Lipoxygenase as a Versatile Biocatalyst

Harold W. Gardner*
NCAUR, ARS, USDA, Peoria, Illinois 61604

ABSTRACT: This review of lipoxygenase and lipoxygenase pathway enzymes focuses on the potential for the efficient production of useful compounds. Although the existence of lipoxygenase has been known for many years, only recently has there been progress toward understanding the conditions required to improve yields and immobilize its activity. Maintaining a high O_2 tension is necessary to obtain good yields of hydroperoxides; whereas, partial anaerobic conditions can lead to hydroperoxide decomposition. Fatty hydroperoxides, obtained from lipoxygenase action, can serve as precursors for further transformation by either enzymes or chemical reactions. Well over one-hundred products from lipoxygenase-generated hydroperoxides of linoleic acid alone have been described. Examples will be given of the formation of fatty acids with epoxide, hydroxy, ketone, cyclic, and multiple functional groups. The cleavage of fatty hydroperoxides into short-chain aldehydes and alcohols also will be described. Many of the products have biological activity, suggesting a significant physiological function for lipoxygenase.
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KEY WORDS: Allene oxide synthase, free radical, hydroperoxide lyase, hydroperoxylinoleic acid, hydroxylinoleic acid, lipoxygenase, oxygenated fatty acids.

LIPOXYGENASE

Lipoxygenase (LOX) was first reported in soybeans almost 65 years ago (1), but it required another 15 years for a soybean isozyme (LOX-1) to be crystallized (2). More decades passed before the enzyme was seriously investigated again. Soybean LOX-1, a soluble nonheme iron enzyme of 94 kDa size, has become a model enzyme, and consequently, it is the most studied. Recently, LOX-1 has been sequenced (3), and the tertiary structure was determined by x-ray diffraction (4). Several other plant LOX have been sequenced, including two others from soybean seed, and all have partial, but significant, homology with soybean LOX-1.

LOX oxygenates methylene-interrupted (*Z,Z*)-pentadiene fatty acids, but structures other than fatty acids are known to be oxidized. The oxidation product is a conjugated hydroperoxy-(*E,Z*)-diene fatty acid. Several recent reviews concerning plant lipoxygenases are available (5–8).

*Address correspondence at NCAUR, ARS, USDA, 1815 N. University St., Peoria, IL 61604.

Sources and specificity. Table 1 lists several LOX from different sources for which the oxidation stereospecificity have been characterized. As can be seen from the table, a variety of specificities were observed. Often, LOX with pH optima near neutrality are specific for 9*S*-oxidations, but pH optimum is not a reliable predictive parameter. Most cereal seeds contain a LOX isozyme that oxygenates linoleic acid at carbon-9 (stereospecificities are only partially determined for cereals), and this constitutes another interesting trend (19).

Soybean lipoxygenase-1 (LOX-1), an enzyme with a pH optimum of 9 to 10, oxygenates polyunsaturated fatty acids at the ω-6 carbon with *S*-stereospecificity (20), and this enzyme represents a large class of other less-studied LOX of this type. Thus, the prevalent plant polyunsaturates, linoleic and linolenic acids, are oxygenated into (13*S*,9*Z*,11*E*)-13-hydroperoxy-9,11-octadecadienoic acid (13*S*-HPODE) and (13*S*,9*Z*,11*E*,15*Z*)-13-hydroperoxy-9,11,15-octadecatrienoic acids (13*S*-HPOTE), respectively. With arachidonic acid, a fatty acid primarily restricted to the animal kingdom, the oxi-

TABLE 1
Chiral Analyses of Some Hydroperoxylinoleic Acid Stereoisomers from Action of Various Lipoxygenases (LOX)[a]

LOX source (isoenzyme)	Product regio-/stereo-specificity[b] 13*S*	13*R*	9*S*	9*R*	8*R*	pH[c]	Reference (also see)
Soybean (LOX-1)	94	2	2	2		10.5	9 (10,11)
(LOX-1)	77	3	18	2		7	12 (10,11)
Corn germ	3.5	3.5	89	4		6.5	9 (10)
Tomato fruit	13	2	84	<1		5.5	13 (11,14)
Potato tuber	1.6	2.4	96	0		6.8	15
Barley seed			92	3		7.0	16
Gaeumannomyces graminis					100	7.4	17
Marchantia polymorpha	89	2				9.0	18
Wheat germ	10	5	83	2		6.8	13
Pea seeds (LOX-I)	23	16	32	29		6.8	13
(LOX-II)	87	2	6	5		6.8	13

[a]Data for the hydroperoxy-*E,E*-diene isomers (usually a few percentage and believed to be derived from autoxidation) were ignored; thus, the data are calculated on the basis of 100% of the hydroperoxy-*Z,E*-dienes.
[b]Usually the stereospecificity is determined for linoleic acid, but often the same specificity is observed with linolenic acid as a substrate.
[c]The pH at which the oxidation specificity was determined (not necessarily the optimum).

dation occurs at carbon-15 giving (15*S*,5*Z*,8*Z*,11*Z*,13*E*)-15-hydroperoxy-5,8,11,13-eicosatetraenoic acid.

For soybean LOX-1, relative activity has been studied with various fatty acid substrates. Testing C_{18} fatty acids with methylene-interrupted *Z,Z*-dienes, located at different positions along the chain, it was determined that maximal activity occurred when the position of the first double bond was at C_9 [linoleic acid, (9*Z*,12*Z*)-octadecadienoic acid] (21). Activity decreased on both sides of this optimum placement, except for another smaller maximum of about 50% for the 13,16-diene. Considering the ω-6 oxidation specificity of LOX-1, one might expect the 13,16-diene activity spike. The formation of a 13-hydroperoxy-14,16-octadecadienoic acid might be predicted, but instead, (17*S*,13*Z*,15*E*)-17-hydroperoxy-13,15-octadecadienoic acid was the principal product (22). Activity was virtually gone with the 14,17-diene, and was eliminated with locations of double bonds less than the 5,8-diene (21). In another study, the dienes were held constant at ω-6 and ω-9, and the length of the fatty acid chain was varied from C_{13} to C_{20} (23). Maximal activity was with C_{18} fatty acids, with decreases in activity on both sides. C_{20} still retained about 75% of activity, but activity was gone with chains less than C_{15}. A similar set of ω-6 and ω-9 diene alcohols gave a different activity maximum at C_{15}, with activity decrease on both sides until there was zero activity at C_{12} and C_{20} (23). With an entire series of ω-3, ω-6, ω-9 trienoic acids and alcohols from C_{12} to C_{20}, similar results were obtained, compared to the diene series described above, except the maximum activity was obtained with the C_{20} trienoic acid (24). Other unusual substrates, reported to be readily oxygenated by soybean LOX-1, were linoleoyl sulfate (25), anacardic acid (26), (9*Z*)-12-oxo-9-octadecenoic acid (27), (12*Z*,15*Z*)-9-hydroxy-10-oxo-12,15-octadecadienoic acid (28), phospholipid (29), tri- and di-acylglycerols (30,31), and various (*Z,Z*)-3,6-dienyl 1-adipates (32).

As seen in Table 1, at pH near neutrality, soybean LOX-1 gave a small, but significant, *S*-stereospecific oxidation at the double bond toward the carboxylic acid side of the fatty chain, which in the case of linoleic acid gave a small percentage of (9*S*,10*E*,12*Z*)-9-hydroperoxy-10,12-octadecadienoic acid (9*S*-HPODE). With fatty acids having an (all *Z*)-octatriene moiety located between ω-6 and ω-12, like arachidonic acid and γ-linolenic acid, this type of specificity results in double dioxygenation to dihydroperoxides, often with triene conjugation (33–35).

The first report of a LOX that oxygenated almost exclusively on the carboxylic acid side of the pentadiene moiety was from corn germ, also *S*-stereospecific, which afforded 9*S*-HPODE from linoleic acid (36). LOX from potato (37) and tomato (38) are most commonly used to produce 9*S*-HPODE and (9*S*,10*E*,12*Z*,15*Z*)-9-hydroperoxy-10,12,15-octadecatrienoic acid (9*S*-HPOTE), probably because tomatoes and potatoes are readily available commodities. In our lab, tomato LOX is used to prepare 9*S*-HPODE, but we find some variability among tomato varieties. Cherry tomatoes are used in our lab because they give consistent results in our hands, and their use ensures less genetic tampering of the plant by breeders. With arachidonic acid, this "carboxylic acid-recognizing" type of LOX usually gives varying ratios of oxidation at carbons 5 and 8 with *S*-stereospecificity. The structures of hydroperoxides obtained with various LOX are shown in Figure 1.

Strategies for obtaining maximum yields. In our lab, we routinely produce 600 mg of hydroperoxides from 800 mg linoleic acid in a volume of about a half liter with 10 mg soybean LOX [type 1 or 1B from Sigma (St. Louis, MO), essentially LOX-1]. The crude product mixture is applied to an ordinary open silicic acid column (2.5 cm i.d.) and separated

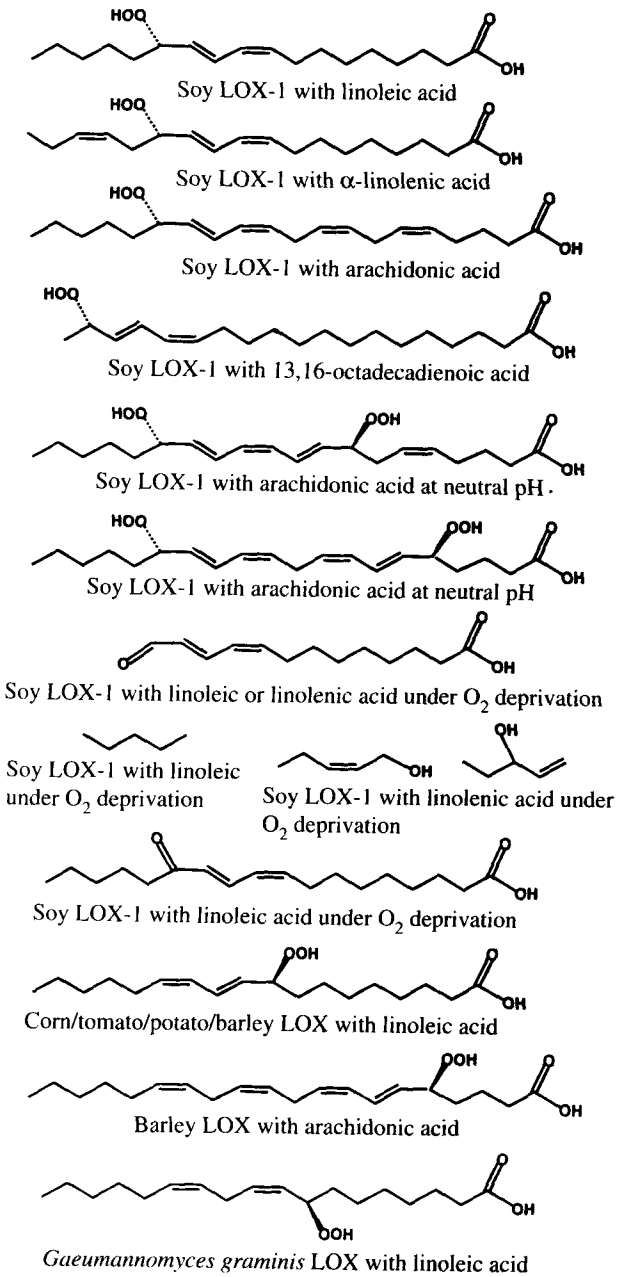

FIG. 1. Structures of fatty acid oxidation products from action of various lipoxygenases (LOX).

(39). The first half of the hydroperoxide peak generally gives 300 mg of 99% 13*S*-HPODE; whereas, the latter half of the peak gives a mixture of hydroperoxides comprised of better than 90% 13*S*-HPODE. The method serves as a convenient preparation of research material, and it is available as a desktop publication (Gardner, H.W., *Step-by-Step Preparation of 13S-HPODE or 13S-HPOTE*). With soybean LOX-1, it is probable that the availability of oxygen and a pH above 10 are among the most important factors in successfully producing hydroperoxides in good enantiomeric purity and yield. Any tendency toward anaerobic conditions resulted in the formation of by-product 13-oxo-9,11-octadecadienoic acid, 13-oxo-9,11-tridecadienoic acid (40), dimers (41), and racemic hydroperoxides (42), not to mention inactivation of enzyme. With linolenic acid as a substrate under partial anaerobic conditions, the result was similar with the exception that chain cleavage was more facile, resulting in 13-oxo-9,11-tridecadienoic acid, (2*Z*)-penten-1-ol, 1-penten-3-ol, and pentene dimers (43). With this in mind, it is not surprising that one successful preparative method used pure oxygen under 4 atmospheres of pressure and 0–4°C aqueous buffer to improve oxygen solubility (44). Thus, incubations should be accomplished (a) at low temperatures, (b) with pure oxygen, not air, (c) with a bubbling device that delivers a "fine mist" of bubbles or vigorous stirring in a container with a large surface area (for convenience, excess foaming should be suppressed), and (d) keeping the enzyme concentration at levels sufficient to permit oxygen replenishment without being too low to accomplish the desired yield. Regarding the latter condition, perhaps the lack of success with low enzyme is caused by product inhibition and/or enzyme inactivation. An optimized large-scale preparation of 13*S*-HPODE has been published recently, and reasonably good product yields were obtained with linoleate concentrations as high as 0.1 M (45). Other optimal parameters included pure oxygen pressure of 2.5 atmospheres, temperature of 5°C, pH 11, and LOX (Fluka, Buchs, Switzerland) concentration of 4 mg/mL with 0.1 M linoleic acid. By comparison with our method (Gardner, H.W., *Step-by-Step Preparation of 13S-HPODE or 13S-HPOTE*), they used a 17.5-fold higher concentration of linoleic acid, and probably much more LOX (comparison between different LOX suppliers is difficult). The much higher concentrations used by these workers may illustrate the importance of the increased O_2 availability supplied by 2.5 atmospheres of pure O_2. Detergents and alcohols are often used to facilitate solubility of substrates, but it must be kept in mind that these substances influence the activity of LOX, suggesting that these reagents be used judiciously (46,47). In this regard, one preparatory method utilizes a 10% ethanol solution; however, 20% dimethylsulfoxide outperformed ethanol and permitted the use of substrate concentrations of up to 100 g/L (48).

A number of workers have immobilized LOX on various supports with the goal of stabilizing and reusing the enzyme in multiple reactions (49–52). Substrate dissolved in organic solvent has been oxidized by some of the immobilized systems (51,53), as well as by soluble LOX (54,55). Improved oxygen solubility in some organic solvents may prove to be an advantage. An octane/borate buffer system reported the use of relatively high substrate concentrations (10–40 g/L), yielding 78 to 30% product, respectively (55). As mentioned above, a miscible aqueous–organic system, comprised of 20% dimethylsulfoxide and soybean LOX, oxidized linoleic acid at a concentration of 100 g/L (0.36 M) in about 75% yield (48).

ALLENE OXIDE SYNTHASE (AOS)

AOS, an enzyme that metabolizes fatty acid hydroperoxides, was first reported by Zimmerman in 1966 (56), but only recently it was found that AOS produces an unstable allene oxide intermediate (57). Thus, 13*S*-HPODE is metabolized by AOS into 12,13*S*-epoxy-9*Z*,11-octadecadienoic acid, which has a half-life of only 33 s at 0°C. Allene oxides can be isolated and manipulated long enough to obtain a nuclear magnetic resonance (NMR) spectrum, provided the sample is methyl-esterified, dissolved in hexane, and kept at or below −15°C (58). AOS is a cytochrome P450 of 55 kDa size (59), and the sequence has been determined with the flaxseed enzyme (60). Also, AOS has been cloned and sequenced from guayule (61), where the enzyme is found in abundance within rubber particles. In the past, AOS has been also named hydroperoxide isomerase, hydroperoxide cyclase, hydroperoxide dehydrase, and hydroperoxide dehydratase.

Occurrence and products. Because AOS is the first enzyme that catalyzes a series of transformations, starting with formation of 12-oxo-phytodienoic acid and ultimately leading to jasmonic acid (62), an important phytohormone, it is likely that the enzyme exists in all plants. The biological activity of the jasmonic acid family has been reviewed recently (63,64). As discussed later, the most usual result of AOS action is the formation of ketols, arising from spontaneous hydrolysis of the allene oxide rather than cyclization to 12-oxophytodienoic acid.

According to Lau *et al.* (65), AOS was the predominant cytochrome P450 in many of the plants they examined. The tissue in which AOS is expressed is not always predictable. For example, AOS was found in abundance in many seeds, such as flaxseed (56) and corn (66), but was completely absent in soybean seed (67). In soybean, activity was predominantly found in immature seed coats and the pericarp or pod (67). AOS also occurs in animal tissue, but currently it has been identified only in coral species (68).

In aqueous buffers, the most usual products obtained are α-ketols (56) and γ-ketols (66) from hydrolysis of the allene oxide. In addition to hydroxyl anion, a large number of other nucleophiles have been known to substitute at the allene oxide (Fig. 2), such as the anions of fatty acids (66,69), sulfhydryls (69), and methanol (57,69). In nonnucleophilic solvents, such as acetonitrile, the allene oxide undergoes an intramolecular attack by the carboxylic acid moiety to form macrolactones (70).

The allene oxide, formed by AOS action on the hydroperoxide of linolenic acid, 13*S*-HPOTE, spontaneously cyclizes

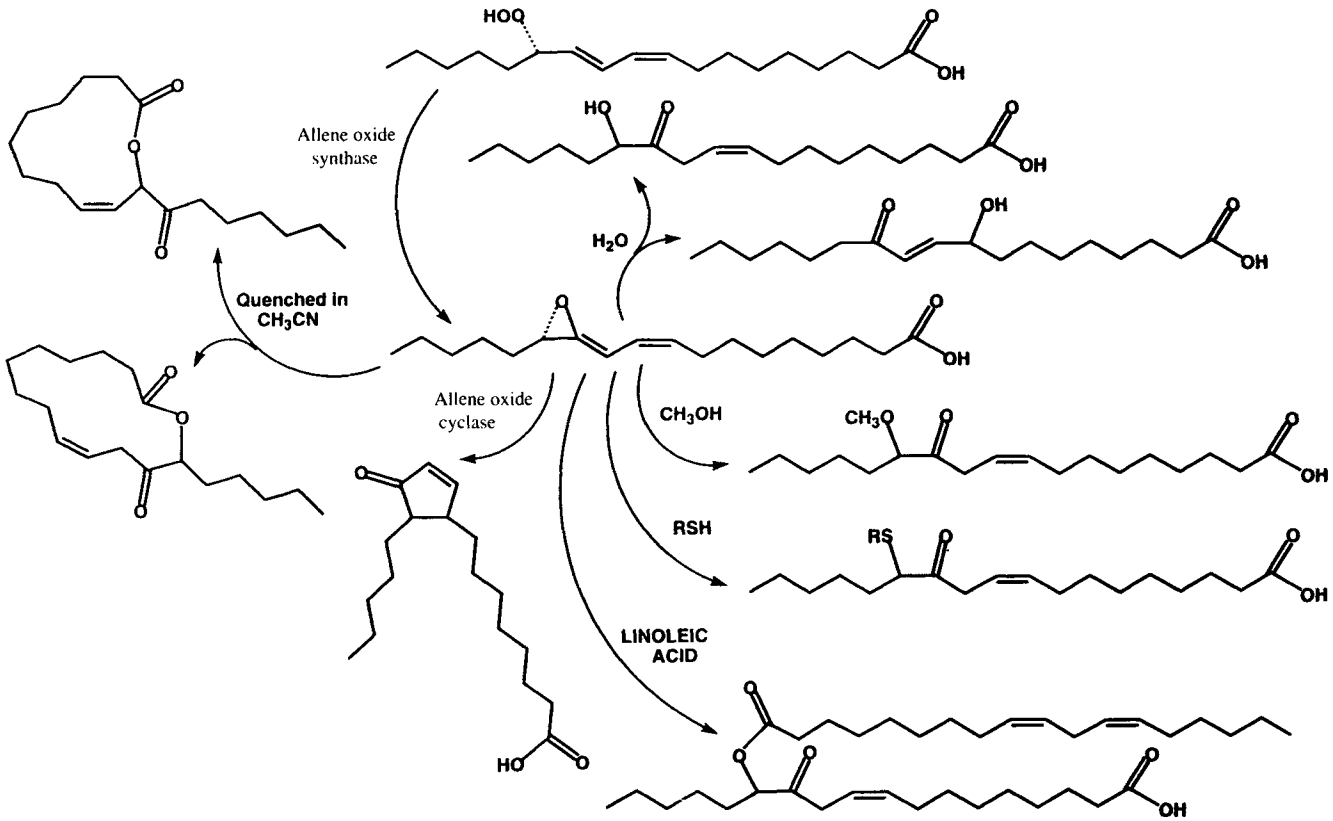

FIG. 2. Conversion of the 13-hydroperoxide of linoleic acid (13*S*-HPODE) into products by allene oxide synthase. RSH, ethanethiol.

into racemic 12-oxo-phytodienoic acid to the extent of about 12%, with the remainder being the ketols (71). The mechanism for this cyclization depends upon the allene oxide opening into a delocalized pentadienol carbocation (68). Although the extra 15*Z*-double bond provided by 13*S*-HPOTE does not participate directly in cyclization, it facilites the reaction (72). 12-Oxo-phytodienoic acid with 9*S*,13*S*-side chains is the "natural" precursor of the jasmonic acid family of phytohormones (73). This stereo-isomer was produced in the presence of both AOS and allene oxide cyclase, and with sufficient allene oxide cyclase activity, the yield of 12-oxo-phytodienoic acid increased greatly at the expense of hydrolysis to ketols (71). Because of the vicinal 12-oxo-11-ene, the 13*S*-side chain readily isomerized into the 13*R* isomer (63,73), giving the configuration needed for the biosynthesis of "natural" (–)-jasmonic acid.

Unlike the facile cyclization of 13*S*-HPOTE, it was long believed that cyclization of 13*S*-HPODE into 15,16-dihydro-12-oxo-phytodienoic acid did not occur (by definition, 12-oxo-phytodienoic acid, being derived from 13*S*-HPOTE, has a 15,16-double bond; therefore, a corresponding product arising from 13*S*-HPODE would be saturated at the 15,16-carbons, i.e., 15,16-dihydro-12-oxo-phytodienoic acid). However, Hamberg and Hughes (74) showed that serum albumin promoted cyclization of 13*S*-HPODE into a racemic mixture of 15,16-dihydro-12-oxo-phytodienoic acid, with side chains predominantly in a *trans* configuration. Of course, serum albumin is hardly an ingredient expected to be found in plants. Recently, Blechert *et al.* (75) reported the formation of 15,16-dihydro-12-oxo-phytodienoic acid from linoleic acid (via 13*S*-HPODE) in flaxseed (Fig. 2), a finding confirmed by us using 13*S*-HPODE with corn germ AOS, spiked with potato tuber allene oxide cyclase (Gardner, H.W., unpublished finding). Presumably, dihydro-jasmonic acid originates by this pathway. Thus, it appears that the jasmonate lipid-signalling has its origins from both of the two major polyunsaturates of plants, linolenic and linoleic acids.

Potential of AOS as a biocatalyst. AOS is an effective enzyme for conversion of fatty acid hydroperoxides with a remarkable activity of about 1000 turnovers per second (59). Thus, LOX plus AOS in the same reaction vessel efficiently convert linoleic acid by sequential reactions into mainly α-ketol and an estolide of α-ketol with linoleic acid; γ-ketol is a relatively minor product (66). Although most AOS are membrane-bound (e.g., see 59), guayule AOS is readily soluble because it lacks the amino-terminal membrane anchor found on many cytochrome P450 (61). Thus, it would appear that there is considerable flexibility for utilization of AOS in either soluble form or as a membrane suspension. In theory, the membrane-type AOS might be adsorbed to reverse-phase supports, but these have not been tested as yet. In our hands, AOS has proven to be a remarkably stable enzyme. AOS ac-

tivity has been found in corn germ stored for at least a decade (Gardner, H.W., unpublished observations).

Inasmuch as 12-oxo-phytodienoic acid and its 15,16-dihydro derivative have biological activity at least as effective as their metabolites, the jasmonates (75), it would seem that AOS would be a promising way to produce valuable compounds that have bioactivity. However, the cyclization process is complicated by lack of the stereospecificity needed for optimum bioactivity and by low yields, especially with 13*S*-HPODE as a substrate. As discussed above, allene oxide cyclase is necessary to solve both of these problems. However, for allene oxide cyclase to function optimally (71), the concentration of the 13*S*-HPOTE substrate must be low (3 μM). Thus, with reasonably high substrate concentrations, one could expect yields of racemic 12-oxo-phytodienoic of only 12%. With 13*S*-HPODE as substrate giving 15,16-dihydro-12-oxo-phytodienoic acid, the yield of racemic product should be small. A suggested method of surmounting the problem might be controlled release of fatty acid from glyceride lipid by lipase, set at low levels of activity in the presence of a 13-oxidizing LOX, AOS, and allene oxide cyclase.

In conclusion, among the hydroperoxide-metabolizing enzymes, AOS seems the most promising because of its stability, high catalytic turnover, and production of a diverse family of oxygenated fatty acids. However, production of the 12-oxo-phytodienoic acid family of cyclic fatty acids requires more work to improve yields and stereospecificity.

HYDROPEROXIDE LYASE

Since the first report of hydroperoxide lyase (HPLS) by Tressel and Drawert in 1973 (76), much research has been accomplished with the enzyme (see reviews, 5,7,77–79). Although the use of hydroperoxide lyase is hampered by its difficulty of isolation, lack of a cDNA clone, and binding to membranes (often chloroplasts), there is still keen interest in the enzyme for the production of volatile aldehydes. This interest is probably fueled by the less stringent regulations regarding enzymically produced biochemicals. Additionally, "naturally" produced chemicals are perceived by the public to be superior to the absolutely identical synthetic chemical, except the petrol-derived chemical would lack ^{14}C-radioactivity.

HPLS cleaves the 9-hydroperoxides, 9*S*-HPODE and 9*S*-HPOTE, into two 9-carbon fragments, 9-oxononanoic acid and either (3*Z*)-nonenal (with 9*S*-HPODE) or (3*Z*,6*Z*)-nonadienal (with 9*S*-HPOTE). The latter two aldehydes have distinctive cucumber-like odors. With the 13-hydroperoxides, 13*S*-HPODE and 13*S*-HPOTE, HPLS action gives (9*Z*)-12-oxo-9-dodecenoic acid and either hexanal (with 13*S*-HPODE) or (3*Z*)-hexenal (with 13*S*-HPOTE). The odor of hexanal is rancid/green, and (3*Z*)-hexenal gives a distinctive odor of freshly mowed grass. The aldehydes are susceptible to further enzymic change, such as reduction by alcohol dehydrogenase, to their corresponding alcohols. Additionally, the (3*Z*)- and (9*Z*)-alkenals are transformed into (2*E*)- and (10*E*)-alkenals, respectively, by isomerase(s), and consequently, modify the odors. For example, (2*E*)-hexenal is spicy green and (2*E*)-nonenal has an "old leather" odor. In certain plants, the (3*Z*)-alkenals are also converted into nonvolatile (2*E*)-4-hydroxy-2-alkenals (80).

It is likely that HPLS are specific for either of the 9- or 13-hydroperoxides, but not both. For example, two HPLS from cucumber were separated chromatographically, one specific for 13*S*-HPODE and one acting only on 9*S*-HPODE (81). Furthermore, two isozymes purified from bell pepper cleaved 13*S*-HPODE and 13*S*-HPOTE, but not 9*S*-HPODE and the (9*E*,11*E*)-diene isomer of the 13-hydroperoxide (82).

The first HPLS isolated to homogeneity was from tea leaves, and this enzyme exhibited specificity for 13-hydroperoxide, especially 13*S*-HPOTE (83). The tea leaf HPLS isozymes were 53 and 55 kDa in size, which was similar to bell pepper HPLS, determined to be 55 kDa (82). Bell pepper HPLS was determined to be heme *b* protein similar to cytochrome P450, but the enzyme did not respond to CO, a diagnostic test for cytochrome P450 (84).

Potential of HPLS as a biocatalyst. As discussed above, HPLS has the potential of providing the flavor industry with odors that are known to provide "fresh" notes, associated with unprocessed fruit and vegetables. Also, there is the possibility of using the aldehydes as antifungals (85,86) and antimicrobials (87).

Compared to AOS, HPLS is more difficult to achieve good product yields. The enzyme tends to inactivate both during purification and reaction with hydroperoxides. Because aldehydes are known to react with proteins, there may be significant product inhibition. Because of the presence of isomerases and (3*Z*)-alkenal oxygenases (80), some purification is necessary to avoid loss of products. However, many difficulties can be surmounted by clever innovation. Workers at Firmenich (88) have utilized an active-yeast sugar fermentation in conjunction with crude HPLS and 13-hydroperoxides to reduce HPLS-produced aldehydes to their corresponding alcohols *in situ*. The reduction by yeast alcohol dehydrogenase accomplishes three goals: (i) the alcohols have similar, but less intense, odors compared to the aldehydes, (ii) converts unstable (3*Z*)-alkenals into alcohols that are stable to double-bond isomerization and further oxidation, and (iii) possibly gives HPLS a longer lifetime. By delaying the addition of fermenting yeast in an HPLS reaction with 13*S*-HPOTE, the Firmenich workers were able to selectively produce (2*E*)-hexen-1-ol, instead of (3*Z*)-hexen-1-ol obtained in the continuous process.

EPOXYHYDROXY- AND TRIHYDROXY-ENE/DIENE FATTY ACIDS

Potential uses and problems with enzymic routes. It is now known that certain plants produce epoxyhydroxy- and trihydroxy-ene/diene fatty acids through the LOX pathway as defensive substances against pathogenic fungi (89–91), and this suggests a specific potential use to investigate.

Epoxyhydroxyoctadecadienoic/enoic acids and their

hydrolysis products, trihydroxyoctadecadienoic/enoic acids, have been formed enzymically through the action of hydroperoxide isomerase of *Saprolegnia parasitica,* a fish parasite (see reviews 5,92), and hydroperoxide-dependent peroxygenase/epoxygenase of soybean or broad bean (see reviews 5,93). These enzymes have disadvantages for applied use. The fungus *Saprolegnia parasitica* requires culture conditions as well as enzyme preparation. With hydroperoxide-dependent epoxygenase/peroxygenase, the enzyme is obtained by high-speed centrifugation of a membrane pellet, and the presence of substrate hydroperoxides leads to inactivation of the enzyme. On the other hand, there are methods for chemical transformation of fatty hydroperoxides available that accomplish similar results more readily (see below).

Acid-catalyzed transformation of hydroperoxides. As outlined in Figure 3, aqueous acid treatment of 13*S*-HPODE is a facile method of producing epoxyhydroxyene and trihydroxyene fatty acids with structural features similar or identical to those fatty acids that have antifungal activity (94). The mechanism of transformation is undoubtedly Hock-Criegee rearrangement to give an epoxyallylic carbocation intermediate, which then undergoes substitution with water solvent (hydroxyl anion) to furnish epoxyhydroxyene fatty acids. Because allylic epoxides are particularly susceptible to hydrolysis, these do not survive acidic conditions and are converted to trihydroxyene fatty acids (Fig. 4). The nonallylic epoxides can be isolated (94). In a different protic solvent, methanol, methoxyl substitution occurred (95) and afforded epoxymethoxyene and hydroxydimethoxyene fatty acids (Fig. 3). Similar to the aqueous-acid model, acidic methanol is responsible for solvolyzing epoxymethoxyene fatty acids of epoxides with an allylic double bond into hydroxydimethoxyene fatty acids.

Free-radical transformation of hydroperoxides. One-electron reduction of hydroperoxides affords alkoxyl radicals. Alkoxyl radicals of 13*S*-HPODE and other conjugated diene hydroperoxides largely rearrange into epoxyallylic radicals (96–98). The epoxyallylic radical subsequently combines with another radical, which is usually oxygen under aerobic conditions, and furnishes intermediate epoxyhydroperoxyene fatty acids (Fig. 3). A number of one-electron catalysts have been used, such as hematin (99,100), hemoglobin (101), ultraviolet (UV) photolysis (102), or a trace of Fe^{2+} that was kept reduced by an excess of cysteine (103). The Fe^{2+}-cysteine catalyst converts 13*S*-HPODE into a mixture of epoxyhydroxyene and epoxyoxoene fatty acids *via* intermediate epoxyhydroperoxyene fatty acids, as shown in Figure 3 (96,97). In contrast, catalysis by hematin (99), hemoglobin (101), and UV photol-

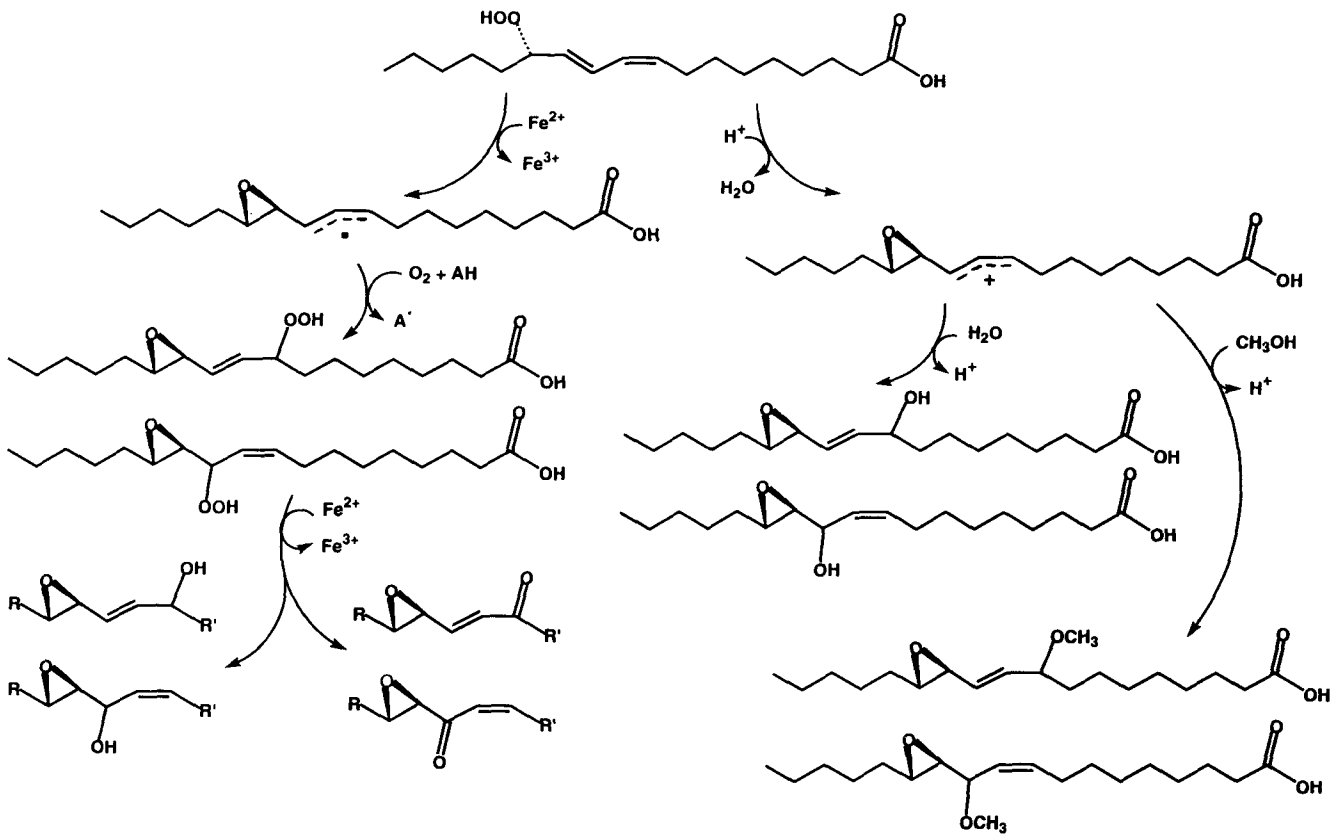

FIG. 3. Transformation of the 13-hydroperoxide of linoleic acid (13*S*-HPODE) *via* an alkoxyl radical route (left) or acid-catalyzed rearrangement (right). The single bond between C_{12} and C_{13} of 13*S*-HPODE rotates into the least hindered (extended) comformer, leading to the formation of mostly *trans*-epoxides. R = $CH_3(CH_2)_4^-$; R′ = $-(CH_2)_7COOH$.

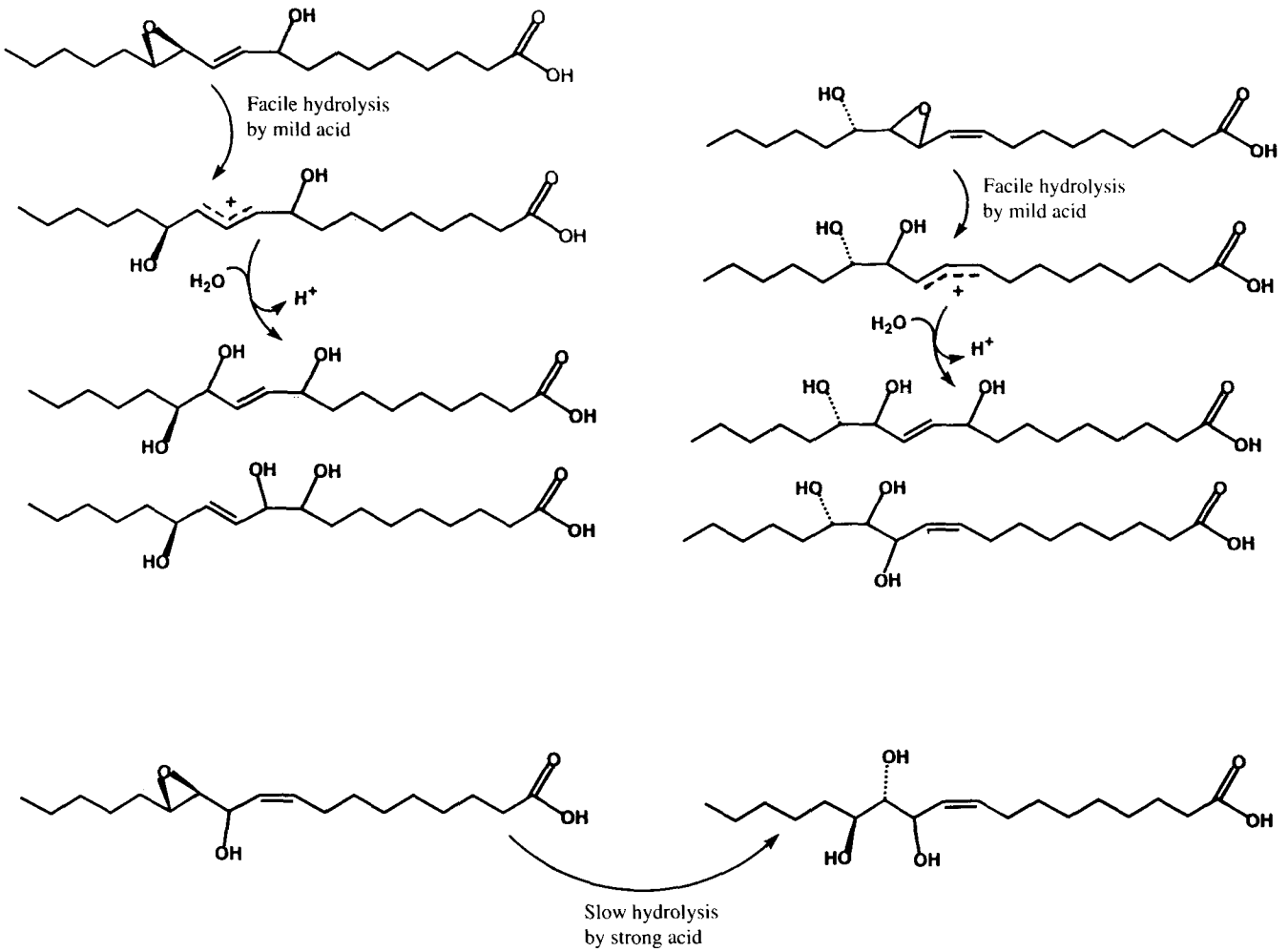

FIG. 4. Hydrolysis of epoxyhydroxyoctadecenoic acids. Allylic epoxides (top) are readily hydrolyzed by mild conditions; nonallylic epoxides (bottom) are more resistant to hydrolysis.

ysis (102) produces mainly epoxyhydroxyene fatty acids in the apparent absence of epoxyoxoene fatty acid. All systems produce significant amounts of 13-oxo-9,11-octadecadienoic acid, except the UV photolysis method.

As observed with the acid-catalyzed products described above, epoxides allylic to a double bond are particularly susceptible to hydrolysis (Fig. 4). Even in the apparent absence of acids, a significant amount of hydrolysis/solvolysis occurs, giving rise to trihydroxyene fatty acids and other solvolysis products (99,102,104).

Intermolecular epoxidation by transition metals. Group IVa, Va, and VIa transition metal ions, such as V, Mo and Cr, coordinate with hydroperoxides, thereby polarizing the hydroperoxide sufficiently to epoxidize double bonds. As shown in Figure 5, an oxyacetylacetonate of V catalyzed transformation of 13*S*-HPODE to products similar to those obtained by enzymes hydroperoxide-dependent epoxygenase/peroxygenase and hydroperoxide isomerase from *S. parasitica* (105). Again, allylic epoxides of this type were also susceptible to hydrolysis into trihydroxyene fatty acids (105, Fig. 4).

REACTION OF HYDROPEROXIDES WITH ALKALI

Hydroperoxides are readily reduced to the corresponding hydroxide by a number of reagents, such as $SnCl_2$, triphenylphosphine, KI, and $NaBH_4$. Interestingly, strong alkali, such as 5 M KOH, will convert 13*S*-HPODE and 13*S*-HPOTE into (13*S*,9*Z*,11*E*)-13-hydroxy-9,11-octadecadienoic acid and (13*S*,9*Z*,11*E*,15*Z*)-13-hydroxy-9,11,15-octadecatrienoic acid, respectively, without loss of stereo-configuration (106,107). The yield was 75%, with the remaining 25% of products originating mainly from 11,12-epoxy-13-oxo-9-octadecenoic acid, which was an early intermediate (107). The latter compound was transformed by alkali into a number of unusual products by Favorskii Rearrangement.

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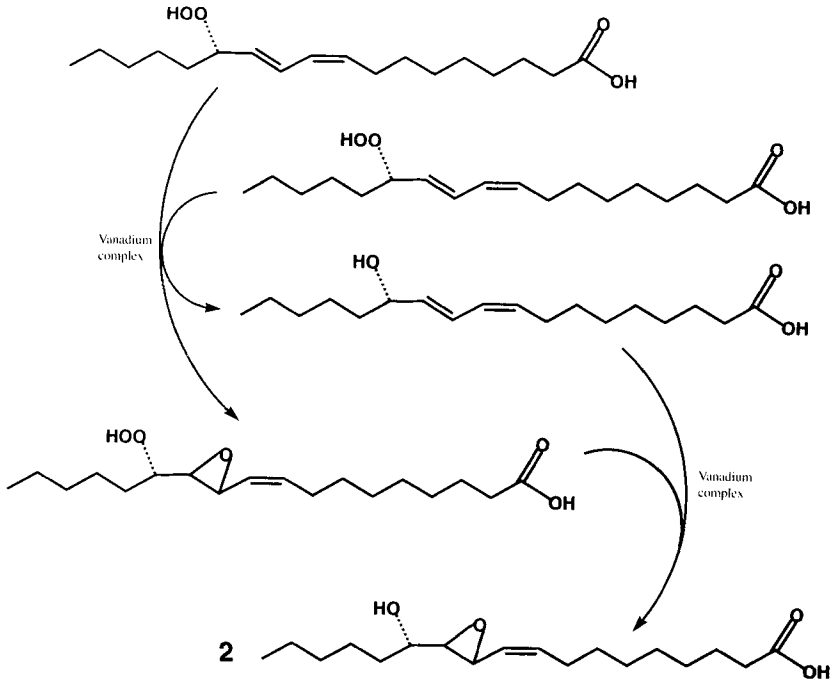

FIG. 5. Mechanism and products of reaction of 13-hydroperoxide of linoleic acid (13*S*-HPODE) catalyzed by vanadium oxyacetylacetonate according to Hamberg (Ref. 105).

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