Dioxygenation of Long-Chain Alkadien(trien)ylphenols by Soybean Lipoxygenase

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Long-chain alkadien(trien)ylphenols, i.e., cardanols, cardols, and anacardic acids, as well as their acetylated and methylated derivatives were used in enzymatic dioxygenation catalyzed by soybean lipoxygenase-1 (LOX-1). Kinetic studies revealed the influence of functional groups, chain length, number of double bonds, and the distance of the double bonds from the terminal end. Free functional groups were found to be a structural prerequisite for high affinity and turnover number; exceptional kinetic parameters were obtained for ω-6 compounds possessing the same chain length as linoleic acid. The regioselectivity and enantioselectivity of LOX-1 catalysis were investigated with 3-[(*Z*,*Z*)-8′,11′-pentadecadienyl]phenol as the representative substrate, yielding 3-[12′-(*S*)-hydroperoxy-(*Z*,*E*)-8′,10′-pentadecadienyl]phenol as the main product. Our extensive chromatographic and spectroscopic studies comprised HPLC−MS/MS for differentiation of the regioisomers, ^{1}H NMR spectroscopy for determination of the double-bond configuration, GC−MS to evaluate the enantiomeric excess, and exciton-coupled circular dichroism for determination of the absolute configuration.

Keywords: *Lipoxygenase; dioxygenation; cardanols; cardols; anacardic acids; exciton-coupled circular dichroism (ECCD)*

INTRODUCTION

Lipoxygenase (LOX) is a non-heme, iron-containing dioxygenase that catalyzes the regioselective and enantioselective oxidation of unsaturated fatty acids containing at least one (*Z*,*Z*)-1,4-pentadienoic moiety. Common substrates such as linoleic acid and linolenic acid are converted to the corresponding (*E*,*Z*)-2,4-conjugated hydroperoxides (Veldink and Vliegenthart, 1984). The actual knowledge in the field of LOX catalysis has recently been summarized (Piazza, 1996).

Many phenolic compounds have been described to act as inhibitors of soybean lipoxygenase-1 (LOX-1), among them the constituents of cashew nut shell liquid (CNSL), i.e., cardanols (**1/2**) and cardols (**3/4**) (Shobha et al., 1994). However, anacardic acid (**5**) has been reported to be a substrate for LOX-1 (Shobha et al., 1992). In addition, as vinylphenols have also been found to be accepted as substrates by LOX-1 (Markus et al., 1992), it was interesting to study in detail long-chain alkadien(trien)ylphenols, exhibiting the (*Z*,*Z*)-1,4-pentadienoic moiety but lacking the fatty acid structure, in order to better understand the structural requirements for LOX-1 catalysis. In this paper, the results of our kinetic and structural studies of the enzymatic dioxygenation of **1-4** as well as **5** and **6**, including their acetylated and methylated derivatives **1a**−**4a, 5a**−**c,** and **6a**−**c**, respectively, are described.

EXPERIMENTAL PROCEDURES

Chemicals. Cardanols (**1/2**) and cardols (**3/4**) were isolated from technical cashew nut shell liquid (CNSL) (Imperial-Oel-Import Handelsgesellschaft mbH, Hamburg, Germany). Anacardic acids (**5/6**) and their methoxy derivatives (**5a/6a**) were kindly provided by Reinhard Zehnter, Laboratory of Organic Chemistry, University of Bayreuth, Bayreuth, Germany. Lipoxygenase (L-8383, Type I−S) was purchased from Sigma, and linoleic acid was obtained from Fluka. All solvents employed were of high purity at purchase and were redistilled before use.

Nuclear Magnetic Resonance (NMR). NMR spectra (cf. Table 2) were recorded on a Bruker WM 400 spectrometer (400 MHz) in $CDCl_3$ and referenced to the solvent signal.

LC−MS and MS−MS Analyses. Analysis of the acetylated compounds **1a**−**4a**, methyl esters **5**−**6b/c**, and regioisomeric hydroperoxides **7** and **8** was performed on a triple quadrupole TSQ 7000 LC−MS/MS system (Finnigan MAT, Bremen, Germany). Data acquisition and data evaluation were carried out on a personal 5000/33 DEC station (Digital Equipment, Unterföhring, Germany) with ICIS 8.1 software (Finnigan MAT). For HPLC, two Knauer 64 HPLC pumps equipped with micropump heads were used.

Isolation of Cardanols (1/2) and Cardols (3/4). Ten grams of CNSL was extracted three times with *n*-hexane, and the organic phase was washed with water and dried over anhydrous sodium sulfate. After evaporation of the organic solvent, the residue was subjected to LC on a silica gel (Merck silica gel 60, particle size 0.2−0.5 mm) column (80 × 4 cm). Elution (3 mL/min) was performed in three steps using mixtures (each 1 L) of *n*-pentane + ethyl acetate + acetic acid, i.e., (i) 90 + 10 + 1, (ii) 80 + 20 + 1, and (iii) 50 + 50 + 1. Fractions (20 mL each) 36−56 were combined, yielding 5 g of cardanols, while fractions 95−125 contained 2 g of cardols. Further separation of the cardanol and cardol isomers was carried out by LC on silica gel (Merck silica gel 60, particle size 0.2−0.5 mm) impregnated with 20% silver nitrate (Evershed et al., 1982). Two grams of cardanols was applied to a silver nitrate-loaded silica gel column (100 g) and eluted with a *n*-hexane + ethyl acetate + acetic acid gradient employing (i) 50 + 2 + 1 and (ii) 90 + 10 + 0.1 mixtures (500 mL each). Fractions (20 mL each) 30−45 and 80−100 were combined, yielding 250 and 1000 mg of **1** and **2**, respectively. One gram of cardol isomers was applied to an equally silver nitrate-loaded silica gel column (100 g) and separated with a chloroform + ethyl acetate gradient using 60 + 40 and 50 + 50

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mixtures. Pooling of fractions 22−35 and 88−110 yielded 50 and 300 mg of **3** and **4**, respectively. The chromatographic and spectroscopic data of **1**−**4** corresponded to that of the literature data (Roth and Rupp, 1995).

Synthesis of Acetylated Compounds 1a−4a. Fifty milligrams of **1a** (**2a**, **3a**, **4a**) was dissolved in 5 mL of dry pyridine, 5 mL of acetic acid anhydride was added, and the mixture was stirred under nitrogen for 24 h at room temperature. The remaining acetic acid anhydride was hydrolyzed by adding 20 mL of cold water, and the solution was extracted three times with diethyl ether. The combined organic extracts were washed three times with 0.1 N hydrochloric acid and twice with a saturated solution of sodium carbonate and water each and dried over anhydrous sodium sulfate. After evaporation of the solvent, purification was carried out by preparative TLC on precoated SIL G-100 UV_{254} TLC plates from Macherey−Nagel (hexane + ethyl acetate, 80 + 20), and the structures of **1a**−**4a** were confirmed by HPLC−MS analysis. HPLC−MS was performed with atmospheric pressure chemical ionization (APCI) interface in the positive mode. The temperatures of the heated vaporizer and inlet capillary in the nebulizer interface were 400 and 200 °C, respectively. The current of the APCI corona needle was set to 5.0 μA, resulting in 4.0 kV of needle voltage. Nitrogen served both as the sheath (50 psi) and auxiliary (10 units) gases. HPLC separations were carried out on an Eurospher 100 C-18 (100 × 2 mm, 5 μm, Knauer) employing different linear gradient programs at a flow rate of 200 μL/min. Solvent A was 0.05% TFA in water; solvent B was methanol. The scan range was 150−450 u.

1a: MS APCI (+) *m/z* 343 $[M + H]^+$, 301 $[343-H_2C{=}C{=}O]^+$, 189. **2a:** MS APCI (+) *m/z* 341 $[M + H]^+$, 299 $[341-H_2C{=}C{=}O]^+$, 217, 203, 189. **3a:** MS APCI (+) *m/z* 401 $[M + H]^+$, 359 $[401 - H_2C{=}C{=}O]^+$. **4a:** MS APCI (+) *m/z* 399 $[M + H]^+$, 357 $[399 - H_2C{=}C{=}O]^+$, 315 $[399 - 2H_2C{=}C{=}O]^+$.

Synthesis of Methyl Esters 5−6b/c. Methylation of the carboxylic group in **5** and **6** as well as **5a** and **6a** was performed in diethyl ether by use of diazomethane; the phenolic OH group of **5/6** remained underivatized. After evaporation of the solvent, purification was carried out by preparative TLC on precoated SIL G-100 UV_{254} TLC plates from Macherey−Nagel (pentane + diethyl ether + acetic acid, 80 + 20 + 1) and the structures of the isolated methyl esters were confirmed by HPLC−MS analysis with APCI interface in the positive mode as described for the synthesis of the acetylated compounds.

5b: MS APCI (+) *m/z* 359 $[M + H]^+$, 327 $[359 - CH_3OH]^+$, 309 $[359 - CH_3OH - H_2O]^+$. **6b:** MS APCI (+) *m/z* 387 $[M + H]^+$, 355 $[387 - CH_3OH]^+$, 337 $[387 - CH_3OH - H_2O]^+$. **5c:** MS APCI (+) *m/z* 373 $[M + H]^+$, 341 $[373 - CH_3OH]^+$, 323 $[373 - CH_3OH - H_2O]^+$. **6c:** MS APCI (+) *m/z* 401 $[M + H]^+$, 369 $[401 - CH_3OH]^+$, 351 $[401 - CH_3OH - H_2O]^+$.

Determination of Kinetic Parameters. LOX-1 activity was measured both polarographically (oxygen consumption) using a Clark-type oxygen electrode and spectrophotometrically at 234 nm (formation of the conjugated double bond; ϵ = 25 000 M^{-1} cm^{-1}). All essays were performed in 0.1 M borate buffer (pH 9.0) using ethanolic stock solutions of the substrates (1.0 mM for substrates **1**−**6/5a** and **6a** and 10.0 mM for substrates **1a**−**4a/5b/5c/6b** and **6c**). In a typical essay, varying amounts (10−200 μL) of the substrate stock solution were added to 2000 μL of buffer in a quartz cuvette. After efficient mixing, 10 μL of enzyme stock solution (1 or 10 mg of LOX/mL of borate buffer, pH 9.0) was added to start the reaction. The substrates under study exhibited an initial lag period which is common for lipoxygenase catalysis. The initial rates were determined over a period of 1−5 min during the linear increase of the reaction course, and the data were processed with a software program (DNRPEASY, version 3.55) on a personal computer (Duggleby, 1981) to give the K_m and k_{cat} values.

Product Isolation from LOX-1 Catalysis of 1. Dioxygenation of 20 mg (0.067 mmol) of **1** by LOX-1 (5 mg; 5×10^{-4} mmol) was carried out in 200 mL of 0.1 M borate buffer (pH 9.0) under oxygen flux (1 mL/min) over 4 h. The reaction was stopped by acidification to pH 3 (hydrochloric acid), then sodium chloride was added up to saturation, and the mixture was extracted three times with diethyl ether. The combined organic extract was dried over anhydrous sodium sulfate and the solvent was evaporated under vacuum. Separation of the regioisomeric hydroperoxides **7** and **8** was carried out by normal phase HPLC on an Eurospher Si 100 column (Knauer; 250 × 4 mm; 5 μm) using pentane + ethanol (100 + 1) at a flow rate of 1 mL/min (UV detection, 234 nm). Separation of the isomeric hydroxides (cf. bottom) was performed analogously.

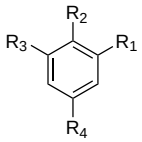
On-Line HPLC−Electrospray Tandem Mass Spectrometry (HPLC−ESI−MS−MS) of Regioisomeric Hydroperoxides. The position of the hydroperoxy group in **7** and **8** was determined by HPLC−ESI−MS−MS in positive mode without derivatization as recently described by Schneider et al. (1997a). The spray capillary voltage was set to 4 kV, and the temperature of the heated inlet capillary was 180 °C. Nitrogen served both as the sheath (482 MPa) and auxiliary gases. MS−MS experiments were performed at a collision gas pressure of 0.24 Pa of argon. HPLC was carried out on an Eurospher 100 C-18 column (Knauer; 100 × 2 mm; 5 μm) with methanol + H_2O (5 mM NH_4Ac) = 85 + 15 as eluent at a flow rate of 0.2 mL/min.

Reduction of Hydroperoxides 7 and 8. To a solution of 5 mg (0.015 mmol) of **7/8** in 50 mL of methanol was added 4.0 mg (0.105 mmol) of $NaBH_4$. The mixture was stirred over 1 h at 0 °C and ambient temperature each, then 0.1 N hydrochloric acid was added, and the solution was extracted three times with diethyl ether. The combined organic phase was washed acid free with distilled water, and the solvent was evaporated under vacuum.

Determination of the Enantiomeric Excess (ee) of 7. $NaBH_4$-reduced **7** was converted into its (−)-menthoxycarbonyl (MC) derivatives, subjected to oxidative ozonolysis and methylated with diazomethane. The obtained diastereomers were separated by gas chromatography (cf. below). Esterification of the chiral hydroxy group was carried out in a mixture of 200 μL of dry toluene, 50 μL of pyridine, and 300 μL of (−)-menthylchloroformate (1 μmol/μL in dry toluene). After 3 h at 60 °C, the surplus (−)-menthylchloroformate was degraded with methanol and the solvent was evaporated. For oxidative ozonolysis, the MC derivatives were taken up in 0.5 mL of dichloromethane and treated with an excess of ozone for 30 min at −20 °C. The solvent was removed under a stream of nitrogen, the residue taken up in 500 μL acetic acid and 100 μL of hydrogen peroxide (30%), and the mixture incubated overnight at ambient temperature. The resulting MC derivatives of 2-hydroxypentanoic acid were extracted from the aqueous solution with diethyl ether and methylated with diazomethane at room temperature. As authentic standard, racemic 2-hydroxypentanoic acid was esterified and methylated analogously. The resulting diastereomers **(9)** were separated by gas chromatography on an achiral column (DB wax; 30 m × 0.25 mm, 0.24 μm). The temperature program was 3 min isothermal at 50 °C and then programmed at 4 °C/min to 240 °C. Mass spectra (70 eV) were recorded in order to identify the MC derivatives of methyl 2-hydroxypentanoate. **9:** EI (+) MS *m/z* (%) 138 (20), 123 (16), 95 (46), 83 (53), 81 (58), 59 (40), 55 (100), 43 (69).

Determination of the Absolute Configuration. The chromophoric derivatives of **7** were prepared as follows: 2-naphthoyltriazole (1 mg, 4.6 μmol) and redistilled 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.7 mL, 4.6 μmol) were added to a solution of sodium borohydride-reduced **7** (1 mg, 3.0 μmol) dissolved in 0.2 mL of dry acetonitrile in a 2-mL conical glass vial under argon atmosphere. The reaction mixture was stirred at ambient temperature overnight, concentrated, and purified by TLC (pentane + diethyl ether = 80 + 20) to yield chromophoric derivative **10**. The UV and CD spectra of **10** were recorded in acetonitrile on a Perkin-Elmer Lambda 4B UV/vis spectrometer and a Jasco J-600 spectropolarimeter, respectively.

Table 1. Structures of Compounds Studied as Substrates for LOX-1 Catalysis and Their Kinetic Parameters[a]



compd	R_1	R_2	R_3	R_4	K_m, μM	k_{cat}, min^{-1}
linoleic acid					19	802
1	(*Z*,*Z*)−8′,11′-pentadecadienyl	H	OH	H	20	76
1a	(*Z*,*Z*)−8′,11′-pentadecadienyl	H	OAc	H	757	68
2	(*Z*,*Z*)−8′,11′,14′-pentadecatrienyl	H	OH	H	21	67
2a	(*Z*,*Z*)−8′,11′,14′-pentadecatrienyl	H	OAc	H	261	64
3	(*Z*,*Z*)−8′,11′-pentadecadienyl	H	OH	OH	23	115
3a	(*Z*,*Z*)−8′,11′-pentadecadienyl	H	OAc	OAc	550	84
4	(*Z*,*Z*)−8′,11′,14′-pentadecatrienyl	H	OH	OH	21	129
4a	(*Z*,*Z*)−8′,11′,14′-pentadecatrienyl	H	OAc	OAc	245	53
5	(*Z*,*Z*)−8′,11′-pentadecadienyl	COOH	OH	H	18	267
5a	(*Z*,*Z*)−8′,11′-pentadecadienyl	COOH	OMe	H	27	170
5b	(*Z*,*Z*)−8′,11′-pentadecadienyl	COOMe	OH	H	136	22
5c	(*Z*,*Z*)−8′,11′-pentadecadienyl	COOMe	OMe	H	186	21
6	(*Z*,*Z*)−8′,11′-heptadecadienyl	COOH	OH	H	22	679
6a	(*Z*,*Z*)−8′,11′-heptadecadienyl	COOH	OMe	H	21	849
6b	(*Z*,*Z*)−8′,11′-heptadecadienyl	COOMe	OH	H	209	58
6c	(*Z*,*Z*)−8′,11′-heptadecadienyl	COOMe	OMe	H	431	40

[a] Data taken from the polarographic assays; photometric results are consistent.

RESULTS AND DISCUSSION

The kinetic parameters determined for the LOX-1 catalyzed dioxygenation of the long-chain alkadien-(trien)ylphenolic substrates under study are summarized in Table 1. Despite their lack of a carboxylic moiety, both long-chain phenols **1** and **2** and resorcinol derivatives **3** and **4** were accepted as substrates by LOX-1. These compounds exhibited K_m values similar to those of linoleic acid (K_m = 19 μM), reflecting the high affinity of LOX-1 toward compounds containing free phenolic groups. The corresponding acetylated compounds **1a**−**4a** showed drastically increased K_m values (K_m > 200 μM). At the pH conditions (pH = 9.0) applied for the enzymatic reaction, phenolic hydroxy groups are deprotonated like the carboxylic group of the unsaturated fatty acids, the common natural substrates of LOX-1. If these functional groups are blocked, e.g., by acetylation, no dissociation is possible and, as a consequence, LOX-1 affinity decreases significantly. Obviously, (a) free phenolic hydroxy group(s) mimic(s) the carboxylic group of fatty acids, which is generally considered as a structural prerequisite for high LOX-1 affinity.

Compounds **1**, **3**, and **5**, possessing free functional groups and bearing the same (*Z*,*Z*)-8′,11′-pentadecadienyl side chain, exhibited comparable K_m values, while the turnover number (k_{cat}) increased from the phenolic (**1**) to the resorcinol (**3**) and the salicylic acid (**5**) compounds. Evidently, the presence of a carboxylic group enhances the catalytic efficiency of the enzyme, and compounds with several free phenolic groups are slightly better substrates of LOX-1 than those with only one free functional group.

Comparison of the kinetic parameters of **1** with **2** and **3** with **4**, respectively, indicates that neither K_m nor k_{cat} was influenced by the presence of two or three double bonds.

Exceptional kinetic parameters were obtained for the salicylic acid compounds **6** and **6a**, reaching not only K_m but also k_{cat} values similar to those of linoleic acid. With these substrates, distinctly higher turnover numbers than with any other substrate under study were observed. As shown in Figure 1, the distance between the aromatic ring (the carboxylic group in the case of linoleic acid) and the pentadiene moiety is fixed for *all* compounds under study, whereas the side chain of linoleic acid and compounds **6/6a**−**c** is two carbon atoms elongated in comparison to all other substrates. Compounds **6/6a**−**c** and linoleic acid are ω-6 compounds, while **1**, **1a**, **3**, **3a**, and **5/5a**−**c** are ω-4 compounds. Our results indicate that next to the presence of free functional groups, two further factors can be considered responsible for the remarkable kinetic parameters of **6** and **6a**, i.e., the chain length and the ω-6 position of the double bond. These observations are in accordance with previous studies carried out by Kühn et al. (1990) and Hatanaka (1993). The first mentioned authors have reported ω-6 compounds to be optimal substrates for LOX-1, exhibiting excellent k_{cat} values and regioselectivity. Hatanaka (1993) has investigated the kinetic parameters of a series of C_{14}−C_{24} carboxylic acids containing a likewise fixed pentadiene carboxyl moiety. His results have revealed the highest LOX-1 activity for linoleic acid and considerably less activity for all longer or shorter carboxylic acids.

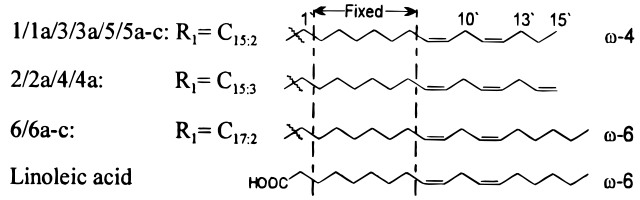


Figure 1. Comparison of the different carbon chains (R_1) of the substrates under study.

The regio- and enantioselectivities of LOX-1 catalysis were investigated with **1** as a representative example (Figure 2). The analytical procedure for the identification of the reaction products is outlined in Figure 3. Separation of the regioisomeric hydroperoxides **7** and **8** was carried out by normal phase HPLC on an Eurospher Si 100 column as shown in Figure 4, and the position of the hydroperoxy group was determined by on-line LC−ESI−MS/MS without derivatization

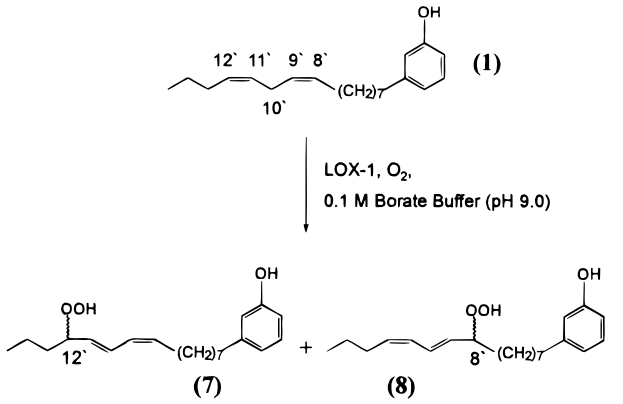


Figure 2. LOX-1-catalyzed dioxygenation of 3-[(*Z*,*Z*)-8′,11′-pentadecadienyl]phenol (**1**).

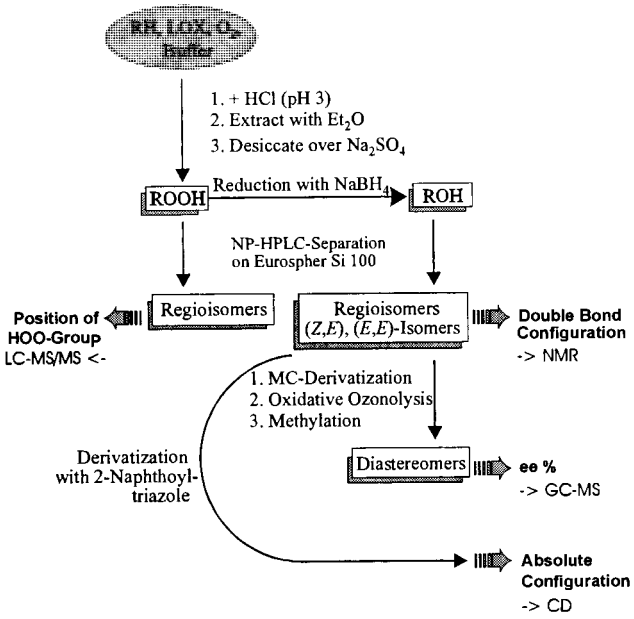


Figure 3. Analytical procedure: isolation and characterization of products formed by LOX-1-catalyzed dioxygenation of 3-[(*Z*,*Z*)-8′,11′-pentadecadienyl]phenol (**1**).

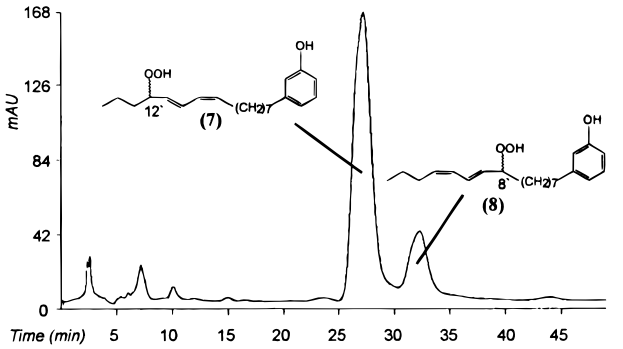


Figure 4. Normal phase HPLC separation of hydroperoxides formed by LOX-1 -catalyzed dioxygenation of 3-[(*Z*,*Z*)-8′,11′-pentadecadienyl]phenol (**1**). Product separation was performed on an Eurospher Si 100 column (250 × 4 mm, 5 μm); flow, 1 mL/min; eluent, pentane + ethanol = 100 + 1, monitored by UV detection at 234 nm.

(Schneider et al., 1997a). Low-energy collision-induced dissociation of precursor ions $[M + NH_4]^+$ with *m*/*z* 350 led to characteristic product ions for both hydroperoxides, due to a cleavage of the carbon bearing the hydroperoxy moiety and the adjoining double-bond carbon. Product ion *m*/*z* 243 was characteristic of the

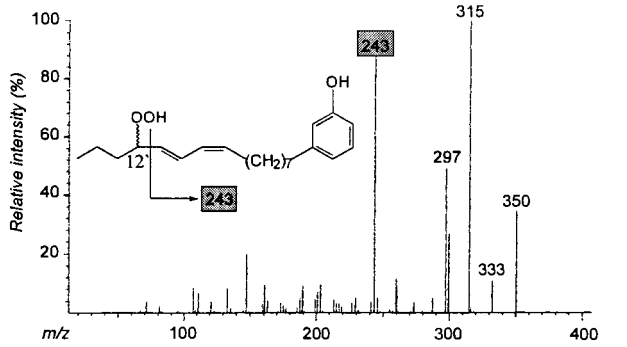


Figure 5. Product ion spectrum of 3-[12′-hydroperoxy-(*Z*,*E*)-8′,10′-pentadecadienyl]phenol (**7**), precursor ion *m*/*z* 350 $[M + NH_4]^+$ (ESI positive mode, −7 eV, 0.24 Pa of argon as collision gas). Inset: fragmentation pattern explaining the characteristic ions observed.

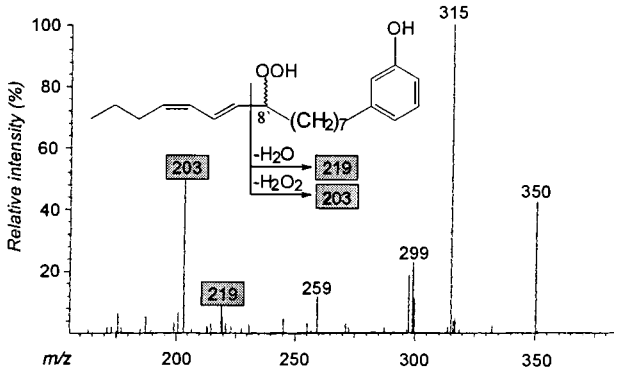


Figure 6. Product ion spectrum of 3-[8′-hydroperoxy-(*E*,*Z*)-9′,11′-pentadecadienyl]phenol (**8**), precursor ion *m*/*z* 350 $[M + NH_4]^+$ (ESI positive mode, −7 eV, 0.24 Pa of argon as collision gas). Inset: fragmentation pattern explaining the characteristic ions observed.

12′-hydroperoxide **7**, resulting from the cleavage of the $C_{11'}-C_{12'}$ bond as shown in Figure 5. In the case of the 8′-hydroperoxide **8**, distinguishing product ions *m*/*z* 219 and 203 arose from the fission of the corresponding $C_{8'}-C_{9'}$ bond and subsequent loss of H_2O and H_2O_2, respectively (Figure 6). Thus, the regioisomers were identified as the 12′-hydroperoxide **7** and 8′-hydroperoxide **8** at an 82:18 ratio.

Further characterization was carried out after reduction of the instable hydroperoxides with sodium borohydride to the corresponding alcohols. In order to determine the configuration of the double bonds, a 400-MHz ^{1}H NMR spectrum of the 12′-hydroxide in $CDCl_3$ was recorded and decoupling experiments were carried out. The ^{1}H NMR data are summarized in Table 2. The 8′-H (5.45 ppm) showed a doublet of a triplet based on its coupling with 9′-H (J = 10.8 Hz) and the two protons of 7′-C (J = 7.9 Hz), 9′-H (5.99 ppm) coupled with 8′-H (J = 10.8 Hz) and 10′-H (J = 11.0 Hz), resulting in a doublet of a doublet. The 10′-H (6.50 ppm) and 11′-H (5.68 ppm) also formed doublets of doublets ($J_{11'-10'}$ = 15.1 Hz). With the coupling constant of 15.1 Hz between 10′-H and 11′-H being characteristic of a trans double bond and J = 11.0 Hz between 8′-H and 9′-H being typical of a cis double bond, the double-bond configuration of **7** was determined as 8′(*Z*), 10′(*E*).

To evaluate the ee, $NaBH_4$-reduced **7** was converted into its (−)-MC derivatives, subjected to oxidative ozonolysis and esterified with diazomethane (Van Aarle, 1993). Separation of the resulting diastereomers, i.e., (−)-MC derivatives of **9**, by GC on an achiral phase

Table 2. ^{1}H NMR (400 MHz) Data of $NaBH_4$-Reduced 3-[12′-Hydroperoxy-(*Z*,*E*)-8′,10′-pentadecadienyl]phenol (Solvent: $CDCl_3$)

δ, ppm	proton	multiplicity	*J*, Hz
7.14	5-H	dd	$J_{5-6} = 7.7$
			$J_{5-4} = 7.7$
6.75	4-H	d	$J_{4-5} = 7.7$
6.66	2-H	d	$J_{2-6} = 1.6$
6.65	6-H	dd	$J_{6-5} = 7.7$
			$J_{6-2} = 1.7$
6.50	10′−H	dd	$J_{10'-11'} = 15.1$
			$J_{10'-9'} = 11.0$
5.99	9′−H	dd	$J_{9'-10'} = 11.0$
			$J_{9'-8'} = 10.8$
5.68	11′−H	dd	$J_{11'-10'} = 15.1$
			$J_{11'-12'} = 7.0$
5.45	8′−H	dt	$J_{8'-9'} = 10.8$
			$J_{8'-7'} = 7.9$
4.20	12′−H	m	n.d.
2.56	1′−H	t	$J_{1'-2'} = 7.7$
2.19	7′−H	m	n.d.
2.12	13′−H	m	n.d.
1.60	2′−H	m	n.d.
1.34−1.24	3′-6′, 14′−H	m	n.d.
0.90	15′−H	t	$J_{15'-14'} = 7.0$

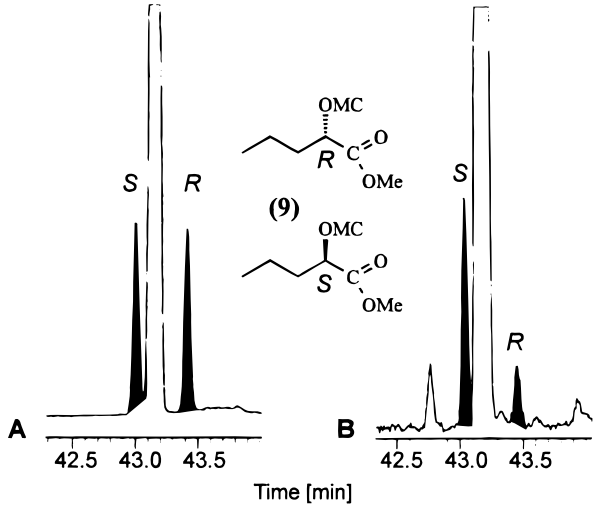


Figure 7. Gas chromatographic separation of the diastereomeric (−)-MC derivatives of methyl 2-hydroxypentanoate. (A) (−)-MC derivatives of racemic methyl 2-hydroxypentanoic acid as the authentic standard; (B) (−)-MC derivatives of enantiomerically enriched methyl 2-hydroxypentanoate, derived from enantiomerically enriched 3-[12′-hydroxy-(*Z*,*E*)-8′,10′-pentadecadienyl]phenol. Column: DB wax (30 m × 0.25 mm, 0.24 μm); temperature program, 50 °C [3 min] → 4 °C/min → 240 °C [10 min].

(Figure 7) revealed an ee of 64%. The identity of the diastereomers was confirmed by GC and GC/MS analyses using derivatized racemic 2-hydroxypentanoic acid as the authentic standard. In addition, the ECCD method (Nakanishi and Berova, 1994) was applied to determine the absolute configuration of **7**, as recently described for similar hydroxylated dienes (Schneider et al., 1997b). In order to introduce an exciton coupling chromophore, the chiral hydroxy group was acylated with 2-naphthoyltriazole. The UV and CD spectra of the 2-naphthoate derivative **10** are shown in Figure 8. The CD spectrum, revealing a positive split CD curve with extrema at 243 nm (Δε +40.0) and 226 nm (Δε −41.0), arises from exciton coupling between the two transition moments of the chromophores. The $\pi \rightarrow \pi^*$ transition of the diene, which is polarized along the long axis of the chromophore, couples with the ^1B_b transition band of the 2-naphthoate chromophore. The first positive Cotton effect at 243 nm and the second negative Cotton effect at 226 nm indicate that the two axes of the 2-naphthoate and diene chromophore adopt a right-handed screw, as is the case for (*S*)-configurated derivatives. Thus, 3-[12′-(*S*)-hydroperoxy-(*Z*,*E*)-8′,10′-pentadecadienyl]phenol was characterized as the main product (67%) of the LOX-1-catalyzed dioxygenation of **1** by extensive chromatographic and spectroscopic studies. The enzyme exhibits distinct regio- and enantioselectivities toward phenolic compound **1**, which can be considered to be a fine substrate for LOX with regard to its kinetic parameters. The selective dioxygenation of the phenolic compounds under study reveals that the carboxylic function in LOX-1 substrates can be replaced by phenolic structures. This finding extends previous studies, in which *N*-linoleoylamides were selectively converted by LOX-1 (Van der Stelt et al., 1997).

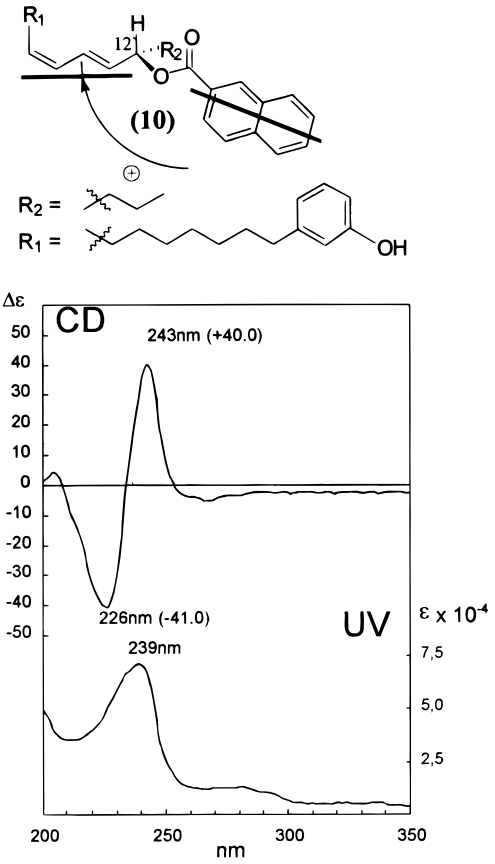


Figure 8. UV and CD spectra of the 2-naphthoyl derivative of 3-[12′-(*S*)-hydroxy-(*Z*,*E*)-8′,10′-pentadecadienyl]phenol in acetonitrile.

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