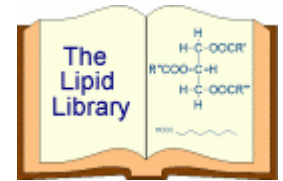


PLANT OXYLIPINS

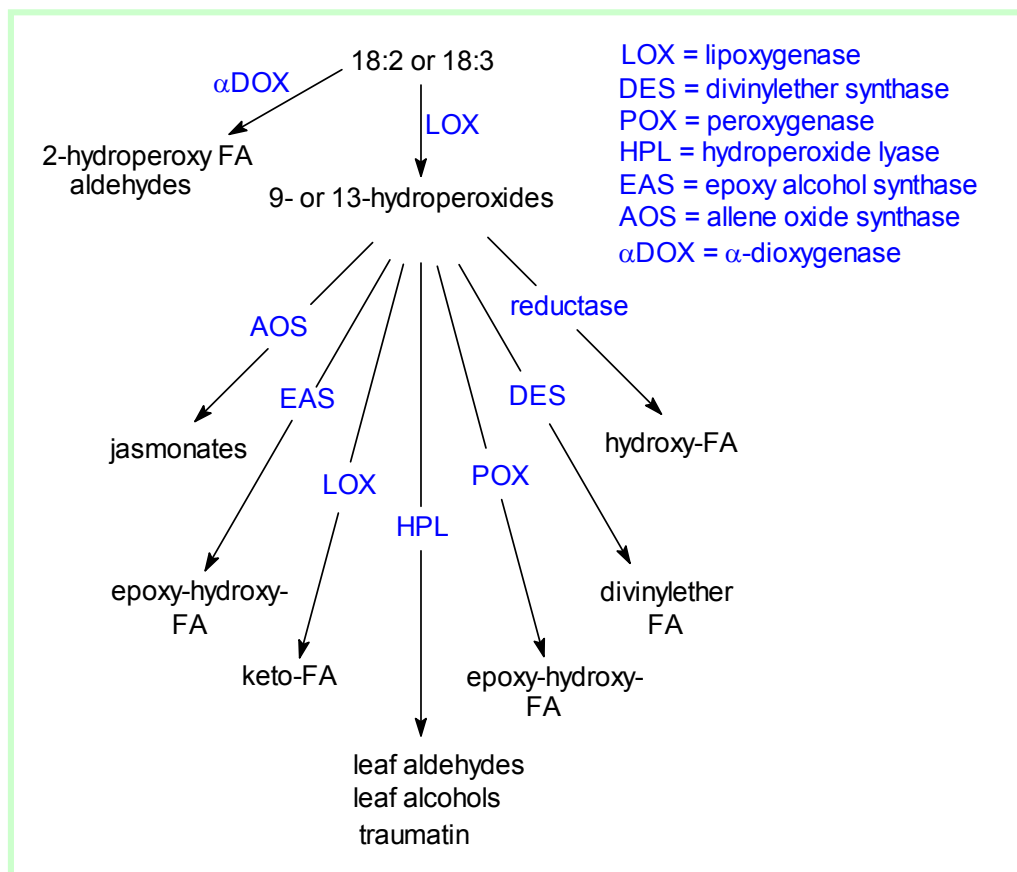
Chemistry and Biology



1. Introduction

Plants lack an immune system in the sense that it exists in animals, but they possess mechanisms that recognize potential pathogens and initiate defense responses. It has become evident that various types of oxygenated fatty acids, collectively termed 'oxylipins' or sometimes 'octadecanoids', are involved in responses to physical damage by animals or insects, stress and attack by pathogens. These compounds are similar in many ways to the eicosanoids derived from arachidonate in animals, which have so many varied functions but especially in the inflammatory process.

There are very few definitive reports of the presence of arachidonic acid in higher plants, and oxylipins are derived from linoleic and more importantly α -linolenic acid, released from their lipid associations by poorly defined acyl hydrolases of various kinds. A first key step in oxidation is the action of lipoxygenases, although cytochrome P450 and pathogen-induced oxygenases have lesser roles. For example, depending on the source of the enzyme, lipoxygenases (EC 1.13.11.12) (LOX) catalyses the oxidation of α -linolenic acid into either 9- or 13-hydroperoxy-octadecatrienoic acids, or a mixture of both. Such compounds are highly reactive, and they are quickly metabolized by various enzymes into series of oxylipins, as summarized in the figure below, with a range of distinct biological activities.



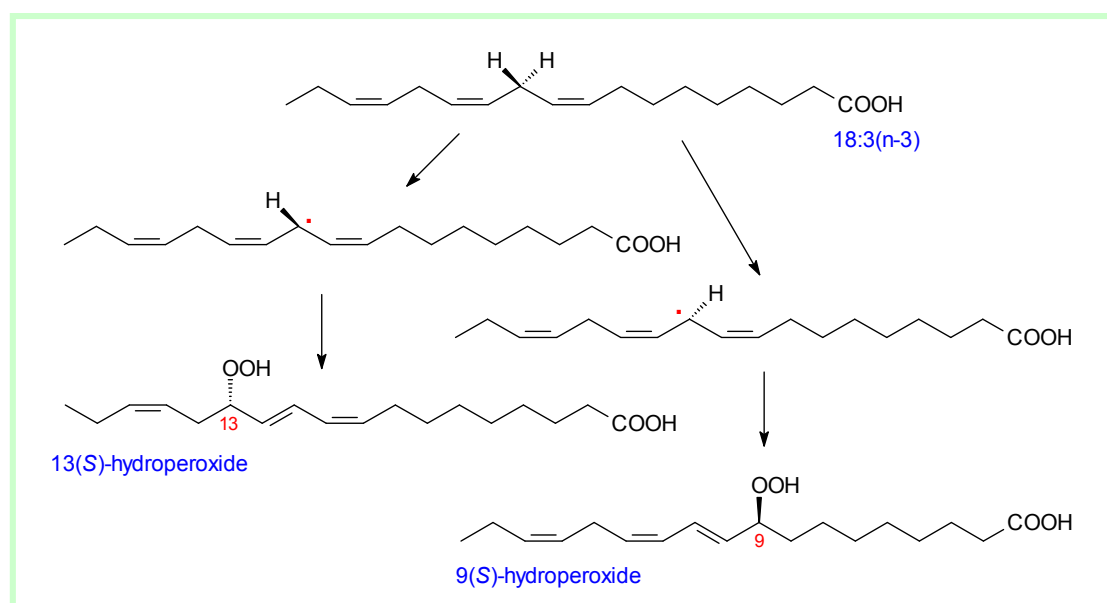
An alternative oxidation reaction utilizes an α -dioxygenase, distinct from the lipoxygenases, to generate 2-hydroperoxy fatty acids, which decay spontaneously to produce an aldehyde one carbon atom shorter, as part of the α -oxidation process in plants.

2. Lipoxygenases

Lipoxygenases are non-heme iron-containing dioxygenases that are widely distributed in plants and animals. That from soybean was among the first to be studied in great detail, including its three-dimensional structure, and the knowledge gained assisted greatly with the understanding of the analogous animal enzymes. Plant LOX consist of a single polypeptide chain with a molecular mass of 94 to 104 kDA. The carboxy-terminal domain harbors the catalytic site of the enzyme, which contains a non-heme iron atom that is coordinated with five amino acids - three histidines, one asparagine and the carboxyl group of the carboxy-terminal isoleucine. The amino-terminal domain may be involved in membrane or substrate binding.

From amino acid-sequence studies of enzymes from a number of plant sources, it is evident that there are two main families of lipoxygenases, designated 'type-1' (mainly extra-plastidial) and 'type-2' (mainly plastidial), but many different iso-enzymes exist depending on the particular species. Soybean lipoxygenase exists in eight different isoforms, for example. They include both soluble cytoplasmic and membrane-bound enzymes. In *Arabidopsis*, lipoxygenase activity is located mainly in the plastidial envelope and stroma of leaf chloroplasts. There are suggestions that isoforms in different subcellular regions may provide different pools of hydroperoxy fatty acids, which serve as substrates for alternative metabolic pathways and physiological functions. The properties of lipoxygenases in general are discussed in the [Introductory web page](#). Here, those properties characteristic of the plant enzymes are discussed.

Lipoxygenases catalyse the addition of molecular oxygen to polyunsaturated fatty acids containing a (*cis,cis*)-1,4-pentadiene system to yield an unsaturated fatty acid hydroperoxide. Oxygen can be added to either end of the pentadiene system with high stereospecificity, and in the case of linoleic and α -linolenic acids, this leads to either the 9(*S*)- or 13(*S*)-hydroperoxy derivatives or both depending on the specific iso-form of the enzyme. For example, the model plant *Arabidopsis* contains two genes encoding for 9-LOXs and four that encode 13-LOXs. Physiological conditions can also affect this positional specificity (regiospecificity), and under conditions of low oxygen concentrations, for example, the soybean LOX-1 produces equal amounts of the two isomers, though normally the 13(*S*) isomer predominates. Photosynthetic tissues tend to produce mainly 13(*S*)-hydroperoxides.

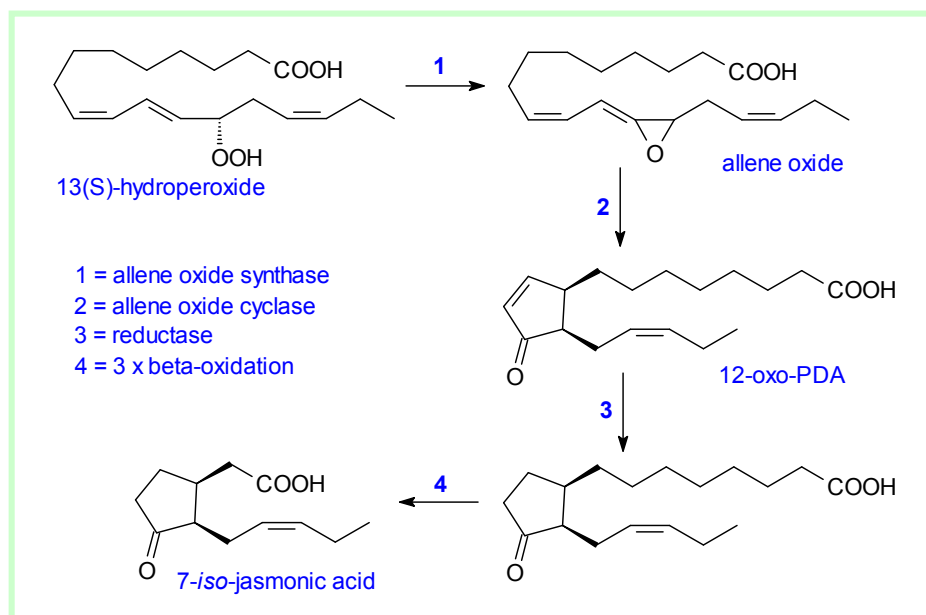


The reaction proceeds in three stages as illustrated above for α -linolenic acid, with the first step the (antarafacial) stereospecific abstraction of a hydrogen atom from the methylene group between the double bonds. The resulting delocalized free radical undergoes an allylic rearrangement before the oxygen molecule adds to form the hydroperoxide. Subsequent steps are specific for either the 9-LOX or 13-LOX products.

Free acids appear to be the preferred substrates, and under conditions of stress in plants, phospholipases are activated that rapidly break down the complex lipids – another analogy with animal systems. However, it is also evident that lipoxygenases can react with esterified fatty acids in lipids and perhaps disrupt the cellular membranes.

3. Jasmonates and Related Compounds

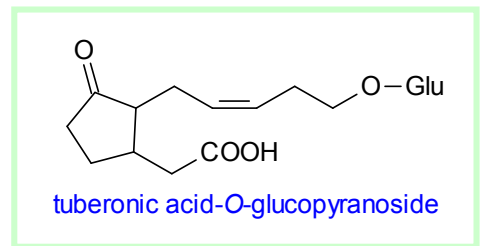
The jasmonates are 12-carbon cyclic fatty acids derived from linolenic acid that have important signalling functions in plants. Allene oxide synthase, an enzyme of the cytochrome P450 family, catalyses the first key step after formation of the LOX product, 13(*S*)-hydroperoxy-9*c*,11*t*,15*c*-octadecatrienoic acid, as the substrate as illustrated below. The product is the allene oxide 12,13(*S*)-epoxy-9*c*,11*t*,15*c*-octadecatrienoic acid. However, this compound is highly unstable, and it can be hydrolysed rapidly to α - and γ -ketols, or it can cyclize spontaneously to form 12-oxo-10,15*c*-phytodienoic acids (12-oxo-PDA), i.e. with a prostaglandin-like structure, in two of the four possible stereoisomeric forms. However, reaction with the enzyme allene oxide cyclase produces only 12-oxo-9(*S*),13(*S*)-phytodienoic acid (the *cis*-(+)-enantiomer), which is the single biologically important isomer. Not only is it the precursor of the jasmonates but it also has distinctive signalling functions of its own. Both the allene oxide synthase and the cyclase are located in the plastids, and they probably operate in concert; they may be even be linked physically in some form of complex, although no direct evidence exists for this.



The product is transferred by an as yet unknown mechanism to the peroxisomes, where a specific 12-oxo-phytodienoate reductase reduces the double bond in position 10, i.e. in the cyclopentenone ring, to 3-oxo-2-(pent-2'-enyl)-cyclopentane-1-octanoic acid. This is a key step in directing the metabolism towards jasmonic acid, as this compound only is able to undergo the three cycles of β -oxidation, catalysed by the multifunctional enzyme complex acyl-CoA oxidase, which are required to give the 12-carbon (-)-7-*iso*-jasmonic acid. This is the main isomer isolated from plant tissues and was long thought to be the active metabolite. However, it is now recognized that (+)-7-*iso*-jasmonic acid or *cis*-(*epi*)-jasmonic acid is in fact the active isomer. As both side chains are on the

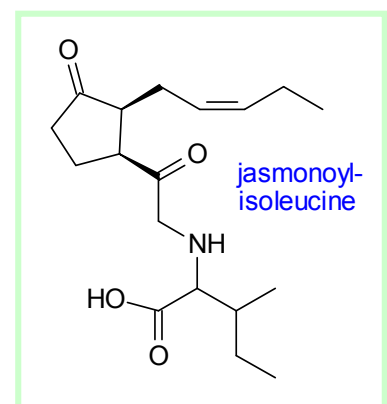
same side of the 3*R*,7*S*-cyclopentanone ring and the keto group at C-6 can tautomerize to an enol, the more stable (3*R*, 7*R*) isomer ((-)- or *trans*-jasmonic acid is usually isolated as the main product (90% of the equilibrium mixture).

Dinor-oxo-phytodienoic acid is a related metabolite derived from hexadecatrienoic acid (16:3(*n*-3)) and has signalling functions in its own right, as well as being a precursor of jasmonic acid. In addition, a number of further metabolites of jasmonic acid are formed in plants and have biological activity including methyl jasmonate, the formation of which is catalysed by *S*-adenosyl-L-methionine:jasmonic acid carboxyl methyltransferase. Decarboxylation gives the volatile *cis*-jasmone. The tuber-inducing factor in potatoes, the glucopyranosyl derivative of tuberonic acid, is derived from jasmonic acid by hydroxylation at C-12; the aglycone is much less potent. It is synthesised in the leaves and transported down to the stolons.



A number of amino acid conjugates of jasmonic acid have been found in plants, and those with leucine and isoleucine have especial importance. For example, jasmonoyl-isoleucine and hydroxylated and carboxylated analogues are formed in plants in response to wounding, and jasmonoyl-isoleucine specifically is now known to be a central element in hormone signalling by jasmonic acid.

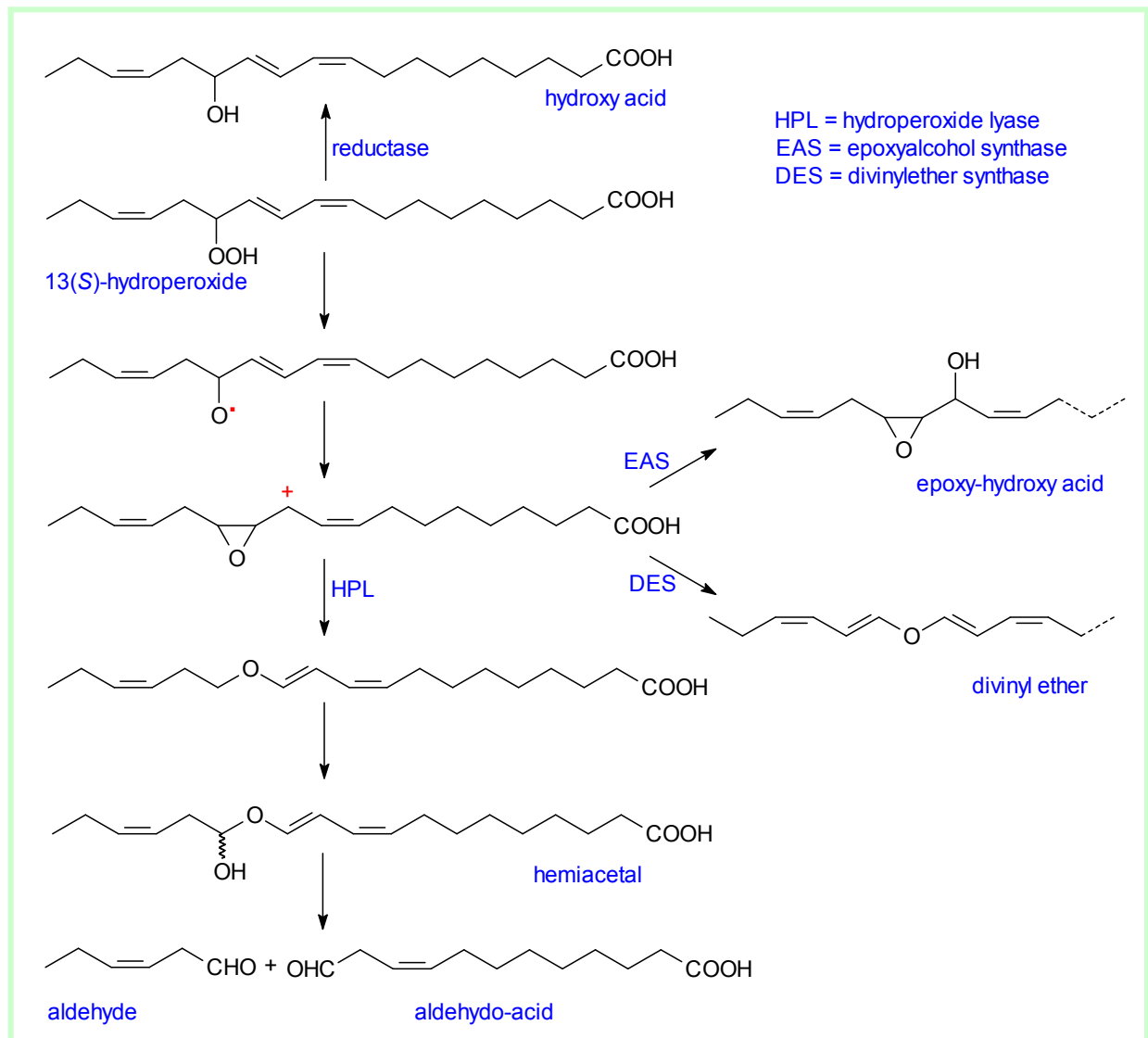
It is now known that the common isomer (-)-7-jasmonoyl-L-isoleucine is not the active isomer, but rather the much less abundant (+)-7-epimer. pH changes promote conversion of the (+)-7-epimer to the inactive (-)-7- form, suggesting that this may be a simple mechanism regulating the activity of the hormone through epimerization.



At least five different mono- and digalactosyldiacylglycerols containing 12-oxo-phytodienoic acid and/or dinor-oxo-phytodienoic acids in position *sn*-1, and termed 'arabidopsides A to E', have been isolated from stressed plants of *Arabidopsis thaliana*. For example, in plants challenged by a bacterial pathogen, a monogalactosyldiacylglycerol containing two 12-oxo-phytodienoate and one dinor-oxo-phytodienoate acyl chain (arabidopside E) accumulated in amounts up to 8% of the total lipids. It was shown to have anti-bacterial properties *in vitro*. However, such compounds were not be detected in species other than *Arabidopsis* in one systematic study.

4. Other Oxylipins

The allene oxide synthase discussed above is one of a family of related cytochrome P450 monooxygenases collectively termed the CYP74 subfamily. These are not typical of the common P450 monooxygenases in that they do not require molecular oxygen nor NAD(P)H-dependent cytochrome P450-reductase. Instead, the new carbon-oxygen bonds are formed by using an acyl hydroperoxide both as substrate and oxygen donor. The enzymes produce three further types of oxidation products of biological importance. Starting again with the 13(*S*)-hydroperoxy intermediate from the lipoxygenase reaction, oxylipins of various kinds result as summarized in the figure below. These reactions all appear to proceed via first free radical and then unstable epoxide intermediates, though many aspects of the mechanisms require clarification or confirmation. It is of interest from an evolutionary standpoint, that two key of the enzymes, hydroperoxide lyase and divinylether synthase, differ only in a few amino acid residues, and there are other close relationships between enzymes of the CYP74 family. Similar enzymes are found in bacteria and lower animals.



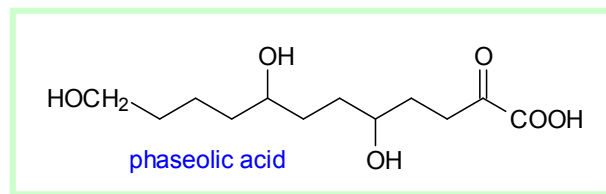
For example, the enzyme hydroperoxide lyase converts the epoxy intermediate into a vinyl ether and thence to a short-lived hemiacetal, which is oxidatively cleaved rapidly to generate *cis*-3-hexenal and 12-oxo-*cis*-9-dodecenoic acid, though the positions and geometry of the double bond may change by chemical or enzymatic isomerization. Traumatin or 12-oxo-*trans*-10-dodecanoic acid, a plant wound hormone, is produced in this way, via isomerization of 10-oxo-*cis*-9-octadecanoic acid, and this can in turn undergo autoxidation to traumatic or 10(*E*)-dodeca-1,12-dicarboxylic acid. The other products of this and related reactions via hydroperoxide intermediates are volatile unsaturated aldehydes, which also have potent biological effects.

The epoxy intermediate can also be converted by an epoxyalcohol synthase into an epoxyhydroxy fatty acid, while in a few plant species, another related enzyme synthesises a divinylether fatty acid.

In alternative pathways, the 13(*S*)-lipoxygenase product can be reduced to a hydroxy acid, while a peroxygenase or pathogen-induced oxygenase pathway is another route to the biosynthesis of epoxy-hydroxy fatty acids.

Di- and trihydroxy-octadecanoids are also produced in some plant species. For example, phaseolic or 2-oxo-5,8,12-trihydroxydodecanoic acid was first found in bean seeds, and has been shown to

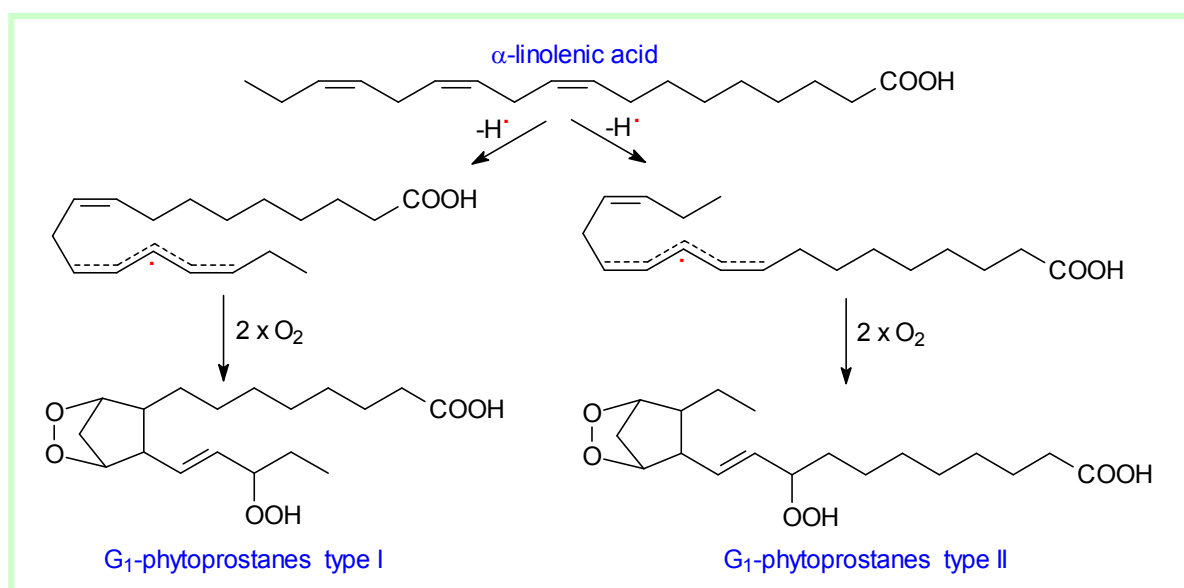
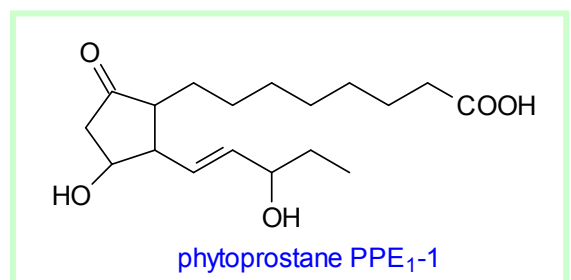
stimulate the growth of pea stem segments, to induce α -amylase synthesis in barley endosperm, and to retarded senescence in barley leaf segments.



Mosses, fungi and yeasts produce a variety of oxylipins, from saturated and unsaturated fatty acids, including arachidonic acid, which can occur naturally in these organisms. For example, 8*R*-hydroxy-octadeca-9,12-dienoic and 5*S*,8*R*-dihydroxy-octadeca-9,12-dienoic acids are produced by *Aspergillus* species. The prostaglandin metabolites PGF₂ and PGF₂-lactone, derived from arachidonic acid, have been detected in yeasts of the Lipomycetaceae family, while PGE₂ is produced by *Candida albicans* and other species. Similarly, the moss *Physcomitrella patens* produces 12-hydroperoxy-eicosatetraenoic acid (12-HPETE) and further metabolites from arachidonate. 12- and 5-HPETEs and a range of further metabolites are produced by red algae. 3-HETEs are important fungal metabolites. Marine diatoms produce many distinctive oxylipins, and some have even been found in prokaryotes. The functions of most of these compounds have yet to be determined, although in fungi at least, they are known to act as hormone-like signals that regulate the development of spores. Also, much remains to be learned of the biosynthetic enzymes involved.

5. Phytoprostanes (Plant Isoprostanes)

Plants utilize linolenic acid to produce C₁₈-isoprostanoids (dinor-isoprostanoids or phytoprostanoids) via non-enzymatic, free radical-catalysed pathways similar to **isoprostan**e synthesis in animals. Singlet oxygen is the most important reactive oxygen species involved. As phytoprostanes are derived from linoleic and linolenic acids, they differ from the animal isoprostanoids in the number of double bonds and the lengths of the side-chains. The phytoprostanoid PPE₁ is illustrated as an example.



An initial abstraction of a hydrogen radical is followed by addition of oxygen with formation of a cyclic endoperoxide. Addition of a further oxygen molecule forms the hydroperoxide. Abstraction of the hydrogen atom at C-14 generates phytoprostanes G_1 (PPG_1) of type I, while hydrogen abstraction at C-11 yields PPG_1 of type II. The endoperoxide group is highly unstable, and PPG_1 forms rearrange spontaneously or are reduced to D_1 , E_1 and F_1 ring analogues of the animal prostaglandins, with further dehydration and isomerization to the J_1 and other types.

Whereas lipoxygenases insert oxygen only at C-9 or C-13 of these acids with defined stereochemistry, reactive oxygen species can produce racemic hydroperoxides at C-10 and C-12 of linoleate and also at C-15 and C-16 of linolenate. Indeed, in the twenty or so plant species analysed to date, various regioisomers of free phytoprostanes of the A_1 , B_1 , D_1 , E_1 , F_1 , G_1 and deoxy- J_1 series have been detected. The phytoprostane PPE_1 is illustrated as an example. Like the isoprostanes, they occur mainly in lipid-bound form in membranes. However, they also occur as the free acids, in contrast to the animal equivalents, while glutathione conjugates of PPA_1 accumulate after pathogen infection in *Arabidopsis thaliana*.

Such non-enzymatically produced lipids are formed continuously in healthy plant tissues, in the range for each component of 0.01 to 6.7 nmol/g dry weight in tomato leaves, for example. Indeed, the concentrations of esterified phytoprostanes can be an order of magnitude higher than those of the equivalent free jasmonates, and two to three orders of magnitude higher than of analogous isoprostanes in animal tissues. Such high levels of oxidized lipids in membranes might be injurious to the plant if there were not efficient repair mechanisms in place.

In evolutionary terms, phytoprostanes are likely to have been developed before the oxylipins produced enzymatically, and they have retained a wide range of biological activities in plants. For example, the cyclopentenone-phytoprostanes PPA and PPB up-regulate gene expression especially for enzymes involved in the response to challenges by foreign organisms or external conditions, while the 7 down-regulate genes involved in cell division and growth. However, these studies are at an early stage and little is known of the effect of specific regio-isomers. Like the jasmonates, they trigger phytoalexin production (see below).

As they are present in vegetable oils and have been detected in plasma, the biological properties of phytoprostanes in terms of potential effects on human cells are under investigation.

6. Biological Activity of Plant Oxylipins

When plants are attacked by bacterial or fungal pathogens, lipases are activated that release the unsaturated fatty acids and trigger the synthesis of a range of oxylipins with diverse roles. Some of these have direct antimicrobial or anti-insect functions, while others, especially the jasmonates and their precursors the oxo-phytodienoic acids, are potent regulators of defense mechanisms, for example by stimulating proteinase inhibitors or by promoting the accumulation of antimicrobial secondary metabolites (phytoalexins).

(9*S*,13*S*)-12-Oxo-phytodienoic and jasmonic acids, but especially jasmonoyl-isoleucine, are phytohormones that are involved in growth and developmental processes, and in immunity. Indeed jasmonoyl-isoleucine is the only jasmonate known with certainty to be active at the molecular level via an identified receptor. It promotes ubiquitination and thence degradation of transcriptional repressor proteins (so-called 'JAZ proteins'), which control genes involved in the physiological responses. The jasmonates have a role in fertility, for example in pollen maturation, and in such varied processes as fruit ripening, root growth and tendril coiling. They are believed to act by mechanisms that are as yet unknown to activate signalling pathways both intra- and inter-cellularly that modulate the expression of a number of genes, and thence the synthesis of many key proteins. The picture emerging is a highly complex one, and many aspects await clarification. For example, there appear to be functional differences between species that have yet to be explained,

and the relationship with other defense mechanisms including those based on auxin, ethylene, abscisic acid and salicylate requires further elaboration.

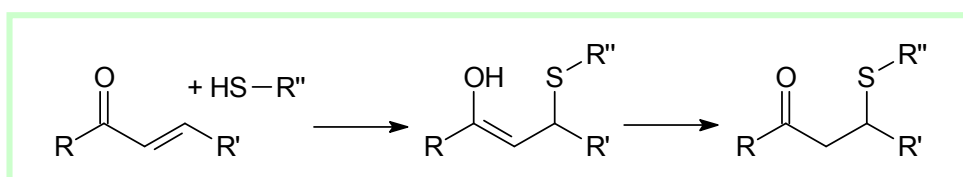
Each of the various jasmonate derivatives, i.e. the free acid, methyl ester and conjugates with amino acids, has distinct biological effects but especially in defense mechanisms. Wound response is one of the most-studied pathways of jasmonates in signal transduction with the tomato often as the model. In brief, local wounding results in break down of cells and release of fatty acids. At the same time, cleavage of the octadecapeptide systemin is initiated from prosystemin, and in turn this stimulates jasmonic acid biosynthesis and the formation of various jasmonic acid conjugates. This is believed to act as a signal leading to systemic expression of genes encoding proteinase inhibitors, and anti-feedant and poisonous compounds, which deter insect herbivores and in effect immunize the plant against further herbivore attacks. At the same time, jasmonates may down-regulate genes involved in the core metabolism of the plant, in effect deciding whether defense or growth are more important.

Volatile jasmonate metabolites, such as *cis*-jasmonone, may regulate the behavior of some insects, for example by deterring herbivorous species or attracting their predators. They may even enable communication between plants.

In addition jasmonates are involved in defense against bacterial pathogens, especially those that feed on necrotic tissue or cells undergoing apoptosis. The bacterial phytotoxin coronatine functions by binding to the receptor for jasmonoyl-isoleucine. In contrast, certain soil-borne microorganisms have beneficial effects by enhancing the defensive capacity of plants, with jasmonic acid as an important regulator of the process. Jasmonates also promote the beneficial interactions between certain mycorrhizal fungi and nitrogen-fixing bacteria.

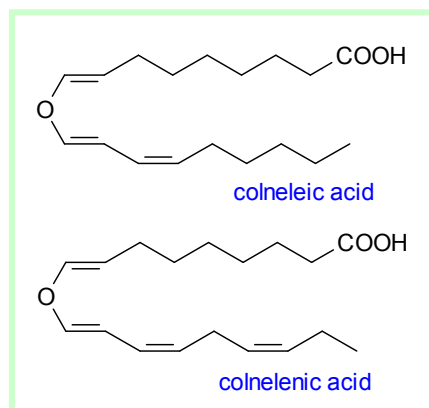
The oxylipins derived from the other branches of the lipoxygenase pathway have characteristic biological activities also. Indeed, even the primary 9(S)- and 13(S)-hydroperoxides have antifungal and anti-microbial properties. They may be part of a short-term local response while the jasmonates operate over a longer time scale. Some of the further metabolites, such as the C₆ volatiles produced by hydroperoxide lyase in damaged tissues may act like methyl jasmonate to elicit defense responses. They also have potent antimicrobial effects and reduce the fecundity of insect pests. As mentioned briefly above, the other product of the enzyme, a C₁₂ fatty acid, is a precursor of traumatic acid and other wound hormones, which also have growth-stimulating effects.

Other natural products of fatty acid oxidation with potent biological activities include unsaturated aldehydes (alkenals) and more highly oxidized derivatives of these. For example, several unsaturated aldehydes, but especially (2*E*)-hexenal, have antimicrobial properties and have defense roles against fungi, bacteria and arthropods. When leaves are wounded, this volatile compound is released transiently into the air and is able to diffuse rapidly through damaged or infected tissues. (2*E*)-4-Hydroxynonenal produced from (3*Z*)-nonenal in plants has anti-fungal properties also. Some of these effects may result from covalent binding to proteins via reaction of an α,β -unsaturated carbonyl structure with free thiol (illustrated) or amine groups. Unsaturated hydroperoxides and 12-oxo-phytodienoic acid may react in the same way. In this manner, they may have signalling functions by reacting with redox-regulated proteins especially.



The epoxy and hydroxy derivatives of linoleic acid resulting from the peroxygenase pathway are toxic to fungal pathogens. Similarly, colneleic and colnelenic acids (divinyl ether fatty acids

originating from 18:2- and 18:3-derived hydroperoxides, respectively) are produced quickly in leaves of potato plants infected by fungi or viruses, and they are believed to have a defensive role against potato blight especially. Indeed, colneleic and colnelenic acids have been found esterified at the *sn*-2 position of phospholipids in potato, suggesting the presence of a preformed pool that would be immediately available in response to challenge by pathogens. Other oxylipins may be stored in the same way and 12-oxo-phytodienoic acid is found esterified to position *sn*-1 of the monogalactosyldiacylglycerol in *Arabidopsis*, for example.



The 3-hydroxy oxylipins in fungi and yeasts are believed to play a role in cell aggregation and spore release.

Although they do not occur naturally in animal tissues, some jasmonate metabolites and methyl jasmonate in particular have been shown to have pronounced cytotoxic effects against human cancer cell lines *in vitro* and appear to have therapeutic potential.

7. Analysis

As with the eicosanoids, methods involving gas chromatography-mass spectrometry or high-performance liquid chromatography allied to electrospray tandem mass spectrometry are preferred for the analysis of the plant oxylipins, with HPLC and UV detection as a useful complementary technique. Internal standards labelled with stable isotopes, such as O^{18} , are essential for quantification.

Recommended Reading

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