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Preparation of Fatty Epoxy Alcohols Using Oat Seed Peroxygenase in Nonaqueous Media

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ABSTRACT: Peroxygenase is an enzyme of higher plants that is capable of using hydroperoxide and hydrogen peroxide for oxidation of a double bond to an epoxide. A microsomal fraction was prepared from dry oat (*Avena sativa*) seeds. The peroxygenase activity of this fraction was tested using fatty acid hydroperoxide **2a** [13(*S*)-hydroperoxy-9(*Z*),11(*E*)-octadecadienoic acid] and its methyl ester **2b** as sources of peroxygen. These were prepared by the action of soybean lipoxygenase on linoleic acid. A high-performance liquid chromatographic assay was used to differentiate between peroxygen cleavage and peroxygen cleavage with accompanying double-bond oxidation. Higher activity was obtained with **2b** compared to **2a**, and peroxygen cleavage activity was observed in both aqueous and organic solvent media. Double-bond oxidation activity was high only in aqueous media and nonpolar organic solvents. Structural elucidation of the epoxidized product showed it to be the oxylipid, methyl *cis*-9,10-epoxy-13(*S*)-hydroxy-11(*E*)-octadecenoate **4b**, demonstrating specificity for epoxidation of the *cis* double bond. Trihydroxy product was not detected, demonstrating that the epoxide was not hydrolyzed.

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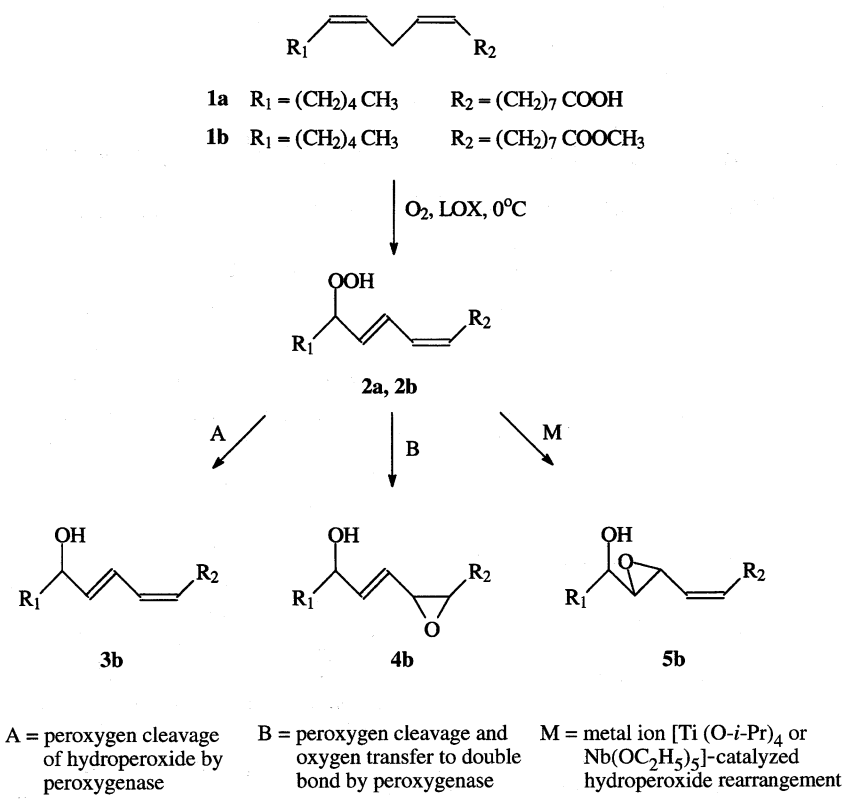
KEY WORDS: *Avena sativa,* epoxide, hydroperoxide, linoleic acid, lipoxygenase, peroxygenase.

Most fats and oils contain only double-bond and ester functionality, and for many nonfood uses derivatization of a fat or oil to modify or increase its chemical functionality is required. The only commercial exception is castor oil, which contains the monohydroxyl fatty acid, ricinoleic acid. The value that this hydroxyl function imparts to castor oil is indicated by its market price, which is higher than that of other common vegetable oils. Much research has been directed toward adding hydroxyl groups or other oxygen-containing functionality such as epoxides to ordinary fats and oils using chemical methodology (1). Although methods have been devised that achieve high levels of oxygenation, the resulting product is not homogeneous because the site of modification cannot be controlled. The use of enzymes or of enzymes in conjunction with other catalysts holds the promise of achieving greater selectivity. For example, when linoleic acid **1a** is treated with soybean lipoxygenase (LOX), its hydroperoxide **2a** is formed (Scheme 1). Treatment of the methyl ester **2b** with the metal catalysts $Ti(O\text{-}i\text{-}Pr)_4$ and $Nb(OC_2H_5)_5$ gives exclusively the alcohol epoxide **5b** in which the alcohol is located at C13 and the epoxide at C11–C12 (2,3).

We have sought additional catalysts that might be used to produce modified fats and oils and have chosen for study the enzyme peroxygenase (also termed epoxygenase) from oat seeds. This enzyme catalyzes the heterolytic cleavage of a peroxygen bond. The liberated oxygen is transferred to an oxidizable functional group. If the oxidizable functionality is a carbon–carbon double bond, the product is an epoxide. Thus in the presence of an oxygen donor peroxygenase isolated from soybean, broad bean, and oat (4–7) converts oleic acid to its 9,10-epoxide. Linoleic acid afforded 9,10- and 12,13-epoxy derivatives. Studies with peroxygenase from soybean and broad bean show that only *cis*-double bonds are substrates (6,7). Peroxygenase can also catalyze internal epoxidation, if the peroxygen is contained in a molecule with a double bond. Thus when the peroxygenases from soybean and broad bean were presented with the hydroperoxide of linoleic acid **2a**, a product was 9,10-epoxy-13(*S*)-hydroxy-11(*E*)-octadecenoic acid (5,7). Oat seed microsomes containing peroxygenase catalyzed the epoxidation of oleic acid using hydrogen peroxide as the oxygen donor (4). Microsomes containing peroxygenase also converted linoleic acid **1a** to a number of oxygenated derivatives, presumably through the hydroperoxide of linoleic acid, formed by the presence of lipoxygenase in the peroxygenase preparations. In this study, the hydroperoxides of linoleic acid **2a** and its methyl ester **2b** were presented directly to oat seed peroxygenase in order to provide direct evidence that this enzyme has the ability to catalyze epoxidation from fatty hydroperoxides. Epoxidation activity was also tested in organic solvents, and the structure of the major oxygenated product was determined.

MATERIALS AND METHODS

Materials. Oats seeds (*Avena sativa* L.) were supplied by Equine Speciality Feed Co. (Ada, MN). Soybean (*Glycine max* L. Merr.) lipoxygenase (Lipoxidase, Type 1-B) and linoleic acid were purchased from Sigma (St. Louis, MO). Organic solvents were reagent grade and were saturated with water. Water was purified to a resistance of 18 mΩ-cm using



SCHEME 1

a Barnstead (Dubuque, IA) NANO pure system. All other reagents were of the highest purity available.

Preparation of oat seed microsomes. Dry oat seeds (10 g) were ground in 5-g batches in a 37 mL Waring Blender (New Hartford, CT) mini-jar for 30 s. The ground oat seeds were transferred to a 110 mL mini-jar containing 90 mL cold 0.1 M potassium phosphate buffer (pH 6.7) and blended for 90 s at high speed. The oat seed slurry was centrifuged at 77 × *g* for 10 min. The pellet was discarded, and the supernatant was centrifuged for an additional 10 min at 77 × *g*. After the second centrifugation, the supernatant was transferred to four 25 × 89 mm centrifuge tubes, 20 mL potassium phosphate buffer was added to each tube, and the tubes were centrifuged at 97,000 × *g* for 70 min in an SW 28 rotor (Beckman, Palo Alto, CA). The supernatant was decanted, and the pellet was suspended in either potassium phosphate buffer or organic solvent.

*Incubation of **2a** or **2b** with microsomes.* Linoleic acid **1a** was enzymatically converted to **2a** using lipoxygenase as described previously (2). Linoleic acid hydroperoxide **2a** was methylated with CH_2N_2 to give its methyl ester **2b** (2). The oat microsomes from one centrifuge tube were dispersed in 0.7 mL organic solvent or 0.1 M potassium phosphate buffer (pH 6.7) containing 0.1% (wt/vol) Tween 20. The microsomal suspension was placed into a 10-mL Erlenmeyer flask containing 10 mg **2a** or **2b**. The mixture was agitated at 20°C for 2 h. At the end of the incubation period, 3.5 mL methanol was added, and the contents were transferred to a 125-mL separatory funnel. The products were partitioned between 30 mL diethyl ether and 25 mL water. After separating the layers, the water layer was reextracted with 25 mL diethyl ether. The ether fractions were combined, dried over sodium sulfate, and taken to dryness under a stream of nitrogen. The products were dissolved in 2 mL dichloromethane and stored at –20°C until analysis.

*Preparation of standard methyl 9,10(11,12)-epoxy-13(*S*)-hydroxy-11(*E*)(9Z)-octadecenoate (mixture of **4b** and **5b**).* Compound **2b** (400 mg, 1.2 mmol) was reduced to the corresponding hydroxy derivative **3b** [methyl 13(*S*)-hydroxy-9(*Z*),11(*E*)-octadecadienoate] as described (8). Compound **3b** (308 mg, 992 mol) was dissolved in 12 mL $CHCl_3$, and an equal molar amount of 3-chloroperoxybenzoic acid was added. The mixture was shaken for 18 h at 20°C. The product was extracted with diethyl ether (50 mL), and the ether was washed with 4 × 100 mL 5% $NaHCO_3$. The ether layer was dried over anhydrous Na_2SO_4, and the ether was removed under a stream of nitrogen to give 246 mg (754 mol) of a mixture of **4b** and **5b**.

Thin-layer chromatography (TLC). Silica-gel preadsorbent high-performance thin-layer chromatography (HPTLC-HL) TLC plates (10 cm × 10 cm, coating thickness 150 m, Analtech, Newark, DE) were dipped in 5% boric acid in methanol and allowed to air-dry prior to spotting. The TLC plates were developed sequentially in the following solvent systems:

toluene/ethyl acetate/diethyl ether/acetic acid (62.5:10.8:8.3:1, by vol); air-drying (10 min); hexane/diethyl ether/formic acid (32:8:1, vol/vol/vol). Products were visualized by charring after spraying the TLC plate with 60% H_2SO_4.

High-performance liquid chromatography (HPLC). Reaction products were separated on a LiChrosorb 5μ diol HPLC column (250 × 10 mm) (Phenomenex, Torrance, CA) installed on a Waters (Milford, MA) LCM1 HPLC instrument. The instrument was equipped with a Waters 996 photodiode array detector in tandem with a Varex evaporative light-scattering detector MK III (Alltech, Deerfield, IL) operated at a temperature of 55°C, and with N_2 as the nebulizing gas at a flow rate of 1.5 L/min. Mobile phase composition and gradient were hexane/isopropanol (97:3) to (94:6) over 29 min using a linear gradient. The flow rate was 2 mL/min.

Gas chromatography–mass spectrometry (GC–MS). Samples were analyzed after treatment with *N,O*-bis(trimethylsilyl)-trifluoroacetamide (BSFTA, Pierce, Rockford, IL). Mass spectra were obtained on a Hewlett-Packard (HP) (Wilmington, DE) 5890 Series II Plus gas chromatograph equipped with an HP 5972 mass selective detector set to scan from *m/z* 10 to 600 at 1.2 scans per second. A capillary column (HP-5MS, 30 m × 0.25 mm) coated with 0.25 μm 5% cross-linked phenyl methyl silicone was used to separate the products. The oven temperature was increased from 80 to 230°C at 10°C per min. The injector port temperature was 230°C, and the detector transfer line temperature was 240°C.

Nuclear magnetic resonance (NMR) spectrometry. Spectra were obtained on a Varian Unity Plus 400 MHz NMR spectrometer in 99 atom-% *p*-dioxane-d_8 (Cambridge Isotope Labs, Woburn, MA). Typical acquisition conditions for the proton spectra were 9,600 data points; 4 kHz spectral width; 2.2 s recycle time. For carbon spectra typical acquisition conditions were 60,000 data points; 25 kHz spectral width; 21.7 s recycle time. The 90° pulse was measured for both proton and carbon spectra prior to acquisition. All spectra were recorded at 30°C.

RESULTS AND DISCUSSION

Reaction characteristics of microsome catalysis. The hydroperoxide of linoleic acid **2a** or its methyl ester **2b** was incubated with oat seed microsomes containing peroxygenase in an aqueous or organic (1,1,2-trichlorotrifluoroethane: CFE) medium. After extraction, products from the reaction of the free acid **2a** were treated with CH_2N_2 before analysis by TLC. As shown in Figure 1, the free acid **2a** produced only low levels of polar products in both media (lanes D and E), but significant levels of polar products were obtained from the methyl ester **2b** (lanes B and C). Greater amounts of polar products were obtained in aqueous media (lane C), compared to the amounts of polar products formed in CFE (lane B). The slowest-running major product (spot d, Fig. 1) had the same mobility on TLC as a component of the chemically synthesized epoxy alcohols (**4b** + **5b**). The other major product (spot c, Fig. 1) had the same mobility on TLC as the alcohol **3b**.

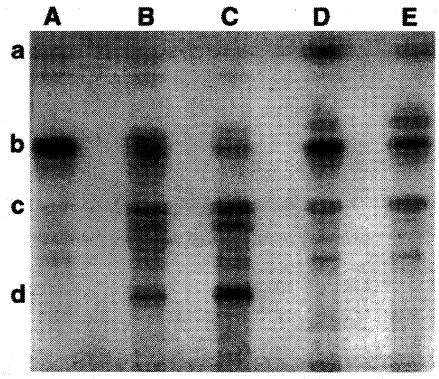


FIG. 1. Thin-layer chromatographic (TLC) analysis of the catalytic conversion of **2a** and **2b** (for structures see Scheme 1) by oat seed microsomes containing peroxygenase in potassium phosphate buffer/Tween 20 or 1,1,2-trichlorotrifluoroethane (CFE). Reactions on lanes A–C contained 10 mg **2b** [methyl 13(*S*)-hydroperoxy-9(*Z*),11(*E*)-octadecadienoate]. Reactions on lanes D and E contained 10 mg **2a** [13(*S*)-hydroperoxy-9(*Z*),11(*E*)-octadecadienoic acid]. The products from reactions D and E were methylated prior to application to the TLC plate. Lanes B–E contained oat seed microsomes (1.5 mg protein). Lane A, 0.7 mL potassium phosphate buffer (0.1 M, pH 6.7)/0.1% (wt/vol) Tween 20; Lane B, 0.7 mL CFE; Lane C, 0.7 mL potassium phosphate buffer/Tween 20; Lane D, 0.7 mL CFE; Lane E, 0.7 mL potassium phosphate buffer/Tween 20. Spots labeled a, b, c, and d correspond to structures **1b**, **2b**, **3b**, and **4b** (Scheme 1).

Another product directly below spot c has not been identified. A notable feature of lane B is that spot b corresponding to the starting material **2b** is significantly broadened (compare lanes A and B). This is due to the isomerization of **2b** to give the methyl 9-hydroperoxy-10,12-octadecadienoate isomer. This reaction is catalyzed by some component of the microsomes, as **2b** incubated under identical conditions but without microsomes did not form significant amounts of the 9 isomer.

Figure 2 shows the influences of aqueous buffer and organic solvent on the formation of alcohol **3b** and epoxy alcohol **4b** from **2b** by peroxygenase. Conceptually the peroxygenase reaction can be divided into two parts, cleavage of peroxygen and transfer of the oxygen to the double-bond receptor. The epoxy alcohol product **4b** cannot be formed unless both cleavage and transfer are operative. It can be seen from Figure 2 that these two steps operated most efficiently in nonpolar, aliphatic solvents: octane, heptane, isooctane, hexane, and dodecane. Within experimental error, the level of alcohol **3b** formed in aqueous buffer was identical to that formed in the nonpolar solvents. However, the level of epoxy alcohol product **4b** that was produced in aqueous buffer was lower than that formed in the nonpolar solvents. In other tested organic solvents (trichlorotrifluoroethane, isopropyl ether, octanol, and toluene) the amount of **4b** diminished. In octanone no **4b** could be detected. Although the levels of epoxy alcohol **4b** produced in these solvents were low, the level of alcohol **3b** formed was higher. In the solvent toluene the highest amount of **3b** was formed.

Figure 3 shows the influence of the volume of heptane on the reaction of hydroperoxide **2b** with oat seed microsomes.

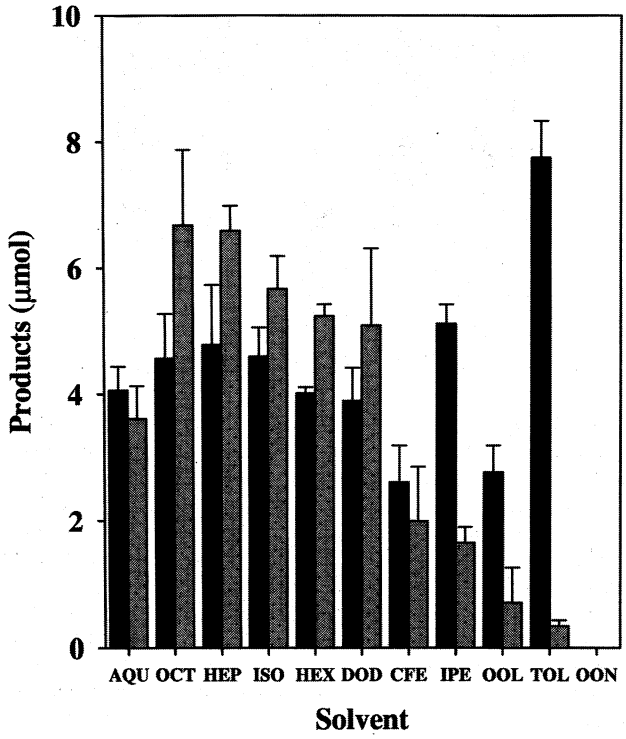


FIG. 2. Effect of solvent on the conversion of **2b** to alcohol **3b** and epoxy alcohol **4b** by oat seed microsomes containing peroxygenase. The levels of **3b** (solid) and **4b** (cross-hatch) were determined from their respective peak areas in high-performance liquid chromatographic analyses (mean ± S.E., n = 3–5). Each reaction contained **2b** (10 mg, 30.6 mol), water-saturated solvent (0.7 mL), and oat seed microsomes (1.5 mg protein). Solvents: AQU, 0.1 M potassium phosphate buffer (pH 6.7) containing 0.1% (wt/vol) Tween 20; OCT, octane; HEP, heptane; ISO, isooctane (2,2,4-trimethylpentane); HEX, hexane; DOD, dodecane; CFE, 1,1,2-trichlorotrifluoroethane; IPE, isopropyl ether; OOL, 2-octanol; TOL, toluene; OON, 2-octanone. For chemical structures see Scheme 1.

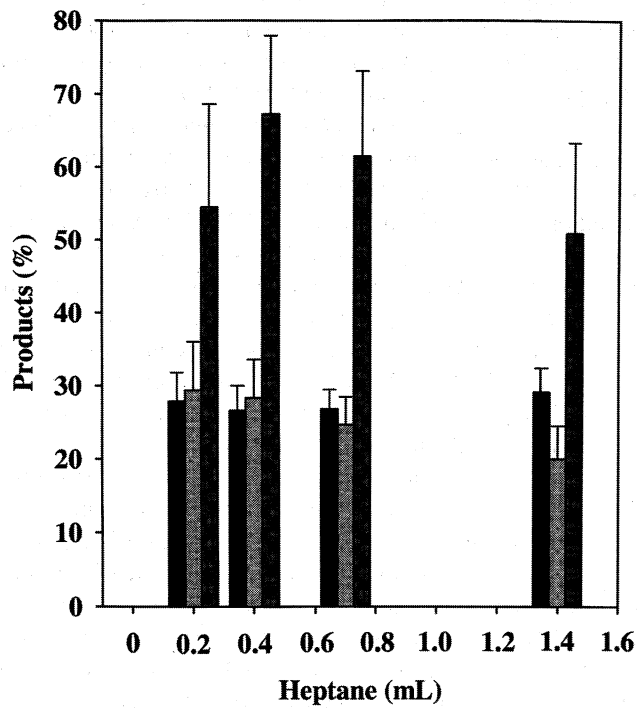


FIG. 3. Influence of the volume of heptane upon the use of **2b** by oat seed microsomes containing peroxygenase. The levels of products were determined by high-performance liquid chromatographic analyses (mean ± S.E., n = 4). Reactions were conducted as given in Figure 2. Products: alcohol **3b**, solid bar; epoxy alcohol **4b**, light cross-hatch bar; all products, dark cross-hatch bar. For structures of **2b**, **3b**, and **4b** see Scheme 1.

Overall conversion of **2b** to all products was insensitive to the amount of heptane added. The results show that epoxygenase was saturated with substrate **2b** at all tested concentrations. Thus K_m of **2b** must be less than or equal to one-tenth of the lowest tested concentration of **2b** or 2 mM. The partitioning of substrate into the peroxygen cleavage pathway to form **3b** or peroxygen cleavage plus epoxidation pathway to form **4b** was nearly insensitive to the initial concentration of **2b** over the tested concentrations.

*Structural elucidation of epoxy alcohol **4b** produced by microsomes.* Monoepoxy alcohol was chemically synthesized from **3b** as described in the Materials and Methods section. The synthesized material showed four peaks on HPLC, with elution times of approximately 15, 18, 23 and 24 min, that had spectral characteristics indicative of monoepoxy alcohol. Prior work showed that the peaks at 15 and 18 min were the *erythro* and *threo* isomers of methyl *trans*-11,12-epoxy-13(*S*)-hydroxy-9(*Z*)-octadecenoate (2). Therefore, it was concluded that the peaks at 23 and 24 min were the 9*R*,10*S*/9*S*,10*R* diastereomers of methyl *cis*-9,10-epoxy-13(*S*)-hydroxy-11(*E*)- octadecenoate (**4b**), respectively.

The products resulting from the action of oat seed microsomes on **2b** showed two main peaks that eluted at approximately 15 and 24 min. The peak at 15 min was shown to be **3b** by comparison of its properties to previously isolated material (2). The peak eluting at 24 min had an HPLC elution time comparable with one diastereomer of chemically synthesized methyl 9,10-epoxy-13(*S*)-hydroxy-11(*E*)-octadenoate. Spectral data obtained on this material were entirely consistent with this assignment. After formation of the $(CH_3)_3Si$ derivative, the mass spectrum of the epoxy alcohol showed ions at *m/z* 398 (M), 383 (M − 15; loss of $\cdot CH_3$), 327 [M − 71; loss of $\cdot(CH_2)_4\text{-}CH_3$], 277 [M − (90 + 31); loss of $(CH_3)_3SiOH$ and $\cdot OCH_3$], 237 (327 − 90), 185 [$O{=}C^+(CH_2)_7\text{-}COOCH_3$], and 173 [$(CH_3)_3SiO^+{=}CH\text{-}(CH_2)_4\text{-}CH_3$]. The ultraviolet/visible spectrum showed absorbance below 210 nm. Thus, the data indicate that the epoxy alcohol is a monounsaturated 18-carbon methyl ester with the hydroxyl group at C-13 and the epoxide at C-9.

The neat infrared spectrum of the epoxy alcohol showed a broad band centered at 3447 cm^{-1} (hydrogen-bonded hydroxyl), 1740 cm^{-1} (ester carbonyl), 967 cm^{-1} (*trans* double bond), and 827, 849 cm^{-1} (oxirane C-O twin bands). No absorption band was observed in the region 850–900 cm^{-1}, thus excluding the presence of a *trans* epoxide group (9)

TABLE 1
Diagnostic ^{1}H NMR Chemical Shifts and Coupling Constants of Methyl 9,10-Epoxy-13- hydroxy-11(*E*)-octadenoate (4b)[a]

Chemical shift (δ)	Number of protons	Appearance	Assignment	Coupling constant (Hz)
2.98	1H	*dt*	H-9	$J_{8-9} = 6.1$, $J_{9-10} = 4.3$
3.33	1H	*dd*	H-10	$J_{9-10} = 4.3$, $J_{10-11} = 7.5$
4.02	1H	*dt*	H-13	$J_{12-13} = 5.3$, $J_{13-14} = 10.8$
5.49	1H	*dd*	H-11	$J_{10-11} = 7.9$, $J_{11-12} = 15.5$
5.91	1H	*dd*	H-12	$J_{11-12} = 15.6$, $J_{12-13} = 6.1$

[a]NMR, nuclear magnetic resonance; for structure of **4b** see Scheme 1.

The decoupled ^{13}C NMR ($C_4D_8O_2$, 400 MHz) spectrum of the epoxy alcohol showed important signals at δ 51.4 (OCH_3), 56.7 (C-9), 58.8 (C-10), 72.1 (C-13), 125.1 (C-11), 141.4 (C-12), and 174.0 [$C(O)OCH_3$]. Because there are only two signals for the epoxide carbons, two signals for the double-bond carbons, and one signal for the alcoholic carbon, the epoxy alcohol consists of one structural isomer.

Important signals from the ^{1}H NMR ($C_4D_8O_2$, 400 MHz) spectrum of the epoxy alcohol are shown in Table 1. The coupling constant J_{9-10} was 4.3 Hz, demonstrating that the configuration of the epoxide is *cis*: $J = 4.3$ Hz for *cis* and 2.1–2.4 Hz for *trans* (10). The coupling constant J_{11-12} was 15.5–15.6 Hz, demonstrating that the double bond is in the *trans* configuration: $J = 5$–14 Hz for *cis* protons and 12–18 Hz for *trans* protons (11). From all the above data, it was concluded that the structure of the epoxy alcohol **4b** is methyl *cis*-9,10-epoxy-13(*S*)-hydroxy-11(*E*)-octadecenoate.

Epoxide hydrolysis. Isolated **4b** was subjected to hydrolysis in H_2O/HCl as described previously (12). After formation of the $(CH_3)_3Si$ derivative, GC–MS analysis showed the presence of two trihydroxy, hydrolysis products. The mass spectrum of the major product showed ions at *m/z* 487 [M − 73; loss of $(CH_3)_3Si\cdot$], 439 [M − (90 + 31)], 259 [$(CH_3)_3SiO^+$=CH- $(CH_2)_7$-$COOCH_3$], and 173 [$(CH_3)_3SiO^+$=CH-$(CH_2)_4$-CH_3]. The mass spectrum of the minor product showed ions at *m/z* 439, 301 {$CH_3(CH_2)_4CH[OSi(CH_3)_3]CH[OSi(CH_3)_3]$-CH=$CH^+$}, 259, 211 (301 − 90), and 173. Comparison of the HPLC characteristics of these hydrolysis products with freshly prepared extracts from a reaction of the microsomes with **2b** in heptane revealed that similar trihydroxy materials were not formed by the microsomes. This result was not expected because prior work using linoleic acid with oat seed microsomes had shown the formation of significant amounts of trihydroxy material (4).

This research has confirmed the presence of the enzyme peroxygenase in oat seeds by demonstrating that the hydroperoxide of methyl linoleate **2b** can be directly converted to oxygenated products. As with other peroxygenase enzymes studied previously, the oat seed peroxygenase is capable of transferring oxygen only to a *cis* double bond. It was demonstrated that oat seed peroxygenase has catalytic activity in hydrocarbon solvents. One advantage of the use of organic solvents for the transformation of lipolytic materials is that the requirement for relatively expensive surfactant in aqueous media is eliminated. Inasmuch as this allows for the transformation of lipids at reduced cost, this finding will increase the utility of this procedure for the synthesis of highly oxygenated lipids with potential for use in industrial products.

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REFERENCES

1. Gardner, H.W., Oxygen Radical Chemistry of Polyunsaturated Fatty Acids, *Free Radical Biol. Med. 7*:65–86 (1989).
2. Piazza, G.J., T.A. Foglia, and A. Nuñez, Enantioselective Formation of an α,β-Epoxy Alcohol by Reaction of Methyl 13(*S*)-Hydroperoxy-9-(*Z*),11(*E*)-octadecadienoate with Titanium Isopropoxide, *J. Am. Oil Chem. Soc. 74*:1385–1390 (1997).
3. Piazza, G.J., T.A. Foglia, and A. Nuñez, Enantioselective Conversion of Linoleate Hydroperoxide to an α,β-Epoxy Alcohol by Niobium Ethoxide, *Ibid. 75*:939–943 (1998).
4. Hamberg, M., and G. Hamberg, Peroxygenase-Catalyzed Fatty Acid Epoxidation in Cereal Seeds, *Plant Physiol. 110*:807–815 (1996).
5. Hamberg, M., and G. Hamberg, Hydroperoxide-Dependent Epoxidation of Unsaturated Fatty Acids in the Broad Bean (*Vicia faba* L.), *Arch. Biochem. Biophys. 283*:409–416 (1990).
6. Hamberg, M., and P. Fahlstadius, On the Specificity of a Fatty Acid Epoxygenase in Broad Bean (*Vicia faba* L.), *Plant Physiol. 99*:987–995 (1992).
7. Blée, E., A.L. Wilcox, L.J. Marnett, and F. Schuber, Mechanism of Reaction of Fatty Acid Hydroperoxides with Soybean Peroxygenase, *J. Biol. Chem. 268*:1708–1715 (1993).
8. Kühn, H., R. Wiesner, V.Z. Lankin, A. Nekrasov, L. Alder, and T. Schewe, Analysis of the Stereochemistry of Lipoxygenase-Derived Hydroxypolyenoic Fatty Acids by Means of Chiral Phase High-Pressure Liquid Chromatography, *Anal. Biochem. 160*:24–34 (1987).
9. Hamberg, M., Fatty Acid Hydroperoxide Isomerase in *Saprolegnia parasitica*: Structural Studies of Epoxy Alcohols Formed from Isomeric Hydroperoxyoctadecadienoates, *Lipids 24*:249–255 (1989).
10. Pierre, J.-L., P. Chautemps, and P. Arnaud, Résonance Magnétique Nucléaire des Petits Cycles. vi. Étude d'α-Époxy-alcools, *Chim. Anal. 50*:494–500 (1968).
11. Pretsch, E., J. Seibl, and W. Simon, in *Tables of Spectral Data for Structure Determination of Organic Compounds*, Springer-Verlag, Berlin, 1981, p. B25.
12. Hamberg, M., Regio and Stereochemical Analysis of Trihydroxyoctadecenoic Acids Derived from Linoleic Acid 9- and 13-Hydroperoxides, *Lipids 26*:407–415 (1991).

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