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Registry No. Glucose, 50-99-7; fructose, 57-48-7; sucrose, 57-50-1; sorbitol, 50-70-4; hexanal, 66-25-1; *trans*-2-hexenal, 6728-26-3; benzaldehyde, 100-52-7; linalool, 78-70-6; γ-decalactone, 706-14-9.

Fatty Acid Hydroperoxide Lyase in Germinating Soybean Seedlings

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A fatty acid hydroperoxide lyase from soybean seeds (*Glycine max* var. Williams) has been partially purified by differential centrifugation, ion-exchange chromatography, and gel filtration. The enzyme preparation, which is free of *cis*-3:*trans*-2-enal isomerase, has an optimun pH in the range 6-7. The lyase cleaves the 13-hydroperoxides of linoleic and linolenic acids to form the volatile aldehydes hexanal and *cis*-3-hexenal, respectively. In both case the ω-oxo carboxylic acid 12-oxo-*cis*-9-dodecenoic acid is also formed. The enzyme does not act on 9-hydroperoxides of these acids. The activity of hydroperoxide lyase was followed for 6 days of germination and found to increase constantly. Lipoxygenase activity (L-2 + L-3) also increased, as did the level of fluorescence in the phospholipids extracted. These facts suggest that lipoxygenase and hydroperoxide lyase may be involved in the formation of fluorescent substances.

Although it is well-established that lipoxygenase are widespread throughout the plant kingdom, their physiological role and the fate of their products, lipid hydroperoxides, are incompletely understood. The hydroperoxides derived from polyunsaturated fatty acids are intrinsically unstable. They undergo both enzymatic and nonenzymatic changes, including carbon chain cleavage. The enzymatic cleavage of the polyunsaturated fatty acid

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hydroperoxides is catalyzed by a hydroperoxide lyase. This enzyme was found for the first time in banana fruit (Tressl and Drawert, 1973) and later demonstrated in cucumber fruit (Galliard and Phillips, 1976) and in watermelon seedlings (Vick and Zimmerman, 1976).

It appears from the accumulated reports that there are lyases of varying specificity. Some attack 13-hydroperoxy-*cis*-9,*trans*-11-octadecadienoic acid (13-LAOOH) and 13-hydroperoxy-*cis*-9,*trans*-11,*cis*-15-octadecatrienoic acid (13-LNAOOH) to form hexanal and *cis*-3-hexenal, respectively, as well as 12-oxo-*cis*-9-dodecenoic acid (Galliard and Matthew, 1977). On the other hand, while the enzyme from pear fruit is specific for 9-hydroperoxy-*trans*-10,*cis*-12-octadecadienoic acid (9-LAOOH) and 9-hydroperoxy-*trans*-10,*cis*-12,*cis*-15-octadecatrienoic acid (9-LNAOOH) (Kim and Grosch, 1981), the enzyme from cucumber acts on both isomers (Phillips and Galliard, 1978). Vick and Zimmerman (1976) demonstrated the stoichiometric production of hexanal and 12-oxo-*cis*-10-dodecenoic acid. The expected oxo acid is 12-oxo-*cis*-9-dodecenoic acid. They attributed their result to the presence of a *cis*-3:*trans*-2-enal isomerase, although no experimental data were offered for its existence. The presence of *cis*-3:*trans*-2-enal isomerase in the juice of cucumber fruit has been established by Phillips et al. (1979) who found it to be active against *cis*-3-hexenal and 12-oxo-*cis*-9-dodecenoic acid, interalia. It is resonable that the initial oxo acid is 12-oxo-*cis*-9-dodecenoic acid, in view of the results of Galliard et al. (1977) who showed that a crude tomato preparation, which contained a 13-LAOOH-specific lyase and was devoid of the isomerase, generated only this isomer.

The soybean is the richest in lipoxygenase of all plant tissues studied and is well endowed with linoleic and linolenic acids. Thus, it has a good potential for generating hydroperoxides that can serve as substrates for hydroperoxide lyase. Nonetheless, little has appeared in the literature concerning the presence of lyase in soybean. Motoba et al. (1985a,b) have related the production of hexanal in soybean homogenates to the content of lipoxygenase enzyme, L-2. They found that homogenates from soybeans that had been soaked overnight generated hexanal from 13-LAOOH but not from 9-LAOOH.

We report here the partial purification and properties of hydroperoxide lyase from soybean seedlings, which acts on 13-LAOOH and 13-LNAOOH, but not on their 9-isomers, to form the expected volatile aldehydes and ω-oxo carboxylic acid.

EXPERIMENTAL SECTION

Plant Material and Growth Conditions. Soybean seeds (*Glycine max* var. Williams) were soaked overnight and placed on paper towels, which was considered to be time zero when developmental times were determined. The seeds were germinated in the dark at 28 °C and 80% relative humidity.

Chemicals. Linoleic and linolenic acids were obtained from Nu-check Prep (Elysian, NY); *cis*-3-hexenol, *trans*-2-hexenal, hexanal, and 2,4-dinitrophenylhydrazine (DNP) were from Sigma Chemical Co. (St Louis, MO). Octadecyl (C_{18}) and aminopropyl bonded-phase disposable extraction columns (6 mL) were obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ); DE-52 cellulose was from Watman (Maidstone, England) and Sephacryl S-200 from Pharmacia (Uppsala, Sweden). The other chemicals were of analytical grade.

13-LAOOH and 13-LNAOOH were prepared from pure soybean lipoxygenase (L-1) (Hamberg and Samuelsson, 1967); 9-LAOOH and 9-LNAOOH were obtained with lipoxygenase purified from potato tubers (Galliard and Phillips, 1971). The 9- and 13-hydroperoxides proved to be pure (greater than 95%) when analyzed by HPLC (Olías and Valle, 1988). The concentration of the hydroperoxides was measured spectrophotometrically at 234 nm with 2.5×10^4 M^{-1} cm^{-1} as the extinction coefficient (Axelrod et al., 1981).

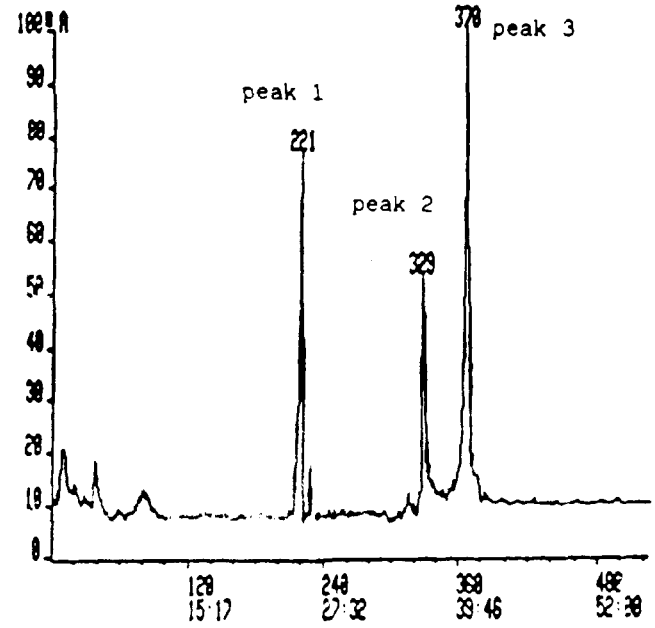

Figure 1. Ion chromatogram of nonvolatile products from cleavage of 13-LAOOH by partially purified hydroperoxide lyase from soybean seed. Conditions as described in the Experimental Section. Gas chromatogram was plotted for total ion abundance vs scan number (upper line) and retention time (lower line).

Methyl 12-oxo-*cis*-9-dodecenoate was prepared from methyl 12,13-epoxy-*cis*-9-octadecenoate, obtained by saponification of vernonia seed oil. The steps were acetylation to open the epoxy group, saponification to obtain diol, and oxidation with periodic acid in acid medium. The ω-oxo carboxylic acid was purified by silica gel TLC and its identity checked by IR, NMR, and GC–MS. Methyl 12-oxo-*trans*-10-dodecenoate was prepared from methyl 13-hydroperoxide-*cis*-9,*trans*-11-octadecadienoate by treatment with BF_3, in anhydrous ether (Gardner and Plattner, 1984). *cis*-3-Hexenal was obtained from *cis*-3-hexenol by chromic oxidation in glacial acetic acid (Nichols and Shipper, 1958).

Methods. *Preparation of Crude Extract.* Soybean seeds were germinated for 6 days at 28 °C in the dark. Cotyledons from these seeds were homogenized in a Waring blender in batches of 100 g, wet weight, in two volumes of grinding buffer, 50 mM sodium phosphate (pH 6.8) containing 0.2 mM EDTA and 0.3 mM dithiothreitol (DTT). Grinding was done in three 20-s periods. The homogenate was passed through four layers of cheesecloth and centrifuged for 30 min at 12000*g*. The supernatant was clarified by passing it through four layers of cheesecloth to exclude lipid material that separated during centrifugation.

Solubilization of the Enzyme. According to a previous report, hydroperoxide lyase is membrane-bound (Phillips and Galliard, 1978). This possibility was examined in soybean seeds, with the operational criterion that the membrane-associated activity should sediment at 150000*g* in 1 h and should remain in the void volume of Sephacryl S-200 column. When the crude extract was centrifuged at 150000*g* for 1 h, more than 70% of the activity was recovered in the pellet. It was also noted that about 50% of the lipoxygenase activity, as measured at pH 6.5, was sediment. When 15 mL of crude extract was applied to a Sephacryl S-200 column (2.4 × 105 cm), both the lyase and lipoxygenase (pH 6.5) appeared in the void volume. It was therefore assumed that the enzymes were adsorbed on membrane fragments or otherwise associated with particulate matter.

To solubilize the enzymes, the nonionic detergents, Tween-20 (0.1–0.4%, w/v), Triton X-100 (0.05–0.4%, w/v), octyl-β-D-glucopyranose (0.8–1.6%, w/v), and 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) (0.49–0.98%, w/v) were tested. All detergents caused some inactivation of the lyase. Triton X-100 (0.2%, w/v) was the most effective in terms of the ratio of solubilisation to denaturation. Less effective, in

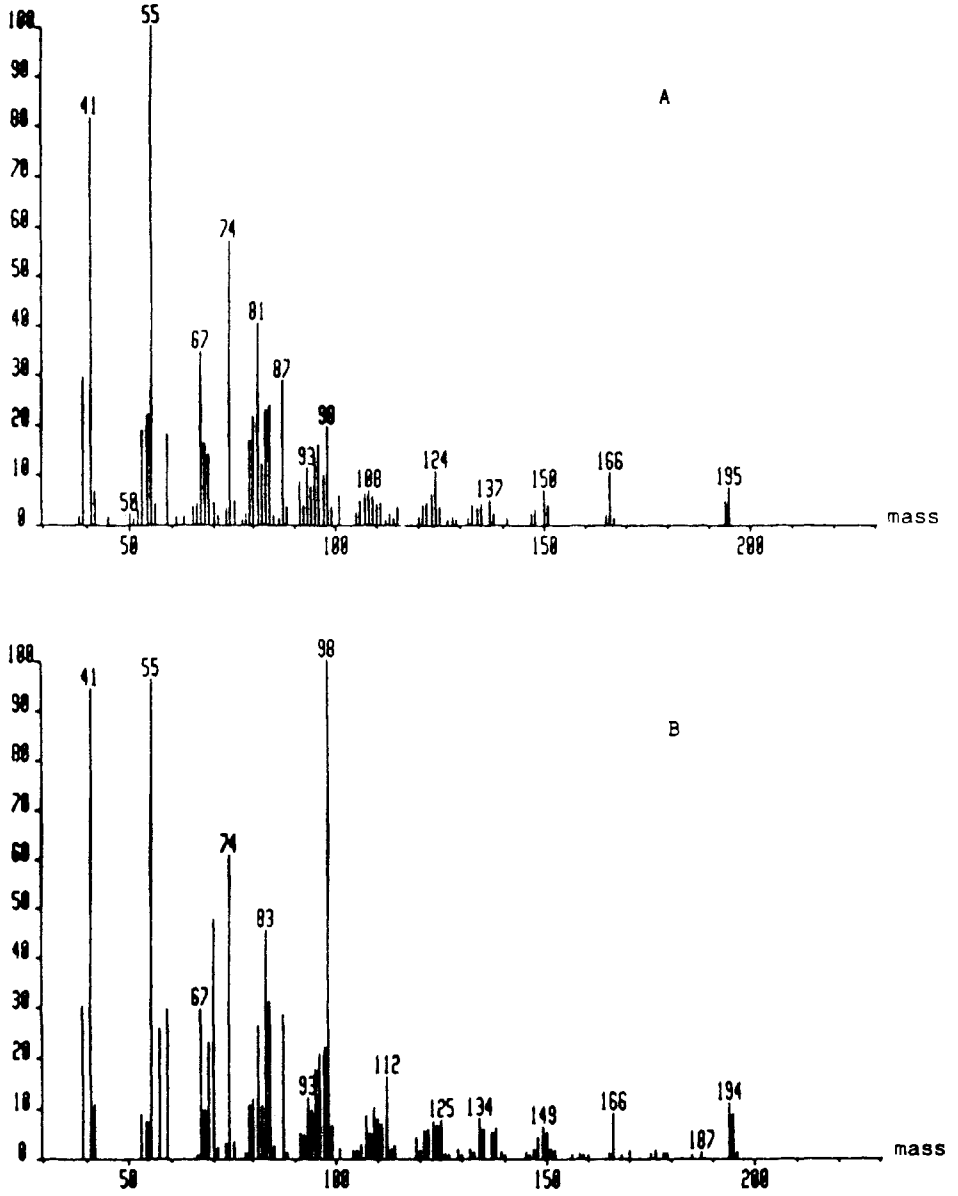

Figure 2. Mass spectra: (A) peak 329 (see Figure 1), the product coincided in R_t and mass spectrum with authentic methyl 12-oxo-*trans*-9-dodecenoate; (B) peak 370 (see Figure 1), the product coincided in R_t and mass spectrum with authentic methyl 12-oxo-*trans*-10-dodecenoate.

the following order, were octyl-β-D-glucopyranose (0.8%, w/v), CHAPS (0.5%, w/v), and Tween-20 (0.15%, w/v).

Fatty Acid Hydroperoxide Lyase Assay. Two methods for assaying lyase were employed in this work. For a rapid location of activity in the eluates of chromatographic systems, hydroperoxide lyase was assayed by the loss in absorption at 234 nm by the hydroperoxide. The oxo acid initially formed, 12-oxo-9-dodecenoic acid, may become isomerized to 12-oxo-10-dodecenoic acid, with a maximum at 226 nm contributing substantially to the absorption at 234 nm. Vick and Zimmerman (1976) overcame this problem by incorporating 10 mM KCN in the assay mixtures to form the nonabsorbing cyanohydrin compounds. The KCN may be omitted when the isomerase content is low or absent, as in the case of purified lyase. The reaction mixture contained $(3 - x)$ mL of 200 mM sodium phosphate (pH 6.8), 20 μM 13-LAOOH, and the enzyme in volume of x mL. KCN (0.01 M) was added when required. Temperature was maintained at 25 °C. Rates were calculated from the initial slopes of the curves obtained. One unit of activity is defined as the quantity of enzyme that decomposes 1 μmol of hydroperoxide/min. The second method is based on the direct analysis of volatile aldehyde in the headspace by gas chromatography. This method is relatively simple, and the results are independent of possible presence of fatty acid hydroperoxide isomerase and fatty acid hydroperoxide cyclase, which could modify the chromophore at 234 nm. The headspace analysis was made on the reaction mixture described above, without KCN in order to avoid cyanohydrin formation. The reaction was carried out in a 10-mL vial fitted with a serum stopper. After 1 min of incubation at 25 °C, the reaction was stopped by lowering to pH 3 with 2 N HCl; then the vial was heated for 10 min at 80 °C and headspace automatically sampled for 5 s. Quantification was made with the aid of a calibration curve prepared with hexanal, between 10 and 500 nmol, under the above conditions. Gas chromatography of the headspace gas was performed on a column (3 mm × 2 m) containing 3% OV-225 on Chromosorb Q (80–100 mesh), operated isothermally at 60 °C; the injector temperature was 250 °C, and the detector (FID) temperature was 275 °C; nitrogen was used as the carrier gas (20 mL/min).

cis-3:trans-2-Enal Isomerase Assay. Measurement of isomerase activity was carried out with *cis*-3-hexenal as substrate. The incubation mixture was made up of $(5 - x)$ mL of phosphate buffer pH (6.8), 1.3 nmol of *cis*-3-hexenal, and x mL of enzymatic extract. The reaction was stopped by lowering to pH 3 with 2 N HCl. The incubation mixture was shaken well with hexane (1 mL), and after centrifugation, a portion (approximately 0.5 mL) of the upper phase was removed and analyzed by GC. A 25-m capillary column OV-1/OV-101 was operated isothermally at 60 °C, flow rate 1.2 mL/min. Under these con-

ditions, *cis*-3-hexenal had a R_t 5.09 min and *trans*-2-hexenal 6.84 min.

Lipoxygenase Assay. Lipoxygenase activities were determined by continuously monitoring the formation of conjugated diene at 234 nm. In addition to detecting L-3 activity, the aerobic keto diene formation was monitored at 280 nm (Axelrod et al., 1981). One unit of activity is defined as the amount of enzyme catalyzing the formation of 1 μmol of the product/min.

Identification of Hydroperoxide Fatty Acid Degradation Products. Volatile aldehydes and oxo acids were determined by HPLC as the 2,4-dinitrophenylhydrazone (DNPH) derivative. In a typical determination, 20 mL of phosphate buffer (pH 6.8) containing 3 mM 13-LAOOH or 13-LNAOOH was incubated with an appropriate amount of enzyme for the desired time at 25 °C. The reaction was stopped by adjusting to pH 3 with 2 N HCl. Next, 0.5 mL of 0.4% DNPH in 4 N HCl was added. The mixture was shaken for 1 min, allowed to stand for 10 min, and extracted three times with 5-mL portions of hexane. The combined hexane was removed by evaporation and the residue dissolved in a known volume of methanol. DNPHs were analyzed by HPLC (Hewlett-Packard 1090). A microbore (2.1 × 250 mm) column packed with 5-μm ODS-Hypersil C_{18} was used. Elution was done with a increasing gradient (1%/min) formed between 70% and 100% methanol in water. Under these conditions it was possible to resolve DNPHs of *cis*-3-hexenal (R_t 8.7 min), *trans*-2-hexenal (R_t 9.5 min), and hexanal (R_t 10.4 min), but DNPHs of 12-oxo-*trans*-10-dodecenoic acid and 12-oxo-*cis*-9-dodecenoic acid had identical retention times (3.5 min).

Oxo acid isomers were identified by GC–MS. The nonvolatile products were extracted from the incubation mixture on a reversed-phase C_{18} microcolumn and eluted with methylene chloride. The concentrated products were separated by silica gel TLC, developed with diethyl ether–hexane–acetic acid (70:30:1). The zone of R_f 0.40–0.49 was scraped off, eluted with diethyl ether, esterified with ethereal diazomethane, and subjected to GC–MS analysis (MS-30-VG) on a 25-m capillary column, OV-1/OV-101, temperature-programmed from 80 to 180 °C at 3 °C/min.

Protein Determination. During chromatography, the concentration of protein was measured by absorbance at 280 nm. For more accurate measurements, protein was determined by the Lowry method (1951), with BSA as the standard.

Extraction of Lipids. Extraction of total lipids from cotyledons was carried out as described by Fletcher et al. (1973). To separate lipid mixtures present in chloroform extract into individual classes, aminopropyl bonded-phase columns were used as described by Kaluzny et al. (1985). An aliquot of the extract equivalent to 100 mg of cotyledons was applied to a disposable column (500 mg) and eluted with 4 mL of chloroform–2-propanol (2:1), 4 mL of ethyl ether with 2% acetic acid, and finally 4 mL of methanol. This eluate (phospholipids) was saved for fluorescence measurement.

Fluorescence Measurements. Excitation and emission spectra were measured with a Perkin-Elmer LS-5 fluorometer, with entrance and exit slits set at 5 nm. The spectrofluorometer was standardized against 0.1 mg/mL quinine sulfate in 0.1 M H_2SO_4.

RESULTS AND DISCUSSION

Partial Purification. The crude extract obtained as stated in the Experimental Section was centifuged at 150000*g* for 1 h. The resulting pellet was suspended in 50 mL of grinding buffer to which Triton X-100 (0.2%, w/v) had been added. After being stirred gently for 30 min, the homogenate was centrifuged as before. The supernatant (40 mL) was applied to a column of the anion exchanger DE-52 cellulose (5 × 15 cm), which had been equilibrated with grinding buffer. Elution (1 mL/min) was performed with a linear gradient established between 400 mL of starting buffer (grinding buffer) and 400 mL of final buffer (200 mM sodium phosphate (pH 6.8) containing 0.2 mM EDTA and 0.3 mM DTT). Hydroperoxide lyase and the pH 6.5 activity lipoxygenase (L-2 + L-3) appeared almost together in an early peak (V_e 115 mL) while lipoxygenase-1 (assayed at pH 9.0) appeared in the later peak (V_e 180 mL). The fractions containing the lyase and pH 6.5 lipoxygenase were combined (50 mL), concentrated 5-fold by ultrafiltration through an Amicon filter (PM 30), and chromatographed on a Sephacryl S-200 column (1.8 × 100 cm). Elution (0.2 mL/min) was performed with 50 mM sodium phosphate (pH 6.8) containing 0.2 mM EDTA and 0.3 mM DTT. This column resolved the hydroperoxide lyase (V_e 74 mL) and pH 6.5 lipoxygenase (V_e 105 mL). The specific activity of lyase was 0.38 U/mg, with a purification factor of 26-fold and recovery of 14%.

Soybeans are the richest known source of lipoxygenase. They contain at least three major isoenzymes, L-1, L-2, and L-3, which are kinetically distinguishable (Axelrod et al., 1981). The facts that L-2 and L-3 have somewhat similar pH–activity ranges and their resolution is not sharp in conventional ion-exchange chromatography have made it difficult to prove the lipoxygenase isoenzyme in our preparation. To establish their identity, an aliquot of the pooled fractions containing pH 6.5 lipoxygenase activity was subject to HPLC ion-exchange chromatography (Ramadoss and Axelrod, 1982). The major peak (65% of protein eluted, measured by the absorbance at 280 nm) catalyzed the aerobic formation of keto diene from linoleic acid hydroperoxide. Such activity is typical of lipoxygenase-3 (L-3) (Axelrod et al., 1981). There was a minor peak (20% of protein eluted) of pH 6.5 activity tentatively identified as lipoxygenase-2 (L-2).

Enzyme Properties. The partially purified enzyme preparation was used to determine the optimum pH. Hydroperoxide lyase had its maximum activity in the range of pH 6–7, losing more than 50% of activity bellow pH 5 and above pH 8. The preparation showed loss of activity during the purification procedure, and the enzyme was unstable even when stored at 4 °C, losing about 30% of its activity in 48 h, and was inactive in 7 days. The purified enzyme activity was extremely heat labile. Loss of 80% of activity was incurred by heating at 50 °C for 5 min. Heating at 70 °C for 1 min had the same effect. Similar results were found with partially purified fatty acid hydroperoxide cleaving enzyme from cucumber (Phillips and Galliard, 1978).

Gel filtration on Sephacryl S-200 indicated at MW of 240 000–260 000 for lyase, assuming a uniform spherical shape for the molecule. SDS gel electrophoresis of the lyase in the presence of mercaptoethanol provided a value of 62000 Da, suggesting that the protein exists as a tetramer in its native state.

In order to study substrate specificity, the purified enzyme was incubated against isomeric 9- and 13-hydroperoxides of linoleic acid linolenic acids. The enzyme showed no activity with the 9-isomers; the 13-hydroperoxides were shown to be effective substrates. Other plants in which specific cleavage of 13-hydroperoxides has been detected include watermelon seedlings (Vick and Zimmerman, 1976), tomato fruits (Galliard and Matthew, 1977), alfalfa seedlings (Sekiya et al., 1979), tea leaves (Hatanaka et al., 1982), cultured tobacco cells (Sekiya et al., 1984), and soybean seeds var. Suzuyutaka (Motoba et al., 1985b). The kinetic values, obtained from a Lineweaver–Burk representation, showed that the 13-hydroperoxide of linoleic acid (K_m = 5.5 × 10^{-2} mM, relative V_{max} = 3.7 × 10^{-2} mM/min) is a better substrate than the corresponding isomer of linolenic acid (K_m = 0.16 mM, relative V_{max} = 2.7 × 10^{-2} mM/min). Motoba et al. (1958b) have found similar kinetic parameters of

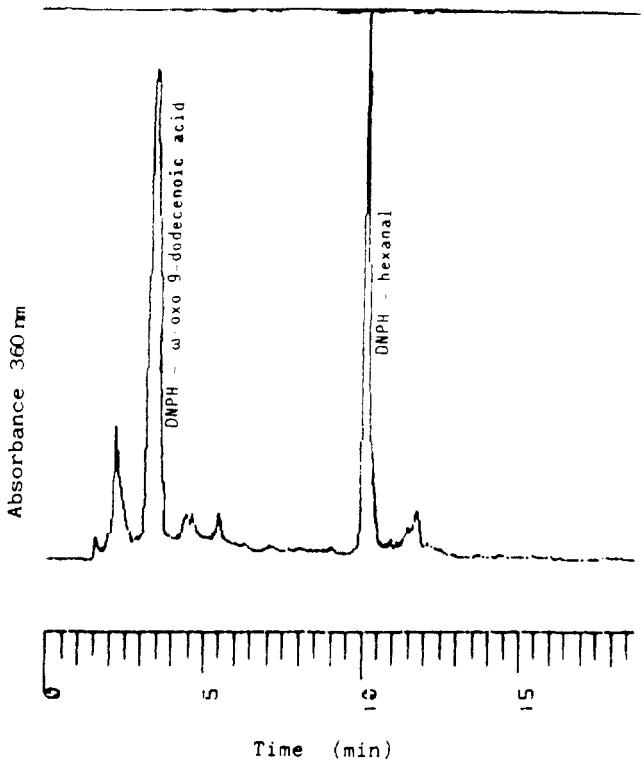

Figure 3. HPLC analysis of DNPHs of products from cleavage of 13-LAOOH by partially purified hydroperoxide lyase from soybean seed. Peak R_t 3.5 min was coincident with that of authentic DNPH of ω-oxo-9-dodecenoic acid; peak R_t 10.4 min, with DNPH of hexanal.

hexanal formation from 13-hydroperoxide of linoleic acid in homogenates of soybean var. Suzuyutaka and three mutants of this seed.

Products Formed by Lyase Action. The isomeric structure of the metabolites from hydroperoxides could be a good indication of the degradation mechanism. Thus, it was necessary to know whether a *cis*-3:*trans*-2-enal isomerase enzyme was present in the partially purified extract or whether the final structure was a consequence of the lysis mechanism itself. The measurement of isomerase activity was carried out with *cis*-3-hexenal as substrate. A clear isomerase activity was demonstrated in the crude extract, which was not seen in the partially purified extract. A confirmation was carried out incubating purified enzyme with 13-LNAOOH; the volatile carbonyls were identified by preparation in situ of DNPHs followed by HPLC analysis. When the crude enzymatic preparation was used, the carbonyl compound was *trans*-2-hexenal, while in the case of the partially purified preparation it was *cis*-3-hexenal. We conclude that the purified extract of hydroperoxide lyase was free of *cis*-3:*trans*-2-enal isomerase enzyme. When partially purified enzyme was incubated with 13-LAOOH, the only volatile carbonyl found was hexanal.

Nonvolatile products from cleavage of 13-LAOOH and 13-LNAOOH were obtained as described in the Experimental Section and analyzed by GC–MS. Figures 1 and 2 show gas–liquid chromatography and mass spectra, respectively, of oxo acids from 13-LAOOH. Peak 2, scan 329, was identified as methyl 12-oxo-*cis*-9-dodecenoate and peak 3, scan 370, was methyl 12-oxo-*trans*-10-dodecenoate. Peak 1, scan 221, was the BHT antioxidant used as stabilizer. Because the expected product was the cis-9-isomer and, as previously shown, the enzyme is free of *cis*-3:-*trans*-2-enal isomerase, the presence of both isomers leads us to consider nonenzymatic isomerization

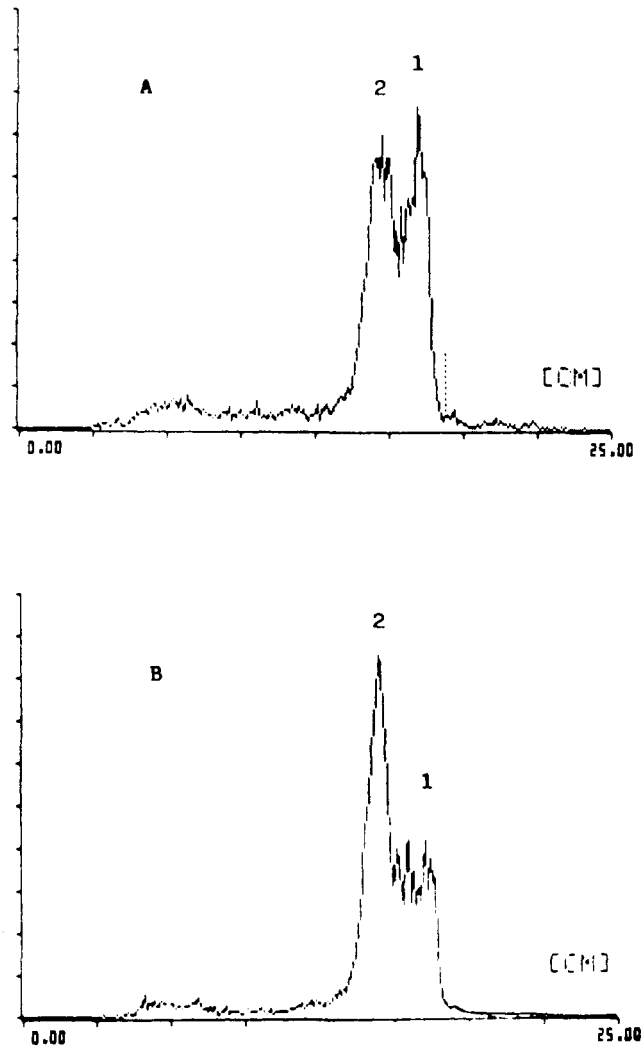

Figure 4. Radioactivity scan of TLC separation of ^{14}C-labeled products formed from [1-^{14}C]-13-LAOOH by partially purified enzyme from soybean seed. The scans represent results using (A) incubation at 25 °C for 2 min and (B) incubation at 25 °C for 6 min. Peak 1 was due to the unreacted substrate; peak 2 was identified as mixture of 12-oxo-*cis*-9-dodecenoic acid and 12-oxo-*trans*-10-dodecenoic acid.

occurring during the manipulation. Hatanaka et al. (1977) using isolated chloroplasts of *Thea sinensis* reported similar results but suggested that the trans-10-isomer is enzymatically generated.

To establish the stoichiometry of cleavage, partially purified enzyme was incubated with 250 nmol of radioactive [1-^{14}C]-13-LAOOH. After 5 min the hexanal formed was quantified by headspace GC (150 nmol, 60% of starting concentration of 13-LAOOH). The percentage of recovery of radioactive oxo acid as determined with a radioisotope scanner on TLC plate was 61%. This value was confirmed with the aid of the DNPH derivatives. As incubation mixture of identical characteristics was subjected to the formation of derivatives and then analyzed by HPLC. The DNPH of oxo acid (R_t 3.5 min) had an area of 4500 units, and the corresponding hexanal (R_t 10.4 min), 4300 (Figure 3). Thus, it can be concluded that the cleavage of one molecule of 13-LAOOH by lyase leads to one molecule each of hexanal and 12-oxododecenoic acid.

To elucidate the mechanism of the cleavage reaction, Gardner and Plattner (1984) have studied model chemical reactions that mimic hydroperoxide lyase activity. Oxygen isotope labeling experiments with hydroperox-

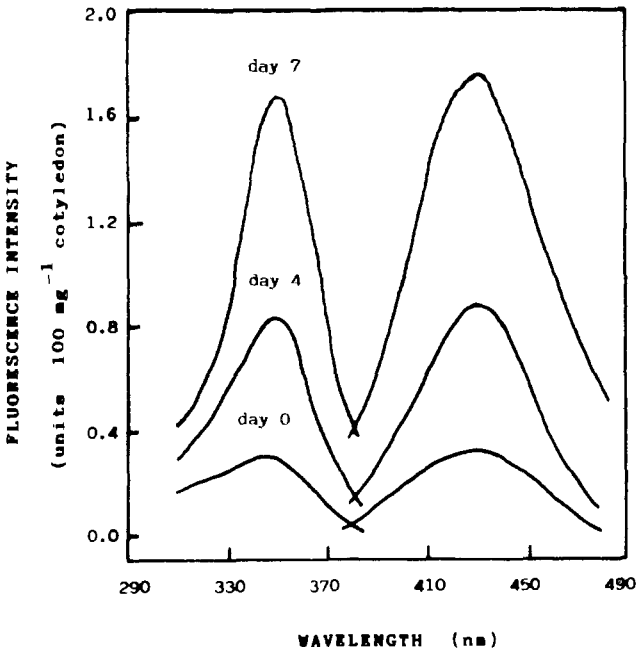

Figure 5. Representative excitation (350-nm) and emission (420-nm) spectra for phospholipid fraction from cotyledons of soybean seed at various stages of germination. The phospholipid fraction was isolated from chloroform lipid extract by aminopropyl bonded-phase minicolumn, eluted with methanol, as described in the Experimental Section.

ide lyase from tea chloroplasts (Hatanaka et al., 1986) supported the proposed mechanism, but no evidence of any intermediate was shown. In seeking possible intermediates in the decomposition mechanism, incubation mixtures identical with those in the previous section were prepared, using radioactive substrate, and were allowed to react for 2 and 6 min. TLC radioactive scanning (Figure 4) showed that already by the second minute more than 50% of the oxo acid (peak 2) has been produced; no other radioactive band that was not the remains of nondecomposed substrate (peak 1) was observed. The zone of radioactivity were scraped off, eluted with diethyl ether, esterified with ethereal diazomethane, and subjected to GC–MS analysis. Under the conditions used, the proposed intermediate (Gardner and Plattner, 1984; Hatanaka et al., 1986) was not detected.

Enzyme Development during Germination. When development of the hydroperoxide lyase was followed during germination of soybean seed, it was found that between days 0 and 6 there was an increase of more than 60% over initial specific activity followed by a decrease; a concordant behavior was observed in lipoxygenase activity, measured at pH 6.5 (L-2 + L-3). A similar development has been reported in the germination of watermelon seed (Vick and Zimmerman, 1976).

The fluorescence intensity of lipid extracts can be used to quantitatively assess accumulated peroxidative damage (Tappel, 1975). Lipid extracts from soybean cotyledons at various stages of germination, prepared by the method of Fletcher et al. (1973), were fractioned by aminopropyl bonded-phase columns (Kaluzny et al., 1985); the generically denominated phospholipid fraction (eluted with methanol) was found to exhibit a distint increase in the fluorescence intensity during the germination period (Figure 5). The fluorescence spectra obtained have excitation and emission maxima that are very similar to those measured previously for compounds that accumulate in ripening fruits (Maguire and Haard, 1975), senescing leaves (Wilhelm and Wilhelnova, 1981), and senescing bean cotyledon tissue of *Phaseolus vulgaris* (Pauls and Thompson, 1984).

There is good evidence that these fluorescent products are formed by the reaction of compounds containing free amino groups with products from peroxidized lipids (St. Angelo and Ory, 1975; Kikugawa and Bippu, 1987). In model membranes it has been shown that lipid peroxidation of liposomes containing phosphatidylethanolamine leads to the formation of fluorescent chromolipids (Shimasaki et al., 1984). We investigated the reaction of ethanolamine with hexanal and obtained fluorescent compounds with excitation maxima at 350–390 nm and emission maxima at 420–470 nm (manuscript in preparation).

The increase and decrease in activity of the two soybean enzymes during germination suggest a function in the early stage of plant growth. The physiological roles of the aldehydes and ω-oxo acids that result from the action of both enzymes are not well understood. The 12-oxo-*trans*-10-dodecenoic acid has been identified as the active component of the wound hormone traumatin (Zimmerman and Coudron, 1979). A possible role of aldehydes in the defense mechanism of the plant has been also suggested (Vick and Zimmerman, 1987). The concomitant increase of lipoxygenase-hydroperoxide lyase activities and fluorescence intensity of the lipid extracts in senescing cotyledons, suggests to us the possibility that these enzymes could be involved in modification of the membrane lipids via formation of liposoluble fluorescent compounds by supplying carbonyls that react with the nitrogenated bases of phospholipids. At present we are moving on to study of compounds of this fraction to chemically characterize the fluorescent products, which have to be studied in much more detail.

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Registry No. 13-LAOOH, 23017-93-8; 13-LNOOH, 28836-09-1; fatty acid hydroperoxide lyase, 71833-11-9; 13-hydroperoxide lyase, 116515-34-5; hexanal, 66-25-1; *cis*-3-hexenal, 6789-80-6; 12-oxo-*cis*-9-dodecenoic acid, 124536-99-8; lipoxygenase, 9029-60-1; methyl 12-oxo-*cis*-9-dodecenoate, 60485-40-7; methyl 12,13-epoxy-*cis*-9-octadecenoate, 18652-40-9; methyl 12,13-dihydroxy-*cis*-9-octadecenoate, 29714-28-1; methyl 12-oxo-*trans*-10-dodecenoate, 63024-87-3; methyl 13-hydroperoxide-*cis*-9,*trans*-octadecadienoate, 60900-56-3; *cis*-3-hexenol, 928-96-1.