

Special (Secondary) Metabolites from Wood

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10.1 Introduction

Flavonoids, lignans, terpenes, phenols, alkaloids, sterols, waxes, fats, tannins, sugars, gums, suberins, resin acids and carotenoids are among the many classes of compounds known as 'secondary metabolites'. This daunting array of substances, having different chemical, physical and biological properties, presents numerous challenges in the utilization of forest products. Do they also present opportunities?

Secondary metabolites are often defined on the basis of what they are not. To wit, primary metabolites are usually described as those substances that are the fundamental chemical units of living plant cells, such as nucleic acids, proteins and polysaccharides. Secondary metabolites may therefore be defined as being everything else that the organism produces. Intuitively, this definition is a little difficult to accept: why would a plant expend so much energy to produce materials that it does not need? Especially because some of these 'secondary' compounds are vital to its very existence.

In the realm of wood processing and utilization, there is a very pragmatic definition of secondary metabolites: they are everything that is not a structural polysaccharide or lignin. In this sense, secondary metabolites are often referred to as 'extraneous components' because they are mostly extraneous to the lignocellulosic cell wall and are concentrated in resin canals and cell lumina especially those of ray parenchyma cells. These types of compounds are however actually found in all morphological regions and this definition cannot be strictly applied.

While such a definition emphasizing the structural components of wood is very functional, it can give the impression of demeaning the role of these "extraneous components". That is unfortunate, for if polysaccharides and lignins are the bones and flesh of woody tissue, it is the secondary metabolites that give woody plants their blood, soul and character. These components endow woods with their many colours and hues, scents and beauty. An excellent suggestion for recognizing their roles and impact is to refer to these materials as 'special metabolites' (Gottlieb, 1990).

Although much is known about the formation, composition and function of special metabolites, a question repeatedly heard is: 'Why are they formed?' Although not very satisfying, perhaps the best answer may be that the reason that many of these compounds are formed is a conundrum. It has been commonly taught that while animals have developed efficient systems to excrete unwanted by-products, plants have few such mechanisms and usually must alter and then store their wastes ('secondary metabolites') (Manitto, 1981). Others do not believe that the high energy cost of biosynthesizing most special metabolites is consistent with waste product removal. In any case, such processes have led to successful, serendipitous 'strategies'. For example, heartwood extractives retard wood decay, resin formation protects wounded tissues and toxic and antifeedant compounds in foliage and bark minimize insect and animal browsing through their poisonous, unpalatable or emetic properties. It is worth noting that these compounds may be toxic to the producing plant and the wide variety of phytochemicals produced may be part of a selection process to minimize plant toxicity while maximizing protection (Gottlieb, 1990).

Varying by species, woods may contain as little as 1 per cent or as much as one-third of their dry weights as special metabolites. Tropical and sub-tropical species typically contain greater amounts of extractives than do temperate zone woods. The concentration of special metabolites in trees is not uniform; generally, higher amounts occur in bark, heartwood, roots, branch bases and wound tissues. Variations also occur among species, from tree to tree, and from season to season. Most of the special products may be extracted with neutral solvents, and such extracts are sometimes the source of useful materials. It would be impossible to review adequately here the structures and properties of the thousands and thousands of compounds isolated and identified from trees. Instead, a brief overview is presented to sample the breadth and variety of special metabolites. Biosynthesis isolation, purification, characterization, stereochemistry, reactions and derivatives generally will not be included. Fortunately, a number of treatises have been written on these subjects and the reader is referred to these sources, and the citations therein, for in-depth information. Some aspects of special metabolites, such as their production for use as medicinals, adhesives and preservatives, are discussed in other chapters.

10.2 Terpenes (Terpenoids)

Thousands of terpenes (terpenoids) have been isolated, purified and structurally elucidated, and this group represents the largest class of chemical compounds that occur as special metabolites. Terpenes are found throughout nature and occur in almost all plants. As a consequence, terpenes have been exploited since antiquity as perfumes, flavouring agents, waterproofing materials, insect repellents, fungicides and medicinals. Many continue in traditional uses while others provide the raw materials for the commercial synthesis of numerous high-value products.

Terpenes are derived from isoprene (isopentane) C₅ budding blocks. By definition, a monoterpene is a compound of two isoprene-derived units totalling at least 10 carbon atoms. Hence, and perhaps a little confusing, the simplest terpenes, those

with a C₅ skeleton, are termed hemiterpenes. Hemiterpenes, such as isoprene, and prenyl and isoprene alcohols, generally do not accumulate in large amounts in woody plants and are not exploited as forest products.

Systematic chemical nomenclature is generally not used for terpenes due to the history of their isolation and their complexity; 'trivial' or common names are used instead. Monoterpenes may be either cyclic or acyclic (Figure 10.1). Examples of some of the acyclic monoterpenes (Dev, 1989) include citronellol (used in perfumes and isolated from *Chamaecyparis lawsoniana*), citronellal (the major component of citronella oil; used in soap perfumes and as an insect repellent) and linalool (which also may be synthesized from the more readily available β -pinene; used in the synthesis of fragrances and vitamins). Isomeric geraniol and nerol have sweet rose-like odours and are used as perfume bases.

An example of a single ring cyclic monoterpene is limonene, the major component of turpentine from some *Pinus species* (*P. pinea*, *pinceana* and *lumholtzii*). Other examples are α -terpinene, from *Pinus sylvestris*, terpinolene, a minor component in some heartwood oils and in some *Pinus* turpentine, and β -phellandrene, the major component of *Pinus contorta* turpentine (Figure 10.1). Bicyclic monoterpenes (Figure 10.1) are widely distributed; some occur in significant amounts and are extensively utilized. Abundant in turpentine from coniferous woods, α - and β -pinene, camphene and 3-carene are common examples of bicyclic monoterpenes (Dev, 1989). The composition of turpentine is species dependent (Fengel and Wegener, 1984). For example, the monoterpene fraction of *Pinus balfourniana* contains 81 per cent α -pinene and only 1.9 per cent β -pinene whereas that from *Pseudotsuga menziesii* contains 31 per cent α -pinene and 36 per cent β -pinene. Even greater variation is displayed by *Pinus heldreichii* which yields 82.8 per cent limonene and only 11.5 per cent of the pinenes. Other examples of monoterpenes include thujene, from *Boswellia serrata*; chaminic acid, present in the heartwood of *Chamaecyparis nootkatensis*; borneol, first isolated as crystals deposited by *Dryobalanops camphora*; and camphor, from *Cinnamomum camphoru* (Dev, 1989) (Figure 10.1).

Considering all natural sources, over 2500 sesquiterpenes (three isoprene units, C₁₅) have been identified, forming the largest structural subclass of terpenes with at least 120 different skeletal types (Klyne and Buckingham, 1978; Dev, 1989). Cedrene, which is tricyclic, occurs in cedar wood oil (*Juniperus virginiana*). Eudesmol, bicyclic with two six-membered rings, has been isolated from the heartwood of *Callitropis araucriodes*. *x*-Santalol (Figure 10.1), which is tricyclic, is the major component of sandalwood oil (Srinivasan *et al.*, 1992) - an essential oil which is especially valuable in perfume formulations. Widdrol, bicyclic with six- and seven-membered rings, occurs in cedar woods (*Cedrus*, *Chamaecyparis*, *Juniperus*. etc.). Guaiol (Figure 10.1) is bicyclic with five- and seven-membered rings and comprises 30 per cent of the oil from guaiacum wood. Abscisic acid is a relatively simple monocyclic sesquiterpene but it has been found to be a natural plant growth regulatory hormone with widespread occurrence in the higher plants. A number of sesquiterpenes are primarily responsible for the odour of wood and have been tabulated (Imamura, 1989). Farnesene, which has an acyclic structure, is one of a suite of odiferous sesquiterpenes that are responsible for the characteristic aroma of *Juniperus* species.

Classes of special metabolites from trees have often been described on the basis of historic or common uses and isolations. 'Essential oil' is one such group. Generally obtained by steam distillation, these compounds are the source of the distinctive

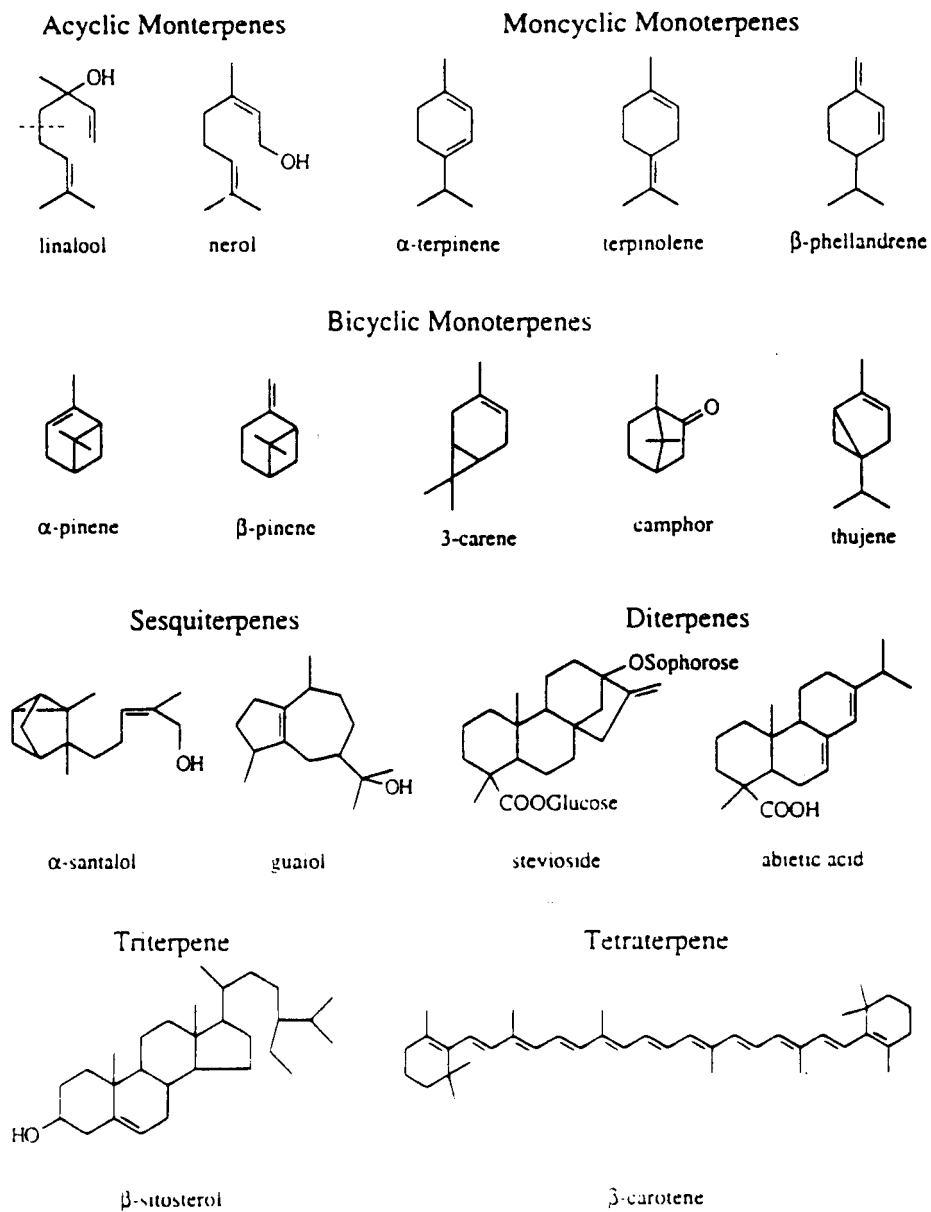


Figure 10.1 Terpenes are comprised of isoprene units which are generally linked in 'head to toe' fashion, as indicated by the dashed line on the structure of linalool. Although only a few examples of terpenes are presented, their wide range of composition and structural features are readily apparent

odour, or essence, of the tree. Essential oils that are particularly fragrant are valued as ingredients in perfumes. The variation in composition of these oils allows some wood identifications by odour (or analysis) and has seen limited utilization in taxonomic studies.

Given this relatively non-selective method of isolation and the awareness of the wide range of types of special metabolizes, it might be a little surprising to learn that most essential oils are comprised mainly of terpenes. Although some essential oils are solids at room temperature, the use of the term 'oil' may be somewhat misleading as most of the oils are mixtures of low molecular weight, non-viscous, highly volatile compounds, typically mono- and sesquiterpenes, for example, 'oil of turpentine'. Monoterpenes, especially, are used in the synthesis of commercially important products.

Comprised of four isoprene units (C_{20}), diterpenes occur commonly in plants and woods. Phytol, an acyclic diterpene with a simple structure, is particularly ubiquitous as it comprises about one-third of the molecule chlorophyll and is found in the leaves of all green plants. Stevioside (Figure 10.1), isolated from the leaves of the shrub *Stevia rebaudiana* (Kohda *et al.*, 1976), is a terpene glucoside with the unique characteristic of being 300 times sweeter than sucrose (Noller, 1961). Stevioside-containing extracts have long been used as sweeteners and the pure compound is approved for use in food in several countries. Gibberellic acid, a tetracyclic lactone, belongs to a class of diterpenes (gibberellins), which occur widely in plants and function as phytohormones. Many of the gibberellins have one less carbon and are C_{19} compounds. Abietic, pimaric, communic and lambertianic acids are representative of a class of diterpenes known as resin acids (Figure 10.1). These acids commonly occur in rosins from gymnosperm woods, particularly the pines. Resin acids, which are obtained as by-products from the Kraft pulping of wood, are commonly used as sizing agents to adjust the absorption of water in paper products.

'Naval stores' is another of the historic categories of special metabolizes (Zinkel and Russell, 1989). This term is derived from the use of pine tar and pitch to waterproof and caulk wooden boats and ships. Traditional sources of naval stores were from gums (pines were wounded and the exudate, called pine gum or oleoresin, was collected) and the destructive distillation or extraction of the heartwood from resinous pine stumps. These processes have been largely supplanted by the isolation of 'tall oil' from the Kraft (sulphate) pulping process which is used to produce paper-making fibres. The term 'tall oil' comes from the Swedish '*tallolja*' which means pine oil: care needs to be exercised not to confuse tall oil with terpeneol which is commonly called pine oil. Naval stores chemicals are fractionated to yield turpentine and rosin. Turpentine composition from the same wood species varies depending on whether the isolation is from the exudate, wood or tall oil (Zinkel, 1981).

Sesterpenes (five isoprene units, C_{25}) occur uncommonly and initially were isolated from insect waxes and as fungal metabolites. They rarely occur in higher plants and are not commercially significant. In contrast, triterpenes (C_{30}) are widely distributed, especially among angiosperm plants (Mahato *et al.*, 1992). Examples of this class are the tetracyclic dammarenediol, isolated from *Shorea vulgaris* resins (Dev, 1989), and the pentacyclic betulin, a white pigment present in birch bark (*Betula alba*) and isolated from many other plants (Hayek *et al.*, 1989). Sterols, which are biosynthesized from squalene oxide, are found in a number of gymnosperm and angiosperm woods (*Larix*, *Abies*, *Picea*, *Pinus*, *Gmelina*, *Fagus*, *Quercus* and *Ulmus*). β -Sitosterol (Figure 10.1) is a commonly occurring sterol and is the major component of the sterol fraction from a number of conifers; campesterol, dihydrobrassicasterol, 24-methylstanol and 5- α -sitosterol often co-occur with β -sitosterol (Nes, 1989). Sterols may also occur as fatty acid esters (Dyas and Goad, 1993) or as glycosides. Some (termed saponins) contain several glycosides and

produce a lather in water; others (functionally defined as cardiac glycosides) have particularly strong effects on the heart muscle and can function either as a medicine or as a poison. Although the concentration of sterols in heartwood is low (often less than 0.1 per cent) relatively large amounts of sterols may be isolated as by-products from Kraft pulping (tall oil).

Tetraterpenes (C_{40}) are comprised of the carotenoids, which include the red and yellow plant pigments (Robinson, 1991). The carotenoids, such as β -carotene (Figure 10.1), occur in leaves, flowers, seeds and fruits, but not in wood. Carotenoids are used as food colorings, in vitamin synthesis, and in cosmetics as suspensions, emulsions, lotions, lipsticks and powder bases.

Rubber and gutta-percha are polymeric isoprenes (containing 3000-6000 monomer units). The elastic properties of rubber are attributed to its all *cis* structure, whereas the all *trans* structure of gutta confers rigidity (Barlow, 1989; Robinson, 1991). The annual consumption of natural rubber is about 5.9 million tonnes (Reisch, 1996).

10.3 Phenolic Compounds

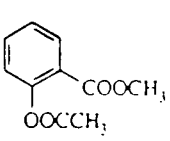
A number of simple phenols (Figure 10.2) have been isolated from various tissues or trees (Theander and Lundgren, 1989). Generally, members of this class have little or no value as individual isolated, commercial products but some, for example, safrole (oil of sassafras), eugenol (oil of cloves), methyl salicylate (oil of wintergreen) and cinnamic acid (oil of cinnamon), are relatively volatile, have characteristic aromas, and contribute significantly to the overall properties and qualities of essential oils. Some of these simple phenolics may also occur as glycosides, such as salicin, a bitter antipyretic which is found in relatively large amounts in many *Populus* and *Salix* species (Lee *et al.*, 1993). Coniferyl and sinapyl alcohols also occur as glucosides and it is this form that may be the immediate precursor to lignin.

Condensation reactions of simple phenolics are likely to be responsible for the formation of biphenyls. Ellagic acid is a dimer of gallic acid and is an important constituent of hydrolyzable tannins. Stilbenes, probably formed from cinnamic acid, are exemplified by pinosylvin (Figure 10.2) and resveratrol. Stilbenes are most commonly found in the heartwood of *Pinus* species and may occur with phenolic hydroxyls, methylated or as glycosides (Norin, 1989). Dimers, trimers and oligomers of stilbenes have been identified and have complex structures and stereochemistries. Many of the phenolics are implicated in various defence mechanisms.

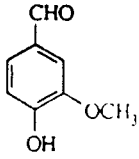
Over 4000 different flavonoids have been isolated from plants and they commonly occur in foliage, bark, sapwood and heartwood in trees. Their function is thought to be that of providing resistance to attack by fungi and insects. This subclass name is derived from the flavan (2-phenylchroman) skeleton which gives rise to a number of structural variations. The flavonoid group includes chalcones, aurones, flavanones, flavones, isoflavones, flaveronoids, flavanols, flavan-3,4-diols (leucoanthocyanidins), flavan-3-ols (catechins) and anthocyanidins.

Isoflavonoids have a unique structural variation: their basic skeleton is a 1,2-diphenylpropane in contrast to the 1,3-diphenylpropane skeleton of the flavonoids. Most common in legumes, isoflavonoids have been reported in the Pinaceae (Tahara and Ibrahim, 1995). Aurones and chalcones rarely occur in wood (Zavarin

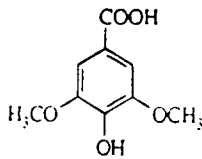
Simple Phenolics



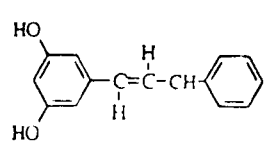
methyl salicylate



vanillin

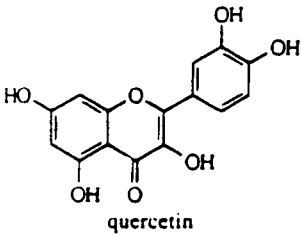


syringic acid

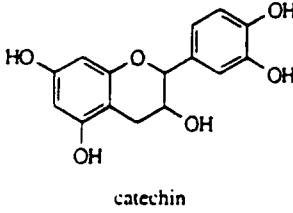


pinosylvin

Flavonoids

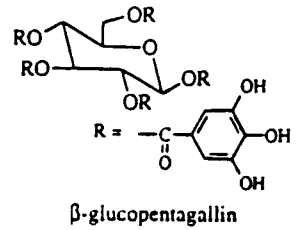


quercetin



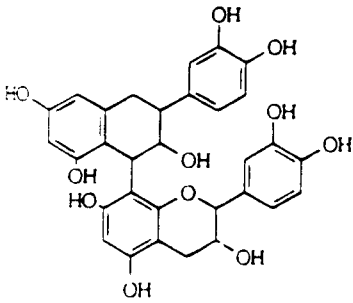
catechin

Hydrolysable Tannin

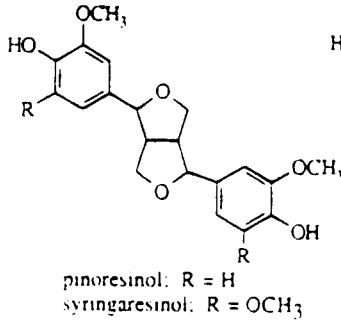


β -glucopentagallin

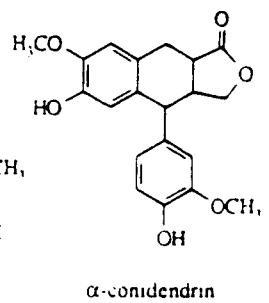
Condensed Tannin
(Proanthocyanidin)



Lignans



pinoresinol: R = H
syringaresinol: R = OCH₃



α -condendrin

Figure 10.2 Many of the special metabolites of wood are phenolic in nature. Examples of a few of the various classifications are shown

and Cool, 1991). Red and blue pigments in flowers and fruits are due to anthocyanidins, but this group is absent in wood. Flavonoids reported in wood and bark have been summarized by Harborne (1989). Quercetin (Figure 10.2) is one of the most common flavonoids isolated from the bark of conifers. Some flavonoids, such as catechin (Figure 10.2), occur in both angiosperm and gymnosperm woods. Flavonoids may couple oxidatively to form dimers and oligomers (Hemingway, 1989).

10.4 Lignans (Neolignans and Norlignans)

The number of isolated and identified lignans has grown exponentially since the 1930s. In a review of lignans in 1936, only 14 compounds were described; it took

nearly two decades to double that. The number of identified compounds jumped to 164 in 1978 and then to 440 in 1987 (Ayres and Loike, 1990). It may be estimated now that it is likely that about 1000 structures have been identified (or double that because lignans are chiral!).

A major reason for the increasing interest in lignan isolation and characterization is that lignans display a remarkable range of biological activity. These activities include fungal enzyme and growth inhibition; fish toxicity; and insect antifeedant properties, growth inhibition and juvenile hormone functions (Gottlieb and Yoshida, 1989). Of course, much of the interest in the physiological properties of lignans is related to mammal and human activity.

Lignans have been defined on a structural basis as those metabolites which are formed by the oxidative coupling of propylphenols (C_3C_6) resulting in linkages between the middle carbons of the propyl side chains (beta-beta bridges). Other linkages between the units may occur subsequently. Extensive listings of lignans have been compiled (Gottlieb and Yoshida, 1989; Ayres and Loike, 1990). Some examples from these lists are pinoresinol, lariciresinol, liovil, matairesinol, α -conidendrin, syringaresinol and thomasic acid. The latter two lignans occur in woody angiosperms, *Quercus rubra* and *Ulmus thomasi*, respectively. Pinoresinol has been isolated from *Pinus* sp. and *Picea abies* and syringaresinol from *Salix sachalinensis* (Figure 10.2) (Lee *et al.*, 1993). Lariciresinol occurs in *Pinus*, *Larix leptolepis* and *Picea abies*. Liovil was found in *Abies nephrolepis*, *Picea ajanensis* and *Larix decidua*. Matairesinol has been shown to occur in *Pinus*, *Abies amabilis* and *Tsuga* sp. Conidendron was isolated from *Picea abies*.

Lignans, and their glycosides, are found in varying proportions in bark, wood, roots, leaves, fruits and seeds. Heartwood is a much richer source of lignans than is sapwood. In some cases, lignans are not present in all tissues, but may occur, for example, in heartwood but not elsewhere. Trees that are wounded, through physical injury or insect attack, often produce resins rich in lignans.

Neolignans are also defined on a structural basis: they result from the coupling of propylphenols linked at positions other than the beta-beta coupling of the side chains. Although there is great opportunity for the formation of more varied and complex structures for neolignans, their occurrence is not widespread. Cedrusin, isolated from *Pinus*, is an example of a neolignan having a bridge from the beta-side chain carbon of one propylphenol to the 5-position of the aryl ring of the other (Castro *et al.*, 1996).

Norlignans (and norneolignans) are structurally similar to lignans but have one less carbon atom and are C_{17} structures. Norlignans are common in the Taxodiaceae (Castro *et al.*, 1996). Coupled through the beta-carbons and having one side chain with only two carbon atoms, yateresinol, found in *Libocedrus*, is a simple example of a norlignin. Norlignins are also sometimes termed coniooids or sequirins (Zavarin and Cool, 1991).

10.5 Tannins

Tannins, which are oligomeric and polymeric phenolics, are widely distributed and are common both in gymnosperms and angiosperms. Species of *Acacia*, *Quercus*, *Betula*, *Salix* and *Pinus* are examples of tannin-containing trees. Considered to have antifeedant properties, tannins are found in bud and foliage tissues, seeds, bark,

roots, sapwood and heartwood. Bark and heartwood are often the regions that contain the highest levels. In common with other plant phenolics, tannins are formed via the shikimic acid pathway.

The term 'tannin' is based on historic uses of plant materials to produce leather from animal skins. Because a wide variety of phenolics can act as tanning agents, more rigorous definitions have been employed. As a result, tannins have been separated into two classes based on their chemistry and origin. One class is the hydrolyzable tannins, which is further categorized as gallotannins (Figure 10.2) and ellagitannins; gallic and ellagic acids, respectively, are essential components. Such esters are easily hydrolyzed, hence the pertinent term 'hydrolyzable' tannins. Gallotannins consist of a saccharides core (usually glucose) with multiple esters of gallic acid, which in turn may be esterified by other gallic acids. Ellagitannins, which occur in dicotyledonous angiosperms, are monomeric and oligomeric, often containing gallate groups. Over 500 ellagitannins have been characterized (Quideau and Feldman, 1996). Hydrolyzable tannins have been described, classified, and their taxonomic significance discussed (Haslam, 1989; Okuda, *et al.*, 1993).

In contrast to hydrolyzable tannins are the 'condensed tannins'. These materials are not significantly hydrolyzed under the mild conditions used to degrade the hydrolyzable tannins. However, emphasizing their origin rather than their behaviour, this group is more correctly referred to as proanthocyanidins (or polyflavanoids) as they are derived from flavonoids (Porter, 1989). The proanthocyanidins are the most commonly found class of tannins and are composed of over a hundred different oligomeric and polymeric structures (Lewis and Yamamoto, 1989). Like many of the special metabolites, this class of compounds is proposed to be involved in plant defence mechanisms (Stafford, 1988). An example of a representative proanthocyanidin structure is presented in Figure 10.2 (Lewis and Yamamoto, 1989).

Discussions of the condensed tannins usually include mention of phlobaphenes. These are water-insoluble, condensed phenolics related to proanthocyanidins but have differing functionality, including methoxyl groups (Foo and Karchesy, 1989). As a class of natural products, phlobaphenes are poorly defined chemically. They are generally obtained as crude, heterogeneous products with complex structures and vary depending on the source. Phlobaphenes from Douglas fir (*Pseudotsuga menziesii*) bark may be reaction products of proanthocyanidins, carbohydrates, flavanoid methyl ethers, lignans and dihydroquercitin (Foo and Karchesy, 1989).

10.6 Carbohydrates

Although most of the carbohydrates in woody plants occur as structural polymers (cellulose and hemicelluloses), numerous other compounds such as sugars, oligosaccharides, alditols, cyclitols, and polysaccharides, are commonly found. Starch is ubiquitous in woody tissues, except in heartwood, as it functions as a food and chemical raw material reserves for trees. With the exception of the sago palm, trees have not been exploited as commercial sources of starch (BeMiller, 1989). However, non-structural polysaccharides in gums are utilized - as food additives and binding agents, and adhesives, paints and varnishes. Such gums are not food reserves: they are formed in response to wounding and usually also contain terpenes and other compounds.

Gum arabic, a pale to orange-brown, slightly acidic polyaccharide solid, is used extensively in food products. It is frequently used as an emulsifier and to prevent the crystallization of confectionary sugar. It is also used to encapsulate flavours and as a glaze (Coppen, 1995). Gum arabic is obtained from *Acacia* species and its composition and properties vary with sources. D-Galactose, L-arabinose, L-rhamnose, and D-glucuronic acid are formed on acid hydrolysis (Robinson, 1991). The Sudan is the major source of gum arabic, producing 33 227 tonnes in 1994. Other forest products gums include karaya (*Sterculia* species), carob (*Ceratonia siliqua*) and tara (*Caesalpinia spinosa*) (Coppen, 1995).

As intermediates in numerous biosyntheses, monosaccharides occur widely, but only in small amounts as they do not accumulate. Tree sap is a good source of oligosaccharides, particularly sucrose. Sugar maples (*Acer saccharum*) are tapped commercially and the sap is concentrated for use as a food (maple syrup) and flavour ingredient in various products. Interestingly, maple sap has almost no flavour. The unique flavour is developed after boiling the sap in air. While the sap contains mainly sucrose, many other components are present in small quantities including carboxylic acids, phenols, phenylglycosides, oligosaccharides, vanillin, syringaldehyde, proteins and amino acids. This mixture apparently undergoes reactions, including oxidation, to form the flavour constituents. Some of these have been identified as methyl-, dimethyl-, and ethyl-pyrazines (Kermasha *et al.*, 1995).

10.7 Cutin and Suberin

Cutin and suberin are often discussed together as they have similar waxy, water-repellent properties. Cutin impregnates the epidermal walls of leaves and stems and also forms a separate layer on the outer wall (cuticle) making the surface impermeable to water as well as forming a barrier to pathogens. Suberin is found in the cork tissue of bark.

Cutin is composed mainly of hydroxy and dihydroxy hexadecanoic and octadecanoic acids whereas suberin is composed of ω -hydroxy C₁₆-C₂₄ acids, α , ω -dicarboxylic fatty acids, and C₂₀-C₃₀ fatty acids and alcohols (Wallace and Fry, 1994). Phenolic compounds appear to be associated with both, but especially suberin. Suberized tissue has been described as being comprised of aliphatic domains, similar to cutin, linked to aromatic 'lignin-like' domains (Kolattukudy, 1984). However, suberin cannot be isolated intact and degradative analyses so far have not provided sufficient structural details. Thus, it has been stated that not only are the inter-unit bonding patterns unknown, but the monomer composition is also essentially unknown (Davin *et al.*, 1992). Although some efforts have been made in the past to isolate and utilize waxes from bark, cutin and suberin are generally unsuited for commercialization.

10.8 Other Metabolites (Aliphatic Compounds, Fatty Alcohols and Acids, and Nitrogenous Compounds)

Many of the simple chemicals do not accumulate in woody tissues. Compounds such as glycolic, glyceric, pyruvic and malonic acids are generally intermediary

along pathways to form other products. However, other simple acids, such as oxalic, lactic and tartaric acids may be metabolic end-products. A product of photosynthesis and carbohydrate biosynthesis, shikimic acid is an important intermediate in the biosynthesis of aromatics (phenolics, lignin precursors, aryl amino acids, flavonoids, etc.)

Alkanes occur in woody tissues and sometimes accumulate. For example, *n*-heptane is a significant component in oleoresin turpentine from *Pinus subiniana* (Zinkel, 1989). Long-chain hydrocarbons such as fatty alcohols and acids, fats (mono-, di- and triglycerides of fatty acids), and waxes are produced as special metabolites and accumulate. The role of fats and fatty acids is to provide food reserves, whereas waxes, comprised mainly of esters of saturated fatty acids and alcohols, and long-chain alkanes (C₂₁-C₃₅, primarily are present on foliage surfaces to reduce the evaporation of water. Common fatty acids are found in both angiosperm and gymnosperm woods, but linolenic and linolenic acids are found in higher concentrations in angiosperms. An isomeric form of linolenic acid, the *cis* -5,9, 12 acid, occurs in Pinaceae woods (Zinkel, 1989). A commercially significant source of fatty acids is tall oil.

Numerous nitrogen-containing compounds occur in wood and related tissues. That this type of compound is not present in large amounts is illustrated by nitrogen contents of about 0.1 per cent for wood. Some of this nitrogen is due to proteins and enzymes which are residuals left from cell growth and development. The occurrence of amino acids and proteins has been summarized by Durzan (1989). Although foliage and bark from some trees have been used as animal feeds, generally such applications are few and have limited potential.

There is however significant interest in an important class of compounds known as the alkaloids (Pelletier, 1983; Sakai *et al.*, 1989). Alkaloids, many of which have very complex structures, are relatively common. They occur in about 10 per cent of plant genera and have taxonomic relevance (Hegnauer, 1988). Alkaloids typically are found in higher concentrations in bark, seeds, roots and leaves than in wood. The traditional definition of alkaloids is broad and encompassing: they are alkali-like (basic) substances. However, this definition falls short because the functionality and chemistry of some alkaloids produce neutral and even acidic compounds. The alkaloids are comprised of a range of compounds with various functional groups including acids, amides, phenols, urethanes, pyrroles, piperidines, quinolizidines, acridones and indoles. Alkaloids have long been known both as medicines and poisons.

10.9 Application of Biotechnology

Changing the characteristics of plants to improve their cultivation, quality, growth rate, hardiness, and yield has been practised for thousands of years. Over the last century, an understanding of plant genetics and breeding transformed the simple practice of saving and sowing superior seeds into the sciences of agriculture, horticulture and silviculture. A quantum leap forward occurred just two decades ago as the ability to create transgenic plants was accomplished. Can this technique, or other methods of biotechnology, be applied to enhance the bounty and potential of special metabolites?

Cell and tissue cultures grow rapidly, compared with the many, many years of growth required for most trees. Cultures are renewable and could yield forest products without logging - which is sometimes controversial - and the resulting products could be labelled 'natural'. Thus, at first glance, it seems that plant cell cultures have significant potential for exploitation to produce useful quantities valuable phytochemicals. While laboratory cell cultures can produce compounds such as terpenes, coumarins, anthraquinones, flavonoids, alkaloids and tannis (Tanaka *et al.*, 1995), there has been very limited commercial application in the production of special metabolizes by this method (Charlwood and Rhodes, 1990).

There are a number of difficulties encountered in cultures from cambial and bark tissues. A major one is that plant cultures do not often produce the same compounds, or in the same abundance, as the mature plant. Most 'secondary' products are not formed in developing cells; few undifferentiated cell cultures are likely to form meaningful amounts of special metabolites. This problem can sometimes be addressed by introducing agents that act as inducers or elicitors. In trees, stress, such as wounding or infection, will usually trigger increased production of special metabolites. It might therefore be desirable to mimic this reaction in artificial systems. One interesting *in-vivo* application of this principle was to use bacteria to induce gall formation in *Taxus brevifolia* which resulted in the production of a higher level of taxanes (Stahlhut, 1994).

While many special metabolizes have significant value, especially the industrially important terpenes and the unique essential oils, culture production methods are not likely to be economic in the near future. A significant barrier to commerialization is that it is estimated that the production of special metabolizes becomes feasible only when selling costs are above \$1000/kg (DiCosmo and Misawa, 1995). Some of the rare and structurally complicated metabolites that possess great medicinal value, such as the anticancer drug taxol, are however candidates for production via cell cultures.

Biotechnology in forestry and forest products is usually thought of in terms of forest tree improvement, micropropagation, plant breeding, herbicide tolerance, resistance to pathogens, improved pulp properties, bio-pulping and bleaching, and mill effluent treatment. There is great interest in employing genetic engineering to enhance plant resistance to insects since such an approach can be environmentally benign: it targets specific pests without the introduction of toxic materials into the environment. However, pest control by this method generally results in transgenic plants producing insecticidal proteins from gene transfers from bacteria (Boulter, 1993) and not from modifying amounts or types of special metabolizes in the plant. Most of these efforts however have been with agricultural crops which are not particularly well-suited for emphasizing the potential role of special metabolites, pest resistant forest trees are being sought, and although it is complicated to manipulate biosynthetic pathways to change special metabolize production. The development of pest resistance may still occur.

The utilization of most trees harvested for non-fuel purposes fails essentially into two categories: solid products (lumber, plywood, particle board, etc.) and paper products (writing and printing papers, corrugating and packaging papers, tissue, etc.). Because these are major uses special metabolize applications are likely to be adjuncts to them, as, for example, the isolation of tall oil from pine Krait pulping liquors. It would seem likely that if genetic transformations are to be applied to trees, the goal will be to improve their utilization as building and construction

materials and as an improved raw material for the production of paper and paper products. That does not preclude increasing the concentration and/or distribution of special metabolizes at the same time. Even when wood is used for construction and manufacturing, there is an opportunity since bark, which may be enriched in targeted special metabolizes, can be obtained economically as a by-product of wood harvesting.

Wood pulping offers greater potential to isolate metabolizes either through pre-extraction, especially if pulping with organic solvents becomes a reality, or post-separation or extraction. However, the value of pulp and paper is an overriding concern. Biotechnology in this industry is concerned almost exclusively with a few major research areas (Srebotnik and Messner, 1996). These are biopulping and bi-bleaching, paper deinking and recycling, effluent treatment, and fermentation of waste/by-product hemicelluloses; all employ microorganisms. Additionally, there are efforts being made through genetic engineering to modify lignin to create trees more amenable to pulping. Although research on gene identification to prevent disease and transformation of trees to impart disease and insect resistance is being conducted, biotechnology related to special metabolizes is mainly restricted to fungal removal of pitch in paper making (Srebotnik and Messner, 1996). In this regard, it is important to recognize that all schemes to increase the production of special metabolizes must not adversely impact the processing or use of wood in its major utilization for solid products and paper.

The production of natural rubber, latexes, gums, essential oils, and some other products from forest trees is generally not dependent on wood harvesting for other uses. However, these industries and applications are declining. These industries are often labour intensive, have limited markets, and usually are in direct competition with other materials or those synthesized from petrochemicals. It could however be argued that genetically improved stocks not only could ensure the supply of renewable products of quality, but also maintain a way of life and improve the economic prospects of many workers. Alternatively, it could be argued that to offset the costs in the application of biotechnology, large, integrated and mechanized harvesting and processing systems would result, displacing a number of unskilled and semi-skilled labourers.

The attraction of applying biotechnology to special metabolizes is that it would be a natural, renewable, 'green technology'. The disadvantages are the economics of new technologies which would be in direct competition with the existing processes, and with the chemical and petrochemical industries. The niche that forest products could provide is the utilization of special metabolites as by-products and/or as high-value, generally small volume, applications.

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