The Origin of Natural Aromatic Materials

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Plants contain a number of chemicals based on simple sugars and carbohydrates. These include fatty acids, lipids, amino acids, nucleotides, nucleic acids and proteins, etc. Some chemicals act as primary metabolites which are vital to a plant’s life and survival or act as precursors for secondary metabolites and are concerned with the plant’s interactions with the surrounding eco-system, principally acting as deterrents and attractants to pathogens. Some of these chemicals make up essential oils which comprise of materials from a number of chemical groups. Only as recently as fifty years ago the isolation of natural materials had to be undertaken physically, by chemical reactions to identify compounds. This limited the ability to identify more than just a few compounds in essential oils. The advent of spectroscopic methods created a revolution in natural product chemistry which enabled the identification of trace constituents and more detailed understanding of the chemistry of plants.

The spectrum of odorous substances is very narrow where only materials with a molecular weight below 300-400 and an appreciable vapour pressure at room temperature have noticeable odours to humans. Relatively few organic materials have pleasant odours, with the majority of materials diffusing acetic, propionic, butyric and lactic odours.

Essential oils are not the only chemical substances found in plants. Metabolites, like fats, fatty acids, waxes, oils, coumarins, anthraquinones and alkaloids are also soluble in ethanol and other solvents and can be extracted by distillation. Thus, materials extracted from plants contain both volatile aromatic and odourless substances.

Generally, essential oils can be physically distinguished from other compounds because a drop of a volatile oil on paper will completely evaporate, unlike fatty oils. Essential oils are generally a pale to clear or slightly yellowish liquid, mostly insoluble in water, with specific gravities between 0.8 to 1.2. The odour of an essential oil will resemble the source flora,
made up of a large number of constituents, sometimes into the hundreds. Some essential oil odours are dominated by a single constituent, like citral in lemon grass oil, but most oils rely on a complex mixture of constituents to provide the overall odour profile.

Constituents in essential oils can be put into three classes:
- Those greater than 1%, which are main constituents.
- Those present in parts per thousand, which are minor constituents.
- And those less than one part per thousand, which are trace constituents.

Given the vast number of different odours and chemical structures in essential oils, most compounds are biosynthesised by a small number of metabolic pathways. Although these pathways are common to all plants, small genetic differences introduce important differences in these pathways, thereby producing variances in biosynthesis. These numerous reactions and transformations create exotic fragrance blends, which we call essential oils.

**The plant metabolism**

A plant is metaphorically like a "chemical factory", producing a wide range of complex compounds to promote growth and health (primary metabolites), some for defence and co-existence with the surrounding ecosystem (secondary metabolites) and some chemicals for reasons that are still unknown to science. Plants are complex, open systems with both positive and negative entropy. Plants, through processes not fully understood, are dynamic systems under continual change, utilising sunlight, carbon dioxide, oxygen, moisture and soil nutrients in the synthesis of various chemical compounds (see Fig. 1).

A plant metabolism is the set of metabolites which can be categorised as primary and secondary. The primary metabolites are concerned with the basic life functions of the plant and provide precursors for the production of secondary metabolites. Secondary metabolites are concerned with the plant’s interactions with the surrounding eco-system and principally act as deterrents and attractants to insects.

The two most important primary metabolite processes are the glycolysis process and the Krebs or TCA (tricarboxylic acid) cycles. Through the breakup of glucose into other compounds, energy is also produced. The glycolysis process oxidises glucose produced in the plant by photosynthesis, releasing both energy and a series of chemicals, of which pyruvate (pyruvic acid) is of prime importance as an intermediate for the Krebs cycle. This process can be carried out both aerobically and anaerobically. Pyruvate is carried through to the Krebs cycle which is a series of enzyme catalysed chemical reactions, not exclusive to plants, but all living cells. Pyruvate is combined with coenzyme A, to form acetyl CoA, carbon dioxide and nucleic acids, through the route of nicotinamide adenine dinucleotide (NAD), a coenzyme and FADH2, an energy-carrying molecule to form macromolecules comprising deoxyribonucleic acid (DNA) and ribonucleic acids (RNA), known as ATPs. ATPS play a role in signalling and carrier molecules for amino acids in protein synthesis. Mevalonic acid is also formed from acetyl CoA through the route of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA), which goes to form terpenoid and steroid compounds. Coenzyme A breaks down to form malonyl CoA which plays a role in fatty acid and polyketide synthesis.

Another pathway, the pentose phosphate pathway also processes sugars through oxidation and synthesis. This is an alternative pathway to glycolysis, although it also involves the oxidation of glucose. Through different processes of dehydrogenation, hydrolysis, oxidative decarboxylation and isomerisation assisted by enzymes, a number of precursors are produced for fatty acid and amino acid synthesis and the production of hydroxy-benzoic acid through the shikimate pathway, discussed later in this section. Finally a pathway, polyamine biosynthesis through 6-deoxyxylulose is known to produce a number of precursors for the synthesis of terpenoids.

The factors that influence these complex reactions very briefly summarised above are still the subject of research and thorough understanding. Primary metabolites provide the precursors for aromatic chemical production in plants. They are also the precursors of a number of other compounds that are present in plants. These types of compounds include:
- Carbohydrates.
- Lipids.
- Amino acids.
- Nucleotides.
- Lectins.
- Chitinases.
- And various enzymes.

**The metabolic pathways**

One of the functions of primary metabolites is to provide feed-stocks for secondary metabolite production. This is undertaken through three primary pathways. The mevalonic acid pathway, sometimes called the terpenoid pathway, is responsible for the synthesis of a wide range of metabolites and terpenoids.
The metabolites produced through this pathway include the phytol chain found in chlorophyll and plant growth regulators or pseudo hormones, gibberellins and abscisic acid, discussed above. The mevalonic acid pathway is an enzymically controlled route to the formation of mevanolic acid, which through a number of steps synthesises into isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) where they are oxidised, reduced or hydrated into a wide range of terpenoids and steroids. Recently, a mevalonic acid independent pathway to IPP and DMAPP has been found utilising deoxyxylulose phosphate and methylerythritol phosphate as precursors. The following groups of terpenoids are produced by these methods:

- Sesquiterpenoids: consist of three isoprene units. Example: farnesol.
- Diterpenoids: composed of four isoprene units. Example: cafestol.
- Sesterterpenoids: comprise of five isoprene units. Example: cencereane.
- Triterpenoids: comprise of six isoprene units. Example: squalene.
- Polyterpenoids: long chains of many isoprene units. Example: polysoprene in rubber.

Chemically, terpenes, which are very important in flavour and fragrances, are very similar to terpenoids, where methyl groups are adjusted or removed or oxygen atoms added.

The shikimic acid pathway produces precursors for a number of metabolites, amino acids, plant regulators, alkaloids and phenolic compounds. This pathway is unique to plants and continues on from the pentose phosphate pathway, where carbohydrate precursors derived from glycolysis as erythrose-4-phosphate react with phosphoenol pyruvate to form shikimic acid. The shikimic acid pathway breaks into a number of branches as shown in Figure 2. Approximately 20% of all carbon fixed in a plant’s leaves is processed through this pathway, which makes up around 30% of a plant’s dry weight. Shikimic acid undergoes hydrolysis to break off pyruvic acid and through a number of other steps converts to chorismic acid, a precursor for a number of compounds. Chorismic acid is a precursor of salicyclic acid, a plant hormone. Chorismic acid also undergoes a Claisen type rearrangement to form prephenic acid, which converts into tyrosine and phenylalanine. Tyrosine, an amino acid, is believed to be involved in the photosynthesis process, acting as an electron donor in the chloroplasts to reduce oxidised chlorophyll, the signal transduction process in proteins, and to assist in producing insect deterring glucosinolates. Tyrosine is also a precursor of salicyclic acid.

Figure 3: A plant’s defence system.
to the pigment melanin. Phenylalanine, also an amino acid derives a number of flavonoids, lignin and coumarins, and also assists in producing insect-deterring glucosinolates, similar to tyrosine.

Through another path, anthranilate and its derivatives are produced. Little is known about the purpose of anthranilates in plants, although they occur as both methyl and acid forms in many plants. Anthranilates are precursors for alkaloids and tryptophan. Tryptophan is another amino acid and is a building block for proteins and a precursor for niacin, a number of alkaloids and indole. Finally through the shikimic acid pathway, a number of phenyl propanoid compounds are formed through cinnamic acid by elimination of ammonia phenylalanine. Common phenyl propanoids include methyl chavicol, methyl eugenol, eugenol, methyl cinnamate and vanillin. Phenyl propanoid accumulation in the plant metabolism is still an area where little is known.

Flavonoids and anthocyanins are pigments and phenolic compounds responsible for the colours of flowers in higher plants. Flavones provide the yellow and orange colours and the anthocyanins are the source of red, violet and blues. Flavonoids play some role in attracting insects to feed and pollinate, while others have bitter tastes and repel insects like caterpillars. Flavonoids are also considered antioxidants. Another important small group of polyphenols are the tannins that bind and precipitate proteins and may assist in the repair of damaged plant tissue, in conjunction with phytoalexins, which are reported to have antimicrobial properties. Tannins are very important flavonoids in teas, wines and some fruits. Tannins are used in the preparation of leather, the manufacture of colours and as dietary supplements.

Saponins are glycosides of steroids, steroid alkaloids or triterpenes found primarily in the outer tissue of plants as a waxy protective layer, although they are also found in other parts including the roots. Many saponins are toxic and thought to be part of the metabolism to deter insect predators. Saponins are not found in all plants. Within the last few years a number of medical and industrial uses have been found for saponins. Some of the existing uses include foaming agents in soft drinks and beer, fire extinguishers, photographic emulsions, and food sweeteners. In the medical field, saponins are used for cough medicines and cholesterol. Research is ongoing utilising saponins to fight cancer.

Remnants and artefacts from the pathways and degradation of fatty acids, amino acids, nitrogen and sulphur compounds and also from storage of foliage after harvesting lead to the formation of a number of compounds. These trace compounds, some desirable while others undesirable will contribute to the odour profiles of a number of essential oils. Unsaturated C6 aldehydes can arise in green tissue wherever they are cut, damaged or attacked by insects, through enzymic degradation of linolenic acid. The degradation of lipids in plants leads to the formation of short chain alcohols and aldehydes, such as the n-hexanol and cis-3-hexanol, compounds that provide green notes to an essential oil. Only with much more sensitive analytical equipment over the last few last years have volatile constituents contributing to the flavour of fruits and vegetables been discovered. However, their enzymic pathways are often still unknown. In some plants, essential oil constituents are free within or bound with glycosides within the plant.

The pathways create the through-fare from which the primary metabolites produce a set of secondary metabolites primarily concerned with the plant’s interaction with the immediate environment. The secondary metabolite compounds concern themselves with defences against predators, parasites, diseases, interspecies competition and facilitate the reproductive processes. It is from the secondary metabolites that the constituents of essential oils originate and also a number of other economic products. The secondary metabolites are unique to each plant species, unlike the primary metabolites which are common across the flora genus.

**Secondary metabolites and plant defence systems**

The secondary metabolites, primary terpenoids, alkaloids, phenolics and nitrogen compounds evolve within the metabolism and are utilised to improve a plant’s chances of survival against herbivory, primarily insect predators. Plants utilise a number of attributes against predators which include physical characteristics, such as surface protections, the production of complex polymers that inhibit digestion of the plant, the production of insoluble terpenoids to inhibit digestion, the production of toxins through the alkaloids, and the production of volatiles to attract predators of the insect herbivores. Conversely, insect herbivores utilise a number of counter measures to overcome plant defences such as detoxification of toxic compounds, avoidance mechanisms, sequestration of poisons and adulteration of the gene pattern. Multiple defence systems are required because of different parts of a plant and different types of herbivory. These
defences also assist the plant during times of stress due to droughts, water logging, intensive UV radiation and plant damage. In the reproductive cycle, plants emit aromatic odours to attract insects to assist in pollination. An overview of the plant defence system is depicted in Figure 3.2

Within the eco-system, plants and insects interact, co-exist and compete continuously in very complex ways. This is an evolutionary process as both plants and insects modify their defences and attack strategies continually. Therefore from the plant perspective, different chemical defences will be utilised at different times to meet these evolving threats and stresses.23 Thus as plants grow, they change in leaf, branch and other physical characteristics, including the growth of flowers and fruits which involves certain chemical changes within the plant metabolism, where certain insects can take advantage.24 This is an important consideration in essential oil production as the desired oil constituent profile may only develop during a particular part of the plant lifecycle.25

Aromatic compounds found in plants
With the exception of compounds that are products of catabolic breakdowns of lipids, amino acids, fatty acids and terpenoids, the plant metabolism is directly responsible for producing aromatic compounds within a plant through a limited number of pathways discussed above. Specific and unique extensions of these pathways exist in particular plants, yielding specific aroma compounds found in certain plants. In addition there are also a number of aromatic compounds found in fruits and vegetables.

Through the plant metabolism, essential oils comprise a large number of volatile terpenoid and non-terpenoid compounds which are based on hydrocarbons and oxygenated derivatives, although some contain nitrogen or sulphur derivatives. The hydrocarbons are connected by single, double or triple bonds to form higher molecular weight hydrocarbons, through rings or chains. Oxygen, hydrogen, nitrogen, sulphur, and other carbon atoms attach themselves to form various aromatic compounds. Methane, a colourless and odourless gas, is the simplest hydrocarbon. Some hydrocarbons are non-terpenoid and exist as short chain alcohols and aldehydes formed through degradation of phospholipids and fatty acids. Saturated homologous straight chain structures are alkanes and their unsaturated forms alkenes. Alikeens can form as isomers, a molecule with the same chemical formula with the same bonds between atoms, but arranged differently. These are mostly as stereo isomers in the cis- and trans- form, where the two molecules appear as a “mirror” image of each other. Molecules with three carbon atoms form straight chains, where those with four carbons or more can form either straight or branched chains. Terpenoids usually have a carbon base of 10, 15, 20 or 30 atoms, where five carbon atoms are called an isoprene unit. Various types of terpene compounds can be classified according to the number of isoprene units they contain as Table 1. lists.

A single isoprene unit is a hemiterpene, when two isoprene units link together they form a monoterpene, three form a sesquiterpene, four form a diterpene, and so on.

The types of aromatic compounds found in plants are:

Terpene hydrocarbons
Terpenes are a very large group of plant hydrocarbons formed by polymerisation of five carbon atom units (isoprenes) that form in both chains and rings. They may be reduced and oxidised into a vast array of other compounds including alcohols, lactones, acids and aldehydes, thus the starting point of synthesis of the majority of aromatic compounds. Terpenes are present in the resinous foliage of leaves. Terpene compounds are heavier than diterpene and do not contribute to the odour of essential oils, although they may be present.

Monoterpenes
Monoterpene compounds are found in nearly all essential oils and have a structure of ten carbon atoms (two isoprene units) with at least a double bond. They quickly react to air and heat and consequently lack stability and long shelf life as they are quickly oxidised. Monoterpenes are present in citrus, conifers, herbaceous plants as well as vegetables and fruits. Monoterpenes are formed through the mevanolate pathway by the conversion of methylallyl pyrophosphate with isopentenylpyrophosphate (IPP) to form geranyl pyrophosphate, the precursor of monoterpenes (Fig. 4).2 Compounds like alpha-pinene and beta-pinene are formed through cyclisation from geranyl pyrophosphate through linalool pyrophosphate. A large number of monocyclic compounds, like myrcene are also derived through this route.

Sesquiterpenes
Sesquiterpenes consist of 15 carbon atoms or three isoprene units linked to each other, head to tail. This formation can produce more than 300 different hydrocarbon sesquiterpenes. Sesquiterpenes have great diversity in

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Table 1. Classification of terpenoids according to isoprene units.

<table>
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<th>Terpene Classification</th>
<th>Carbon Atoms</th>
<th>Isoprene Units</th>
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Figure 5: The acetyl coa pathway for the biosynthesis of aldehydes, alcohols and esters.
construction, containing up to four carbocyclic rings. Sesquiterpenes are synthesised from farnesylpyrophosphate which is condensed from geranylpyrophosphate, derived from isoprenylpyrophosphate (IPP) and dimethylallylpyrophosphate (DMAPP), along the mevalonate pathway. Through further oxidative transformations, a number of other terpene compounds are formed (Fig. 4).2

I-ionones
I-ionones and damascones are potent aroma compounds derived from degrading of high molecular weight terpenes or carotenoids through oxidation. Carotenoids are found in a variety of plants and fruits, especially berries.

Oxygenated compounds
Oxygenated compounds contain oxygen molecules within their structures. These include alcohols, aldehydes, amides, carboxylic acids, esters, ketones, nitro compounds and oxides.

Phenols
Phenols are one of the three major chemical groups in plants along with terpenoids and alkaloids. Phenols originate from phenylalanine or tyrosine through the shikimic acid pathway. Phenylalanine ammonia-lyase removes ammonia from phenylalanine to produce trans-cinnamic acid. Cinnamic acid itself is not an important odourant but acts as a precursor for numerous aromatic metabolites including aldehydes, alcohols, lactones, and esters, outside the phenolic group.

Phenols are defined as any compound having molecules with one or more hydroxy group bonded to a benzene ring. As such, many compounds including flavonols, catechins, anthocyanins, isoflavones, dihydroflavonols, chalcones, quercetin, ellagic and tannic acids, vanillin, caffeic acid, curcumin, coumarins and lignans are also defined as phenols. Phenols oxidise easily and are partly the reason why plant material darkens after cutting due to this reaction. Phenols in essential oils also darken on exposure to air and tend to oxidise. Phenols are acidic due to the –OH group in the molecule. In plants, phenolic compounds usually couple themselves with glucosyl compounds.

Alcohols
Alcohols are very similar to phenols and aldehydes in structure. Alcohols are derived from aldehydes through dehydrogenase activation. They are also produced through amino acids through oxidative decarboxylation in ripening fruits. Coenzyme A esters may also be transformed in some way to alcohols.26 Fatty alcohols C8 (octyl, caprylic) occur in citrus fruits with their corresponding esters and aldehydes. Alcohol C9 (nonyl, pelargonic) is found in orange and oakmoss. Alcohol C10 (decy, capric) is found in orange and ambrette seed. Alcohol C11 (undecylenic) is found in the leaves of Litsea odorifera, and alcohol C12 (lauric, docecyl) is found in lime. Alcohols do not have the same pungency as their corresponding aldehydes, although as alcohols get higher in molecular weight their odour intensity increases, until nonanol C9, when they start to weaken again. Hydroxy hydrogen atoms of alcohols tend to carry some of the odour characteristics of aldehydes, while maintaining the smoother alcohol notes.27 Due to the polarity of alcohols they tend to be more soluble in water than most other aromatic compounds. Alcohols transform into other compounds including their corresponding aldehydes, acids and esters through methanol dehydrogenase catalysis.26

Aldehydes
Aldehydes are found in fruits and many plants with their corresponding alcohols and esters. Aldehydes have more pungent odours than their corresponding alcohols. Long chain or aliphatic aldehydes are much more pungent than the other aldehydes of the homologous group, which are extensively used in perfumery, i.e., benzaldehyde.

Aldehydes are relatively unstable materials which are prone to oxidation, polymerisation and acetal formation within essential oils. Aldehydes, esters, alcohols and acids can be converted and can revert within plants through transformation and oxidation. These reactions are thought to be controlled through coenzymes. There is a close interrelationship between acids, aldehydes, alcohols and esters within the Krebs cycle originating from branched amino acids aldehydes are derived from corresponding acids through δ-oxidation where it is decarboxylated. Through further reduction the aldehyde will convert to its corresponding alcohol and later undergo esterification. This can reverse where the aldehyde can oxidise to a corresponding acid, later leading to the conversion to odd-chain esters.28 This process is capable of producing a wide range of aromatic compounds (Fig. 5).2

Esters
Esters are formed from acids and alcohols, usually benzenoid, carboxylic and monoterpenic acids to form esters in essential oils. Esters are also found in fruit
and vegetables. Examples of esters in essential oils are linalyl acetate, benzyl benzoate and benzyl isobutyrate. Esters are formed through exiting the β-oxidation acting on acetyl CoA during the Krebs cycle.  

Ketones  
Ketones are often present in small quantities in plants and provide fruity flavours in fruits. They are highly reactive to air and heat and will easily convert to their corresponding acetals and alcohols. Ketones are formed through the Krebs cycle through β-oxidation of alcohols or with acyl CoA activation from carboxyl acids by hydrolysis causing decarboxylation.

Lactones  
Lactones exist in essential oils as γ-lactones, a five membered cyclic ring and δ-lactones, six membered cyclic rings. Lactones are found in herbs, coffee, fruits, dairy products, with fruity, nutty and hay like odour profiles. Macrocyclic lactones also exist in a number of essential oils and are known as musk lactones. Coumarins, a lactone in tonka bean and hay is formed through hydroxylation of trans-cinnamic acid to coumaric acid and then glycosylation, which is stored in cavities of plant tissue where it undergoes light-dependent isomerisation on rupture of the plant tissue, crystals form. Further synthesis of coumaric acid will give rise to dihydrocoumarin. Coumarins are widely used in fragrances for grassy, hay-like green spicy notes. Another forms of lactones are benzofluran derivatives found as butylphthalides in celery and angelica.

Nitrogen compounds  
Methyl anthranilate is a very freshly scented citrus-floral odour compound. As secondary metabolites, amines maybe generally involved in growth regulation of plants and, along with other aromatic chemicals, methyl anthranilate has been found to be both a bird attractant and repellent. Amines are very reactive to air and darken on exposure, as well as being photosensitive. They can also react with aldehydes to form aldimines. Amines are produced through a degradation pathway controlled by amine oxidase enzymes, within the amino acid pathway. Indole and skatole are two other nitro compounds, aromatic compounds that are found in plants. They are heterocyclic compounds and act as hormones in plants.

Sulphur compounds  
A few plants are known to contain volatile sulphur compounds such as dimethyl sulphide, dimethyl disulphide and dimethylthiophene in garlic, onion, leek and shallots. Blackcurrant (Ribes nigrum) and buchu (Agathosma betulina) also possess sulphur compounds, as well as some citrus fruits, coriander, ylang ylang, rose, peppermint, pepper, geranium, chamomile, hops and davana. Little is known about the purpose of sulphur compounds in plants except they play some role against pathogens and nitrogen detoxication of plants. Little is also known about the sulphur pathways in plants, even though sulphur is a necessary compound for amino acid, proteins, polysaccharides, lipids and other sulphur containing secondary metabolites. Sulphur compounds are believed to be derived through a sulphur reduction pathway (Fig. 6).

Volatile aromatic compounds are found within the Apiaceae (Umbelliferae), Asteraceae (Compositae), Cupressaceae, Hypericaceae, Lamium, Lauraceae, Myrtaceae, Pinaceae, Piperaceae, Rutaceae, Santalaceae, Zingeraceae and the Zygophyllaceae families. The wide
variety of different plant metabolisms producing a diverse range of aromatic compounds makes it difficult to meaningfully classify volatile oil plants according to these families. Due to the current accepted methods of plant taxonomy chemical constituents play very little role in plant family designation.

Note: A version of this article was first published by the ASCC.

References
8. Ananthakrishnna TN. Induced responses, signal little role in plant family designation.