Terpenoids: Higher

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Terpenoids (or isoprenoids/isopentenoids) are a diverse group of compounds that are found throughout nature and are biosynthesized through the successive condensation of the five-carbon compound isoprene. Higher terpenoids are complex structures that possess minimally between 20 and 40 carbon atoms. Although higher terpenoids are found in all organisms, plants produce the largest array of higher terpenoids in terms of number, structure and function. These plant-derived higher terpenoids can be utilized by humans for purposes such as drugs to fight diseases or as feedstocks for biofuel production. The genes responsible for producing these plant of higher terpenoids are just now beginning to be identified and these studies are showing that higher terpenoids are essential for plant growth, development and defence against pathogens.

Introduction

Living organisms, whether they are plants, animals or microbes, are composed of similar compounds, derived from a few basic biochemical pathways operating in cells, including the terpenoid pathway. The unity of life, apparent from the uniformity in structural constitution of biomolecules, indicates that phenotypic change and genetic evolution are coordinately regulated with the function played by natural products during ontogenetic and phylogenetic maturation. Terpenoids are lipids that have played a central role in growth and development of living systems. Additionally, plant-derived terpenoids are used by humans for many purposes such as flavourings in food, scents in perfume and drugs to fight diseases such as cancer. This article examines the synthesis, distribution and function of higher terpenoids as well as the regulation of the genes responsible for the production of these molecules.

Nomenclature and Biosynthesis of the Isoprenoid Unit

Terpenes and terpenoids constitute a very large family of natural products. Approximately 20 000 different terpenes and terpenoids have been identified, embracing a bewildering but fascinating array of skeletal types that contribute to the many varied functions of these molecules in nature. The term terpene was originally used to describe $C_{10}H_{18}$ hydrocarbons occurring in turpentine oil, a complex mixture of terpenes. The term terpenoid has, over the years, acquired a generic significance and is used almost interchangeably with isoprenoids (*oid*, meaning like) and isopentenoids, with no universal agreement on terminology in this area. An isoprenoid unit (or isoprene unit) is a five-carbon-atom structure with the isopentane carbon skeleton of isoprene, a product of the dry distillation (pyrolysis) of natural rubber. A terpene unit is considered to comprise 10 carbon atoms, from which all designations originate: hemi (C_5), mono (C_{10}), di (C_{20}), sester (C_{25}), tri (C_{30}), tetra (C_{40}) and poly ($> C_{40}$) (**Table 1**).

In the biological unit, the branched end of isoprene is known as the tail and the other end is known as the head (**Figure 1**). During biosynthesis of terpenoids, the tail of the isoprene intermediates is bound to a diphosphate group (or pyrophosphate; **Figure 1**). The terms prenyl, for example a prenyl alcohol, and polyisoprenoid are both in common use to refer to linear (open-chain) isoprenoids. An example of a prenol is phytol, the C_{20} diterpene side-chain of chlorophyll. Some authors have used terpene to refer only to hydrocarbons based on an integral number of C_5 units as shown in **Table 1**, and 'isoprene' (or more properly, isoprenoid) to designate the whole class of natural products derivable from a $(C_5)n$ structure. Terpenes have also been defined as compounds whose carbon skeleton can be dissected into

Table 1 Classification of terpenes and terpenoids

Classification	No. of carbon atoms	No. of isoprene units	Example
Diterpenes	20	4	Gibberellic acid (**Figure 2**)
Sesterterpenes	25	5	Ophiobolin
Triterpenes	30	6	β-Amyrin (**Figure 3**)
Sterols	27–30	6	Stigmasterol (**Figure 3**)
Tetraterpenes	40	8	β-Carotene (**Figure 2**)
Polyterpenes	>40	>8	Rubber (**Figure 2**)

isoprenoid units joined in a regular linkage (e.g. head-to-tail), or in an irregular linkage (e.g. head-to-head).

The basic C_5 isoprene unit for terpenoid biosynthesis can originate from three different pathways: (1) the mevalonate (MVA) pathway, (2) the nonmevalonate pathway or methylerythritol phosphate (MEP) pathway and (3) the mevalonic acid shunt. The MVA pathway occurs in the cytosol of animals and plants, whereas the MEP pathway occurs in bacteria and the plastids of plants. It appears that green algae only contain the MEP pathway localized in the plastid. In plants, monoterpenes, diterpenes, carotenoids, ubiquinones and phytol are produced in the plastid via the MEP pathway. All other plant terpenoids (sesquiterpenes, triterpenes and polyterpenes) are produced using the MVA pathway (Rohmer, 2003). **See also**: Terpenoids: Lower

The MVA pathway utilizes three molecules of C_2 acetyl–CoA to form C_6 HMG (3-hydroxy-3-methylglutarate)–CoA, which is reduced to C_6 MVA. MVA is then phosphorylated twice to produce MVA diphosphate (MVAPP), which undergoes decarboxylation to give rise to the C_5 biological isoprene unit isopentenyl diphosphate (IPP). IPP is then isomerized to dimethylallyl diphosphate (DMAPP; **Figure 1**).

In the MEP pathway, IPP is produced by the condensation of glyceraldehyde-3-phosphate and pyruvate to form 1-deoxyxylulose-5-phosphate, which is reduced and isomerized to MEP for which the pathway is named. MEP then undergoes a series of reactions, the last of which produces both IPP and its isomer DMAPP (**Figure 1**; Rohdich et al., 2002).

In both the MVA and MEP pathways, IPP is combined with DMAPP in a head-to-tail condensation to form C_{10} geranyl diphosphate (GPP). Successive head-to-tail additions of IPP can then produce terpenoid intermediates such as C_{15} farnesyl diphosphate (FPP) and C_{20} geranylgeranyl diphosphate (GGPP) that are used to form diterpenoids, triterpenoids and tetraterpenoids, respectively. Coupling reactions that can lead to the biosynthesis of acyclic 30 carbon atom molecules, triterpenes (e.g. coupling of FPP, to give squalene), and of acyclic 40 carbon molecules, tetraterpenes (e.g. coupling of GGPP to give phytoene), involve head-to-head condensations. Cyclization and oxidation reactions follow coupling reactions to give the desired end product. **See also**: Cholesterol, Steroid and Isoprenoid Biosynthesis

The MVA shunt is a bidirectional pathway that allows carbon from amino acids to be incorporated into IPP and thus higher terpenoids and carbon from IPP to be incorporated into amino acids.

Diterpenes

Diterpenes are C20 compounds formed from C_{20} GGPP mainly produced by plants. Plant diterpenes are used for defence against pathogens and herbivoures, for hormone production, and by humans as drugs. For example, the diterpene taxol, produced by the Pacific yew tree, *Taxus brevifolia*, is well known as an anticancer agent. GGPP itself can be covalently attached, posttranslationally, to proteins and act as a membrane anchor. This is common for nontransmembrane proteins involved in membrane-associated signalling such as Rab guanosine triphosphatase (GTPase) proteins. **See also**: Membrane Targeting: Methods; Proteins: Postsynthetic Modification – Function and Physical Analysis; Taxol

Conifer trees secrete a resin in response to insects that create a hole in the tree. The resin is made up of equal amounts of monoterpenes and diterpenes and small amounts of sesquiterpenes that can bury the invading insect. The diterpenes in the resin act to seal the hole as well as have a role in defence (Keeling and Bohlmann, 2006). Although the resin in conifers is constitutively present in resin ducts, wounding or herbivory has been shown to form traumatic resin ducts as well as induce the expression of diterpene synthesis genes, which is mediated by the wound-induced production of the signalling compound methyl jasmonate. Typical diterpenes in resin include abietic and isopimaric acids. Castor bean also produces the diterpene, casbene, that function as an antifungal agent.

Gibberellins (GAs; **Figure 2**) are diterpene plant hormones that were initially isolated from the fungus *Gibberella fujikuroi* and were subsequently found to be widespread among higher plants. GAs are produced by the cyclization of GGPP into the kaurene skeleton, which is then further metabolized to over 50 different GAs with a C_{20} ent-gibberellane or C_{19} ent-20-nor gibberellane skeleton. GAs regulate many growth aspects of plants including shoot elongation, fruit set and the *de novo* synthesis of α-amylase. *G. fujikuroi* is a pathogen of rice and takes advantage of the growth-stimulating properties of GAs by secreting GAs into the rice host causing unnatural growth, which results in infertile flowers and plant death. **See also**: Plant Growth Factors and Receptors
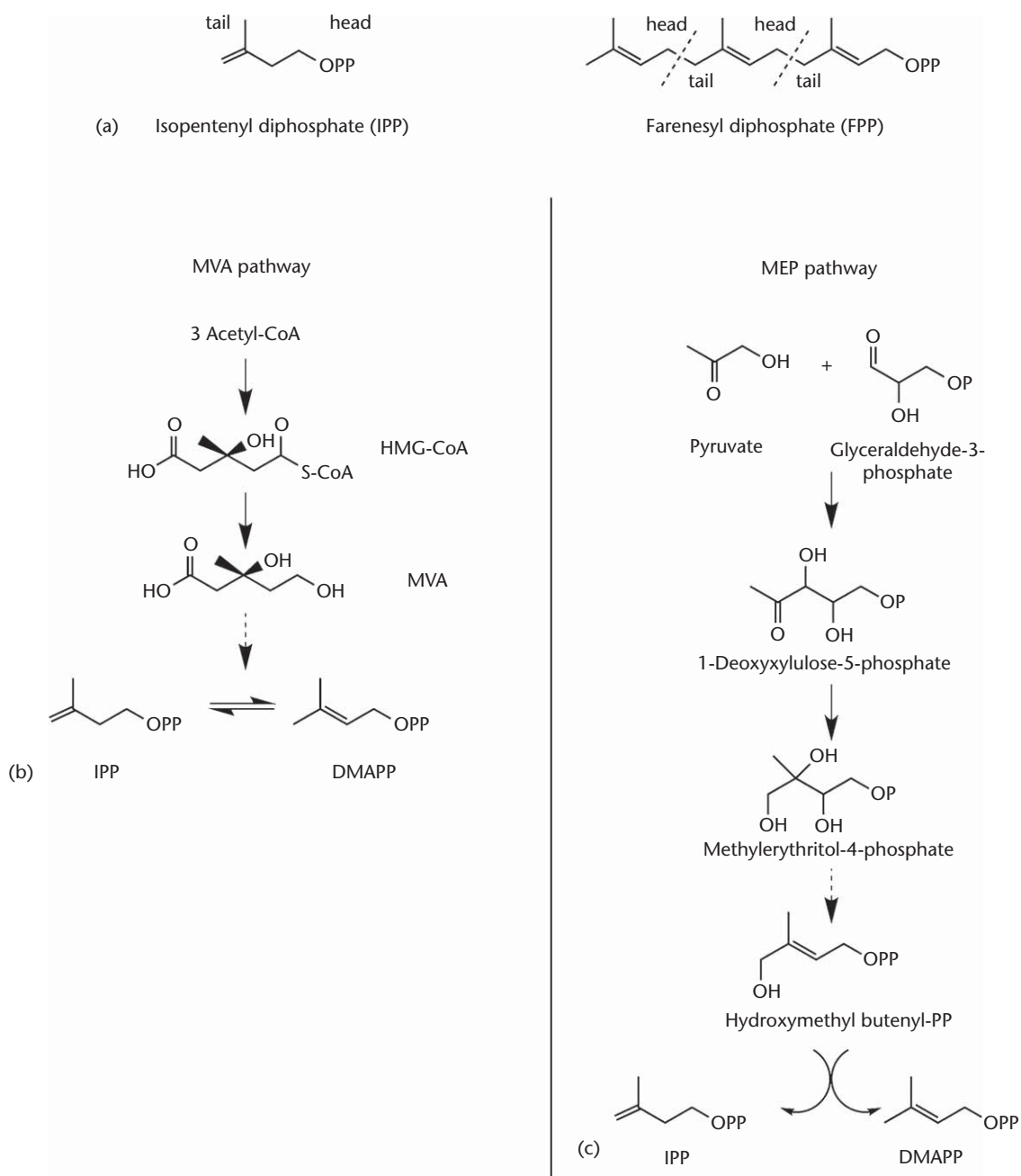
Figure 1 Head and tail positions of the basic isoprene unit and pathways for producing IPP. (a) Head and tail position for IPP and FPP. (b) The MVA pathway utilizing acetyl–CoA for production of IPP and DMAPP. (c) The MEP pathway utilizing pyruvate and glyceraldehyde-3-phosphate to produce IPP and DMAPP. Dashed lines indicate multiple steps.

Triterpenes

Triterpenoids constitute a large and diverse class of higher terpenoids that can be cyclic or acyclic. Cyclic triterpenoids usually contain four or five membered ring systems. There are over 1500 known triterpenoids, most of which are produced by plants where they often function as defence compounds against fungal and insect pathogens. These plant-derived triterpenoids are also currently investigated for activity against cancer and other human diseases. Triterpenoids are classified as C_{30} terpenoids, but, sterols and steroids, which contain less than 30 carbons, are also classified as triterpenoids since they originate from the same reaction mechanisms as C_{30} triterpenoids.

Eukaryotic life on earth is intimately tied to the production of oxygen from photosynthesis. The rise in
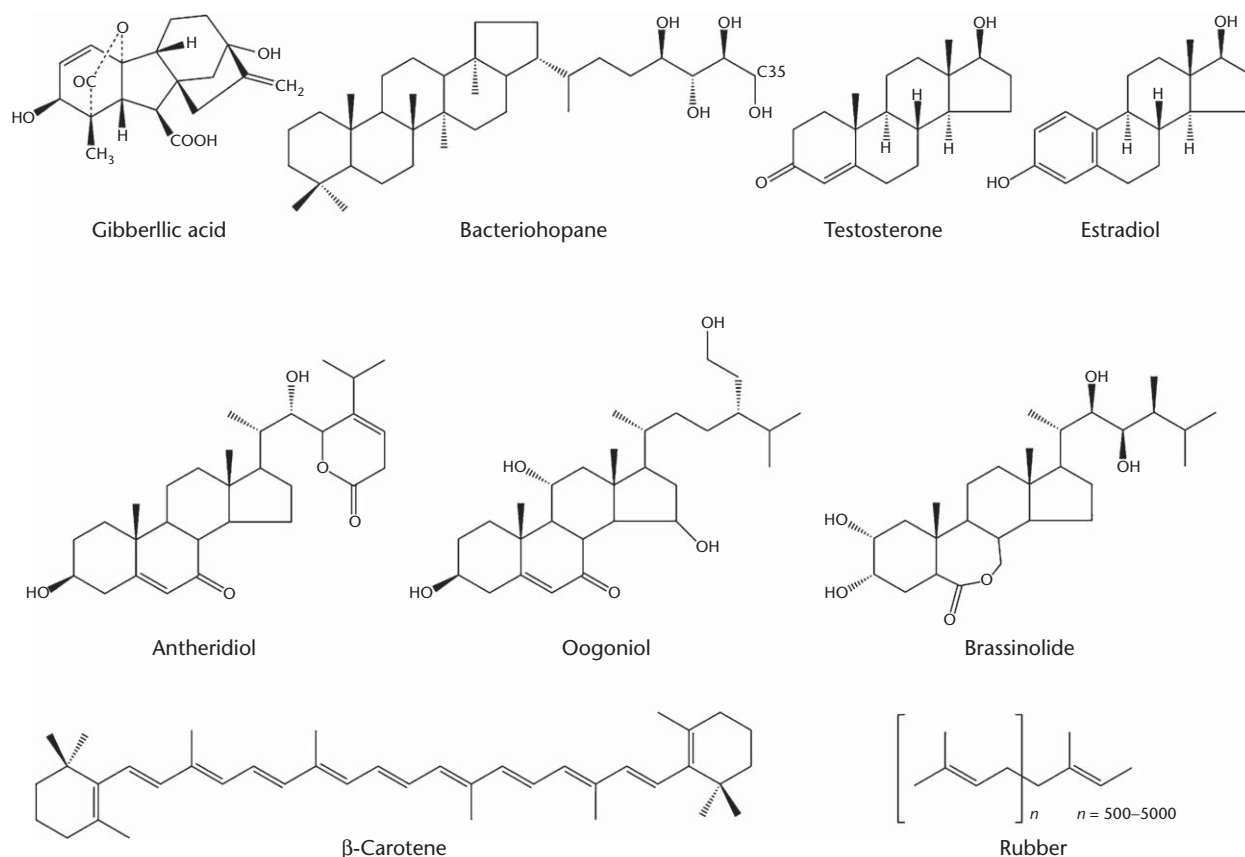
**Figure 2** Structures of some common higher terpenoids.

oxygen levels of both the oceans and the atmosphere have been suggested to have lead to the evolution of macroscopic multicellular organisms as well as the production of triterpenes since triterpene production is oxygen intensive (Summons *et al.*, 2006). For example, the production of one molecule of cholesterol requires 11 oxygen molecules. Thus, the presence of triterpenes in sediments can be used as a marker on oxygenated atmosphere. Additionally, since sterols and steroids are limited to eukaryotic organisms, the presence of sterols and steroids in the geologic record is being used to determine when the last common eukaryotic ancestor was present.

Triterpenoids are derived from the head-to-head condensation of two molecules of C_{15} FPP which forms the C_{30} compound squalene (**Figure 3**). In the biosynthesis of eukaryotic cyclic triterpenoids, squalene is then oxidized to form 2,3-oxidosqualene. At this point, oxygen-containing cyclic triterpenoids diverge depending on the enzyme that carries out the cyclization reaction. For example, cycloartenol synthase produces cycloartenol, the first cyclized product in plant sterol biosynthesis, whereas lanosterol synthase produces lanosterol, the first cyclized product in mammalian sterol biosynthesis. These sterol intermediates are then further processed by the loss of three carbons to form C_{27} sterols such as cholesterol. In fungi

and plants, sterols are methylated on the acyclic side-chain to form C_{28} and C_{29} sterols such as ergosterol and stigmasterol, respectively. In both plants and animals, sterols are utilized for the production of steroid hormones. Nonsterol oxygen-containing cyclic plant triterpenoids, such as saponins, are also produced by the cyclization of 2,3-oxidosqualene and are often found conjugated to sugar molecules. The biosynthesis of prokaryotic cyclic triterpenoids such as hopanoids does not require oxygen and thus they are formed by the direct cyclization of squalene (**Figure 3**). Acyclic triterpenoids are produced by the modification of the squalene backbone through oxidations, halogenations and additions of molecules such as acetate and amino acids. **See also**: Cholesterol, Steroid and Isoprenoid Biosynthesis

Sterols and steroid hormones

Sterols are the main terpenoid product of the isoprenoid pathway when cells are actively proliferating. The main function of sterols is to regulate membrane fluidity and plasticity. Other functions of sterols include the production of steroid hormones. Sterols have been reported to occur in animals, fungi, plants and bacteria. There are differences between the sterols produced by animals and those of
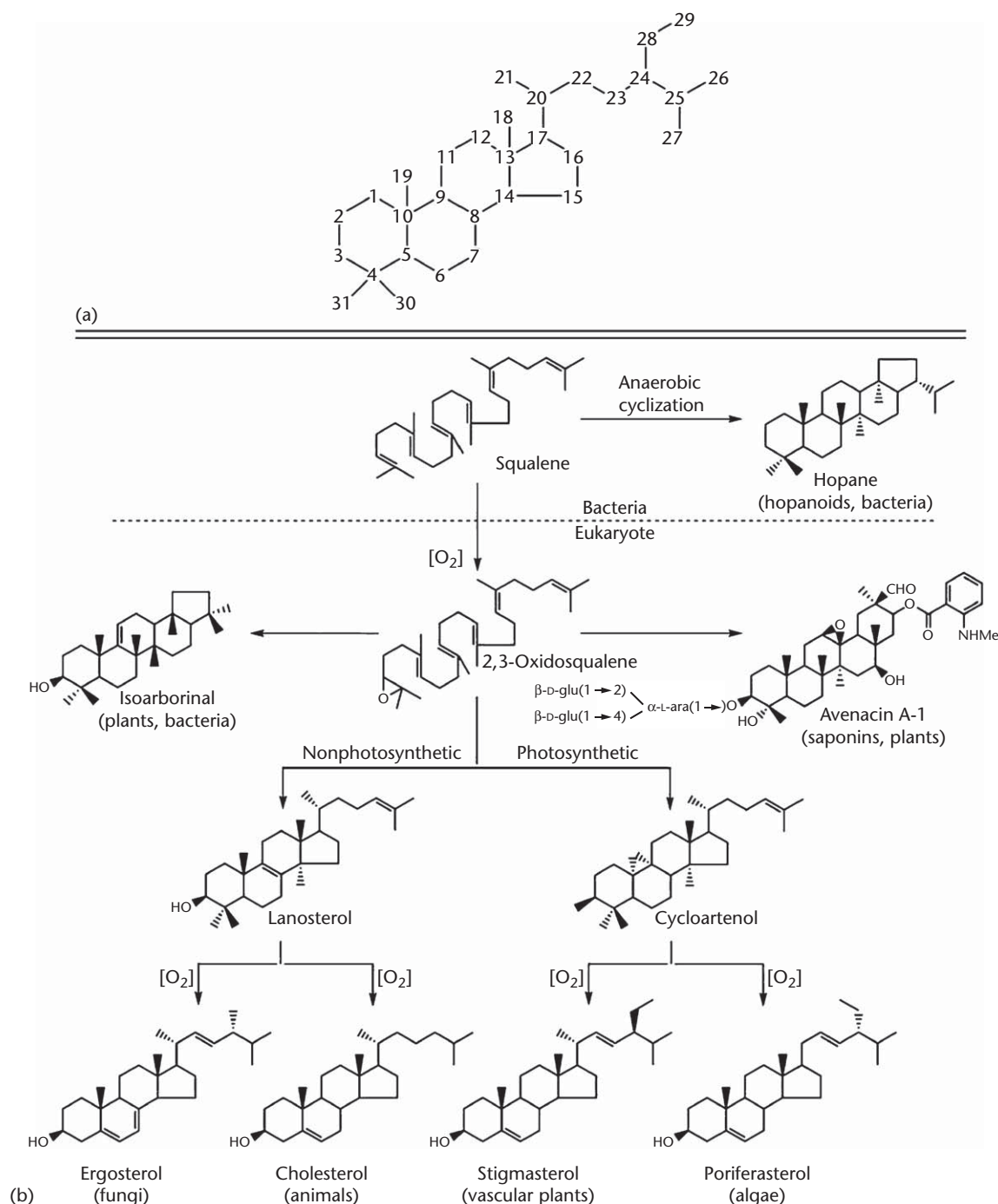
Figure 3 Cyclic triterpenoid production beginning with squalene. (a) The sterol numbering system. (b) The tetracyclic-pentacyclic isopentenoid bifurcation to sterol and sterol-like molecules.

plants, bacteria and fungi. Animal and bacterial sterols are C_{27} triterpenoids, whereas plant and fungal sterols have the addition of one or two methyl groups at the C24 position on the acyclic side-chain giving C_{28} or C_{29} sterols (**Figure 3**). The presence of sterols in cyanobacteria has been reported. However, this has been shown to be due to contaminating fungi in the cyanobacterial cultures. Additionally, genome

sequences of cyanobacteria do not indicate sterol biosynthetic genes (Summons *et al.*, 2006).

In contrast to animals and fungi that predominantly produce cholesterol and ergosterol as the main sterol, respectively, plants produce a wide array of sterols (phytosterols) with one or two methyl groups added to the C24 position (**Figure 3**). In fact, there are over 200

known naturally occurring C24-methylated sterols produced by plants. Phytosterols can exist in cells as either a free sterol or esterified to a fatty acid at the C3 hydroxyl group. Phytosterols are thought to be esterified so as to maintain proper membrane composition of free sterols (Schaller, 2004). Alternatively, the esterification of phytosterols may target them for transport out of the cell of production and to the site of incorporation into the membrane, or for further metabolism. Recently, two genes responsible for the esterification of phytosterols were isolated from *Arabidopsis*. The *At*SAT1 gene has been shown to preferentially esterify cycloartenol using 16 or 18 carbon saturated fatty acyl–CoA as a fatty acid donor (Chen *et al*., 2007). The *At*PSAT gene produces phytosterol esters with unsaturated fatty acids utilizing 18:2 phosphatidylethanolamine as the acyl donor for esterification to a broad range of phytosterols including intermediates and end product phytosterols (Banas *et al*., 2005).

A role for phytosterols in plants beyond cell membrane composition has been difficult to determine. But, with the advancement of *Arabidopsis* as a model plant system for plant studies, the roles phytosterols play in plant development are beginning to be identified. *Arabidopsis* mutant screens for developmental defects have revealed that loss of sterol biosynthetic gene function can have drastic effects on plant development (Schaller, 2004). Mutations in the sterol biosynthetic genes *At*FACKEL (sterol C14 reductase) and *At*HYDRA (Δ^8–Δ^7 sterol isomerase) have embryonic lethality. AtHYDRA also is defective in ethylene signalling. Mutants in the two C24 sterol methyltransferase genes, SMT1 and SMT2, have reduced plant stature. SMT1 mutants also have defects in embryonic patterning and polar auxin transport. Additional SMT2 mutants, *cvp1* and *frill1*, show defects in leaf vascular patterning and sepal and petal formation, respectively. Mutations in the *Arabidopsis* cycloartenol synthase gene CAS1 are male lethal, have cessation of meristem formation and defects in chloroplast development (Babiychuk *et al*., 2008). The explanations for these loss of phytosterol biosynthetic gene phenotypes vary. One school of thought says that mutations in phytosterol biosynthetic genes alter the composition of end product phytosterols and thus the make up of membrane sterols. This, in turn, modifies membrane properties leading to altered function of membrane proteins associated with the described functions. The second school of thought suggests that a sterol(s) may actually act as a signalling molecule to regulate plant developmental processes. In support of a sterol(s) acting as a signalling molecule are the *Arabidopsis* PHABULOSA (PHB) and PHAVOLUTA (PHV) genes which have a role in determining leaf polarity. Both PHB and PHV encode deoxyribonucleic acid (DNA)-binding proteins containing sterol-binding domains. Mutations in the PHB and PHV sterol-binding domains have effects on abaxial and adaxial leaf cell fates suggesting a sterol(s) as a modulator of PHB/PHV activity (McConnell *et al*., 2001).

Sterols are also metabolized to steroid hormones in animals, plants and fungi. In the aquatic saprophytic fungi *Achlya bisexualis*, the C_{29} sterol fucosterol is converted to the C_{29} steroid sex hormones antheridiol and oogoniol (**Figure 2**; Brunt *et al*., 1990; Brunt and Silver, 2004). In mammals, cholesterol is converted to the sex hormones testosterone and estradiol by removal of the acyclic sterol side-chain and replacement with a hydroxyl group giving C_{19} (testosterone) and C_{18} (estradiol) steroids (**Figure 2**). Although these hormones are typically referred to as male (testosterone) and female (estradiol) sex hormones, testosterone is found in female ovaries and functions in sexual drive as well as red blood cell production. Estradiol can also be found in males and functions in both sexes for sexual functioning as well as bone development. **See also**: Cholesterol, Steroid and Isoprenoid Biosynthesis; Hormones and Behaviour; Sex Hormones in Vertebrates

The presence of steroid hormones in plants was not confirmed until 1979 when the structure of the plant growth promoting substance brassin was positively identified as a steroidal lactone and renamed brassinolide (BL; (**Figure 2**)). Brassinolide was first isolated from *Brassica napus* but has been shown to be present in all plants that have been analysed to date including dicots, monocots, gymnosperms, algae and ferns. Approximately 40 different BLs have been identified and as a group are termed brassinosteroids (BRs). BRs have many effects on plant development including promotion of cell growth, elongation, division, tracheary cell differentiation, senescence advancement and responses to biotic and abiotic stresses. The discovery of the BR-deficient *Arabidopsis* mutant *det2* De-etiolated 2 helps to confirm the role of BRs as a hormone and determine the pathway for BR biosynthesis (Li *et al*., 1996). The DET2 gene encodes a sterol $\Delta^{4,5}$ reductase and carries out the second step in the conversion of the C_{28} phytosterol campesterol to BRs. An intense area of BR research has been BR perception and signal transduction leading to gene expression (Gendron and Wang, 2007). These studies in *Arabidopsis* have revealed a complex signalling mechanism initiated by the perception of BR by the transmembrane receptor kinase BRI1. On BR binding, BRI1 heterodimerizes with another transmembrane receptor kinase, BAK1, and together they function to initiate BR signal transduction. Through a cascade of phosphorylation and dephosphorylation, the transcription factors BZR1 (brassinazole resistant 1) and BZR2/BES1 (BRI1-EMS-suppressor 1) are activated and bind promoters of BR-responsive genes to alter gene expression and bring about BR responses. **See also**: Brassinosteroids

Hopanoids

Hopanoids are a group of sterol-like pentacyclic triterpenoids mainly produced by bacteria, but are found in lower plants including ferns, lichens and mosses. Hopanoids are produced through the direct cyclization of squalene and the simplest hopanoid is the C_{30} diploptene. But, most bacterial hopanoids are based on the C_{35} bacteriohopane (**Figure 2**) and can have many additions at the C35 position such as hexoses, amino acids and fatty acids (Rohmer,

2008). Hopanoids are predicted to function in membranes to modulate fluidity in a manner similar to sterols, but may have other functions such as stress reduction and maintenance of an oxygen-free environment for some enzymes (Poralla *et al.*, 2000; Berry *et al.*, 1993). Hopanoids do not appear to be ubiquitous in bacteria since the *Myxococcales* bacteria produce C_{27} sterols (Bode *et al.*, 2003) and analysis of sequence data from the Sorcerer II Global Ocean Sampling suggests that the presence of squalene hopene cyclase genes is not ubiquitous in marine bacteria (Pearson and Rusch, 2008).

Saponins

Saponins comprise a large and diverse class of largely pentacyclic triterpenoids produced mainly by dicot plants that have the basic C_{30} structure of the hopanoid skeleton. However, unlike hopanoids that utilize squalene for cyclization, saponins are produced by the cyclization of 2,3-oxidosqualene by an oxidosqualene cyclase (OSC) gene (**Figure 3**). Thus, saponins are oxygenated at C3, which is utilized for glycosylations. Saponins have been reported to have many different activities within plants including

allelopathic activity and as defence compounds against pathogens and herbivores. Saponins have been utilized by humans for many different applications such as drugs, sweeteners and bittering agents just to name a few (Vincken *et al.*, 2007). In this respect, yeast are currently being engineered to express plant OSC genes to produce β-amyrin, a saponin precursor that can be converted to many different saponins (Kirby *et al.*, 2008). **See also**: Glycosides: Naturally Occurring; Secondary Metabolites: Killing Pathogens

Phytoecdysteroids

Ecdysteroids are steroid hormones found in arthropods that regulate moulting cycles. Insects are incapable of producing sterols and thus obtain sterols through their diet of plant material. These alkylated (C_{28} and C_{29}) plant sterols are dealkylated to a C_{27} sterol core by the insect and converted to cholesterol which is then used for membrane sterols and the production of ecdysteroids. The most common insect ecdysteroid is 20-hydroxyecdysone (**Figure 4a**). Ecdysteroids, including 20-hydroxyecdysone, are also found in plant species. They are termed
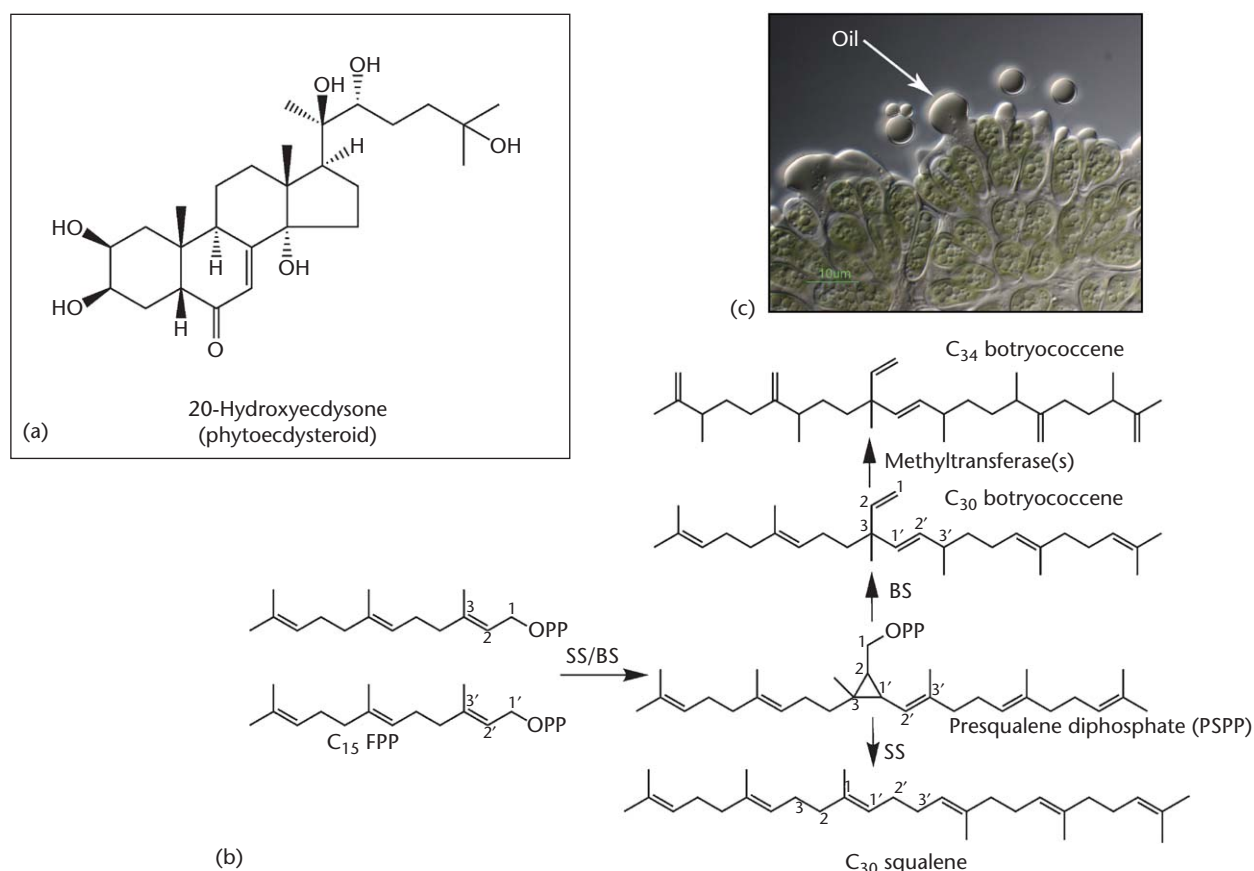
Figure 4 Phytoecdysteroid structure and biosynthesis of squalene and botryococcene. (a) 20-Hydroxyecdysone, a common phytoecdysteroid. (b) The reaction mechanism of the enzymes squalene synthase (SS) and botryococcene synthase (BS). (c) Botryococcene liquid hydrocarbon expelled from a colony of *B. braunii* by pressure.

phytoecdysteroids and are structurally identical to insect ecdysteroids. Phytoecdysteroids have been identified in 29 families of nonflowering plants (ferns, club mosses and gymnosperms) and in 78 families of flowering plants. But, it appears that the ability to synthesize phytoecdysteroids is not highly conserved within these families as varying species in each family contain ecdysteroids. However, this analysis in not complete and more conservation of phytoecdysteroids among families may become evident as more species are analysed. Unlike in insects, phytoecdysteroids biosynthesis in spinach does not utilize cholesterol as a precursor, but rather has been shown to proceed through the sterol intermediate lanosterol (Grebenok and Adler, 1993). This also appears to hold true for phytoecdysteroids found in corn (Devarenne *et al.*, 1995).

The role of ecdysteroids in plants appears to be defensive as they do not appear to have a hormonal role. In feeding assays, phytoecdysteroids can have effects ranging from antifeeding activity to mortality depending on the phytoecdysteroids level and larval growth stage. Evidence has been obtained in spinach to support a defensive role for phytoecdysteroids. Wounding and jasmonic acid treatments have been shown to rapidly increase spinach 20-hydroxyecdysone levels. Root herbivory by both the dark-winged fungus gnat *Bradysia impatiens* and weevil larvae of *Otiorhynchus sulcatus* has been shown to induce production of spinach 20-hydroxyecdysone (Schmelz *et al.*, 2002).

Botryococcenes

The liquid hydrocarbon triterpene known as botryococcene (**Figure 4b**) is produced by the B race of the green fresh and brackish water colony forming microalga *Botryococcus braunii*, which has been found on all continents. The cells of a *B. braunii* colony are held together by an extracellular matrix that is made up of a crosslinked hydrocarbon polymer core. The liquid botryococcenes are found within the extracellular matrix and can be expelled using pressure (**Figure 4c**). *B. braunii* has gained particular attention since botryococcenes can accumulate up to 86% of their dry weight (usually approximately 40%), caustic hydrolysis and distillation of botryococcenes results in the generation of hydrocarbon fuels suitable for internal combustion engines with a yield comparable to petroleum, and botryococcenes have been shown to occur in large amounts in currently used petroleum deposits.

Botryococcenes are produced by the head-to-head condensation of two FPP molecules to produce C_{30} botryococcene, which is then methylated four times to form the final product C_{34} botryococcene (**Figure 4b**). The reaction forming C_{30} botryococcene is predicted to follow a reaction mechanism similar to that of squalene production using FPP and proceeding through the presqualene diphosphate (PSPP) intermediate (see the section on Regulation and organization of triterpenoid genes; **Figure 4b**). The difference between the C_{30} botryococcene reaction and the squalene reaction is the breakage of the PSPP cyclopropane ring; in C_{30} botryococcene, the two FPP molecules are connected by the 1′ and 3 carbons, whereas in squalene the two FPP molecules are connected by the 1′ and 1 carbons (**Figure 4b**; Okada *et al.*, 2004). The enzyme producing C_{30} botryococcene has been termed botryococcenes synthase (BS). However, the gene encoding BS, as well as the methyltransferase gene(s) responsible for producing C_{34} botryococcene, have yet to be characterized from *B. braunii*.

Regulation and organization of triterpenoid genes

The production of squalene has been one of the most studied triterpenoid reaction mechanisms due to the association of squalene with cholesterol biosynthesis in humans. This reaction is catalysed by the enzyme squalene synthase (SS) and represents the first enzymatic step committing carbon exclusively to triterpene production. The SS reaction is a two-step reaction that requires nicotinamide–adenine dinucleotide phosphate reduced form (NADPH). In the first step, the two FPP molecules are condensed, head-to-head, to form the reaction intermediate PSPP, which contains a cyclopropane ring between carbon 1 of one FPP molecule and carbons 2 and 3 of the second FPP molecule. The second step converts PSPP to squalene by cleaving the cyclopropane ring, creating a connection of the two FPP molecules through the 1–1′ carbons. This is followed by bond rearrangement and reduction utilizing NADPH (**Figure 4b**). **See also**: Cholesterol, Steroid and Isoprenoid Biosynthesis

The regulation of mammalian SS has been well-studied in relation to cholesterol biosynthesis. SS gene transcript levels have been shown to be coordinated with the amount of cellular sterol in a feedback regulation mechanism. In the presence of high sterol levels, SS messenger ribonucleic acid (mRNA) levels are decreased up to 95% while in low-sterol conditions, SS mRNA levels are increased. This regulation mechanism ensures that additional sterols are not produced when total sterol levels are high. Given the negative implications of high cholesterol levels in humans, this type of regulation will help to minimize the effects of high cholesterol levels. **See also**: Cholesterol Metabolism Regulation

In plant systems, SS has been studied in the context of pathogen interactions as well as during normal cellular development. Unlike mammalian SS, plant SS does not appear to be regulated by a feedback mechanism in response to high sterol levels. However, these studies are not complete and lack the in-depth analysis of the mammalian studies. Plant SS is highly regulated in response to pathogen. Treatment of *Solanaceous* plants such as tomato and tobacco with fungal pathogens causes a suppression of sterol biosynthesis and accumulation. Coordinately, a rapid and dramatic decrease in SS enzyme activity is observed in response to pathogen. Additionally, in tobacco this rapid drop in SS enzyme activity is accompanied by an equally rapid decline in SS mRNA levels. However, the SS protein levels are maintained for

almost 24 h after SS enzyme activity and mRNA levels have decreased, after which the SS protein levels decrease to undetectable levels (Devarenne *et al.*, 2002). This would suggest that in response to pathogen, SS activity is regulated through a combination of transcriptional and posttranslational mechanisms in response to pathogen. Suppression of plant sterol biosynthesis in response to fungal elicitor appears to be a conserved event to divert carbon from general metabolism and towards defence compound production. Other, non-*Solanaceous*, plant species decrease sterol biosynthesis through the suppression of different sterol biosynthetic enzymes such as cycloartenol synthase (*Tabernaemontana divaricata*) or the C4 demethylation step (*Ammi majus*).

It is well known that in prokaryotic genomes, genes with related functions are organized to operons so that expression of all genes in the operon can be regulated as one. In eukaryotic genomes, genes with related functions, such as genes involved in a biosynthetic pathway, are conventionally thought to be randomly dispersed throughout the genome. However, two triterpene operon-like gene clusters have recently been discovered in plants. In oat, there is an operon-like gene cluster for the biosynthesis of the triterpenoid saponin avenacin (**Figure 3**); and in *Arabidopsis thaliana*, there is an operon-like gene cluster for the biosynthesis of the triterpenoid thalianol (Qi *et al.*, 2004, 2006; Field and Osbourn, 2008). Both of these plant 'operons' contain the necessary OSC gene and genes required for the subsequent processing of the cyclized product to end product triterpenoid. Unlike in bacteria where operons are thought to be obtained by different species through horizontal gene transfer, the oat and *Arabidopsis* triterpene 'operons' are predicted to have independently evolved and originated by gene duplication events, gene specialization and genome reorganization.

The great diversity of plant triterpenoids appears to be a consequence of the large number of OSC genes present in plants. For example, the genome of *Arabidopsis* appears to contain 13 OSC genes whereas rice contains 9 (Phillips *et al.*, 2006). Presumably, individual OSC genes are utilized for the production of different triterpenoids such as sterols and the many different saponins found in plants. Interestingly, *Arabidopsis* contains an OSC for the production of lanosterol, the sterol precursor in animals and fungi (Kolesnikova *et al.*, 2006). Other plants have been identified with a functional lanosterol synthase suggesting that, while cycloartenol has been shown to be the predominant precursor for plant sterols, some sterol metabolites can only be produced through lanosterol. The gene responsible for producing 2,3-oxidosqualene, squalene epoxidase (SQE), also has multiple copies in plants; *Arabidopsis* contains 3 SQEs, *Medicago* 2 SQEs and *Populus* 4 SQEs. This suggests that a single SQE may feed 2,3-oxidosqualene to multiple OSC enzymes for triterpene production. The multiple SQE and OSC genes are predicted to have arisen by gene duplication, which may have been driven by the requirement of plants to respond to multiple biotic and abiotic stresses in different tissues.

Thus, different SQE and OSC genes would be expressed in response to specific stresses and in distinct tissues. This is supported by the fact that many of the upstream terpenoid pathway genes, such as FPP synthase and 3-hydroxy-3-methylglutaryl–CoA reductase (HMGR) are represented by multiple gene copies that are expressed in specific tissues, developmental phases or in response to biotic stresses.

Tetraterpenoids

Tetraterpenoids consist of only the C_{40} carotenoids, which are produced by the head-to-head condensation of two molecules of C_{20} GGPP forming phytoene. This reaction is carried out by the enzyme phytoene synthase (PS) and proceeds in a two-step reaction nearly identical to that of SS. The PS reaction intermediate, prephytoene diphosphate, contains a cyclopropane ring identical to PSPP. Phytoene is then further metabolized to produce acyclic or cyclic carotenoid end products. Oxygenated cyclic carotenoids are referred to as xanthophylls and hydrocarbon carotenoids are known as carotenes. Carotenoids are found in plants where they function against photodynamic damage as well as the pigments giving colour to carrots (β-carotene; **Figure 2**) and tomatoes (lycopene). Carotenoids are also found in other nonphotosynthetic organisms such as bacteria and fungi.

Carotenoids are also used a base for the production of other compounds. In humans, the ingested β-carotene is converted to vitamin A. In fungi, the hormone trisporic acid is produced from β-carotene. In plants, violaxanthin is cleaved and converted to the plant hormone abscisic acid. Seeds of parasitic plants have been shown to germinate only when exposed to isoprenoid strigolactones produced by the host plant. Strigolactones have long been thought to be sesquiterpenes. However, recently, strigolactones were shown to be produced from carotenoids and also function to inhibit plant shoot branching (Matusova *et al.*, 2005; Umehara *et al.*, 2008). Based on these data, strigolactones have been suggested as a new class of plant hormones.

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