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FOCUSED REVIEW

Variation on a theme: the structures and biosynthesis of specialized fatty acid natural products in plants

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SUMMARY

Plants are able to construct lineage-specific natural products from a wide array of their core metabolic pathways. Considerable progress has been made toward documenting and understanding, for example, phenylpropanoid natural products derived from phosphoenolpyruvate via the shikimate pathway, terpenoid compounds built using isopentyl pyrophosphate, and alkaloids generated by the extensive modification of amino acids. By comparison, natural products derived from fatty acids have received little attention, except for unusual fatty acids in seed oils and jasmonate-like oxylipins. However, scattered but numerous reports show that plants are able to generate many structurally diverse compounds from fatty acids, including some with highly elaborate and unique structural features that have novel bioproduct functionalities. Furthermore, although recent work has shed light on multiple new fatty acid natural product biosynthesis pathways and products in diverse plant species, these discoveries have not been reviewed. The aims of this work, therefore, are to (i) review and systematize our current knowledge of the structures and biosynthesis of fatty acid-derived natural products that are not seed oils or jasmonate-type oxylipins, specifically, polyacetylenic, very-long-chain, and aromatic fatty acid-derived natural products, and (ii) suggest priorities for future investigative steps that will bring our knowledge of fatty acid-derived natural products closer to the levels of knowledge that we have attained for other phytochemical classes.

Keywords: fatty acid biosynthesis, natural products, specialized metabolism, polyacetylenes.

INTRODUCTION

Although plants share a set of building blocks (amino acids, sugars, fatty acids) with virtually all organisms on Earth, they are remarkable in that they can modify these simple compounds into highly elaborate and unique chemicals. Such compounds (often called secondary metabolites, specialized metabolites, or natural products) occur in lineage-specific patterns and help plants thrive in their specific, local environments. Research over the past decades has provided considerable insight into the structures and biosynthesis of plant specialized metabolites, particularly those derived from amino acids (e.g., certain alkaloids) and branch pathways of sugar metabolism (the shikimate pathway, which leads to phenylpropanoids, and the mevalonate/MEP pathways, which lead to terpenoids).

In addition to amino acids and sugars, plants derive specialized metabolites from fatty acids. Perhaps the

structurally simplest group of fatty acid-derived natural products are those whose structures are essentially long-chain fatty acids but with a small number of unusual unsaturations (i.e., double or triple bonds) or a single oxygen-containing functional group (Figure 1). These ‘unusual fatty acids’ are only found in certain species, typically in seed oils where they modify the physical properties of the oil and thus its potential applications (Cahoon & Li-Beisson, 2020; Jaworski & Cahoon, 2003; Ohlrogge et al., 2018). Another well-studied group of fatty acid-derived natural products in plants are those compounds related to jasmonic acid, or ‘jasmonate-type oxylipins’. These compounds regulate a wide variety of plant processes (Howe & Schilmiller, 2002; Mosblech et al., 2009) and can also be found in species-specific patterns (de León et al., 2015; Gachet et al., 2017). Thus, unusual fatty acids and jasmonate-type oxylipins are two fatty acid-derived natural products that are relatively

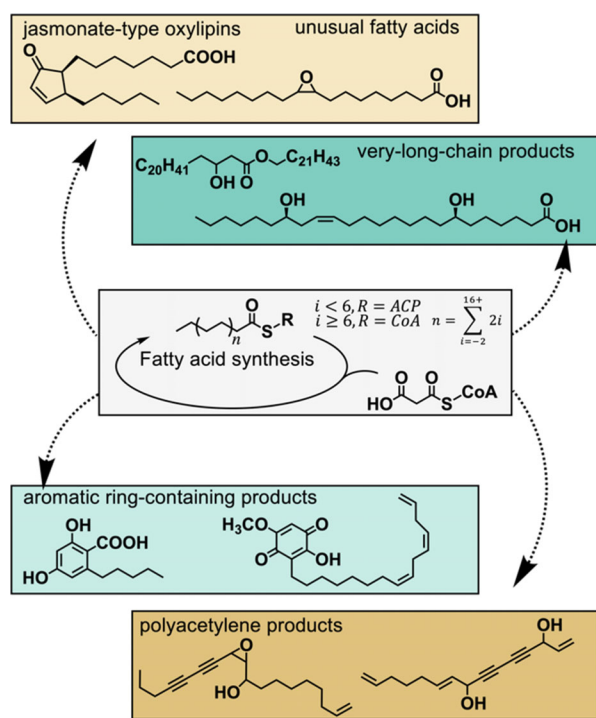


Figure 1. Fatty acid biosynthesis and categories of fatty acid-derived natural products.

Fatty acid biosynthesis is represented in the central box. At certain chain lengths, and in select species, fatty acids can be converted into more elaborate natural products, including polyacetylenes (dark brown box), aromatic ring-containing products (light green box), and very-long-chain products (dark green box). These classes of compounds are covered in this review. Using fatty acids, plants can also create jasmonate-type oxylipins and unusual fatty acids (light brown box). By comparison, the structures and biosynthesis of those compounds have been well covered in other reviews (see main text) and are not included in this work.

well studied and widely known. The many known structures that belong to these two groups of compounds, as well as their underlying biosynthetic pathways, have been documented and studied to the point that developing and experimenting with methods for pathway engineering in those areas is now a research focus (Ledesma-Amaro & Nicaud, 2016; Napier, 2007; Yu et al., 2012).

Despite the considerable attention that has been paid to unusual fatty acids and jasmonate-type oxylipins, plants produce a wide array of fatty acid-derived natural products that do not belong to either of those categories. These include polyacetylenes, very-long-chain products, and fatty acid natural products with aromatic rings. Many of these function as defense chemicals (Greger, 2016), compounds with apparent structural roles (Buschhaus & Jetter, 2011), or mediators of plant-insect interactions (Ibrahim et al., 2016). Humans have found uses for these compounds as lubricants (Li et al., 2018), potential medicines (Stasiuk & Kozubek, 2010), and health-related foodstuffs (Andersson et al., 2011; Kaur et al., 2014). Despite their important functions in both plant biology and industry,

these compounds have been the subject of only modest research compared to unusual fatty acids or jasmonates, but the past decade has seen several major advances in this area. This review summarizes our knowledge of the structures and biosynthesis of relatively understudied fatty acid-derived natural products and highlights priorities for future research in this area.

MAIN TEXT

To synthesize fatty acids, plants use a plastid-localized, multi-enzyme fatty acid synthase complex that acts on fatty acyl-intermediates linked to acyl carrier protein (ACP). This complex starts with acetyl-CoA and then condenses it with malonyl-ACP via release of carbon dioxide. The resulting 3-keto intermediate is then modified through three subsequent reactions to form a two carbon-extended saturated hydrocarbon chain (Figure 1, central box). Additional fatty acid synthase cycles generate saturated C_{16} and C_{18} fatty acid chains. Accordingly, fatty acid-derived natural products can often be identified by the presence of carbon chains in their structures that originate from the fatty acid synthase. Several other structural features can be used to differentiate subgroups of fatty acid-derived natural products, including the presence of (i) carbon-carbon double and triple bonds, (ii) oxygen-, nitrogen-, or sulfur-containing functional groups, and (iii) ring structures, particularly aromatic rings. Using these structural features as guides, it is generally possible to classify fatty acid-derived products into a small number of categories (Figure 1), including polyacetylene products (compounds with one or more carbon-carbon triple bonds), very-long-chain products (compounds with ≥ 20 carbons), and aromatic products (compounds with aromatic rings). Here, we give a general overview of the major types of structures and biosynthetic mechanisms known in each of these categories. We note that some alkamide products (reviewed in Greger, 2016) are structurally similar to fatty acids and may appear to be derived from such; however, recent evidence indicates that many of these compounds may be type I polyketide synthase products and thus not derived from fatty acids (Buitimea-Cantúa et al., 2020). Such alkamides are therefore not included in this review.

Polyacetylenic fatty acid-derived products

Polyacetylenes are a diverse class of fatty acid derivatives that contain one or more carbon-carbon triple bonds. They are found in diverse branches of the tree of life, including marine algae and invertebrates (Zhou et al., 2015), insects (Haritos et al., 2012), and fungi (Negri, 2015). Plant polyacetylenes are particularly interesting since they form a component of the human diet. Accordingly, the bioactivities of these compounds have been the subject of considerable study, which has led to the discovery that they have a variety of nutraceutical properties (Christensen & Brandt, 2006). Several reviews document the diverse structures of

polyacetylenes, including structures of polyacetylenes from across the plant kingdom (Negri, 2015), specific core areas of the chemical space (Santos et al., 2022), and structures from specific groups such as the Asteraceae (Konovalov, 2014), the Apiaceae (Chen et al., 2015), and, for example, the genus *Daucus* (Dawid et al., 2015) and the genus *Bupleurum* (Lin et al., 2016). Here, we provide a general overview of those findings and describe polyacetylene structures and biosynthesis from across the plant kingdom.

Many polyacetylenes have at least two carbon–carbon triple bonds that are adjacent to one another on a linear hydrocarbon backbone that generally ranges from C₁₀ to C₁₈ (example structures are shown in Figure 2). These often contain one or more carbon–carbon double bonds, as well as several primary or secondary oxygen-containing functional groups, including hydroxyl, keto, epoxy, carboxylic acid, acetyl, and methoxy groups, among others. Recent reports of new polyacetylenes sometimes provide missing details or describe variants on this theme (for example, Murata et al., 2017, Figure 2). Other newly reported molecules are relatively extreme examples within these general structural constraints, such as compounds with four or more carbon–carbon double or triple bonds (for example, Appendino et al., 2009; Liu et al., 2018, Figure 2) or highly acetylated compounds (for example, Mi et al., 2019; Figure 2).

On top of the structural features described for the linear compounds above (approximately C₁₀–C₁₈, double/

triple bonds, oxygen-containing functional groups), polyacetylenes can also contain rings within their structures (Figure 2, blue ovals). These rings vary from five-member sulfur-containing rings (thiophenes, for example, those recently reported by Blagojević et al., 2017 and those reviewed in Ibrahim et al., 2016) and five-member oxygen-containing rings (furans, such as those recently reported from the carrot family; Zheng et al., 2018) to lactones, spiroketals, and macrocycles (for example, the structures reported by Pollo et al., 2013; Figure 2; reviewed in Negri 2015). In addition to heterocyclic rings, polyacetylenes can also bear functional group modifications (Figure 2, green ovals). These can include, for example, acetylation (structures reported by, e.g., Chen et al., 2015; Mi et al., 2019; Figure 2) and glycosylation (structures from, e.g., Guo et al., 2017; Pollo et al., 2013; Figure 2), as well as esterification and amidation (such as structures recently reported in Althaus et al., 2017; Blagojević et al., 2017; Figure 2; reviewed in Negri, 2015). The addition of ring arrangements and functional group modifications to the repertoire of polyacetylene variability considerably increases the structural space occupied by polyacetylenes. Overall, more than 2000 polyacetylene structures have been described (Minto & Blacklock, 2008; Singh et al., 2020), making this one of the most diverse classes of fatty acid-derived natural products, based on current knowledge.

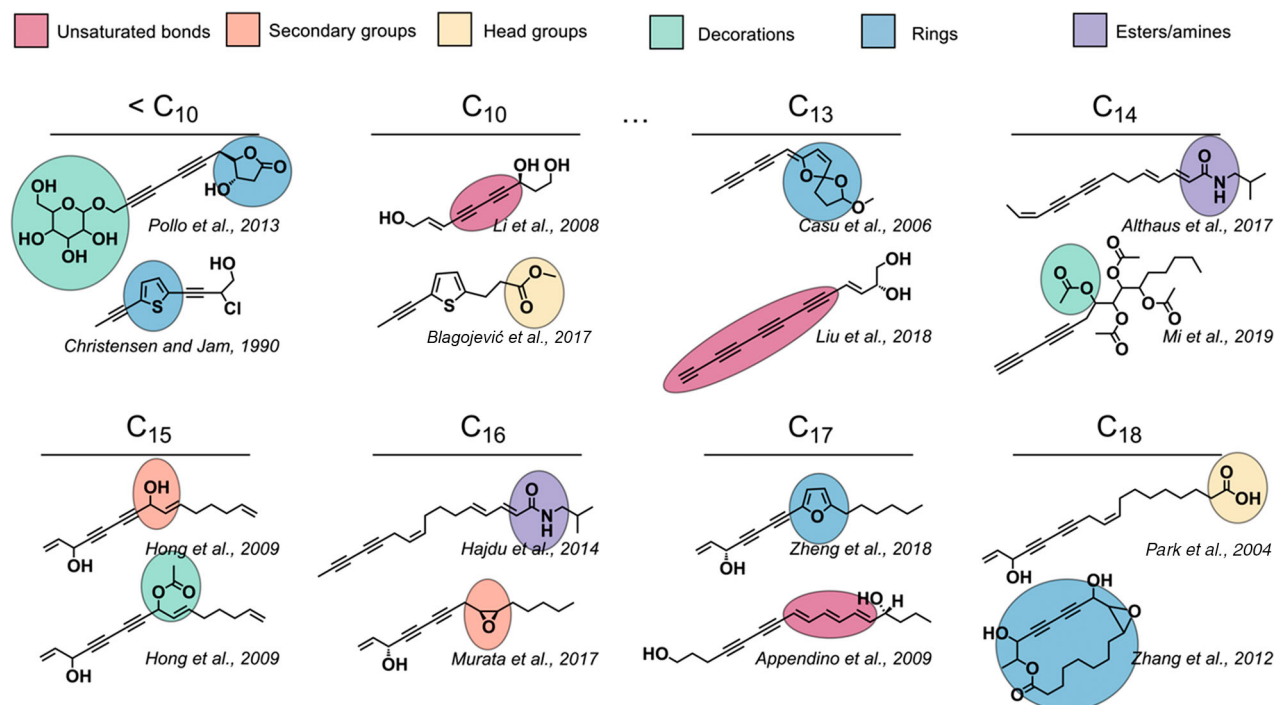


Figure 2. Examples of diverse polyacetylene structures found in plants. Representative structures are shown for the most encountered chain lengths so far, <math>< C_{10}</math> to C₁₈, as shown in the column headers. Colored circles indicate types of structural variation found in polyacetylene compounds, as indicated in the legend.

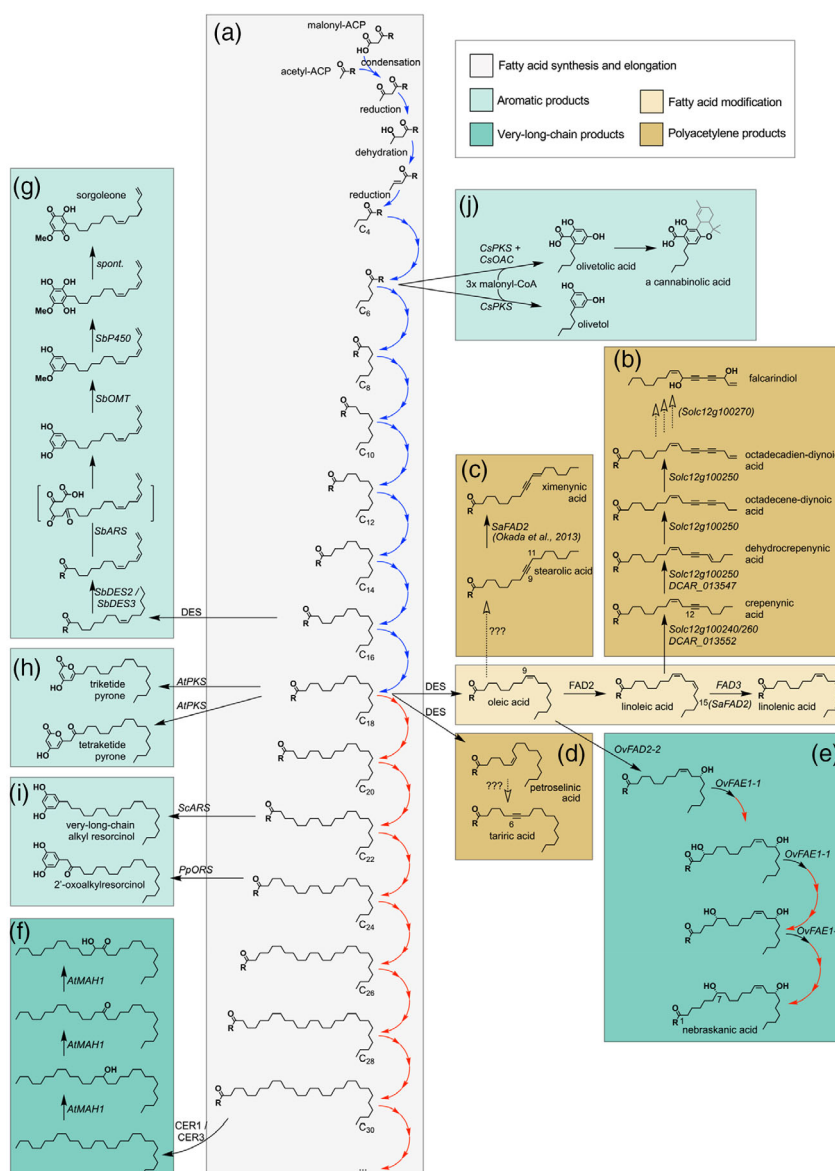
The structures described above, combined with evidence from radiotracer studies (Knispel et al., 2013), suggest that polyacetylenes are anabolically derived from fatty acids. This means that simple, monoacetylenic compounds precede more complex polyacetylenic molecules. So far, three monoacetylenic fatty acids (tariric acid, $C_{18:1\Delta 6}$; stearic acid, $C_{18:1\Delta 9}$; and crepenynic acid, $C_{18:1\Delta 12}$) have been found, making it seem likely that there are at least three metabolic entry points into the structural space occupied by polyacetylenes. These have been dubbed the tarirate, stearoleate, and crepenynate pathways, respectively (Li et al., 2020; Minto & Blacklock, 2008; Negri, 2015).

Of the three pathways into polyacetylene space, the crepenynate pathway has been studied the most. Several groups, working with various Asterales or Apiales species,

have identified *FAD2* family genes that encode enzymes with oleic acid $\Delta 12$ acetylenase (i.e., a triple bond-forming desaturase) activity and thus have the ability to produce crepenynic acid (Figure 3b) (Cahoon et al., 2003; Chen et al., 2020; Lee et al., 1998; Nam & Kappock, 2007). Further work revealed that certain *FAD2* genes encode enzymes with crepenynic acid $\Delta 14$ desaturase activity, yielding dehydrocrepenynic acid (DCAR_013547; Figure 3b) (Busta et al., 2018). Finally, an additional study found that tomato (*Solanum lycopersicum*) possesses an acetylenase that can generate crepenynic acid, as well as an additional desaturase that appears to catalyze multiple desaturation steps, leading to octadecadiene-diyonic acid (Solc12g100240/260, Solc12g100250; Figure 3b; Jeon et al., 2020). That same study also found that tomato

Figure 3. Biosynthesis of fatty acid-derived natural products in plants.

(a) Fatty acid biosynthesis and elongation, in which blue arrows indicate fatty acid synthase (FAS) steps and blue arrows indicate fatty acid elongase (FAE) steps. Also shown are other biosynthetic pathways, including: (b) falcarindiol, (c) ximenynic acid, (d) tariric acid, (e) nebraskanic acid, (f) very-long-chain secondary alcohols, ketones, and diols, (g) sorgoleone, (h) long-chain pyrones, (i) 2'-oxoalkylresorcinols, and (j) olivetolic acid. Classes of products are shown with colored boxes as indicated in the legend.



plants lacking a gene encoding a putative decarboxylase (Solc12g100270) are unable to produce falcarindiol, the primary polyacetylene found in that species. This suggests that a decarboxylase acts somewhere near the end of the pathway to falcarindiol.

Though most of our knowledge about polyacetylene biosynthesis comes from studies of the crepenynate pathway, there is some information available about one of the other entry point pathways into polyacetylene space, the stearoleate pathway. Working with *Santalum acuminatum*, investigators identified several *FAD2*-like genes that catalyzed both $\Delta 15$ desaturation of linoleic acid (an activity usually associated with *FAD3*-like genes) and $\Delta 11$ desaturation of stearic acid to form ximenynic acid (*SaFAD2*; Figure 3c; Okada et al., 2013). Considering this multifunctionality together with the multifunctionality observed for some enzymes on the crepenynate pathway, it is evident that *FAD2*-like enzymes can be rather promiscuous.

Despite our knowledge of several features of the crepenynate and stearoleate pathways, our knowledge of neither pathway is complete. Genes encoding hydroxylase activities on the pathway to falcarindiol have yet to be identified (Figure 3b), as are gene(s) that enable the creation of stearic acid. Based on the way in which triple bonds are installed in the crepenynate pathway (i.e., existing double bonds are oxidized into triple bonds), it seems likely that stearic acid is synthesized by $\Delta 9$ acetylation of oleic acid and that the tarirate pathway (also a yet uncharacterized pathway) comprises $\Delta 6$ acetylation of petroselinic acid (Negri, 2015). Genes encoding these activities are important targets for future research because they will lay a foundation upon which we can investigate biochemical pathways to the more complex polyacetylenic compounds that plants produce.

Very-long-chain fatty acid-derived natural products

Fatty acyl chains up to C_{18} are synthesized *de novo* in the plastids, and while in select species these are converted into unusual fatty acids or polyacetylenes, a major fate for these acyl chains is extension in the endoplasmic reticulum (ER). In the ER, fatty acyl chains are extended to very-long-chain fatty acids (C_{20} – C_{38+}) by an enzyme complex called the elongase that uses chemistry analogous to *de novo* fatty acid biosynthesis in the plastid (Haslam & Kunst, 2013; Yeats & Rose, 2013). This pathway is initiated by β -ketoacyl-CoA synthase activity that transfers two carbons from malonyl-CoA to the elongating fatty acid chain. Though this elongation process happens in virtually all plant species (as very-long-chain fatty acyl units are needed for sphingolipids, suberin, and cuticular waxes), some species use very-long-chain acyls as starting points for the creation of specialized metabolites. Here, we briefly describe the structures and

biosynthesis of the major types of known very-long-chain fatty acid-derived natural products.

One source of very-long-chain fatty acid-derived natural products are seed oils. Many very-long-chain seed oil components that are synthesized by select species have been systematically documented on a large scale at plantfadb.org (Ohlrogge et al., 2018). These include, for example, very-long-chain fatty acyls bearing one or two oxo, hydroxyl, or methoxy groups, as well as double bonds (orange and red ovals, respectively, Figure 4a; for example, Mikolajczak et al., 1965; Smith, 1966). Often, these very-long-chain fatty acid-derived natural products are minor components of a species' seed oil, though there are some exceptions. Examples of such are the recently discovered hydroxy fatty acids from *Orychophragmus violaceus* that contain two hydroxyl groups and at least one double bond (Li et al., 2018).

In addition to seed oils, plant cuticular waxes are another source of very-long-chain fatty acid-derived natural products. Species-specific wax compounds were relatively recently and comprehensively reviewed (Busta &

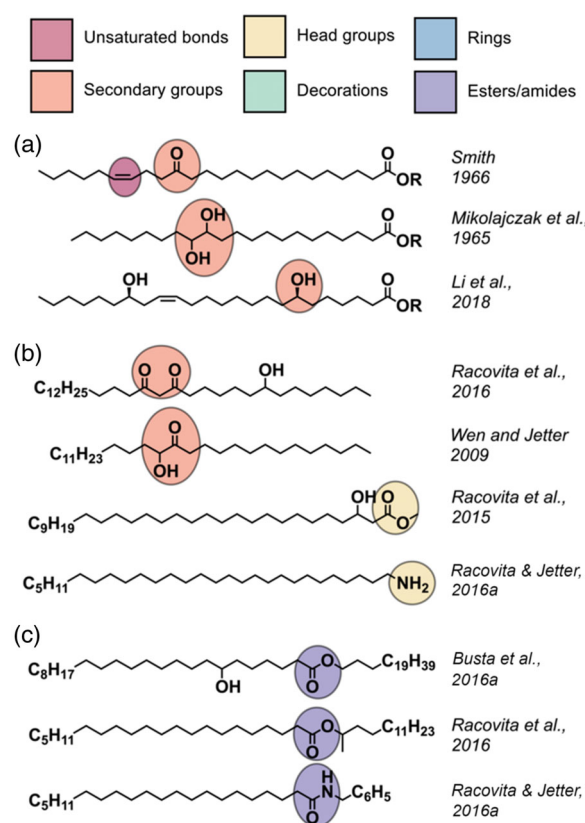


Figure 4. Examples of very-long-chain fatty acid-derived natural products. Representative structures are shown for the major types of structural variants. Colored circles indicate types of structural variation found in very-long-chain fatty acid-derived compounds, as indicated in the legend. (a) Glycerol-bound compounds. (b) Compounds with and without head groups. (c) Ester compounds.

Jetter, 2018), but we provide a brief overview here. Cuticular waxes are found on virtually all plants and contain very-long-chain fatty acid-derived compounds with or without a single oxygen-containing terminal functional group. These ubiquitous wax compounds are fatty alkanes, alcohols, aldehydes, and acids. However, certain taxa produce lineage-specific compounds, which vary in chain length and may contain one or more secondary hydroxyl or oxo groups on adjacent or distal carbons (orange ovals, Figure 4b; Racovita et al., 2015, 2016; Racovita & Jetter, 2016a; Wen & Jetter, 2009). These specialized wax compounds can also bear unusual head groups such as methoxy or amine groups (yellow ovals, Figure 4b) (Racovita et al., 2015; Racovita & Jetter, 2016a), or they may contain no head group at all, such as in methyl ketones and 2-alcohols (Perera et al., 2010; Racovita et al., 2015). Finally, while fatty acid–fatty alcohol esters are ubiquitous wax compounds, some lineage-specific wax esters have been discovered, including hydroxy fatty acid esters, secondary alcohol esters, and fatty acid amides (Figure 4c, purple ovals; for example, Busta et al., 2016a; Racovita et al., 2016; Racovita & Jetter, 2016a). Thus, though very-long-chain fatty acid-derived natural products are not as structurally complex as polyacetlenes, they still occupy a rather large structural space, with more than 600 compounds having been described (Busta & Jetter, 2018).

The fatty acid elongase (Figure 3, red arrows) that extends long-chain fatty acids (C_{16} , C_{18}) to very-long-chain lengths ($\geq C_{20}$), like the fatty acid synthase complex, is composed of enzymes with condensation, reduction, dehydration, and reduction activities. Thus, one of the ways in which plants can generate very-long-chain natural products is to alter the extent to which or order in which elongase enzymes act. Indeed, several groups provided evidence that very-long-chain compounds with secondary functional groups on exclusively odd-numbered carbons (as opposed to even-numbered carbons) might be synthesized by elongase complexes with modified substrate preferences (Balzano et al., 2019; Busta & Jetter, 2018; Schulz et al., 2000). Recently, work with *O. violaceus*, a species in the Brassicaceae, revealed that such pathways indeed exist and can generate molecules with structures like those which had been hypothesized (Li et al., 2018). Specifically, this work found that a modified fatty acid elongase condensing enzyme can use as substrate an incompletely reduced elongation cycle intermediate, that is, a compound with a hydroxyl group on C-3 (*OvFAE1-1*; Figure 3e). This results in that hydroxyl group being retained in the products of subsequent rounds of elongation, eventually resulting in a compound with 1,7 functional group geometry, ‘nebraskanic acid’, via a pathway referred to as ‘discontinuous elongation’. Based on this precedent, it seems likely that other species harboring very-long-chain compounds with secondary functional groups on odd-

numbered carbons may also possess fatty acid elongation complexes with modified activity. This seems likely to include a wide variety of plants, including gymnosperms (Wen & Jetter, 2007), ferns (Guo et al., 2018; Jetter & Riederer, 1999, 2000; Zhang et al., 2011), and mosses (Busta et al., 2016a, 2016b), raising questions about whether very-long-chain fatty acid-derived compounds with elongase-derived secondary functional groups found across the plant kingdom are retained from a common ancestor or if the pathways arose in a convergent manner.

In contrast to the example of discontinuous elongation pathway described above, which generates compounds bearing secondary functional groups on odd-numbered carbons, other pathways have been described that can generate compounds with functional groups on both even- and odd-numbered carbons along the hydrocarbon backbone. Such compounds have been described from a variety of species, particularly those in the Brassicaceae (for example, Tassone et al., 2016), most commonly from the well-studied model *Arabidopsis thaliana* (Wen & Jetter, 2009). Working with the genetic resources available for *Arabidopsis*, investigators identified a cytochrome P450 enzyme (AtMAH1) that oxidizes carbon chains to create such compounds (Figure 3f) (Greer et al., 2007). Based on structural patterns, it seems likely that other species, including some outside of the Brassicaceae, such as wheat (*Triticum aestivum*) (Racovita & Jetter, 2016b) and pea (*Pisum sativum*) (Wen et al., 2006), have similar enzymes that oxidize very-long-chain compounds to create lineage-specific natural products.

Although progress has been made toward understanding the biosynthesis of very-long-chain fatty acid-derived natural products in a variety of species, a number of unanswered questions remain. Some of these include the characterization of biosynthetic pathways in crop species that are likely homologous to known pathways in model plants (such as AtMAH1 homologs in Brassicaceous crops). However, other work includes characterizing yet unknown pathways to very-long-chain fatty acid-derived natural products, particularly those in crop species and those to industrially important products such as lactones and methyl ketones. It has been suggested that very-long-chain methyl ketones are formed in an analogous manner to medium-chain methyl ketones, in which 3-oxo-acyl elongation intermediates are intercepted and then decarboxylated (Ben-Israel et al., 2009; Kalinge et al., 2018; Yu et al., 2010). Perhaps chief among yet undescribed biosynthetic routes is the pathway to β -diketones in the grain crops wheat and barley (*Hordeum vulgare*). Considerable attention has been dedicated to these pathways, and it seems that both species contain gene clusters that mediate β -diketone biosynthesis (Hen-Avivi et al., 2016; Schneider et al., 2016). Still, the exact nature of these pathways has yet to be determined.

Fatty acid-derived natural products with aromatic rings

In addition to polyacetylene and very-long-chain products, plants can also use fatty acids to generate products with aromatic rings. These compounds generally consist of a hydrocarbon chain and an aromatic ring, and include the alkyl resorcinols, which have been noted for their bioactivities (Kozubek & Tyman, 1999; Stasiuk & Kozubek, 2010). Here, we briefly cover aromatic fatty acid natural product structural diversity, including alkyl resorcinols, their major derivatives, and other aromatic fatty acid-derived compounds as well.

Some of the structurally simplest aromatic fatty acid-derived natural products are those with a hydrocarbon chain and aromatic ring with one or two oxygen-containing functional groups (Figure 5a). When these groups are hydroxyl substituents located in meta positions relative to the hydrocarbon tail, the compound is called an alkyl resorcinol. These may vary in chain length and can be built with rather short-chain (approximately C₆), long-chain (C₁₆/C₁₈), or very-long-chain substrates (C₂₀₊) (Kozubek & Tyman, 1999). Some alkyl resorcinols have modified hydrocarbon tails, often bearing double bonds or oxygen-containing primary or secondary functional groups (see examples in Figure 5a; Cabanillas et al., 2015; Stasiuk & Kozubek, 2010). Compounds with modified aromatic rings have also been encountered, such as glycosylated hydroxyl groups, additional methyl groups on the ring itself, the absence of one of the meta position hydroxyl groups, or pyrone ring structures (see examples in Figure 5a; Đorđević et al., 2020; Kardar et al., 2014; Kim et al., 2010; Lorenz et al., 2017).

Several fatty acid-derived natural products with aromatic rings have been the subject of particular scientific inquiry, some of which are structurally related to alkyl resorcinols. One of these is olivetolic acid, a precursor to cannabinoids, which is comprised of a five-carbon hydrocarbon tail as well as an aromatic ring with two hydroxyl groups meta to the chain and a carboxyl group ortho to the chain (Figure 5b; Raharjo et al., 2004). Another is sorgoleone, an allelopathic compound made up of a hydrocarbon chain with three double bonds and an aromatic ring bearing two hydroxyl groups and two carbonyl groups (Figure 5b; Kagan et al., 2003). Another important class of aromatic fatty acid-derived natural products are the urushiols, the active ingredients in *Toxicodendron* spp. (i.e., poison ivy, oak, and sumac), which are composed of a hydrocarbon tail with up to three double bonds and an aromatic ring with two hydroxyl groups, ortho and meta to the chain (Figure 5b, ElSohly et al., 1982). Sometimes found alongside urushiols are anacardic acids, in which the functionality ortho to the hydrocarbon chain is a carboxyl group (Kubo et al., 1986). Both urushiols and anacardic acids vary in their chain lengths (usually C₁₅ and C₁₇) and the number

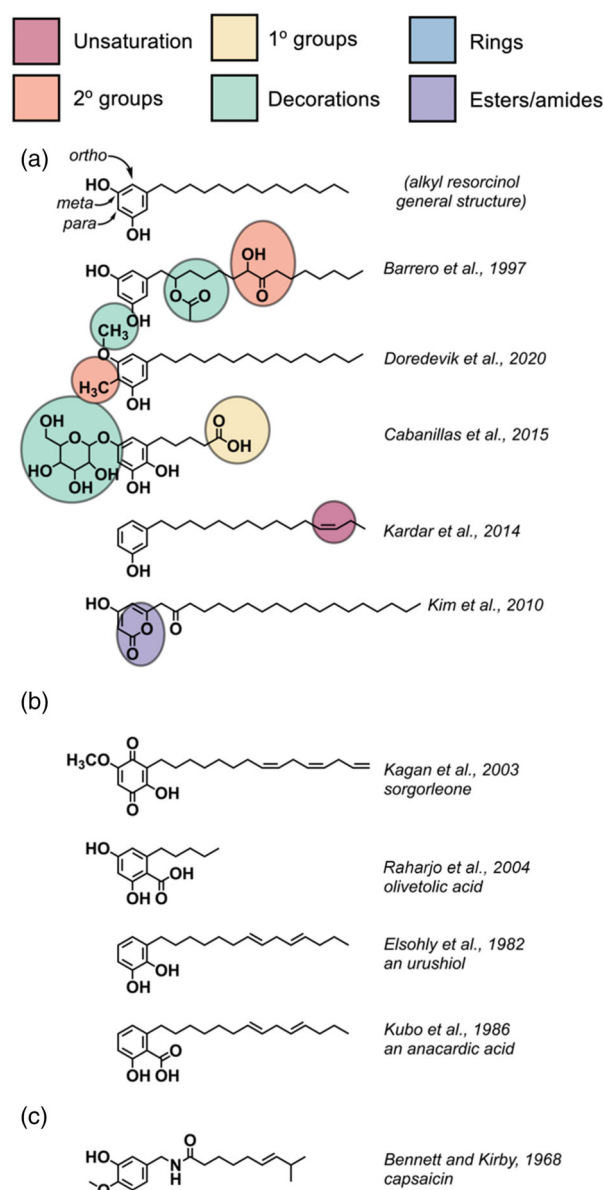


Figure 5. Fatty acid-derived natural products with aromatic rings. (a) Representative structures are shown for the major types of functional group variation using colored circles as shown in the legend. (b) Structures of some of the best-studied aromatic ring-containing fatty acid-derived natural products. (c) Structure of an aromatic ring-containing fatty acid-derived natural products where the ring is not derived from the fatty acid portion of the molecule.

and stereochemistry of the double bonds in their hydrocarbon chains (Du et al., 1986; Gross et al., 1975). Finally, fatty acid-derived aromatic compounds also include the capsaicin family of molecules, which give chili peppers (*Capsicum annuum*) their spice. These are from an amide-linked short-chain fatty acid (<C₁₀) and aromatic ring-containing amine (Figure 5c; Bennett & Kirby, 1968). Thus, fatty acid-derived products with aromatic rings occupy a

major structural space, with more than 100 structures having been described (Kozubek & Tyman, 1999), and include molecules of potential economic importance.

Similar to the classes of fatty acid-derived natural products described in the previous sections, biochemical research is also beginning to shed light on the enzymatic processes that create fatty acid-derived compounds with aromatic rings. In particular, previous studies have shown that type III polyketide synthase (PKS) enzymes can use fatty acids as substrates with which to generate aromatic ring-containing compounds. For example, work with *Sorghum bicolor*, which produces sorgoleone, revealed that a type III PKS can repeatedly condense a fatty acid substrate with malonyl CoA-linked building blocks to create a tetraketide intermediate that is then cyclized and decarboxylated to yield an end product with both a fatty acid-like hydrocarbon tail and an aromatic ring in its structure (Cook et al., 2010; Figure 3g). In the case of the sorgoleone pathway, the substrate used by the type III PKS is an unsaturated fatty acid (C16:3) generated from palmitic acid by two desaturases (SbDES2 and SbDES3; Pan et al., 2007). Subsequent action by an *O*-methyltransferase (Baerson et al., 2008) and a P450 (Pan et al., 2018) complete the pathway to sorgoleone, and all these enzymes together can be used to produce the compound heterologously (Pan et al., 2021), opening an avenue to engineering root-microbe interactions. Like those in *Sorghum*, PKS enzymes in *Arabidopsis* have been shown to act on long-chain fatty acid substrates, though they use a slightly different cyclization mechanism and thereby generate tri- and tetraketide pyrones (AtPKS; Figure 3h; Dobritsa et al., 2010; Kim et al., 2010). Interestingly, those studies also suggested that those *Arabidopsis* PKS enzymes may, *in vivo*, prefer fatty acid substrates with secondary or terminal hydroxyl groups, expanding the substrate space that appears to be usable by type III PKS enzymes.

Not all type III PKS enzymes that generate aromatic fatty acid natural products require substrates with unsaturated or functionalized hydrocarbon chains. For example, a type III PKS from *Physcomitrella patens* uses saturated fatty acids to produce 2-oxo-alkylresorcinols (PpORS; Figure 3i; Kim et al., 2013), and one from *Secale cereale* uses very-long-chain fatty acids to produce very-long-chain alkyl resorcinols (ScARS; Figure 3i; Sun et al., 2020). Yet other type III PKSs use short-chain fatty acids. In particular, the biosynthesis of the C₆ alkyl resorcinol moiety of cannabinoids (psychoactive components of *Cannabis*) has been studied in this regard. Initial investigations of type III PKS enzymes from *Cannabis* revealed an enzyme (CsPKS) that acted on C₆ substrates, generated a tetraketide intermediate, and cyclized and decarboxylated it, yielding olivetol, an alkyl resorcinol (Figure 3j; Taura et al., 2009). However, the compound that is used for cannabinoid synthesis is olivetolic acid, not olivetol (Figure 3j), which raised

questions about whether a plant type III polyketide synthase can generate a product without decarboxylating it. Subsequent work revealed that a *Cannabis* protein (CsOAC) with homology to a bacterial polyketide cyclase can act, together with CsPKS, to generate olivetolic acid (Figure 3j; Gagne et al., 2012). Since this discovery, the entire pathway to natural (as well as unnatural) cannabinoids has been reconstituted heterologously (Luo et al., 2019).

That the combination of CsPKS and CsOAC is required for the formation of an alkyl resorcinol derivative raises the possibility that other species may bear type III PKS enzymes that act in combination with other proteins to generate unusual alkyl resorcinol-like products. It will be interesting to see if this is the case for aromatic fatty acid natural products of yet undescribed biosynthesis, such as the urushiols and anacardic acids of Anacardiaceae species such as *Toxicodendron* spp. Expanding our knowledge of aromatic fatty acid biosynthesis in this way and others, such as understanding PKS substrate chain length specificity, will bolster our ability to produce these compounds in biotechnologically tractable systems and rationally design non-allergenic Anacardiaceae crops.

CONCLUSIONS AND OUTLOOK

Plants use fatty acids as starting points to synthesize a wide variety of natural products, the structures of which we reviewed here. These include polyacetylenes, very-long-chain lineage-specific products, and compounds with aromatic rings. Some of these compounds currently or potentially have considerable economic importance. As such, discovering more structures of this sort will likely be a worthwhile endeavor. Research in other areas of plant specialized metabolism has shown that close relatives of a specialized metabolite-producing species of interest can contain structurally similar metabolites of interest (Boachon et al., 2018). This raises the question of whether plants that are closely related to known producers of fatty acid-derived natural products of interest can also synthesize the same compounds or related compounds, perhaps of similar importance (Figure 6a). Future studies could consider targeted analyses of this sort.

In addition to their chemical structures, we also reviewed here the biosynthesis of fatty acid-derived natural products. For several classes of these products, we noted that key members of their biosynthesis pathways exhibited considerable promiscuity – they could accept multiple substrates, generate multiple products, or sometimes both. For example, depending on the chain length of the substrate used and the presence of certain active site mutations, the *Physcomitrella* PKS involved in 2'-oxoalkylresorcinol synthesis could also generate tri- or tetraketide pyrones (Kim et al., 2013), and several of the FAD2-like enzymes involved in polyacetylene biosynthesis

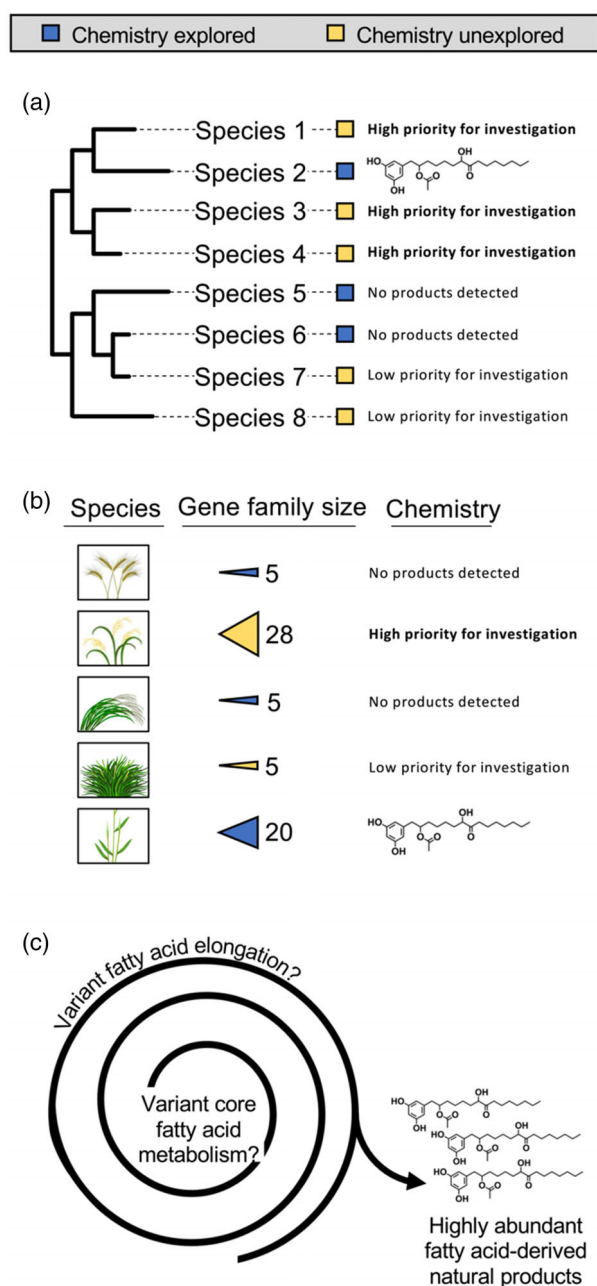


Figure 6. Schematic diagram of proposed approaches to future fatty acid natural product research.

(a) Using phylogenetic relationships to identify structural variants of known products. (b) Using gene family expansion as a marker for lineages with undiscovered fatty acid-derived natural products. (c) Investigating upstream, core fatty acid synthesis and elongation pathways for variants that may facilitate enhanced fatty acid-derived natural product accumulation.

appear to catalyze multiple or different reactions depending on the substrate they encounter (Cahoon et al., 2003; Jeon et al., 2020; Okada et al., 2013). Since enzyme promiscuity has been suggested as a characteristic that

may enable the evolution of new metabolic pathways (Kruse et al., 2020), it could be that PKS and FAD2 promiscuity, in addition to gene duplication and neofunctionalization (Feng et al., 2019), has played a role in the evolution of pathways leading to fatty acid-derived natural products. This also suggests that, as a complement to the close relative-based searches proposed above, genome-based screening for species with expanded fatty acid metabolism gene families, particularly those known to exhibit promiscuity, could also be an approach by which to conduct wide screens for yet unstudied lineages that contain fatty acid-derived natural products (Figure 6b).

Finally, we note that several research groups have found, in select species, what appear to be modifications to core (primary) metabolic pathways that support downstream fatty acid-derived product synthesis (Figure 6c). For example, a unique acyl-activating enzyme has been reported to divert C₆ fatty acid units to the fatty acid modification pathway leading to cannabinoids (Stout et al., 2012), and a specialized condensing enzyme appears to supply branched fatty acid precursors for capsaicin biosynthesis (Aluru et al., 2003). These findings suggest that similar supply modifications may exist to generate other branched fatty acid products, such as very-long-chain branched alkanes (Busta & Jetter, 2017; Vogg et al., 2004). Together, these observations suggest that flux through pathways to fatty acid-derived natural products may be enhanced by modifying various aspects of upstream core metabolism, including protein–protein interactions (Busta et al., 2022), as has been suggested for other plant natural product pathways (Lopez-Nieves et al., 2018; Schenck et al., 2017). This notion, together with the fact that fatty acid-like compounds can be obtained on industrial scales from engineered yeast, field-grown oilseeds, and crop vegetative mass, makes it seem likely that even relatively complex fatty acid-derived natural products may be well suited to bio-based production on a large scale.

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AUTHOR CONTRIBUTIONS

SS and LB performed literature searches and prepared figures. EBC contributed to the conception of this review and provided critical input. All authors wrote and edited the manuscript.

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

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