Table II. Crystallographic Data for 16a

(a) Crystal Parameters

formula	$C_{24}H_{18}N_2O_2$	V, Å^3	1893 (1)
formula wt	366.38	Z	4
crystal system	monoclinic	D(calcd), g cm^{-3}	1.286
space group	$P2_1/c$	μ(Mo Kα), cm^{-1}	0.77
a, Å	14.802 (4)	color	yellow
b, Å	7.409 (2)	size, mm	0.35 × 0.35 × 0.40
c, Å	17.723 (5)	T, K	293
β, deg	103.12 (3)		

(b) Data Collection

diffractometer	Nicolet R3m/μ	data collected	3258
radiation	Mo Kα	indpdt data	2893
wavelength, Å	0.71073	R(int), %	3.5
monochromator	graphite	obs data ($5\sigma F_o$)	1761
2θ limits	$4 \le 2\theta \le 48$	decay, %	<1

(c) Refinement

$R(F)$, %	5.63	Δ/σ(firal)	0.05
$R(wF)$, %	6.27	$\Delta(\rho)$, e Å^{-3}	0.24
GOF	1.544	N_o/N_v	7.6

The solvent was evaporated on a rotary evaporator, using a bath temperature not greater than 27 °C. The residue was checked by ^{1}H NMR and was chromatographically separated (methanol–ethyl acetate–petroleum ether, 0:1:25 at the beginning and 1:3:7 at the end, using a 20 × 1.5 cm column with 50 g of silica gel G, F842). The solution was allowed to stand until **16a** and **16a′** crystallized from the eluant. The total yield of the intermediates was 68%, and the ratio of the isomers **16a** to **16a′** was 4.23:1. ^{1}H NMR ($CDCl_3$, TMS as internal standard) for mixture: δ 2.24 (s, CH_3, for **16a**), 2.56 (s, CH_3, for **16a′**), 6.27 (s, C8-H, for **16a**), 6.57 (s, C8-H, **16a′**), 7.13–7.82 (m, all Ar H for two isomers). A pure sample of **16a** was obtained by separating by chromatography (hexane–ethyl acetate/95–5), employing the method of Taber,[12] and recrystallizing from diethyl ether, mp 151–152 °C. ^{1}H NMR ($CDCl_3$) for **16a**: 2.39 (s, 3 H, CH_3), 6.28 (s, 1 H, C8-H), 7.21–7.95 (m, 14 H, Ar). ^{13}C NMR ($CDCl_3$) for **16a** (numbering system is shown in Scheme I): 17.40 (C9-CH_3), 107.82 (C8), 120.63, 125.58, 128.00, 128.21, 128.82, 128.96, 128.28, 130.00, 130.65, 134.62 (C3, C4, C5, C6, C12, C13, C14, C16, C17, C18), 123.63 (C9), 135.30 (C10), 138.02, 140.19 (C2a, C6a), 147.88 (C7), 158.44, 159,32 (C11, C15), 166.25 (C2). MS, m/z (rel intensity): 366 (M^+, 4), 351 (16), 185 (21), 184 (100), 183 (18), 128 (28), 127 (10), 105 (29), 102 (10), 77 (38), 51 (19). Exact mass calcd for 366.1368, found 366.1368. The X-ray result for **16a** is reported.

Conversion of 16a (16a′) into 18a. Conversion of **16a** (**16a′**) into product **18a** was accomplished by 3 h of heating in dry toluene, employing the procedure described in General Reaction paragraph above. The recovered yield of **18a** was 83% (70%) and was identical with **18a** obtained employing the general reaction, given above.

Crystal Structure Determination for 16a. Crystallographic data are summarized in Table II. The ORTEP diagram is depicted in Figure 1. A well-formed specimen of **16a**, recrystallized from ether, was mounted on a glass fiber. Film data and unit-cell parameters uniquely determined the monoclinic space group, $P2_1/c$. No correction for absorption was required. The structure was solved by direct methods. To conserve data, the two terminal phenyl rings were constrained to rigid hexagons. All non-hydrogen atoms were refined with anisotropic thermal parameters. All hydrogen atoms, except for H(3) (numbering on Figure 1), were located to verify stereochemistry, were isotropically refined, and were treated as idealized, updata isotropic contributions. All computations used SHELXTL (5.1) software (G. Sheldrick, Nicolet XRD, Madison, WI). Bond distances and angles are given in Table II.

Supplementary Material Available: Atomic coordinates and isotropic thermal parameters (Table 1S), anisotropic thermal parameters (Table 2S), H-atom coordinates and isotropic thermal parameters (Table 3S), ^{1}H NMR (Table 4S), ^{13}C NMR (Table 5S), and mass spectral data (Table 6S) (6 pages). Ordering information is given on any current masthead page.

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Preparative, Enzymatic Synthesis of Linoleic Acid (13*S*)-Hydroperoxide Using Soybean Lipoxygenase-1

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Lipoxygenase-1 (EC 1.13.11.12) is a non heme iron dioxygenase which catalyzes the incorporation of dioxygen into polyunsaturated fatty acids possessing a (1*Z*,4*Z*)-pentadienyl unsaturation to yield *E*,*Z* conjugated diene hydroperoxides[1] as depicted in Scheme I.

Plant lipoxygenases are one of the most available enzymes of this class and serve as models for the study of mammalian lipoxygenases, relevant to leukotriene biosynthesis and, to a lesser extent, to prostaglandin synthase.[1]

We describe herein an easy method for preparative scale production of the (13*S*)-hydroperoxide of linoleic acid, using commercial lipoxygenase-1 from soybean as catalyst.

Studies on lipoxygenases are generally done in dilute solutions of fatty acids in aqueous buffers because of their poor solubility in water. Fatty acid solutions up to 5 × 10^{-3} M are usually obtained with added agents such as surfactant (Tween 20, 80) and cosolvent (ethanol).[1] Only few examples describe the preparative synthesis of hydroperoxides by enzymes (continuous process, 10^{-4} M;[2] batch process, 2 L, 1 × 6 10^{-3} M[3]).

We have observed that oxidation of 0.1 M fatty acid emulsions (28 g/L) in 0.1 M (pH 9) sodium borate buffer in the presence of a commercial preparation of the enzyme (2 mg/mL) proceeds rapidly. Indeed, using a Schlenck tube under weak O_2 pressure (i.e. 4 atm) at 0–4 °C with vigorous magnetic stirring, we obtained a clear solution of linoleic acid hydroperoxides (yield 80%) after 1 h. Normal- and chiral-phase HPLC analyses showed that one major chiral component was obtained (96% isomer purity, 95% ee). This compound was identified as (9*Z*,11*E*,13*S*)-13-hydroperoxy-9,11-octadecadienoic acid according to the literature,[4,5] and the structure assignment is confirmed by NMR spectroscopy.

The selectivity of soybean lipoxygenase-1 is not affected by our conditions in terms of regio-, stereo-, and enantioselectivity.[1] The acid, as a monomer in aqueous solution, is known to be the enzyme substrate.[6] Under these conditions, this form is probably present in sufficient quantity to initiate catalysis. The fact that soybean lipoxygenase-1 has its optimum pH in the basic range is an advantage, since fatty acids are more soluble as salts in aqueous solution. The hydroperoxide formed solubilizes more linoleic acid as noted by Haining et al.,[7] allowing the reaction to progress. The reasons why the enzyme is not rapidly deactivated in this reaction are under investigation. However, the presence of great quantities of dissolved oxygen seems to prevent the total self-deactivation of the enzyme during catalysis, allowing high yields of hydroperoxide to be obtained.

In conclusion, this report describes a simple procedure by which soybean lipoxygenase-1 catalyzes formation of

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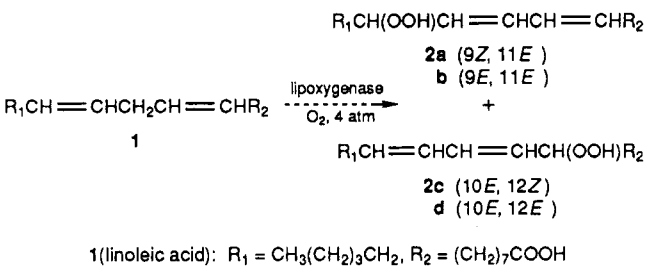
Scheme I



1(linoleic acid): $R_1 = CH_3(CH_2)_3CH_2$, $R_2 = (CH_2)_7COOH$

linoleic acid (13*S*)-hydroperoxide with good selectivity and in satisfactory yields on preparative scale. Work is underway to extend this simple method to the hydroperoxidation of different polyunsaturated fatty acids (i.e. α-linolenic and arachidonic acid) using soybean lipoxygenase-1, and to different oxygenases (i.e. other lipoxygenases, prostaglandin synthase).

Experimental Section

Linoleic acid (ca. 99% pure) and soybean lipoxygenase-1 (5.2 units/mg) were purchased from Fluka A.G. The enzyme was used without any purification. ^1H and ^{13}C NMR spectra were run on a Bruker AC 300 spectrometer.

(9*Z*,11*E*,13*S*)-13-Hydroperoxy-9,11-octadecadienoic Acid (2a). Linoleic acid (280 mg; 1 mmol) was introduced into a Schlenck tube (60 mL) placed in an ice bath (0–4 °C). Sodium borate buffer (10 mL; 10^{-1} M) was added under gentle magnetic stirring in order to emulsify the mixture. The reaction was initiated by the addition of 20 mg of the enzymatic powder and pressurizing of the reaction vessel with pure oxygen (4 atm). Stirring was then accelerated, and the temperature was kept between 0 and 4 °C during the whole reaction. The reaction progress can be followed by UV analysis after dilution of an aliquot of the solution in ethanol (50 μL/100 mL). Measurements were carried out at 234 nm, assuming a molar extinction coefficient for the hydroperoxide of $\epsilon = 25\,000$ mol L^{-1} cm^{-1}.[8] After 1 h, hydroperoxide formation stabilized, giving an 80% yield (250 mg) based on the UV absorption.

Isomer determination was based on the HPLC analysis[4] and comparison of the retention times with authentic samples obtained by controlled autoxidation of linoleic acid with methylene blue as sensitizer as described in the literature.[9] The four hydroperoxide isomers (**2a–d**) were separated using the following procedure: 100 μL of the reaction mixture was dissolved in 5 mL of water (pH 3) and rapidly extracted with 5 mL of diethyl ether. The organic phase was dried ($MgSO_4$). Ten microliters of the organic phase was then subjected to HPLC (Waters 441 HPLC; Interchim L5-25F, 5 μm, 250 × 4.6 mm silica column; hexane/diethyl ether/acetic acid, 1000/200/1, flow rate 1 mL/min; UV detection at 229 nm). Retention times and isomer distribution: **2a** (16.0 min, 95%), **2b** (20.3 min, 1%), **2c** (23.3 min, 3%), and **2d** (27.6 min, 1%).

Reduction of 2a to (9*Z*,11*E*,13*S*)-Hydroxy-9,11-octadecadienoic Acid (3). To 10 mL of the aqueous hydroperoxide solution (25 mg/mL) were added 10 mL of ethanol and 100 mg of $NaBH_4$. After 1 h (25 °C, pH 9), the solution was diluted with water, acidified to pH 3, and extracted twice with diethyl ether. The combined washed organic phases were dried ($MgSO_4$) and evaporated to dryness. The product was purified using the HPLC procedure described above (flow rate 2 mL/min),[4] affording 0.20 g (65%). Retention time: **3** (21.2 min). $[\alpha]^{20}_D = +10.75°$ (c = 0.4, EtOH). ^{13}C NMR spectra showed the same chemical shifts as reported by Van Os et al.[10] ^1H NMR (300 MHz, $CDCl_3$): δ 6.48 (dd, J_{11-12} = 15 Hz, J_{10-11} = 11 Hz, H-11), 5.97 (t, J_{10-11} = J_{9-10} = 11 Hz, H-10), 5.66 (dd, J_{11-12} = 15 Hz, J_{12-13} = 7 Hz, H-12), 5.44 (dt, J_{9-10} = 11 Hz, J_{8-9} = 7 Hz, H-9), 4.18 (q, J_{12-13} = J_{13-14} = 7 Hz, H-13), 2.34 (t, J = 7 Hz, H_2-2), 2.17 (q, J = 7 Hz, H_2-8), 1.7–1.5 (br m, H_4-14, 3), 1.32 (m, H_{14}-17, 16, 15, 7, 6, 5, 4), 0.89 (t, J = 7 Hz, H_3-18).

Enantiomeric Determination.[5] Enantiomeric excess analysis was performed on the methyl ester of **3**. The purified dienol acid was esterified in ether with diazomethane (1 h at room temperature). The product was dissolved in hexane and submitted to chiral phase HPLC analysis (Pirkle type 1A column, hexane/2-propanol, 99.5/0.5, 0.8 mL/min). The racemic mixture of **2a** derived from reduction of the corresponding hydroperoxide obtained by controlled autoxidation.[9] Enantiomeric excess determination was based on comparison of retention times and the areas between the enzymatic mixture and the racemic ones, giving 95% ee. The *S* absolute configuration was assigned by cochromatography with an authentic sample of **2a**, obtained by enzymatic oxidation of linoleic acid with lipoxygenase-1 from soybean under conditions known to produce the *S* enantiomer.[11]

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