BIOSYNTHESIS OF CHEMICAL SIGNALS – *DE NOVO* SYNTHESIS AND SECONDARY METABOLITES

J. G. Romagni,
Dept. of Biology, University of St. Thomas, Houston, TX USA

Keywords: Polyketide, shikamate, mevalonate, terpenoids, flavenoids, lignans, prostaglandins

Contents

Summary

The basic pathways that produce plant secondary products, *i.e*. polyketide, shikimic acid and mevalonic acid, appear to have been relatively well defined. However, they are constantly being modified as novel compounds are discovered. Other pathways that produce defense compounds, such as phytoalexins, are still poorly understood. New technologies, such as functional genomics may help to elucidate the sequence of events that leads to the production of secondary compounds [see The methodology and approach of Chemical Ecology]. The use of DNA microarray technology allows the rapid monitoring of thousands of genes in response to treatment, such as an elicitor. This, in theory, should yield a gene expression profile that may give clues to what enzymes are involved and in what order they are expressed. Molecular biology

techniques using mutants can also help to identify the actual pathway by providing valuable clues depending upon the mutant or missing enzyme.

Several new technologies will make the discovery process for secondary metabolites and their intermediates much easier. Tandem analytical instrumentation (*e.g.* GC-MS; GC-MS-MS; NMR-MS; LC-MS) has improved and is continuing to improve identification of these compounds. With new secondary metabolites being identified every day, comes new and interesting biochemical pathways that produce them. Additionally, like isoprenoid and abscisic acid biosynthesis, we are finding several examples of compounds that are produced in an entirely different manner than we originally thought. With the discovery of each new compound, new questions arise as to how they are made, and why they are produced, the potential roles in the ecosystem, and their potential exploitation by mankind.

1. Introduction

Organisms vary widely in their ability to interconvert and transform organic compounds. Plants and algae are particularly effective at synthesizing organic compounds through the process of photosynthesis. Other organisms, such as animals and most microorganisms, must obtain raw materials from their diet. In this way, metabolic pathways interact using the catabolic degradation of food, while anabolic pathways biosynthesize specialized molecules. These two processes together define 'metabolism'. Metabolism can be further divided into processes called primary and secondary metabolism. Primary metabolism involves processes that are basically the same in most organisms, such as pathways for modifying and synthesizing carbohydrates, proteins, fats and nucleic acids. However, there are several organisms that also possess the ability to synthesize specialized compounds that are often unique to that species. This is defined as secondary metabolism. These processes are also loosely defined as those not essential for life.

Secondary metabolites are not produced under all conditions, and in many cases, their actual function is unknown. However, due to the high investment in energy and carbon, it follows that these compounds probably have important ecological roles, either as protection against biotic factors such as herbivory, predation and competition or abiotic factors such as UV light. Other potential roles, especially in plants, may be as volatile attractants. Whatever their specific functions, they probably play some role that benefits the producing organism. It is within this area of secondary metabolites that most biologically active compounds are found.

Although we make the distinction, there is no clear boundary between primary and secondary metabolism. There are several 'natural' products that might be classified either way. The best examples of these include fatty acids and sugars. These normally are described as primary metabolites, however, as will be discussed briefly in this paper, several of them can function as secondary metabolites as well.

The primary pathways involved in the production of secondary metabolites are the acetyl-polymalonyl (polyketide), shikimate and mevalonate pathways. Each of these will be addressed separately. The initial steps of the polyketide pathway consist of the usual addition of 3 malonyl-CoA to either acetyl-CoA or other suitable acyl-CoA starting unit, yielding a linear tetraketide backbone that can cyclize via either an orsellinic acid-type or phloracetophenone-type mechanism. This pathway produces fatty acids, phenols, quinones and prostaglandins. The shikimate pathway also produces a variety of phenols, cinnamic acid derivatives, lignans and alkaloids. Mevalonic acid is produced from a series of three actyl-CoA units, but synthesizes different compounds than the polyketide pathway. The mevalonaic acid pathway is responsible for a variety of terpenoid and steroid compounds.

Secondary metabolites are often synthesized using repeated monomers, as in the case of terpenoids, or by using a mixture of different, but structurally simple, building blocks. This expands structural diversity and allows for different types of substitutions. Some natural products may be a combination of subunits from two or more of the common pathways. Many secondary products contain mono- or polysaccharide units either by themselves or as part of a modified molecule. However, it must be noted that most of these compounds are multi-functional in their effects. Many compounds that may serve as inhibitors to herbivory, may secondarily serve as antimicrobial agents. An example can be seen in some lichen secondary metabolites, specifically, the compound, usnic acid. The antimicrobial activity of usnic acid is well documented and several antibacterial creams have been developed with usnic acid as a primary ingredient. However, more important ecologically, it is a 4-hydroxyphenyl pyruvate dioxygenase inhibitor (HPPD). This enzyme indirectly inhibits carotenoid biosynthesis. Carotenoids dampen excess energy caused by high light conditions, thus preventing the production of free radicals. Inhibition of this pathway causes photodestruction of membranes in competing seedlings found in the canopy. Other examples of secondary metabolites with multiple actions are protoporphyrinogen oxygenase (Protox) inhibitors. Protox is the last common enzyme of the porphyrin pathway involved in the biosynthesis of heme and chlorophyll. It is dangerous to both animals and plants. Disruption of this enzyme causes an extreme sensitivity to light, peroxidation of membranes and the production of free radicals. Organisms producing diphenyl ether(s) as secondary product may prevent competition or predation.

Biosynthesis of secondary metabolites involves a series of reactions that are enzymatically catalyzed using several common mechanisms such as electrophilic additions in alkylation reactions, aldol reactions, transamination reactions, decarboxylations, glycosylations and redox reaction. The synthesis of these metabolites is costly, requiring a steady flow of precursors from primary metabolism together with energy-rich cofactors like ATP and NAD(P)H. Plants can compensate for production of secondary compounds high in carbon through photosynthesis. However, for those metabolites high in limiting compounds, like nitrogen in alkaloids, secondary metabolism can compete with primary pathways, such as protein biosynthesis. Allocation of nutrients and carbon to growth and differentiation is a physiological trade-off. One current hypothesis suggests that organisms, and perennial plants in particular, may be divided into two groups: first, growth-dominated organisms, or those with rapid growth and a poor chemical defense system, but with a highly inducible resistance

system; and second, the differentiation-dominated organisms, or those with a slow growth rate, an excellent chemical defense, but a poorly inducible resistance similar to lichens.

Usually secondary metabolites are produced continuously by the organism and are housed in specific storage structures such as glands and/or trichomes. Formation of these compounds is determined by allocation of carbon and nitrogen and most of these compounds are produced by the pathways described below. However, some products, such as phytoalexins, are produced upon elicitation from an external signal. Elicitors for these plant compounds include microorganisms and fungi. Most phytoalexins are low molecular weight compounds not found in healthy plants. Phenylalanine ammonia lyase, a key enzyme in the synthesis of isoflavenoids, is one of the primary enzymes involved in the production of phytoalexins. Many phytoalexins are also produced in the shikamate pathway.

Although the definitive roles of most secondary compounds are not known, there are several studies that suggest the more probable functions within the ecosystem. Plants and fungi, because they lack the ability to escape herbivores and competition, and are successional or pathogenic species, have a wide variety of secondary compounds. Many of these compounds are localized at the surface of the plant/fungi. This is especially true in those compounds functioning in an anti-herbivory or competitive role. Animals without physical defense structures also often produce secondary compounds.

A complex and rather bizarre example of production of plant anti-herbivory secondary metabolites is that of the wild potato species *Solanum berthaultii*. This defense system is localized at the surface of the plant in two morphologically different types of trichomes, or leaf/glandular hairs. The type B trichomes contain the volatile compound, *E*-β-farnesene, a sesquiterpene. Farnesene is an aphid alarm pheromone and the release of this volatile compound causes a disturbance in aphid feeding. The second type of trichome (type A) consists of a phenolic-containing exudate linked to a phenolase/peroxidase enzyme system. When an aphid disrupts this defense structure, the phenolic substrate reacts with the enzyme and a sticky brown residue is formed. The aphid becomes immobilized and is prevented from feeding, eventually starving to death [see Foraging and Food choice in Phytophageous Insects].

Examples of secondary compounds produced by animals include the prostaglandins. Prostaglandins display a wide variety of bioactivity including contraction and relaxation of smooth muscle in the uterus, the cardiovascular system and the intestinal tract. They also may be important in regulating blood pressure and suppressing blood platelet aggregation. These compounds are found extensively in soft corals, with the coral *Plexaura homomalla* (sea whip) in the Caribbean having an extremely high prostoglandin content [see Chemical Ecology in Aquatic Ecosystems].

In the following sections, the major biosynthetic pathways of plant secondary products are discussed. Each pathway gives an overview of biosynthesis of the backbone molecules and a few specific examples.

2. Polyketide pathway: fatty acids and polyketides

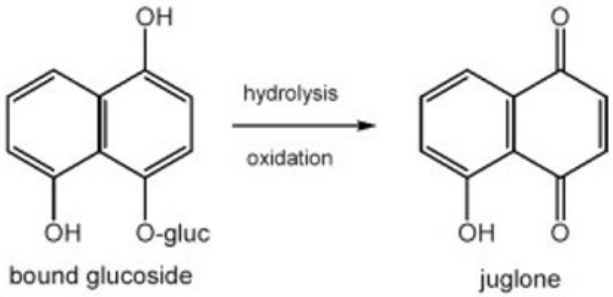

Figure 1. Bound toxin from *Juglans nigra* (black walnut) undergoes hydrolysis and oxidation in the leachate to form the allelopathic naphthoquinone, juglone

The acetyl/polymalonyl (polyketide) pathway leads to some plant quinones, particularly naphthoquinones and anthraquinones, and various side-chain elongated phenylpropanoids such as some flavenoids. This is one of the most important pathways in the production of plant phenolics. The classic example in this pathway includes the allelopathic naphthoquinone, juglone glucoside (Figure 1) found in members of the Juglandaceae. Juglone, the toxic final product, is known to supress growth of competing plants. Anthraquinones occur frequently in bacteria, fungi and lichens, as well as in higher plant families. Of all the pathways producing quinones, the polyketide is found mostly in micro-organisms.

2.1 General Biosynthesis

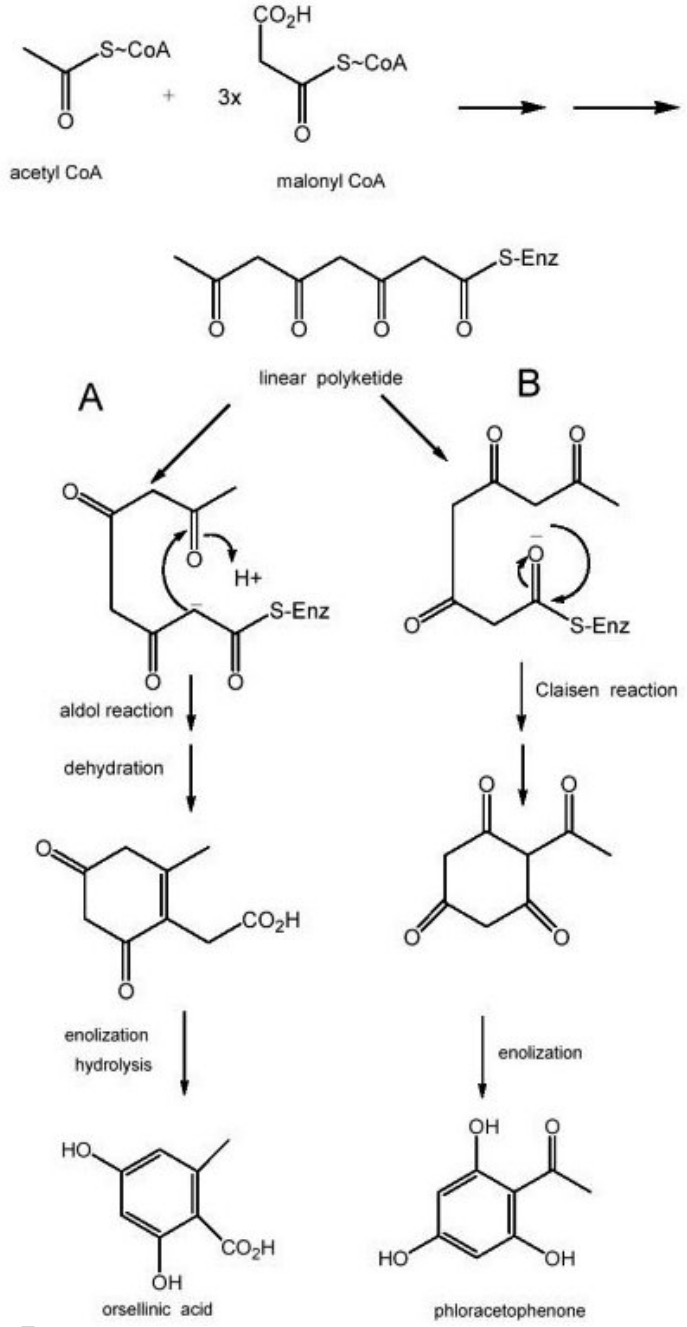

Figure 2 . Formation of beginning linear tetraketide structure and the subsequent cyclization to form either orsillinic acid or phloracetophenone involving a carbonyl group and a CH_2 [C2 and C7 (*e.g.* in lichens) or C3 and C8 (common in higher plants)]. Acetyl CoA is the starting material for most compounds in this pathway. Direct condensation and cyclization gives various aromatic structures. The initial steps consist of the usual addition of 3 malonyl-CoA to either acetyl-CoA or other suitable acyl-CoA starting unit, yielding a linear tetraketide backbone (Figure 2). This structure can cyclize via an orsellinic acid-type mechanism through hydrolysis of the thioester grouping, via a Claisen condensation or by phloracetophenone-type (aldol) mechanism (Figure 2). Both reactions result in the formation of a phenyl ring, the only difference being the

position of the nucleophilic attack. Orsellinic acid-type cyclization and the Claisen condensation involve the thioester group and a CH_2 (C1 and C6).

2.1.1 Anthraquinones

Anthraquinones derivatives such as emodin, endocrocin and parietin, are also derived by the cyclization of linear polyketides (Figure 3). Endocrocin is found in species of Deutermycetes, including *Penicillium* and *Aspergillus*. The further metabolite, emodin, is found in fungi, lichens and higher plants and is formed from endocrocin by simple decarboxylation reactions. These anthraquinone secondary compounds are known to inhibit both photosynthesis and respiration in competing seedlings.

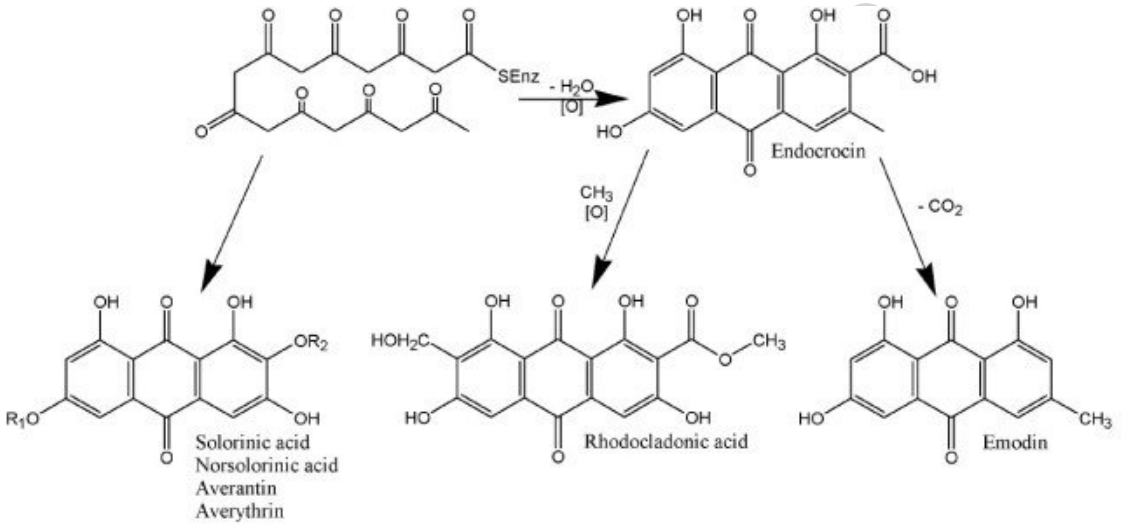

Figure 3. Biogenesis of anthraquinones derived from the polyketide pathway

Variations in some phenolic structures are derived from the initial acyl-CoA unit (e.g. proprionyl-CoA,malonyl-CoA, etc.), while other variations are due to the number of malonyl CoA units involved. There can be further modifications by redox reactions or through the addition of substituents or conjugations (e.g. glycolsylations). Although the backbone molecule remains the same, small modifications in substitutions often cause a major change in the mode of action ofthe molecule.

-
-
-

Bibliography

Chapell, J. 1995. Biochemistry and molecular biology of the isoprenoid biosynthetic pathway in plants. Annual Review of Plant Physiology and Plant Molecular Biology 46: 521-547.

Dewick, P.M. 1997. Medicinal Natural Products: a biosynthetic approach. John Wiley & Sons, New York. 466 pp.[An excellent source for information on natural products with pharmaceutical applications]

Dey, P.M. and Harborne, J.B. 1997. Plant Biochemistry. Academic Press, London. 554pp. [This is a good general text for all metabolic pathways in plants]

Gang, D.R., Wang, J., Dudareva, N., Hee Nam, K., Simon, J.E., Lewinsohn, E. and Pichersky, E. 2001. An investigation of the storage and biosynthesis of phenylpropenes in sweet basil. Plant Physiology 125: 539-555.

Katz, L., and Donadio, S. 1993. Polyketde synthesis: prospects for hybrid antibiotics. Annual Review of microbiology 47: 875-912.

Kaufman, P.B., Cseke, L.J., Warber, S., Duke, J.A., Brielmann, H.L. 1999. Natural Products from plants. CRC Press, London. 343pp. [A good general, non-technical source]

Ketchan, T.M. 1995. Alkaloid biosynthesis – the basics for metabolic engineering of medicinal plants. Plant Cell 7: 1059-1070.

Lawrey, J.D. 1986. A biological review of lichen substances. The Bryologist 89: 111-122.

Lichtenthaler, H.K. 1999. The 1-deoxy-D-Xylulose-5-phospate pathway of isoprenoid biosynthesis in plants. Annual Review of Plant Physiology and Plant Molecular Biology 50: 47-65.

Tellez, M, Dayan, F, Romagni, J, Schrader, K and S. Duke. 2002. Terpenoid-based defense in plants and other related organisms. *In* Lipid Biotechnology, (Tsung, MK & Gardner, HW, eds), Marcel Dekker, New York.pp. 319 - 355.

Biographical Sketch

Joanne G. Romagni, received her PhD in 1998 from Arizona State University. She worked for the USDA-ARS as a plant physiologist in the Natural Products Utilization Research Unit and currently is on faculty at the University of St. Thomas in Houston, TX. When she grows up, she wants to be a painter.