The major part (90%) of the fatty acid hydroperoxide isomerase activity present in homogenates of the fungus, *Saprolegnia parasitica*, was localized in the particle fraction sedimenting at 105,000 × g.

13(S)-Hydroperoxy-9(Z),11(E)-octadecadienoic acid and 9(S)-hydroperoxy-10(E),12(Z)-octadecadienoic acid were both good substrates for the particle-bound hydroperoxide isomerase. The products formed from the 13(S)-hydroperoxy acid were identified as α,β- and a γ,δ-epoxy alcohol, i.e., 11(R),12(R)-epoxy-13(S)-hydroxy-9(Z)-octadecenoic acid and 9(S),10(R)-epoxy-13(S)-hydroxy-11(E)-octadecenoic acid, respectively. The 9(S)-hydroperoxide was converted in an analogous way into an α,β-epoxy alcohol, 10(R),11(R)-epoxy-9(S)-hydroxy-12(Z)-octadecenoic acid and a γ,δ-epoxy alcohol, 12(R),13(S)-epoxy-9(S)-hydroxy-10(E)-octadecenoic acid.

9(R,S)-Hydroperoxy-10(E),12(E)-octadecadienoic acid and 13(R,S)-hydroperoxy-9(E),11(E)-octadecadienoic acid were poor substrates for the *S. parasitica* hydroperoxide isomerase. Experiments with 13(R,S)-hydroperoxy-9(Z),11(E)-octadecadienoic acid showed that the 13(R)-hydroperoxy enantiomer was slowly isomerized by the enzyme. The major product was identified as α,β-epoxy alcohol 11(R),12(R)-epoxy-13(S)-hydroxy-9(Z)-octadecenoic acid.

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A pathway for sequential degradation of certain polyunsaturated fatty acids in the primitive fungus, *Saprolegnia parasitica*, was identified in recent work (1–3). Thus, arachidonic acid added to homogenates of the fungus was converted into 15(S)-HETE by a lipoxygenase. The hydroperoxide did not accumulate, but was rapidly converted by action of a hydroperoxide isomerase into a pair of epoxy alcohols—11(S),12(R)-epoxy-15(S)-hydroxy-5(Z),8(Z),13(E)-eicosatrienoic acid and 13(R),14(R)-epoxy-15(S)-hydroxy-5(Z),8(Z),11(R)-eicosatrienoic acid. The two epoxy alcohols possessed an allylic epoxide group and therefore underwent spontaneous hydrolysis into a number of isomeric trihydroxyeicosatrienoates. Interestingly, relatively large amounts of trihydroxyeicosatrienoates and trihydroxoyctadecenoates could be isolated from the culture medium of the fungus, thus showing that the lipoxygenase/hydroperoxide isomerase pathway is active in the growing organism (1).

The present paper describes further studies of the hydroperoxide isomerase of *S. parasitica* using a number of isomeric hydroperoxy-octadecadienoates derived from linoleic acid as substrates.

**MATERIALS**

13(S)-[1-14C]HPOD (specific radioactivity, 1.4 kBq/μmol) was prepared by incubation of [1-14C]linoleic acid with soybean lipoxygenase as previously described (4). 9(S)-[1-14C]HPOD (specific radioactivity, 1.3 kBq/μmol) was prepared by incubation of [1-14C]linoleic acid with whole homogenates of tomato, essentially as described by Matthew et al. (5). 11(R)- and its diastereomer, methyl 11(S),12(S)-epoxy-13(S)-hydroxy-9(Z)-octadecenoate, were prepared by epoxidation of the methyl ester of 13(S)-HETE using vanadium oxyacetylacetonate as catalyst (6). Methyl erythro- and threo-12,13-dihydroxyoctadecenoates were prepared by OsO4 and performic acid hydroxylations, respectively, of methyl 12(Z)-octadecenoate (6). Methyl erythro- and threo-9,10-dihydroxyoctadecanoates were prepared, in a similar way, starting with methyl oleate.

9(R,S)-Hydroperoxy-10(E),12(E)-octadecadienoic acid and 13(R,S)-hydroperoxy-9(E),11(E)-octadecadienoic acid. Linoleic acid (100 mg) was kept under O2 at 37°C for 15 hr. The product was subjected to silicic acid chromatography (column, 5 g). Elution with diethyl ether/hexane (2:8, v/v) yielded a mixture of hydroperoxyoctadecadienoates (ca. 16 mg, according to UV spectrometric analysis). This material was subjected to preparative reversed-phase high performance chromatography (HPLC) using a column of Polygosil C18 5 μ (300 × 8 mm) and acetonitrile/water/acetic acid (68/32/0.02, v/v/v) at a flow rate of 1.5 mL/min. Three peaks of material showing strong absorption at 230–235 nm appeared, i.e., I (38.9–41.9 ml effluent), II (42.3–44.1 ml) and III (44.6–46.5 ml). The UV spectra of material forming peaks II and III showed an absorption band with λ_max = 231 nm, indicating an E,E diene structure. Further proof of the presence of one pair of conjugated E,E double bonds in compounds forming peaks II and III was provided by the IR spectra which showed an absorption band at 995 cm⁻¹ (7). Catalytic hydrogenation of II and III afforded 13-hydroxy- and 9-hydroxyoctadecanoic acids, respectively. Thus, material present in peak II was identical to 13(R,S)-hydroperoxy-9(E),11(E)-octadecadienoic acid, whereas peak III was due to 9(R,S)-hydroperoxy-10(E),12(E)-octadecadienoic acid.

9(R,S)-HPOD and 13(R,S)-HPOD. Material forming peak I was subjected to straight-phase HPLC using a column of Nucleosil 5 μ (250 × 4.6 mm) and isopropanol/Water/
hexane/acetatic acid (1.5:98.5:0.02, v/v/v) at a flow rate of 3 ml/min. Two peaks appeared, i.e., 13(R,S)-HPOD (23.4–26.1 ml effluent) and 9(R,S)-HPOD (34.5–37.2 ml). Catalytic hydrogenation, which afforded 13-hydroxyoctadecanoic acid from the former hydroperoxide and 9-hydroxyoctadecanoic acid from the latter, demonstrated the positions of the hydroperoxy groups. The presence of a conjugated E,Z diene structure in the two compounds was shown by UV spectrometry, which showed an absorption band with \( \lambda_{\text{max}} = 234 \) nm, and by IR spectrometry, which showed bands at 990 and 955 cm\(^{-1}\) (ratio of intensities, 0.9:1) (7).

Methyl 10(R),11(R)-epoxy-9(S)-hydroxy-12(Z)-octadecenoate. The methyl ester of 9(S)\(^{14}\text{C}\)HPOD (3 mg) was treated for 1 hr at room temperature with 3 ml of hexane saturated with vanadium oxyacetylenecarbonate. Thin layer radiochromatography showed two peaks of radioactivity due to a pair of diastereomeric \( \alpha,\beta \)-epoxy alcohols (6). The more polar epoxy alcohol (\( R_\ell = 0.49 \); solvent system ethyl acetate/hexane [3:7, v/v]) was collected and characterized as follows: UV spectrometry did not show any specific absorption. The infrared spectrum showed bands at 3620–3350 cm\(^{-1}\) (hydroxyl), 1735 cm\(^{-1}\) (ester carbonyl) and 890 cm\(^{-1}\) (trans epoxide). No absorption band appeared in the region 950–1000 cm\(^{-1}\). Thus, the presence of \( E \) double bond(s) could be excluded. Analysis of the trimethylsilyl (Me\(_3\)Si) derivative by gas chromatography–mass spectrometry (GC–MS) showed a peak with a retention time corresponding to C 22.5. The mass spectrum showed prominent ions at \( m/e = 383 \) (M – 15, \( R_\ell = 0.36 \)), 367 (M – 31, \( R_\ell = 0.10 \)), 308 (M – 90, \( R_\ell = 0.08 \)), [tentatively ascribed to migration of Me\(_3\)Si from C-9 to epoxy oxygen followed by elimination of OH–(CH\(_2\))\(_7\)-COOCH\(_3\)] , 212 (M – 186, \( R_\ell = 0.16 \)), indicating a methyl octadecenoate carrying a hydroxyl at C-9, as well as a second oxygen function (epoxide according to the IR analysis). KMnO\(_4\) oxidation performed on the MC derivative yielded the MC derivative of methyl 10,11-dihydroxyoctadecenoate, demonstrating that the hydroxyl group of the epoxy alcohol had the \( S \) configuration. Catalytic hydrogenation yielded a major component that was identified as methyl \( \text{threo}-9,10,12,13\)-tetrahydroxyoctadecanoate by thin layer chromatography (TLC) carried out with NaAsO\(_2\)-impregnated silica gel using the authentic \( \text{threo} \) (\( R_\ell = 0.72 \)) and \( \text{erythro} \) (\( R_\ell = 0.63 \)) isomers as references. The presence of a hydroxyl group at C-9 (mass spectrometry, KMnO\(_4\) oxidation), coupled with the results of the catalytic hydrogenation, located the epoxy group at C-10:C-11. The absolute configurations of C-10 and C-11 followed from the established configuration of C-9 ("S") coupled with the findings that the relative configuration of C-9/C-10 was \( \text{threo} \) and that the configuration of the C-10/C-11 epoxy group was \( \text{trans} \). On basis of these data, the epoxy alcohol was assigned the structure methyl 10(R),11(R)-epoxy-9(S)-hydroxy-12(Z)-octadecenoate.

**METHODS**

**Enzyme preparation.** Homogenates of *S. parasitica* in 0.1 M potassium phosphate buffer pH 7.4 (1:2; w/w) (2) were centrifuged at 750 \( \times \) g for 10 min. The supernatant was further centrifuged at 105,000 \( \times \) g for 60 min. The sediment thus obtained was resuspended in potassium phosphate buffer (if not otherwise indicated, protein concentration was adjusted to 0.16 mg/ml). Protein was determined according to Bradford (8).

**Spectrophotometric assay of hydroperoxide isomerase activity.** Suspensions of the sediments obtained by centrifugation at 750 \( \times \) g and 105,000 \( \times \) g, as well as the clear 105,000 \( \times \) g supernatant, were diluted with potassium phosphate buffer as needed. Samples of 2 ml were transferred to cuvettes and treated with 10–20 \( \mu \)g of the different hydroperoxides (temperature, 20°C). The absorbance at 236 nm was followed, vs time. The initial slopes (0–30 sec) of the decrease in absorbancy were taken as a measure of hydroperoxide isomerase activity.

**Incubations and isolation of epoxy alcohols.** [1-\(^{14}\text{C}\)]Linoleic acid, 13(S)/[1-\(^{14}\text{C}\)]HPOD and 9(S)/[1-\(^{14}\text{C}\)]HPOD (0.25 mg) were shaken with suspensions of the 105,000 \( \times \) g particle fraction (5 ml; 0.16 mg of protein/ml) at 22°C for 30 min. The mixtures were acidified to pH 3 and rapidly extracted with two portions of diethyl ether. The residue obtained after evaporation of the solvent was treated with diazomethane and subjected to thin layer chromatography (TLC) (solvent system, ethyl acetate/hexane [3:7, v/v]). With the solvent system used, the \( R_\ell \) values of methyl 11(S)/12(S)-epoxy-13(S)-hydroxy-9(Z)-octadecenoate, \( \Pi_{\text{13S}} \) and \( \Pi_{\text{12S}} \) were 0.63, 0.54 and 0.49, respectively.

Linoleic acid and 13(S)/HPOD were found to yield a common pair (ratio, ca. 3:1) of epoxy alcohols. The \( R_\ell \) values of the methyl esters of the major and minor epoxy alcohols were 0.54 (\( \Pi_{\text{13S}} \)) and 0.42 (\( \Pi_{\text{12S}} \)), respectively. Incubation of \( 9(S)-\text{HPOD} \) resulted in the formation of another pair (ratio, ca. 2:1) of epoxy alcohols. The \( R_\ell \) values of their methyl esters were 0.49 (\( \Pi_{\text{12S}} \), major epoxy alcohol) and 0.39 (\( \Pi_{\text{13S}} \), minor epoxy alcohol). Unlabeled 13/14/15:1-HPOD (0.25 mg) was incubated in the same way, although in this case 10 ml of enzyme preparation was used. Analysis of the esterified product by TLC showed two major spots, i.e., epoxy alcohol \( \Pi_{\text{13S}} \) (\( R_\ell = 0.54 \)) and \( \Pi_{\text{13S}} \) (\( R_\ell = 0.63 \)), as well as fainter spots of more polar material that was not further characterized.

**Methods for structure determination.** TLC was carried out with precoated plates (Kieselgel 60, 0.25 mm) from E. Merck (Darmstadt, FRG). Analysis of diastereomeric methyl 9,10- and 12,13-dihydroxyoctadecanoates was carried out using plates coated with silica gel G–NaAsO\(_2\) (9:1, w/w) and methanol/chloroform (1:99, v/v) as solvent. Material was located by spraying with 2',7'-dichlorofluorescein and viewing under UV light. Gas liquid chromatography (GLC) was performed with an F&M Biomedical gas chromatograph model 402 using a column of 5% QF-1 on Supelcoport. GC–MS was carried out with an LKB 9000S instrument equipped with a column of 3% OV–210 on Supelcoport. The electron energy was set at 22.5 eV, and the trap current was 60 μA. Ultraviolet spectra were recorded with a Hewlett-Packard model 8450A UV/VIS spectrophotometer. Radioactivity was determined with a Packard Tri-Carb series 4000 liquid scintillation counter. A Berthold Dünnschichtscannert II was used for determination of radioactivity on TLC plates.

**Methods for catalytic hydrogenation (2), oxidative ozonolysis (9), KMnO\(_4\) oxidation (3), and preparation and analysis of MC (9) and CC (3) derivatives were as previously described.