specific compounds that offer protection against the carcinogenic effects of a wide variety of natural and synthetic chemicals. A few compounds have been shown to actually reverse the carcinogenic process in animals. As might be imagined, the field of anticarcinogenesis is one of the most exciting areas in nutritional toxicology and cancer research.

Natural Plant Toxins in Foods

The following is a survey of some of the most well-studied and characterized plant toxins.

Canavanine

Despite the notion that they are the ultimate health food, alfalfa sprouts contain up to 15,000 ppm canavanine. Canavanine is produced in other legumes as well, such as the jack bean. It is an analog of arginine and, as such, can substitute for this amino acid in cellular proteins, thereby compromising their function. Canavanine inhibits the enzyme nitric oxide synthetase and
induces heat-shock proteins in human cells in vitro. Due to its action as an antimitabolite, it is under current consideration as an antitumor drug in combination with other antimitabolites such as 5-fluorouracil, but has not yet been tested for carcinogenicity. Canavanine is suspected of causing autoimmune disorders in people, such as lupus erythematosus. Primates fed alfalfa sprouts develop a severe toxic syndrome resembling human lupus.

Cyanogenic Glycosides

Cyanogenic glycosides are cyanide-containing compounds naturally present in seeds from apples, apricots, cherries, peaches, pears, plums, quinces, and also in almonds, sorghum, lime beans, cassava, corn, yams, chickpeas, cashew nuts, and kicin. High cyanide varieties, distinguished by their bitter taste, may contain over 600 ppm cyanide on a dry weight basis, while "sweet" varieties contain much less. There are several such cyanogenic glycosides, of which linamarin, amygdalin, and dhurrin are examples (Figure 6.1). In the 1970s, amygdalin, as laetrile, gained notoriety as a fad remedy and preventative for cancer and other ailments. Cyanogenic glycosides are toxic by virtue of the release of free hydrogen cyanide which occurs when the plant tissue is disturbed as during chopping, processing, or ingestion. These conditions initiate the hydrolysis of the glycoside by the action of β-glucuronidases and other enzymes naturally present in the plant tissue and in the intestinal lumen. The process also can be initiated by acid, but this doesn't appear to occur in the digestive tract to any great extent despite the acid environment in the stomach. Hydrolysis by β-glucuronidases produces the sugar and a cyanohydrin, the latter spontaneously or enzymatically degrades to form free hydrogen cyanide. The scheme of release of hydrogen cyanide is depicted in Figure 6.1.

Cyanide is one of the most acutely toxic chemicals. It binds to and inactivates heme enzymes, the most critical of which is mitochondrial cytochrome oxidase, resulting in an acute, life-threatening anoxia. The two-step therapy is initiated with sodium nitrite, which induces methemoglobinemia permitting the release of cyanide from heme proteins, followed by sodium thiosulfate, which acts as a substrate for rhodanese, an endogenous hepatic enzyme that catalyzes the conversion of free cyanide to the less toxic thiocyanate.

Cases of acute human poisoning from the cyanide released from certain varieties of lime beans, cassava, and bitter almonds are a regular occurrence. Due to the importance as a subsistence crop in Africa and South America, cyanogenic glycosides in cassava probably represents the greatest health risk. Traditional methods of processing cassava, such as sun-drying, soaking, boiling, and fermenting, eliminate most of the cyanide. In addition to regular cases of human deaths, cyanogenic glycosides in cassava may be responsible for birth defects, endemic goiter, and “konzo,” an upper myelopathic motor neuron disease endemic to East Africa. Cyanogenic glycosides also have been implicated as a causative agent of diabetes. The risk associated with
cyanide poisoning due to cassava is negligible in the U.S. because associated products (such as tapioca pudding) are rarely consumed here.

**Allyl Isothiocyanates**

Allyl isothiocyanates are a group of major naturally occurring compounds that confer a pungent flavor to foods, such as mustard and horseradish, where it is present at about 50 to 100 ppm. It is also present at much lower levels in Brassica vegetables such as broccoli and cabbage, and in cassava and other tropical staple foods. In high doses, it is carcinogenic in rats, but it is nonmutagenic in bacteria. Isothiocyanates occur in cruciferous vegetables as glucosinolate conjugates that are hydrolyzed when the plant releases enzymes such as dialing chervil (Figure 6.2). Isothiocyanates are toxic goitrogens which inhibit binding of iodine in the thyroid gland. Because iodine is required for the formation of the critical thyroid hormones thyroxine (T₄) and triiodothyronine (T₃), isothiocyanate-induced hyperthyroidism (goiter) mimics iodine deficiency. Hyperthyroidism is a physiological response as the thyroid attempts to compensate for reductions in both T₄ and T₃ production.

Normal dietary exposures to isothiocyanate-containing foods releases milligram amounts of isothiocyanates. As in the case of cyanogenic glycosides, normal processing steps (chopping, rinsing, milling) results in a safe product.
Hydrazines and Other Toxins in Edible Mushrooms

The three most commonly eaten mushrooms are the cultivated mushroom (Agaricus bisporus), the shiitake mushroom (Cortinarius dimitatus), and the false morel (Gyromitra esculenta). All contain substantial amounts of compounds in the hydrazine family (Figure 6.3), many of which are potent liver toxins and animal carcinogens. N-methyl-N-formylhydrazine is commonly found in concentrations of 500 ppm and causes lung tumors in mice. It is carcinogenic in hamsters as well. People consuming a 100 g serving and, therefore, ingesting 50 mg would be getting very nearly the same dose on a per kilogram (kg) body weight basis as that giving cancer to mice upon sustained daily exposure.

Shiitake mushrooms and the cultivated mushroom contain up to 3000 ppm agaritine. A metabolic product of agaritine (a diazonium derivative) is a

![Diagram showing enzymatic formation of goitrigenic isothiocyanate from glucosinolate.](image)

**FIGURE 6.2**  
Enzymatic formation of goitrigenic isothiocyanate from glucosinolate.

Endemic goiter is seen in geographical areas like India and Africa, where consumption of poorly processed foods is coincident with iodine deficiency.
potent carcinogen and a mutagen. Gymnostigma (acetaldehyde-N-methyl-N- formylhydrazonone), the major carcinogenic hydrazone in the false morel, also is present in similar concentrations. Other carcinogenic hydrazines include p-hydrazinobenzoate (present in A. bisporus at 10 ppm) and 4-(hydroxymethyl) benzenediylazoate (HMDB), the latter shown to induce DNA strand breaks presumably through a carbon-centered, free-radical intermediate, a possible mechanism of the carcinogenic action of hydrazines in general. Another carcinogenic hydrazine, methylhydrazine, is present in smaller concentrations (14 ppm). Whole mushrooms have been shown in numerous studies to cause cancer in laboratory animals, but whether they are a significant cause of cancer in people is uncertain. Recently, a diet of whole A. bisporus mushrooms (30% of total diet) did not cause a significant increase in tumors compared to controls in rats.\textsuperscript{10}

**Toxic Substances in Spices and Flavoring Agents**

Saffrole, estragole, myristicin, β-asarone, piperine, and isoasafrole (Figure 6.4) are closely related alkylbenzenes found in many spices, essential oils, and herbs. They also are present, in much lower levels, in parsnips, parsley, and sesame seeds. All are weak to moderate rodent hepatocarcinogens.

Saffrole is found in sassafras tea and makes up 85% of oil of sassafras (Sassafras albidum), which was once used to flavor root beer. It has been banned as a flavor additive since 1960, but is a minor, natural component of nutmeg, mace, star anise, cinnamon, and black pepper. Sassafras bark is an ingredient in file powder used to make gumbo, a spicy Cajun dish. Estragole, a related aromatic flavor agent, is found in tarragon, basil, and fennel, and is likewise a weak carcinogen. Saffrole is bioactivated to a DNA-binding species via hydroxylation of benzyl carbon, conjugation with sulfate, and then alkylation of DNA with displacement of the sulfate group. Another route of bioactivation involving a rearrangement to electrophilic quinone methides has been identified for saffrole and is presumed to occur with related flavor compounds. Epoxidation at the allylic side chain is another activation route identified for saffrole, estragole, and eugenol. Epoxide intermediates of these compounds degrade to form covalent adducts with guanine in vitro. Isoasafrole, a component of ylang-ylang (Cananga odorata) oil, a flavorant and scent, is carcinogenic in mice. Many of these alkylbenzenes interact with cytochrome P-450 (CYP) mediated metabolism. For example, both isoasafrole and saffrole are powerful inducers of 1A family CYP enzymes. Saffrole and isoasafrole also inhibit CYP 2E1 enzymes and, in so doing, protect against carbon tetrachloride liver toxicity in mice. Piperonyl butoxide, a related synthetic alkylbenzene, is a commercial CYP inhibitor used as a synergist with pyrethroid and carbamate insecticides.

β-asarone is a major component of oil of calamus (derived from the Acorus calamus root which is a folk remedy for indigestion), and was once used to flavor vermouth and bitters. It causes intestinal tumors in rats.
Myristicin is a major flavor component of nutmeg, derived from the dried, ripe seed of the tree *Myristica fragrans*. Approximately 2% of nutmeg is myristicin, which is present in the volatile oil distilled with steam from the dried seeds. Mace, a closely related spice, is derived from the arilode, or outer coating of the seed. The world's principal commercial supply of nutmeg is grown in the Malay peninsula. Myristicin is found in black pepper, parsley, celery, dill, and carrots as well. While not thought to be carcinogenic, large amounts of nutmeg, equivalent to two whole nutmeg seeds (ca. 15 g) are intoxicating and allegedly hallucinogenic. However, large doses also are associated with undesirable side effects, such as tachycardia, flushed skin, and dry mouth. Pure myristicin is not as hallucinogenic as nutmeg. Thus, it is assumed that other components in nutmeg may contribute to its potential psychoactive properties.

Piperine, an alkaloid present in high concentrations (10%) in black pepper (*Piper nigrum* and other sp.), is largely responsible for the pungent "bite" of
this condiment. Powdered \textit{P. cubeba} berries are added to cigarettes and smoked as a remedy for throat irritation, and oil derived from these berries is added to some throat lozenges. Reports of the cancer-causing ability of this compound are conflicting. Extracts of black pepper caused cancer in mice at several sites in skin painting tests, while orally injected pipernine did not.\footnote{Furthermore, pipernine is not mutagenic in a number of in \textit{vivo} screening assays.} However, under appropriate conditions, pipernine is chemically converted to potentially carcinogenic intermediates. In the presence of nitrite, pipernine is nitrosated to form highly mutagenic nitrosamine intermediates in \textit{vivo}, which may have potential carcinogenic activity. Like the related \textit{alkaloid} benzoic acid, pipernine also affects CYP expression and activity. For example, pipernine specifically inhibits CYP 2E1, while specifically inducing the expression and activity of CYP 1A1 and 2B.\footnote{Capsaicin is the extremely pungent ingredient (up to about 0.5\% in red and yellow chili peppers: \textit{Capsicum frutescens}, \textit{C. conside}, and \textit{C. annuum}. Due to its irritating qualities to the eyes and mucous membranes, a solution of capsaicin in an aerosol spray is a popular dog repellent for mail carriers. Typical creams containing capsaicin (0.025\%) are commercially available as an analgesic. Although its pain relieving qualities are debatable, it has been shown to cause a local depletion of \textit{substance P}, an endogenous neuropeptide known to transmit pain impulses. Thus, even though the physiological conditions causing pain may persist, capsaicin prevents pain impulses from reaching the brain. Some evidence suggests that capsaicin is a weak carcinogen. It is a bacterial mutagen in the Ames test and causes benign digestive tract adenomas in mice with life-long dietary exposure at 0.03\%.\footnote{Intraperitoneal injections of capsaicin causes the formation of sister-chromatid exchanges and micronuclei in mice.\footnote{Sister-chromatid exchanges and micronuclei are genotoxic endpoints presumably associated with cancer risk. One possible toxic mechanism is that CYP 2E1 converts capsaicin to an active phenoxyl radical intermediate that has the potential for alkylating tissue macromolecules such as DNA and protein.} Glycyrhrizin is a saponin-like glycoside derived from the dried roots of \textit{Glycyrrhiza glabra}, popularly known as licorice. Licorice is one of the oldest folk medicines traditionally used as an expectorant, flavoring agent (also used to mask the bitter taste of medicines), and demulcent. Cuneiform tablets dating to about 4000 B.C. mention the medicinal use of licorice by the Sumerians, and pieces of licorice root was found in King Tut's tomb. The one caveat to the many benefits of licorice is that it promotes hypertension. Glycyrhrizin is thought to be responsible for the hypertensive properties of licorice, which is brought about by the inhibition of the enzyme \textit{11\beta}-hydroxysteroid dehydrogenase. This enzyme acts as a protective modulator in certain mineralocorticoid receptor-rich tissues — particularly kidneys, colon, and salivary gland — by metabolizing receptor-active glucocorticoids such as cortisol to \textit{11\beta}-derivatives (e.g., cortisone) which are not receptor agonists. A condition of excess glucocorticoids brought about by inhibition}
of the dehydrogenase leads to severe sodium retention, hypokalemia, and hypertension. Licorice reportedly has been responsible for fatal episodes of acute hypertension in people. Consequently, people with heart problems or hypertension should avoid licorice, as little as 100 to 200 g/day can cause persistent, heightened mineralocorticoid activity.

D-limonene is a major constituent of citrus oils and is also found, in much lower amounts, in other fruits and vegetables. The major sources of d-limonene are oils of orange, grapefruit, and lemon. Citrus peel oil can contain as much as 95% d-limonene. d-Limonene per se or citrus oils where d-limonene is the major constituent have been widely used as flavoring agents and/or as fragrances in perfumes and soaps, and in a variety of foods such as ice cream, soft drinks, baked goods, gelatin, chewing gum, and puddings. It is also the active ingredient in “natural” citrus-based degreasing solvents and in insect repellents. Animal studies show that d-limonene is nephrotoxic to male animals. d-Limonene binds specifically, but reversibly to α1-globulin which is the major low molecular weight protein produced by the renal proximal tubules and, hence, excreted in the urine of the male rat. Female rats excrete much less α1-globulin. Accordingly, male rats that do not excrete α1-globulin (NRR strain) do not exhibit nephrotoxicity following d-limonene treatment.

Some animal studies indicate that d-limonene causes renal tumors in rodents. When administered orally, d-limonene induced renal adenomas and carcinomas in male rats, but not in mice. Oral d-limonene also was shown to significantly promote the development of N-nitrosomethylhydroxylamine-induced renal tumors in male rats. However, the toxicity and carcinogenicity of d-limonene appear to be absolutely species- and gender-specific due to the specific binding of this natural compound with α1-globulin. Because humans do not excrete α1-globulin, d-limonene is not thought to be harmful to people. Indeed, several studies have shown d-limonene to possess chemoprotective properties.

**Pyrethridine Alkaloids**

Pyrethridine alkaloids (PAs) are common plant toxins produced by over 200 species of flowering plants, from genera such as Senecio, Crotalaria, and Convolvulus. They are often present at very high levels—as much as 5% of the plant’s dry weight. Pyrethridine-alkaloid-containing plants pose significant health hazards to people who consume some kinds of “natural” herbal teas and traditional folk remedies and those who eat grain-based foods contaminated with PA-containing plant parts. Some PAs have been investigated in clinical trials for their antihypertensive potential.

These chemicals are often carcinogenic, mutagenic, and teratogenic and chronically hepatotoxic. Pyrethridine alkaloids are derivatives of a necine base like retronecine, stenecine, heliotrine esterified to various macromolecular acid substituents (Figures 4-5). Pyrethridine alkaloids are activated by CYPs primarily of the 3A4 subfamily to reactive bifunctional pyrethric electrophilic aldehydes that form covalent cross-links to a variety of cellular nucleophiles, such as DNA and
proteins. Pyrrolic intermediates then reportedly form electrophilic carbonium ions at atoms 7 and 9 and cross-link cellular nucleophiles at these positions. CYP3A4s also convert PAHs to the less toxic and more easily excreted N-oxides that do not interact with cellular constituents (Figure 6.6). Accordingly, animals that metabolize PAHs to produce proportionally more N-oxides (such as sheep) appear to be relatively resistant to the toxic effects of PAHs.

**FIGURE 6.5**
Chemical structures of selected pyrrolizidine alkaloids.

**FIGURE 6.6**
Metabolic fate of pyrrolizidine alkaloids to N-oxides and electrophilic pyrones (nuc = nucleophile).
compared to animals that produce more of the pyrrole (rats and horses). Other hydrolys reactions also may occur that decrease the toxicity of PAs. DNA cross-links are probably a critical event in PA bioactivity in that the cytotoxic, antimitotic, and megalocytic activity of PAs closely correspond with the formation of cross-links in vitro.14,21 Pyrrolizidine alkaloids from both DNA interstrand and DNA-protein cross-links in equal amounts in vitro.20,22 Structure-activity studies have revealed that the presence of a continuous macrocyclic diester and α,β-unsaturation are important structural determinants for DNA cross-link formation.23 Thus, PAs like senecionine are more potent cross-linkers than monocrotaline, which is more potent than open diesters such as latifoline and heliosupeine. Of those examined, the simple necine senecine is the least active cross-linker. The pattern of proteins cross-linked by PAs is similar to those cross-linked by other bifunctional compounds, such as cisplatinum and mitomycin C. actin has been postulated to be one of the proteins involved in the PA-induced cross-links.24

Petasitine is found in Petasites japonicus, a medicinal herb used as an expectorant and cough suppressant. The flower stalks of this herb are used as a food or herbal remedy. When incorporated into the diet, dried stalks are hepatocarcinogenic to rats. Purified petasitine is also hepatocarcinogenic in rats as well as mutagenic in bacteria. Tussilago farfara (coltsfoot) is a common herb used for coughs and bronchitis in Europe and Asia. (Tussilago is the ancient Roman name for “cough suppressor.”) The plant contains the pyrrolizidine alkaloid senkirkine at concentrations as high as 134 ppm, as well as high concentrations of senecionine, another very toxic and carcinogenic PA. Again, both the dried buds of coltsfoot (when ground and mixed) in the diet and purified senkirkine or senecionine cause liver tumors in rats, and both are bacterial mutagens.

Human intoxication by PA-containing plants is well recognized and reported in the medical literature, and is endemic in Jamaica, India, and parts of Africa. Diseases, such as liver cirrhosis, veno-occlusive disease, and liver cancer, are linked to consumption of PA-containing plants. Hispanic and Native American populations in the west and southwest U.S. are at high risk for PA intoxications due to their tradition widespread use of herbs, occasional lack of confidence in traditional medicine, and, more commonly, lack of access to medical care.

Comfrey (Symphytum officinale) is a nearly universal herb commonly sold not only in health food stores and by herbalists, but also in supermarkets. Since ancient Greek and Roman times, both leaves and roots have been used to make teas and compress pastes to treat a variety of external and internal diseases, such as healing of wounds, skin disorders, and respiratory diseases. Numerous vegetarian recipes call for comfrey leaves to make soups, sausages, and breads. Comfrey leaves and roots contain up to 0.3% pyrrolizidine alkaloids such as intermedine, lycoisamine, symphytine, and others. Diets containing powder from dried leaves and roots caused liver tumors in rats.
Additionally, these pure pyrrolizidine alkaloids also are animal carcinogens and bacterial mutagens. There are several cases cited in the medical literature of comfrey-related intoxications in people. The well-demonstrated reported toxicity and carcinogenicity of comfrey is such a significant cause for concern that the governments of Australia, Canada, Great Britain, and Germany either restrict comfrey’s availability or have banned its sale entirely. The U.S. Food and Drug Administration (FDA) has not yet acted to restrict the sale of pyrrolizidine alkaloid-containing foods.

Substances in Bracken Fern

Bracken fern (*Pteridium aquilinum, euculentum*, and others) is widely used as human food in greens or salads in many countries such as New Zealand, Australia, Canada, the U.S., and especially Japan. It is also a forage plant for sheep and cattle. It first attracted the attention of veterinary scientists who noticed severe toxicity — bladder cancer, bone marrow depression, severe leukemia, thrombocytopenia, and a hemorrhagic syndrome — in livestock grazing on this plant. When fed to rodents, bracken is a strong bladder, lung, and intestinal carcinogen. Lactating cows fed bracken fern produced milk that was carcinogenic to rats, showing that human exposure also may occur through cow’s milk. Human consumption of bracken fern has been linked to an increased incidence of esophageal cancer in Japan.

The major carcinogen in bracken is believed to be *ptaquiloside* (Figure 6.7), a potent norsesquiterpenoid glucoside that is present at often high concentrations (up to 1.5% dry weight) in the plant. Ptaquiloside is a potent alkylator of DNA that appears to interact primarily with adenines at codon 61 in the *Ha*-ras oncogene in ptaquiloside-fed sheep.27 The plant also contains quercetin, kaempferol, and other mutagenic compounds of the flavonoid family which may contribute to its carcinogenicity. It also contains toxic tannins.

![Ptaquiloside](image)

**FIGURE 6.7**

Chemical structure of ptaquiloside, the major carcinogen in Bracken fern.
Acetylcholinesterase Inhibitors in Potatoes

Members of the family Solanaceae contain a variety of toxic glycoalkaloids. Potatoes (Solanum tuberosum) are an important food staple in many parts of the world and, under certain conditions, produce a variety of glycoalkaloids. Potatoes that have been damaged, exposed to light (green), or sprouted contain the glycoalkaloids α-solane and α-chaconine (Figure 6.8) that can exceed concentrations of 100 ppm. Like physostigmine, solanine and chaconine are highly potent inhibitors of the enzyme acetylcholinesterase. Higher amounts of solanine and chaconine are present in the potato greens (tops). Healthy potatoes contain negligible amounts of these toxins. Episodes of human poisoning by green potatoes have been documented. Poisoning symptoms — gastric pain, weakness, nausea, vomiting, labored breathing — are consistent with acetylcholinesterase inhibition. These symptoms have been duplicated in clinical trials with human volunteers. Studies have
indicated that the acetylcholinesterase inhibitory activity of solanine is probable insufficient to cause these toxic effects, which are probably due to the combined toxicity of solanine with other cholinesterase inhibitors in the potato, such as chaconine.

Most cases of human poisoning and deaths have occurred in Europe, but are occasionally seen in the Western Hemisphere. Poisoning episodes are not infrequent in animals fed damaged potatoes or peel, greens, or trim. A small number of studies in which animals are fed toxic doses of blighted potatoes or pure glycoalkaloids have indicated that these compounds may have weak teratogenic activity. For example, solanine and chaconine (and their aglycone derivative, solandine) induced craniofacial malformations (exencephaly, cephalocele, and anophthalmia) in Syrian hamsters. In that study, solandine was a much stronger teratogen than solanine and chaconine, which were classified as weakly teratogenic. As is the case with their anticholinesterase activity, the teratogenic and embryotoxic effects of solanine and chaconine appear to be synergistic.31

Tannins

Tannins long have been known as plant materials that confer a dark color when applied to animal hides thereby turning them into "tanned" leather. Although a precise definition is difficult due to their diverse and polymeric nature, one working definition is that tannins are a large group of water-soluble polyphenolic compounds with a molecular weight greater than 500 that have the ability to bind to and/or precipitate proteins. It is their ability to bind to proteins that is of toxicological and nutritional concern. Tannins also strongly bind to metals, such as iron, copper, and zinc, and reduce the gastrointestinal absorption of these metals. The two major classes of tannins are the proanthocyanidins (or "condensed tannins") which are flavonoid polymers, and hydrolyzable tannins, which are polymers of gallic or ellagic acid esterified to either glucose or a polyphenol, such as catechin. As will be discussed later, some polyphenolic compounds also are beneficial in that they can prevent cancer in certain animals.

Tannins occur in nearly every plant-derived food, but they are particularly high in bananas, raisins, spinach, red wines, bracken fern, coffee, and tea. Tea is an especially rich source of tannins. Green tea has about 4%, while black tea may contain as much as 33% tannin; adding milk to tea will bind the tannins so that they will be less absorbable. A normal diet will provide several grams per week from fruits and vegetables. Tannins also are high in traditional herbal stimulant drinks such as those derived from Brazilian guarana (Paullinia cupana), betel nut (Areca catechu), and cola nut (Cola nitida and C. acuminata). In animal studies, tannins cause a diminished weight gain and lowered efficiency of nutrient utilization. The major biochemical basis for these effects appears not to be inhibition of dietary protein digestion but rather a systemic inhibition of the metabolism of digested and absorbed nutrients.32
Tannins are liver carcinogens as well in rats and mice. Habitual chewers of betel nut (primarily in India, Pakistan, and Southeast Asia) have a high incidence of carcinoma of the mouth which has been linked to the high tannin content (10 to 25%) of this nut, although other components may be involved. An extract of betel nut causes cancer in hamsters. A high incidence of esophageal cancer in the Transkei in South Africa has been associated with the consumption of high-tannin varieties of sorghum. Some polyphenolic tannins are also antitumorigenic (see below).

**Caffeic Acid and Chlorogenic Acid**

Caffeic and its quinic acid conjugate chlorogenic acid (Figure 6.9) occur in an extremely wide range of fruits and vegetables. Other minor conjugates of caffeic acid also are known to exist. Upon ingestion, chlorogenic acid is hydrolyzed in the gastrointestinal tract to yield caffeic and quinic acids. In humans, caffeic acid is metabolized to α-methylated derivatives, such as ferulic, dihydroferulic, and vanillic acids, and meta-hydroxyphenyl derivatives, which are excreted in the urine. Caffeic acid and conjugates are present in high concentrations (over 1500 ppm) in many seasonings (thyme, basil, anise, caraway, rosemary, tarragon, marjoram, sage, and dill); vegetables (lettuce, potatoes, radishes, and celery); and fruits (grapes, berries, eggplant, and tomatoes). Coffee is particularly rich in these phenolics, in addition to many other compounds (see below). A cup of coffee contains about 190 mg of chlorogenic acid. Caffeic acid inhibits 5-lipoxygenase which is a key enzyme in the biosynthesis of various eicosanoids, such as leukotrienes and thromboxanes. These eicosanoids are mediators of a wide variety of physiological and disease states and are involved in immunoregulation, asthma, inflammation, and platelet aggregation. At high doses (2% in the diet), caffeic acid caused a significant incidence of forestomach squamous cell papillomas and...

**FIGURE 6.9**

Chemical structures of caffeic and chlorogenic acids, 8-methoxypsoralen and coumarin.
carcinomas in both sexes of rats and mice, renal tubular cell hyperplasia in male rats and female mice, and alveolar type II cell tumors in male mice. Oral caffeic acid also can enhance (or inhibit) the carcinogenic activity of known carcinogens. Chlorogenic acid has been shown to be mutagenic in bacteria, but has not been tested for carcinogenicity.

**Coumarin and Psoralens**

Coumarin (Figure 6.9) is widely found in plants such as cabbage, radish, and spinach, and in plants traditionally used as flavoring agents, such as lavender and sweet woodruff (*Asperula odorata*); the latter is an essential herb for making May wine, which is a popular German drink used to salute the coming of Spring. Coumarin is widely found in herb teas based on tonka beans (*Dipteryx odorata*) and sweet clover (*Melilotus albus* and *officinalis*) called "melilot." The name "coumarin" originates from *coumus*, the Carribean name for tonka beans. Purified coumarin was once used as a food additive, but this use was banned by the FDA after it was discovered that high doses caused liver damage in test animals. Coumarin is a powerful anticoagulant and is, in fact, the active ingredient in many brands of rodent baits. It also is used in human medicines as a blood thinning agent. Coumarin has been reported to cause bile duct carcinomas in rats as well.

Psoralens are a group of phototoxic furcoumarins widespread in a number of plant families such as Apiceae (formerly Umbelliferae — celery and parsnips), Rutaceae (e.g., bergamot, lime, cloves, and Moraceae (e.g., figs). Celery contains 100 pph psoralens, while parsnips contain approximately 40 ppm. When activated by sunlight, psoralens are mutagenic, presumably due to their ability to form interstrand and protein cross-links with DNA. Many members of this chemical family are carcinogenic as well, including 5-methoxypsoralen and 8-methoxypsoralen (also called methoxalen, xanthotoxin, Figure 6.9). The latter, along with UV-A irradiation (PUVA) is used to treat skin disorders such as psoriasis and mycosis fungoides. However, psoriasis patients so treated exhibit a significant increase in premalignant skin lesions as well as malignant melanoma. Methoxalen, in addition to forming DNA cross-links, causes a specific mutation in the tumor suppressor gene p53. Mice treated with PUVA exhibit signature missense mutations in exons 4 to 8. Dietary exposure to psoralens is probably not a significant health risk; however, the margin of safety is thought to be narrow. Human volunteers who ingested 300 g of celery root (with a total phototoxic furcoumarin content of 28 ppm) experience no skin reactions after UVA exposure, and the blood levels of psoralen, methoxalen, and 5-methoxypsoralen were below the analytical detection limit.

**Miscellaneous Flavonoids: Quercetin, Ellagic Acid, Kaempferol, and Rutin**

This family of chemicals is widespread in plant-derived foods, including fruits and fruit juices, vegetables, buckwheat, tea, cocoa, red wine, dill,
soybeans, bracken fern, and others. The estimated average daily intake of flavonoids is 1 g. None of these has yet been conclusively shown to be carcinogenic, but both quercetin and kaempferol are mutagenic. Rutin is not mutagenic in itself, but it can be metabolized by intestinal bacteria to yield quercetin. Quercetin also has some anticarcinogenic properties.

Natural Chemopreventives in Plants

Introduction
Cancer researchers have long ago discovered that a diet rich in some fruits and vegetables can prevent, reduce the severity, or delay the onset of cancer. A survey of approximately 200 studies that examined the relationship between fruit and vegetable intake and the incidence of several cancer types showed that an overwhelming majority (128 of 156) of these studies demonstrated that intake of fruits and vegetables statistically lowered cancer risk. The case was particularly striking for fruits, which showed a statistically significant protective effect in 28 of 29 studies against cancers of the esophagus, oral cavity, and larynx, and in 24 of 25 studies for protection against lung cancer. Given these strong data already available, organizations such as the National Cancer Institute recommend that people eat a balanced diet with five servings of fruit and vegetables daily.

Isolating the individual compounds or phytonutrients with anticarcinogenic properties has proved to be difficult, but many have now been identified. These chemicals, known as chemopreventives, are not chemotherapeutics or cancer anticodons per se, but agents that have been shown in various experimental protocols to somehow interfere with the cancer process rather than cure advanced malignancies. Cancer is a multistage process, with a multitude of biochemical and molecular events that, left unchecked, culminate in cellular malignancy. Although the anticancer mechanisms of many chemopreventives have not been identified, several compounds have been shown to intervene at one or more of the stages of this process. Experimental protocols that have identified anticancer compounds from plants usually involve the administration of the chemopreventive either before, after, or concurrently with some chemical carcinogen such as aflatoxin B1 (AFB1) or benzo(a)pyrene (B(a)P) in laboratory animals. Chemopreventive action is manifested by one of several endpoints, such as a reduction in the tumor incidence of the animal group, a delay in the time in which tumors develop, or a reduction in the number or size of a malignant or premalignant lesion in an animal. Somewhat paradoxically, several of these chemopreventives, such as some of the tannins and isothiocyanates also are known to be toxic. Most chemoprotectives are minor nutrients, but others have nutritional values,
such as vitamins A (and its analogues), E, and C. The latter kind will not be discussed in this chapter. Many antitumor phytochemicals have been identified. Some of the more promising chemopreventives that have been shown in animal studies to inhibit cancer induced by a variety of chemical carcinogens are discussed below.

**Isothiocyanates**

Isothiocyanates are a large group of natural plant compounds (also discussed above as goitrogenic) that exhibit promising anticancer properties. Sulforaphane (Figure 6.10) is a recently discovered powerful chemopreventive found in broccoli and other cruciferous vegetables. It is a powerful inducer of important phase II detoxification enzymes such as glutathione S-transferase and quinone reductase. Sulforaphane is a monofunctional inducer in that it increases activities of phase II enzymes without inducing carcinogen-activating enzymes such as CYP 1A. Phenethylisothiocyanate (PETC) and benzyl isothiocyanate (BITC) are promising constituents shown to inhibit a wide variety of tumor types in experimental animals. These compounds are found also in certain cruciferous vegetables such as cabbage, brussel sprouts, broccoli, and cauliflower. They appear to be particularly effective against lung carcinogenesis in rats induced by the nicotine-derived tobacco carcinogen nitrosamine 4-(methylnitrosamino)- 1-(3-pyridyl)-1-butanone (NNK) and [R(A)]P. In addition to enhancing detoxication by quinone reductase, PETC and BITC also are thought to inhibit CYP-mediated enzymatic activation of these carcinogens.

**Indole 3-Carbinol**

Indole 3-carbinol (I-3-C, Figure 6.10), also present in cruciferous vegetables, is another promising chemopreventive. Indole-3-carbinol inhibits carcinogenesis caused by a number of chemicals in rodents and rainbow trout, most likely by multiple mechanisms. It is thought that I-3-C and derivatives thereof, produced under acid conditions of the stomach, are most likely to be the bioactive compounds. For example, the $\beta$-indolylmethane, a potent noncompetitive inhibitor of rat and human CYP1A1, human CYP1A2, and rat CYP2B1. Indole-3-carbinol and its acid derivatives also have been shown to inhibit AFB, mutagenesis in Salmonella typhimurium in vitro by scavenging the electrophile AFB, 8,9-epoxide. However, another in vivo 1-3-C derivative, indol[3,2-b]carbazole (ICZ), is an arylhydrocarbon (Ah) receptor agonist and CYP 1A1 inducer with a potency similar to that of 2,3,7,8-tetrachlordibenzo-p-dioxin (TCDD). This bifunctional action of 1-3-C (or derivatives thereof) may explain why this phytochemical inhibits hepatocarcinogenesis in trout and rats, when given prior to and with AFB, but actually promotes carcinogenesis in both species when given
continuously following AFB\textsubscript{1} initiation.\textsuperscript{45} Such an observation is just one illustration why extreme caution should be exercised before chemopreven-
tives are considered for human use. In any event, candidate chemopreven-
tives should be rigorously examined in many experimental protocols to
identify potentially adverse effects, such as cancer enhancement.

**Polyphenols**

Many foods are a rich source of chemopreventive polyphenolics, which are a
type of plant tannin. Strawberries, blackberries, cranberries, walnuts, and
pecans are a particularly good source of ellagic acid (Figure 6.10) which is the
hydrolysis product of ellagitannins. Ellagic acid has been shown in numer-
ous studies to be a versatile inhibitor of tumors at a number of sites induced
by several compounds.\textsuperscript{44} Some of the initial studies showed ellagic acid was
effective in preventing Bis(p)-induced lung and skin tumors in mice. Ellagic acid also is active in reversing the skin tumor initiating and promoting activity of Bis(a) and 12-O-tetradecanoylphorbol-13-acetate (TPA).^{56}

Tea is especially rich in several chemopreventive polyphenols that have been the object of intense study. Besides water, tea is the most commonly consumed drink in the world. While there is some, albeit equivocal, association between excessive tea consumption and some forms of human cancer, tea and tea components are now largely recognized to be chemopreventive.^{57} A recent epidemiology study conducted in Shanghai associated green tea consumption with a reduction in esophageal cancer.^{58} The chemopreventive properties of tea have been attributed to several polyphenols which are present in greater quantities in green compared to black tea due to differences in processing of the two products. The major polyphenols in green tea are the epicatechins, (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin-3-gallate (EGCG) (Figure 6.9). (-)-epigallocatechin-3-gallate, which is thought to be the primary protective component in green tea, accounts for over 40% of the total polyphenol content of green tea.^{59} A 200 ml cup of green tea contains about 142 mg EGC, 65 mg ECG, and 17 mg of EC, along with approximately 76 mg caffeine. Black tea typically contains smaller amounts of these catechins because the majority of them are converted to epicatechin polymers, such as theaflavins and thearubigins, during processing. Aqueous extracts of green tea inhibit the mutagenic activity of several heterocyclic amines, in addition to reducing CYP-mediated metabolism of several substrates, suggesting that the chemoprotective properties of green tea are probably due to inhibition of enzymes which activate carcinogens as well as scavenging active metabolites.^{60} Green and black tea were both active in reducing aberrant colonic crypts induced by 2-acetamido-3-methylimidazo[4,5-f]pyrrole (IQ), as well as reducing IQ-DNA adduct formation in rats.^{61}

Miscellaneous Chemopreventives

Chlorophylls (Figure 6.10) and their water-soluble salts called chlorophyllines are ubiquitous pigments found in green and leafy fruits and vegetables. Chlorophyllin (CHL), a copper/sodium salt of chlorophyll, has been given to people for a variety of purposes such as to reduce body, fecal and urinary odor; it has no known adverse side effects. Chlorophyllin derivative has been shown in a number of studies to reduce both in vitro and in vivo endpoints of the cancer process. For example, CHL powerfully inhibits the in vitro mutagenesis of AF<sub>B</sub> in Salmonella, and inhibits the formation of AF<sub>B</sub>-DNA adducts in rainbow trout. The mechanism of inhibition appears to be via complex formation with active AF<sub>B</sub> metabolite, the AF<sub>B</sub>-8,9-epoxide. In addition to AF<sub>B</sub>, CHL also inhibits the carcinogenic action of other procarcinogens such as 3,4-benzoflavone, 3,4-dimethylben[5]anthracene (DMBA), 1,2-dimethylhydrazine (DMH) and the heat-derived foodborne carcinogens
2-amino-1-(methyl-6-phenylimidazo(4,5-b)pyridine (PhIP), and IQ in trout, mice, or rats. While much of the data on the chemopreventive properties of chlorophylls relate to CHL, recent data show that native chlorophylls are equally protective. Pure chlorophyll has equivalent activity as CHL to inhibit DNA adduct formation in rainbow trout (George S. Bailey, personal communication). The consistent protective properties of chlorophylls in animal and in vitro studies has provided the impetus for a newly initiated double blinded, placebo-controlled chemoprevention trial in Daixin, China, conducted by collaborators from Johns Hopkins and Oregon State Universities, to determine whether CHL can reduce biomarkers of AFB, (George S. Bailey, personal communication). People in this region of China have a particularly high intake of AFB, in their diet, and reductions of serum albumin-AFB, and urinary AFB-N7-guanine DNA adducts would indicate that chlorophylls exert chemopreventive properties. Allium plants, such as garlic, onions, leeks, and shallots contain a group of allylsulfur compounds, such as diallyl sulfide (Figure 6.10). Numerous studies have shown that these possess potent anticancer properties in a variety of species and organs caused by many carcinogens. Diallyl sulfide, the most potent of these, induces key detoxifying enzymes, such as GST. A related organosulfur compound from garlic, 5-allylcysteine, induces GSTs in various tissues in mice and strongly inhibits DMH-induced aberrant crypts, but only when given in the initiation phase, further supporting its role as a detoxification promoter. Allicin (Figure 6.10) is another organosulfur compound from garlic that possesses wide-ranging antimicrobial and anticancer properties. Genistin (Figure 6.10) is an isoflavone found in soy beans and soybean products. Genistein appears to act through several mechanisms, but an important one may be through inhibiting angiogenesis or the process through which new blood vessels are formed. Because new blood vessels are important if a tumor is to grow, genistein may act by preventing tumors from growing.

Conclusions
The food supply in the U.S. can be regarded as among the world’s safest, having high nutritional quality and extremely low carryover of agricultural chemicals. However, our food contains many naturally occurring plant compounds that have been shown to be toxic and/or carcinogenic in animals and people. Because it is practically impossible to avoid all plant-derived toxins in a normal diet, the best way to minimize potential hazard would be to eat a wide variety of foods, but not too much of any one dietary item. Because natural chemopreventives are associated with a reduction in risk to many types of cancer, it is also important to include generous helpings of fruits and vegetables in the daily diet. There are several questions to be addressed before
chemopreventives can truly become a practical and safe protective therapy in people. For example, the anticarcinogenic benefits of at least some compounds are seen only when they are a natural part of the food from which they were derived. Thus, their benefits may not be seen when they are given as a supplement. Another concern is that animal studies have shown that under some experimental conditions, some chemoprotective, such as indole-3-carbinol, may actually be carcinogenic in their own right or may promote the carcinogenic effects of another chemical. Lastly, some research has shown that the protective effects of a chemical may be specific to a given carcinogen or a closely related class of carcinogens.

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