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Levels of Oxygenated Fatty Acids in Young Corn and Sunflower Plants[1]

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ABSTRACT

Three oxygenated unsaturated fatty acids were investigated to determine whether they were present in seedlings of corn (*Zea mays* L. cv. NK PX443) and sunflower (*Helianthus annuus* L. cv. Sundak). The three compounds, 13-hydroxy-12-oxo-*cis*-9-octadecenoic acid (I), 13-hydroxy-12-oxo-*cis,cis*-9,15-octadecadienoic acid (II), and 12-oxo-*cis,cis*-10,15-phytodienoic acid (III), were detected and estimated by gas chromatography-mass spectrometry selected ion monitoring of their trimethylsilyloxy, methyloxime derivatives with 20-carbon analogs added as internal standards. In corn, the concentration of III increased between 5 and 10 days, while I and II remained relatively constant. A higher concentration of II was observed in corn seedlings grown in the light than those grown in the dark. Wounding increased the levels of all three compounds. In sunflower seedlings, the concentrations of I, II, and III increased between 6 and 10 days. The intracellular concentration of III in 10-day-old light-grown seedlings was estimated to be 200 nM in corn and 40 nM in sunflower.

The metabolism of polyunsaturated fatty acid hydroperoxides produced by lipoxygenase (EC 1.13.11.12) catalysis has received much attention in recent years. A number of plant enzymes have been characterized which convert fatty acid hydroperoxides to various oxygenated metabolites (1, 2, 6, 10, 11). These investigations were limited to *in vitro* studies of reactions of cell-free extracts or partially pure enzymes with hydroperoxides of linoleic or linolenic acid. Whether these oxygenated fatty acid metabolites have significant *in vivo* roles is not known, and their presence in intact plant tissue has not been demonstrated.

The objective of this study was to determine whether certain oxygenated fatty acid metabolites were present in young plants. Through the use of selected ion monitoring by GC-MS, we estimated the levels of three metabolites in young corn and sunflower seedlings. They were (a) α-ketol-18:1,[2] (b) α-ketol-18:2, and (c) 12-oxo-PDA. The first two metabolites are α-ketol compounds which result from the action of hydroperoxide isomerase on hydroperoxides of linoleic or linolenic acid, respectively. The third metabolite is the product of hydroperoxide cyclase with 13-hydroperoxylinolenic acid.

MATERIALS AND METHODS

Chemicals. Triheptadecanoin, heptadecanoic acid, *cis,cis,cis*-9,12,15-octadecatrienoic acid (linolenic), 20:2(11, 14) and 20:3(11, 14, 17) were purchased from Nu Chek Prep, Inc.,[3] Elysian, MN. Methoxyamine HCl in pyridine and BSTFA were obtained from Pierce Chemical Co., Rockford, IL. TLC separations were accomplished with Anasil HF precoated TLC plates obtained from Analabs, New Haven, CT, and with $KC_{18}F$ reversed-phase TLC plates purchased from Whatman Inc., Clifton, NJ. Gas-Chrom Q (100/120 mesh) and the silicone phase DC LSX-3-0295 for GC were obtained from Applied Science Division, State College, PA. A 30-m glass capillary column containing SP-2330 was purchased from Supelco, Inc., Bellfonte, PA.

Growth Conditions. Corn seeds, *Zea mays* L., var. NK PX443 (Northrup King) and sunflower seeds, *Helianthus annuus* L., var. Sundak, were planted between two sheets of moist paper toweling 2 cm from the top. The moist towels were placed on a sheet of waxed paper, rolled up, and placed upright in a beaker of H_2O. The seeds were germinated at 27°C in the dark or with a 16-h light, 8-h dark cycle at 880 μE m^{-2} s^{-1} light intensity. Seedlings were harvested at various times over a 10-d period.

Preparation of Fatty Acid Substrates. An 8 mM substrate solution was prepared for each polyunsaturated fatty acid according to Surrey (5).

Enzyme Extraction. For corn, the endosperm plus scutellum portion was separated from the seedling portion, and the two parts were weighed and extracted separately. The seedlings were transferred to a mortar, frozen in liquid N_2, and ground to a powder with a pestle. Whole sunflower seedlings with hulls removed were also frozen with liquid N_2 and ground to a powder in a mortar. Enzymes were extracted by grinding the plant tissue with 50 mM K-phosphate (pH 6.0) containing 0.1% Triton X-100 (v/v). For each g fresh weight of plant tissue, 3 ml of buffer were used. The homogenate was filtered through cheesecloth and centrifuged for 10 min at 12,000*g* at 5°C. The lipid material that floated to the top during centrifugation was removed and discarded, and the supernatant was decanted and used as the enzyme source.

Enzyme Assays. Lipoxygenase was measured with a Clark-type polarographic electrode at pH 6 for corn and pH 7 for sunflower. Linolenic acid was the substrate, and the initial O_2 concentration was assumed to be 250 μM.

Hydroperoxide isomerase was assayed spectrophotometrically

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[2] Abbreviations: α-ketol-18:1, 13-hydroxy-12-oxo-*cis*-9-octadecenoic acid; α-ketol-18:2, 13-hydroxy-12-oxo-*cis,cis*-9,15-octadecadienoic acid; 12-oxo-PDA, 12-oxo-*cis,cis*-10,15-phytodienoic acid; BSTFA, *N,O-bis*-(trimethylsilyl)-trifluoroacetamide; α-ketol-20:1, 15-hydroxy-14-oxo-*cis*-11-icosenoic acid; α-ketol-20:2, 15-hydroxy-14-oxo-*cis,cis*-11,17-icosadienoic acid; 14-oxo-DHPDA, 14-oxo-dihomophytodienoic acid; 20:2(11, 14), *cis, cis*-11,14-icosadienoic acid; 20:3(11, 14, 17), *cis,cis,cis*-11,14,17-icosatrienoic acid.

[3] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

at pH 6 as described previously by measuring the decrease in A at 234 nm caused by the disappearance of the conjugated 13-hydroperoxylinolenic acid (11). With this method, a small proportion of the observed activity is due to hydroperoxide cyclase, since the molar absorptivity of its product, 12-oxo-PDA, is about 25% that of the hydroperoxide substrate at 234 nm. However, the cyclase effect in corn and sunflower seedlings is diminished because its rate is about one-fourth that of isomerase. Another assay method, GC-MS selected ion monitoring of individual products and internal standards, gave comparable rates, but was more tedious. The validity of the spectrophotometric procedure must be established for each plant tissue analyzed.

Hydroperoxide cyclase was measured by GC-MS selected ion monitoring of 12-oxo-PDA, formed from 13-hydroperoxylinolenic acid. This assay, which used a 20-carbon analog of 12-oxo-PDA as an internal standard, was described in a recent paper (8).

Internal Standards. The 20-carbon analogs of the metabolites to be measured were prepared by enzymic conversion of 20-carbon polyunsaturated fatty acids with an extract of flaxseed acetone powder. α-Ketol-20:1 was prepared from 20:2(11, 14). 14-Oxo-DHPDA and α-ketol-20:2 were synthesized from 20:3(11, 14, 17). To 600 ml of 50 mM K-phosphate (pH 7) were added 15 ml of centrifuged flaxseed acetone powder extract (1 g in 10 ml of the same buffer). Thirty ml of the appropriate 8 mM fatty acid substrate solution were added, and the mixture was allowed to react for 90 min at room temperature. The pH was adjusted to 4, and the products were extracted by the addition of 500 ml of chloroform:methanol (2:1, v/v). After 3 h with stirring under N_2, the chloroform phase was removed, dried with anhydrous sodium sulfate, and evaporated under reduced pressure. The residue was dissolved in diethyl ether, and the products were purified by TLC on Anasil HF TLC plates with three developments in chloroform:acetic acid (100:1, v/v) solvent.

α-Ketol-20:1 was the single major product from 20:2(11, 14). The major products from 20:3(11, 14, 17) were α-ketol-20:2 and 14-oxo-DHPDA. The pure compounds were scraped from the TLC plates and eluted from the gel with diethyl ether. A second TLC purification in hexane:diethyl ether (65:35, v/v) solvent removed the acetic acid residue from the previous purification. Each compound was eluted from the gel, weighed, and dissolved in isopropanol at a concentration of 2 mg/ml.

Extraction of Oxygenated Fatty Acids from Plant Tissue. Extraction of lipid material from enzyme-inactivated plant tissues was accomplished according to the Hara and Radin method (4). Corn and sunflower plants were kept intact before homogenization, except that the hulls were removed from the sunflower seedlings. For enzyme inactivation, the seedlings (50 g) were added to 360 ml of boiling isopropanol containing 100 μl of 12 M HCl and 25 μg each of α-ketol-20:1, α-ketol-20:2, and 14-oxo-DHPDA as internal standards. After 5 min of refluxing, 540 ml of hexane were added to give a 3:2 composition of hexane-isopropanol extraction solvent. The lipid was extracted by successive 20-s homogenizations at full speed in a VirTis 45 homogenizer until the plant tissue was completely homogenized. The suspension was filtered with suction through a fritted disc Büchner funnel, and the residue was washed three times with 100 ml of hexane:isopropanol (3:2, v/v). A 6.7% (w/v) solution of sodium sulfate (600 ml) was added to the pooled filtrates, and the upper hexane-rich layer was removed and dried with anhydrous sodium sulfate, and the solvent was removed under reduced pressure.

The viscous oily extract was dissolved in 1 to 2 ml of diethyl ether, then applied to three to six 20- × 20-cm Anasil HF TLC plates of 0.5 mm thickness. The TLC plates were developed three times in hexane:diethyl ether:acetic acid (65:35:1, v/v/v) solvent. The area of the developed plate containing the compounds of interest was scraped, and the lipid was eluted from the gel with diethyl ether. This chromatography step aided in the removal of triacylglycerols, unoxygenated fatty acids, and polar lipids. The partially pure lipid fraction still contained substantial amounts of diacylglycerol and some pigment contaminants. The second purification step was a single development on Whatman $KC_{18}F$ reversed-phase TLC plates with acetonitrile:chloroform:acetic acid (90:10:0.2, v/v/v) as the solvent. The metabolites to be measured were scraped from the appropriate area of the TLC plate and eluted from the gel with diethyl ether.

Oxygenated fatty acids in the purified lipid fraction were esterified with diazomethane and then reacted overnight with 0.5 ml of 2% (w/v) methoxyamine HCl in pyridine to convert carbonyl groups to methyloxime derivatives. H_2O (5 ml) was added, and the sample was extracted into diethyl ether. The derivatized fraction was chromatographed a third time on Anasil HF TLC plates, 0.25 mm thickness, with one development in hexane:diethyl ether: acetic acid (65:35:1, v/v/v) solvent. The metabolites were removed from the appropriate area of the TLC plate, then eluted from the gel with diethyl ether, and reacted with BSTFA for 30 min to convert hydroxyl groups to trimethylsilyloxy derivatives. The excess BSTFA was evaporated under a stream of N_2, and the sample was dissolved in 50 μl of hexane. For some samples, a fourth purification by TLC in the above solvent was performed after the silylation step.

At each chromatography step, appropriate standards were co-chromatographed with the sample to ensure that the area of silica gel removed was large enough to include all of the compounds of interest, including both the metabolites to be measured and the internal standards.

In some experiments, the plants were wounded before lipid extraction. Ten-d-old light-grown corn seedlings were wounded by chopping with razor blades, and then the lipids were extracted after 11, 20, and 30 min.

Estimation of Oxygenated Fatty Acid Levels. The sample to be analyzed was injected into a Hewlett-Packard GC-MS with a 2-m × 2-mm i.d. glass column containing 3% DC LSX-3-0295 on 100/120 mesh Gas-Chrom Q. The GC was programmed from 150 to 200°C at 2°C/min. The helium flow rate was 25 ml/min.

Concentrations of α-ketol-18:1, α-ketol-18:2, and 12-oxo-PDA were estimated by comparison of the areas of the chromatographic peaks generated by selected ion monitoring of their characteristic mass fragments with the areas generated by the corresponding mass fragments of the internal standards. Figure 1 shows the mass spectra of the three derivatized metabolites along with the mass spectra of the derivatized internal standards.

The mass fragment monitored for α-ketol-18:1 and for the internal standard (α-ketol-20:1) was m/z 173. the amount of α-ketol-18:1 present in the sample was estimated by the following expression:

$$W_1 = \left(\frac{A_{173}}{A'_{173}}\right)\left(\frac{427}{455}\right)\left(\frac{25{,}000\text{ ng}}{N}\right)$$

In this formula, W_1 is the weight of α-ketol-18:1 in ng/seedling, A_{173} and A'_{173} are the peak areas generated by mass fragment m/z 173 at the known GC retention times of α-ketol-18:1 and α-ketol-20:1, respectively. N is the number of seedlings extracted. The factor 427/455 corrects for the difference in mol wt between the metabolite and the internal standard, and 25,000 ng is the quantity of internal standard added at the beginning of the experiment.

The mass fragment monitored for α-ketol-18:2 was m/z 356; m/z 384 was monitored for the internal standard. The amount of α-ketol-18:2 (W_2) present was estimated by:

$$W_2 = \left(\frac{A_{356}}{A_{384}}\right)(0.33)\left(\frac{25{,}000\text{ ng}}{N}\right)$$

Unlike the mass spectra used for the quantitation of the other two

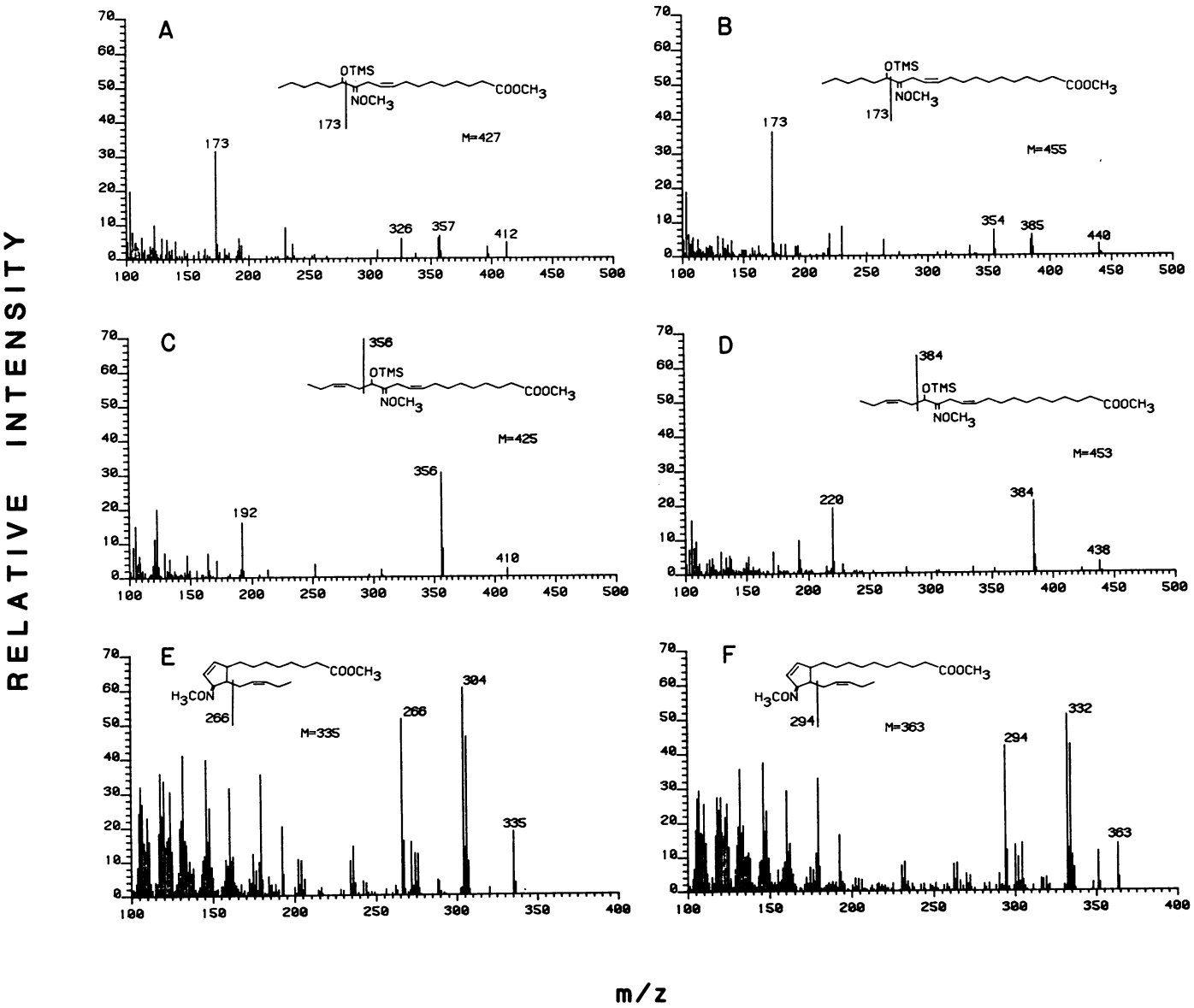

FIG. 1. Partial mass spectra of the trimethylsilyloxy, methyloxime derivatives of α-ketol-18:1 (A), α-ketol-20:1 (B), α-ketol-18:2 (C), α-ketol-20:2 (D), and the methyloxime derivatives of 12-oxo-PDA (E) and 14-oxo-DHPDA (F).

compounds, the fragmentation of masses 356 and 384 did not occur with equal probabilities. Therefore, a factor (0.33) was used in this calculation to correct for the observed difference in fragmentation characteristics. This factor was determined empirically from mixtures of known composition.

The acidic high-temperature conditions necessary for enzyme inactivation during lipid extraction complicated the quantitative measurement of 12-oxo-PDA. These conditions were highly favorable for the isomerization of 12-oxo-PDA from the *cis* to the *trans* configuration of the side chains with respect to the plane of the ring (9). For quantitation, it was necessary to sum the monitored ion peak areas of the *cis* and *trans* conformers, both for 12-oxo-PDA and for the internal standard 14-oxo-DHPDA. In addition, the methyloxime derivative of the *trans* isomer separated into its *syn* and *anti* conformations during GC, making it necessary to sum the areas of three peaks for each mass fragment monitored for 12-oxo-PDA and 14-oxo-DHPDA (*cis* + *trans-syn* + *trans-anti*). The mass fragments which were monitored were *m/z* 304 and 335 for 12-oxo-PDA and *m/z* 332 and 363 for the internal standard. The amount of 12-oxo-PDA (W_3) was estimated by:

$$W_3 = \frac{(A_{304} + A_{335})}{(A_{332} + A_{363})}\left(\frac{335}{363}\right)\left(\frac{25{,}000\text{ ng}}{N}\right)$$

Results from selected ion analysis of a standard mixture of 12-oxo-PDA and 14-oxo-DHPDA were within 12% relative error of the true value.

Some other characteristic mass fragments were occasionally monitored to assure that the correct compound was being measured. These masses were not used in the calculations because they were determined to be unreliable for estimates of metabolite levels.

Each analysis represented an average based on three or more injections of the same sample into the GC-MS. Three separate analyses on seedlings of the same age were averaged to determine the level of metabolite in the seedling at that age.

Linolenic Acid Levels in Corn Seedlings. Ten corn seedlings were grown in the dark, and ten seedlings were grown in the light. After 10 d, each group of seedlings was refluxed in 100 ml of acidic isopropanol containing 5 mg of heptadecanoic acid and 22

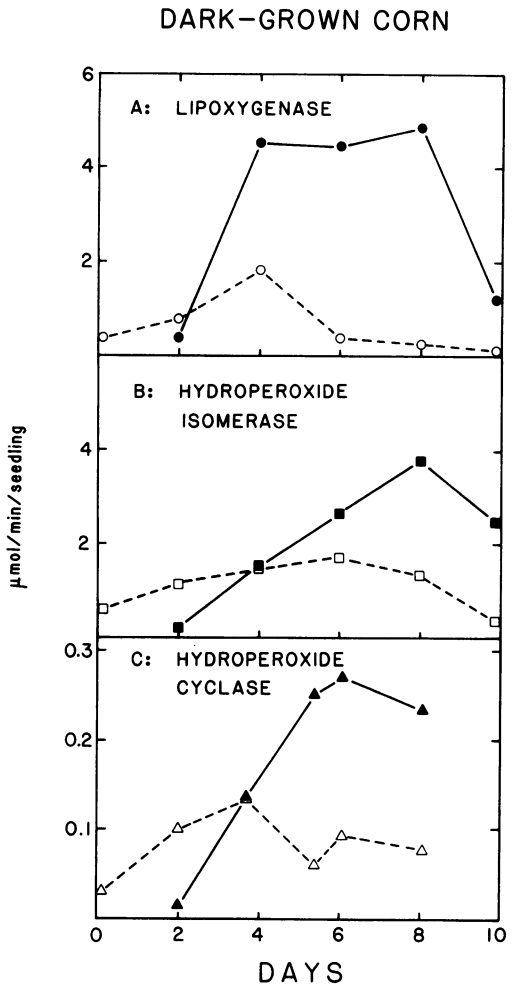

FIG. 2. Effect of age of dark-grown corn seedlings on the activity of lipoxygenase (A), hydroperoxide isomerase (B), and hydroperoxide cyclase (C). The substrate for lipoxygenase was linolenic acid, and the substrate for hydroperoxide isomerase and hydroperoxide cyclase was 13-hydroperoxylinolenic acid. Open symbols, endosperm-scutellum section; closed symbols, root-shoot section.

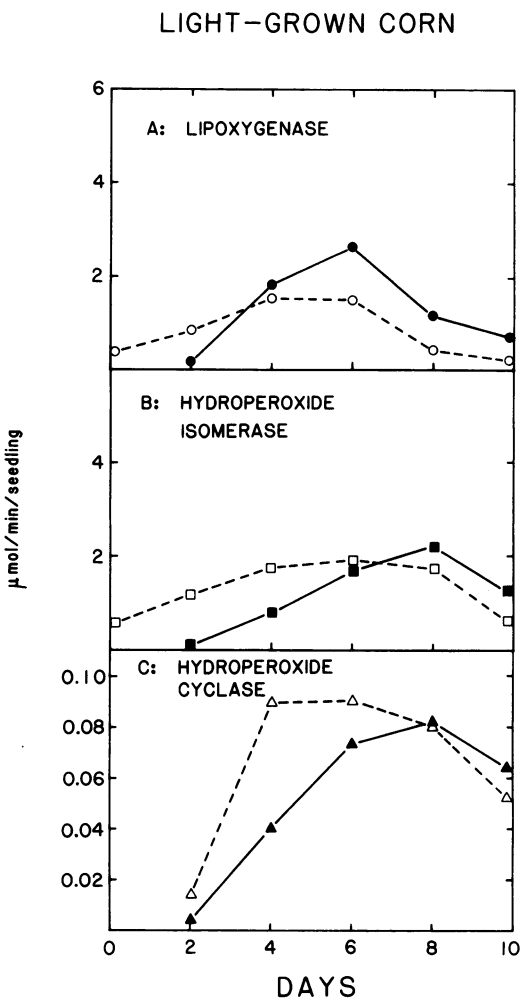

FIG. 3. Effect of age of light-grown corn seedlings on the activity of lipoxygenase (A), hydroperoxide isomerase (B), and hydroperoxide cyclase (C). The substrate for lipoxygenase was linolenic acid, and the substrate for hydroperoxide isomerase and hydroperoxide cyclase was 13-hydroperoxylinolenic acid. Open symbols, endosperm-scutellum section; closed symbols, root-shoot section.

mg of triheptadecanoin as internal standards. After 5 min, 150 ml of hexane were added, and the lipids were extracted as described above. The hexane fraction was concentrated, and a portion was transesterified with 0.5 N sodium methoxide in methanol for the determination of the amount of linolenic acid present in esterified form. A second portion was subjected to TLC analysis with hexane:diethyl ether:acetic acid (65:35:1, v/v/v) solvent for the separation of the fatty acid fraction. The fatty acids were eluted from the gel and esterified with diazomethane. A third portion was separated into its polar lipid components by TLC with acetone:acetic acid:water (100:2:1, v/v/v). The monogalactosyl diacylglycerol, digalactosyl diacylglycerol, and phospholipid fractions were eluted from the gel and transesterified. The linolenic acid concentration in the various fractions was determined by GC with a 30-m × 0.25-mm i.d. glass capillary column containing SP-2330. The GC temperature was 180°C for 6.75 min, then temperature programmed to 210°C at 30°C/min. The helium flow rate was 0.8 ml/min.

RESULTS

Developmental Changes in Enzyme Activity.

Corn. Figure 2A shows the changes in lipoxygenase activity which occurred when corn seedlings were germinated and grown in the dark. Activity in the endosperm plus scutellum increased through 4 d and then declined. Lipoxygenase activity in newly formed tissues (roots plus shoots) was considerably higher from 4 through 8 d, and then decreased. Similar results were observed for hydroperoxide isomerase (Fig. 2B) and hydroperoxide cyclase (Fig. 2C). When corn seedlings were germinated and grown in the light, similar patterns of enzyme activity were demonstrated (Fig. 3), but the levels in the root plus shoot portion were about half the levels of the dark-grown sections.

Sunflower. There was no significant difference between the activity of the enzymes from sunflowers grown in the dark and those grown in the light (Fig. 4). Lipoxygenase, hydroperoxide isomerase, and hydroperoxide cyclase activities were very low in the sunflower seed, but shortly after germination the activities of all three enzymes increased rapidly. Lipoxygenase activity declined after the 4th day, but hydroperoxide isomerase and hydroperoxide cyclase activities remained high through the 10th day.

Developmental Changes in Oxygenated Fatty Acid Levels.

Corn. Lipoxygenase from corn germ is known to catalyze oxygenation of linoleic or linolenic acids primarily at carbon 9. The 9-hydroperoxide isomer formed from (9,12,15)-linolenic acid is not converted to a cyclic product by hydroperoxide cyclase. Gard-

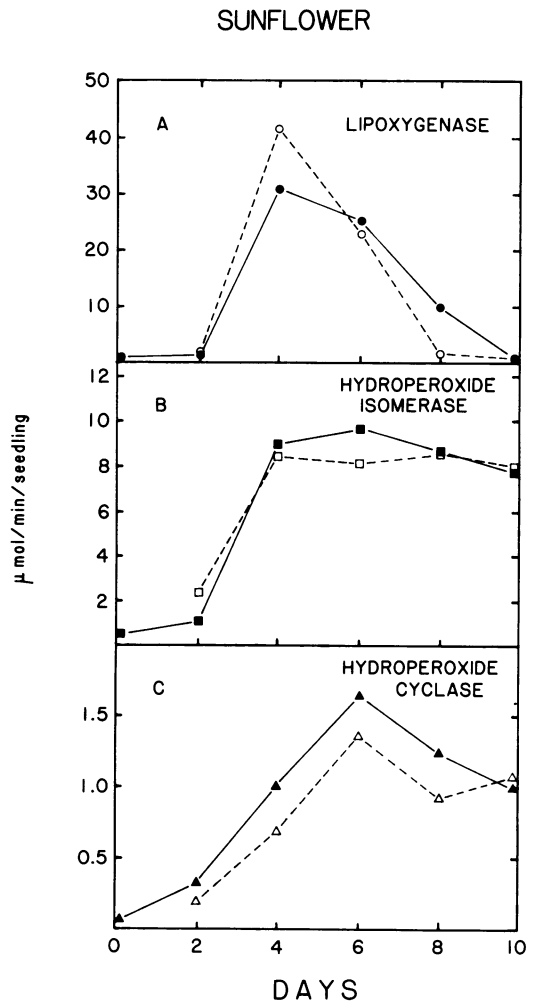

FIG. 4. Effect of age of dark-grown (closed symbols) and light-grown (open symbols) sunflower seedlings on the activity of lipoxygenase (A), hydroperoxide isomerase (B), and hydroperoxide cyclase (C). The substrate for lipoxygenase was linolenic acid, and the substrate for hydroperoxide isomerase and hydroperoxide cyclase was 13-hydroperoxylinolenic acid.

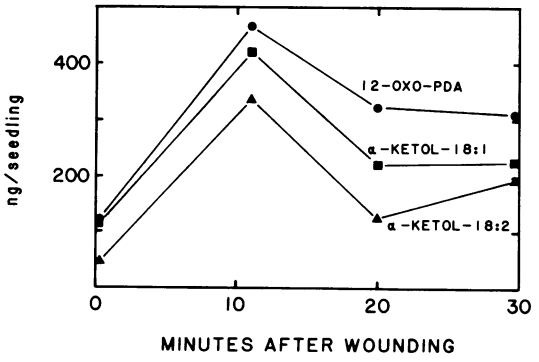

FIG. 5. Changes in the levels of α-ketol-18:1 (■), α-ketol-18:2 (▲), and 12-oxo-PDA (●) in 10-d-old light-grown corn seedlings after the seedlings had been wounded by chopping with a razor blade. The extraction and estimation procedures were those described in "Materials and Methods."

ner (3) reported that at least 83% of the corn lipoxygenase product was the 9-hydroperoxide isomer and only 17% was the 13-hydroperoxide isomer. In this study, no attempt was made to measure the metabolites of the 9-hydroperoxide isomer; only the metabolites of the 13-hydroperoxide isomer were measured. We wondered if the regiospecificity of corn lipoxygenase extracted from the root-shoot portion differed from that of the ungerminated seed. Specificity studies showed that the selectivity for the 13 carbon increased from 4% in the seed to 37% in the root-shoot after 5 d. Apparently, this change in specificity allowed the corn seedling to synthesize adequate amounts of 13-hydroperoxylinolenic acid, the precursor to the compounds measured here.

Table I shows significant increases in 12-oxo-PDA concentrations from 5 to 10 d, but the difference between dark and light treatments were not statistically significant ($p > 0.10$) because of high SEs in some analyses. For α-ketol-18:1, the differences in concentration between samples were also not statistically significant. In this experiment, the concentration of α-ketol-18:2 did not increase between 5 and 10 d, but there was a significant difference ($p < 0.10$) between 10-d dark- and light-grown seedlings. This could be a reflection of the higher levels of the precursor, linolenic acid, in the photosynthetic seedlings. The concentration of 'free' linolenic acid in the dark- and light-grown seedlings was similar, 22 and 23 μg/seedling, respectively, but esterified linolenic acid was, as expected, much higher in light-grown (1190 μg/seedling) than in dark-grown seedlings (157 μg/seedling). In photosynthetic corn, only 15 μg of the esterified linolenic acid was associated with triglycerides; the rest was associated with polar lipids. Fatty acid composition analysis of the two major polar lipids, monogalactosyl diacylglycerol and digalactosyl diacylglycerol, showed that they contained 87% and 55% linolenic acid, respectively. The phospholipid fraction was composed of 12% linolenic acid.

To determine whether wounding the tissue might bring about changes in ketol or 12-oxo-PDA concentrations, we conducted an experiment to measure differences between the amounts of all three metabolites in intact (time zero) and wounded light-grown seedlings. The results (Fig. 5) showed that wounding rapidly increased the levels of all three metabolites more thaan 3-fold in 10-d-old light-grown corn seedlings.

Sunflower. The concentration of 12-oxo-PDA in both dark- and light-grown sunflower seedlings increased from the 6th to the 10th d. In this experiment, the concentration increased faster in the dark-grown seedlings. The level of α-ketol-18:2 increased significantly ($p < 0.10$) between 6 and 10 d in both dark- and light-grown seedlings, but there was no significant difference between concentrations in dark- or light-grown seedlings of the same age. For α-ketol-18:1, some of the replicates were combined and repurified by TLC for a single analysis, and the statistical significance of differences between samples could not be evaluated.

DISCUSSION

To our knowledge, this is the first report that establishes the presence of α-ketols and 12-oxo-PDA in plant tissue. We used selected ion monitoring with internal standards to quantify the amounts of these metabolites in two plants, corn and sunflower seedlings. The deuterated analogs of these compounds are the preferred internal standards for quantitation, but they were not available. Therefore, 20-carbon analogs with similar mass spectral fragmentation patterns were used to estimate the oxygenated fatty acid levels. It was necessary to keep the seedlings intact before homogenization to minimize the effects of wounding. As a result, we were unable to determine the specific location, if any, of these metabolites within the seedling. Only nonesterified, oxidized fatty acids were measured.

When the plant tissues were homogenized in a solvent without prior enzyme inactivation with hot isopropanol, much higher concentrations of oxygenated fatty acids were observed (data not shown). This increase was probably due to enzymic synthesis resulting from a wound response in cells that were damaged and later disrupted during homogenization. Accordingly, rapid syn-

Table I. *Oxygenated Fatty Acid Levels in Corn and Sunflower Seedlings.*

Seedlings were refluxed in isopropanol, and the lipids were extracted with a hexane-isopropanol mixture (3:2, v/v). Oxygenated fatty acid metabolite levels were estimated by GC-MS selected ion monitoring with 20-Carbon analogs of the compounds added as internal standards. Except where indicated, values are the mean of three or more analyses. Values reported are ±SE.

Tissue/ Metabolite	Dark		Light	
	5 d	10 d	5 d	10 d
	ng/seedling			
Corn				
α-Ketol-18:1	104 ± 10[a]	187 ± 78[a]	101 ± 95[a]	158[d]
α-Ketol-18:2	9.6 ± 4.7[a]	8.8 ± 2.2[a]	31.5 ± 25.5[a, b]	37.1 ± 15.3[b]
12-Oxo-PDA	7.1 ± 0.3[a]	26.1 ± 4.8[b, c]	15.4 ± 7.9[a, c]	71.1 ± 42.5[b]
	6 d	10 d	6 d	10 d
Sunflower				
α-Ketol-18:1	5.7[d]	16.7[d]	5.2 ± 2.8	45.4[d]
α-Ketol-18:2	3.3 ± 1.5[a]	19.9 ± 11.0[b]	1.9 ±1.4[a]	20.5 ± 12.2[b]
12-Oxo-PDA	2.5 ± 0.3[a]	41.0 ± 2.0[b]	4.2 ± 2.8[a]	10.5 ± 4.3[c]

[a, b, c] Data within a row with different superscripts are significantly different from each other at the 10% level or less.

[d] Single analysis.

thesis of the three compounds was observed in deliberately wounded corn seedlings (Fig. 5). The decline in metabolite concentration after 15 min was observed in other wounding experiments and suggests there may be a means by which the compounds are further metabolized. It is not known whether these fatty acids are involved in the wound healing process.

In some cases, the variability between replications was high. This may have been due to unintentional wounding of the tissue prior to enzyme inactivation, even though precautions were taken against this. The experiments do, however, establish the order of magnitude of the oxygenated fatty acid concentrations in these plant tissues. For example, the intracellular concentration of 12-oxo-PDA could be estimated from the fresh weight of the seedlings and the results in Table I. Assuming 10% dry weight and a uniform distribution of the metabolite throughout the seedling, the concentration of 12-oxo-PDA in light-grown corn seedlings after 10 d was approximately 200 nM. In light-grown sunflower seedlings, the concentration after 10 d was about 40 nM. However, it may be that 12-oxo-PDA is localized within the seedling (or cell) at higher than the estimated concentrations. There was no clear correlation between enzyme activity and metabolite concentration.

Hydroperoxide cyclase activity was much higher in the sunflower seedlings than in corn seedlings. In an earlier survey of this enzyme (7), we observed that, of the 24 plant tissues analyzed, sunflower seedlings had much higher cyclase activity than any other plant.

Acknowledgment—The technical assistance of Anne Harri is gratefully acknowledged.

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