Keto fatty acids not containing doubly allylic methylenes are lipoxygenase substrates

ARTICLE *in* BIOCHEMISTRY · NOVEMBER 1991

Impact Factor: 3.02 · DOI: 10.1021/bi00106a026 · Source: PubMed

Keto Fatty Acids Not Containing Doubly Allylic Methylenes Are Lipoxygenase Substrates

Hartmut Kühn,*,‡ Lutz Eggert,‡,§ Omitry A. Zabolotsky,∥,⊥ Galina I. Myagkova,∥ and Tankred Schewe‡

Institute of Biochemistry, Medical School (Charitē), Humboldt University, Hessische Str. 3-4, O-1040 Berlin, FRG, and Department of Chemistry and Technology of Fine Organic Compounds, M. V. Lomonosov Institute of Fine Chemical Technology, Vernadsky Prospect, 117571 Moscow, USSR

Received May 2, 1991; Revised Manuscript Received July 18, 1991

ABSTRACT: The soybean lipoxygenase I oxygenates the unusual substrate 12-keto-(9*Z*)-octadecenoic acid methyl ester as indicated by oxygen uptake and spectral changes of the incubation mixture. The main oxygenation products have been isolated by HPLC and identified as 9,12-diketo-(10*E*)-octadecenoic acid methyl ester and 12-keto-(10*E*)-dodecenoic acid methyl ester by UV and IR spectroscopy, cochromatography with an authentic standard, gas chromatography/mass spectroscopy, and ^{1}H NMR. In the formation of both compounds the oxygenase and hydroperoxidase activities of the enzyme appear to be involved. These data and the earlier results on the oxygenation of furanoic fatty acids (Boyer et al., 1979) indicate that the lipoxygenase reaction is not restricted to substrates containing a 1,4-pentadiene structure.

Lipoxygenases are known to oxygenate polyenoic fatty acids containing at least one 1,4-pentadiene system to their corresponding hydroperoxy derivatives (Schewe et al., 1986; Veldink & Vliegenthart, 1984). Saturated or monoenoic fatty acids are not oxygenated by these enzymes but have been shown to be competitive inhibitors of linoleate oxygenation (Holman, 1947; Wisanathan & Cushley, 1981). On the other hand, Boyer et al. reported the oxygenation of 10,13-epoxy-10,12-octadecadienoic acid methyl ester, a furanoic fatty acid not containing a doubly allylic methylene, by the soybean lipoxygenase as indicated by oxygraphic studies and analysis of the reaction products (Boyer et al., 1979). Later on, Wiseman (Wiseman & Nichols, 1988) investigated the conversion of ketopolyenoic fatty acids by lipoxygenases of different positional specificities and observed that soybean 15-lipoxygenase oxygenates 12-keto-(5*Z*,8*Z*,10*E*,14*Z*)-eicosatetraenoic acid (12-KETE)[1] more effectively than the corresponding 12-hydroxy compound. From this observation the authors concluded an initial hydrogen removal from C-13 which is located between a C=C and a C=O double bond and may therefore be regarded as a "quasi-doubly allylic methylene". Unfortunately, the chemical structures of the reaction products have not been identified, so that a hydrogen removal from the doubly allylic methylene C-7 cannot be excluded. Such a hydrogen removal from C-7 has been demonstrated for the oxygenation of 15-H(P)ETE (van Os et al., 1981) by the soybean lipoxygenase.

During a search for lipoxygenase inhibitors we found that 12-keto-(9*Z*)-octadecenoic acid methyl ester, which does not contain a 1,4-pentadiene system, is a substrate for the soybean lipoxygenase. Here, we report the oxygenation of this compound and the identification of the main oxygenation products. The probable mechanism and the possible biological relevance of the reaction are discussed.

Materials and Methods

Chemicals. The chemicals used were from the following sources: linoleic acid, soybean lipoxygenase (grade I, 109 000 units/mg), and deuterated chloroform (isotopic purity >99.8%) from Serva (FRG); triphenylphosphine, sodium borohydride, and sodium dichromate (dihydrate) from Merck (FRG). All solvents used were of analytical grade and were distilled prior to use.

Preparations. 12-keto-(9*Z*)-Octadecenoic acid methyl ester used as substrate was prepared as follows: Castor oil was hydrolyzed under alkaline conditions, the mixture was acidified, and the resulting free fatty acids (more than 90% ricinolic acid) were extracted with diethyl ether. 12-keto-(9*Z*)-Octadecenoic acid methyl ester was obtained by Collins oxidation of methyl ricinoleate and subsequent RP-HPLC purification. Its chemical structure has been confirmed by UV and IR spectroscopy, by GC/MS, and ^{1}H and ^{13}C NMR. An authentic standard of 9,12-diketo-(10*E*)-octadecenoic acid methyl ester was prepared by oxidation of 12-keto-(9*Z*)-octadecenoic acid methyl ester with sodium dichromate (Nichols & Schipper, 1958) and subsequent RP-HPLC purification. Its chemical structure has been confirmed by UV and IR spectroscopy, GC/MS, and ^{1}H NMR.

12-keto-(9*Z*)-Octadecenoic acid methyl ester (200 μM) was incubated with the soybean lipoxygenase (1 μM) in 0.1 M sodium borate buffer, pH 9.0. The oxygen uptake was measured with a Clark-type oxygen electrode. The recorder scale was calibrated by oxidizing known amounts of NADH by submitochondrial particles (Estabrook, 1967). The spectral changes of the reaction mixture were recorded on a Shimadzu SPD 6AV spectrophotometer. After the reaction had ceased (no further oxygen uptake or increase in absorbance at 230 nm), the mixture was twice extracted with diethyl ether. The organic extracts were combined and dried over sodium sulfate.

* To whom correspondence should be addressed.
‡ Humboldt University.
§ Present address: Schwarz Pharma AG, Alfred-Nobel-Str. 10, D-4019 Monheim, FRG.
∥ M. V. Lomonosov Institute of Fine Chemical Technology.
⊥ Part of this work was carried out during a fellowship of D.Z. at the Institute of Biochemistry, Medical School Charitē, Humboldt University.

[1] Abbreviations: 12-KETE, 12-keto-(5*Z*,8*Z*,10*E*,14*Z*)-eicosatetraenoic acid; 15-H(P)ETE, 15-hydro(pero)xy-(5*Z*,8*Z*,11*Z*,13*E*)-eicosatetraenoic acid; 13-H(P)ODE, 13-hydro(pero)xy-(9*Z*,11*E*)-octadecadienoic acid; UV, ultraviolet; IR, infrared; RP(SP)HPLC, reverse-phase (straight-phase) high-pressure liquid chromatography; GC/MS, gas chromatography/mass spectroscopy.

0006-2960/91/0430-10269$02.50/0

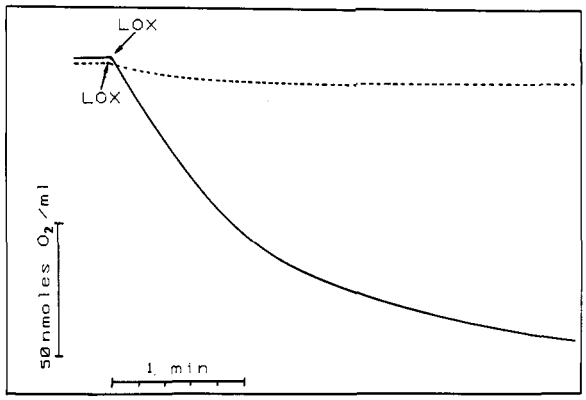


FIGURE 1: Oxygenation of 12-keto-(9Z)-octadecenoic acid and its methyl ester by the soybean lipoxygenase. Soybean lipoxygenase I (1 μM) was incubated with 12-keto-(9Z)-octadecenoic acid (---) and its methyl ester (—) in 0.1 M borate buffer, pH 9.0 (substrate concentration 200 μM), at room temperature. Oxygen consumption was assayed with a Clark-type oxygen electrode; reaction volume was 1.8 mL. The recorder scale was calibrated by oxygenation of known amounts of NADH by beef heart submitochondrial particles.

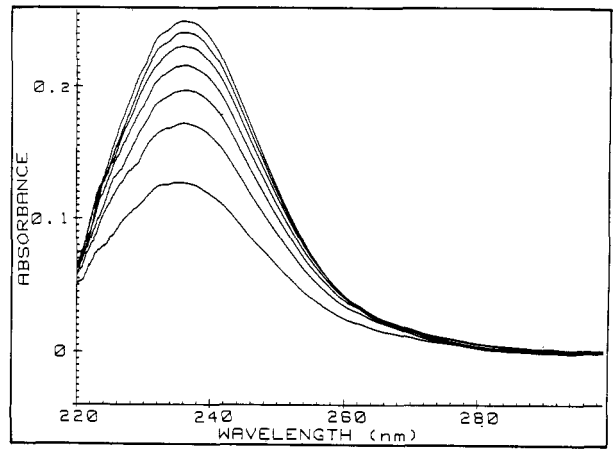


FIGURE 2: Spectral changes of the reaction mixture during the oxygenation of 12-keto-(9Z)-octadecenoic acid methyl ester. Incubation was as described in the legend to Figure 1. Repeated scans were taken on a Shimadzu SPD 6AV spectrophotometer every 5 min. The UV spectrum at the very beginning of the reaction was set as background.

The solvent was evaporated, and the residue was dissolved in 2 mL of methanol and analyzed by RP-HPLC.

The fatty acid derivatives were methylated by treatment with diazomethane at room temperature for 15 min. Compounds were hydrogenated by bubbling hydrogen gas through a solution of 1–5 μg of the keto fatty acids in 200 μL of ethanol (90 s). A total of 2 mg of palladium asbestos was used as a catalyst. Samples were generally stored under argon atmosphere at −20 °C.

Chromatography and Spectroscopy. High-performance liquid chromatography was performed on a Shimadzu instrument coupled with a Hewlett-Packard diode array detector (1040 A). RP-HPLC was carried out on a Nucleosil C-18 column (Macherey-Nagel, KS-system, 250 × 4 mm, 5-μm particle size) with a guard column (30 × 4 mm, 5-μm particle size). Solvent system: methanol/water/acetic acid (85/15/0.1 v/v/v), flow rate 1 mL/min. The UV absorbances at 230 nm and 210 nm were recorded simultaneously. SP-HPLC was carried out on a Zorbax-SIL column (250 × 4.6 mm, 5-μm particle size). Solvent system: *n*-hexane/2-propanol/acetic acid (100/2/0.1 v/v/v); flow rate 1 mL/min.

Gas chromatography/mass spectroscopy was performed on the Shimadzu GC/MS QP 2000 system equipped with a RSL-150 column [poly(dimethylsiloxane)]; 30 m × 0.32 mm). Temperature program: 2 min at 180 °C, then with a rate of 5 deg/min to 250 °C; injector temperature 275 °C, ion source temperature 180 °C. An electron energy of 70 eV was used.

UV spectra of the main products were recorded in methanol on a Shimadzu SPD 6AV spectrophotometer or with the HP 1040 A diode array detector during the chromatographic run. Infrared spectroscopy was carried out on a DIGILAB FTS-15 C Fourier transform spectrophotometer.

^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker AM-300 or a Bruker WM-250 instrument with $CDCl_3$ as solvent at 300 and 250 Hz, respectively. Chemical shifts are reported in relation to tetramethylsilane, and the residual $CHCl_3$ in the solvent was observed at 7.25.

Results

(1) Oxygenation of 12-keto-(9Z)-Octadecenoic Acid Methyl Ester. 12-keto-(9Z)-Octadecenoic acid methyl ester was oxygenated by the soybean lipoxygenase as indicated by oxygraphic measurements (Figure 1). From the oxygraphic trace an oxygenation rate of 200 nmol of O_2/(mg of protein·min) was calculated. This value is more than 1 order of magnitute lower than the rate for methyl linoleate oxygenation [2.2 μmol of O_2/(mg of protein·min)]. In contrast to linoleate, where the free acid (12 μmol of O_2/mg) is a better substrate than the methyl ester (2.2 μmol of O_2/mg), the free 12-keto-(9Z)-octadecenoic acid is scarcely oxygenated (Figure 1). Similarly, 15-HETE methyl ester is a much better substrate for the soybean lipoxygenase than the free acid (Kühn et al., 1986). 12-hydroxy-(9Z)-Octadecenoic acid methyl ester (methyl ricinoleate) was not oxygenated by this enzyme but was shown to be a competitive inhibitor of linoleate oxygenation. This result indicates that substitution of the keto group for a hydroxy grouping prevents the reaction.

The oxygenation of 12-keto-(9Z)-octadecenoic acid methyl ester was also indicated by spectral changes of the incubation mixture. It can be seen from Figure 2 that a product or products with an absorbance maximum at 234 nm were formed. Despite some similarity, the UV spectrum is not identical with that of the usual lipoxygenase products such as 15-HPETE or 13-HPODE (Ingram & Brash, 1988). The λ_{max} value and the shape of the spectrum suggests the formation a 1,4-diketo-2-monoene structure (Abbot et al., 1970; Boyer et al., 1979). Addition of sodium borohydride to the reaction mixture led to the disappearance of the characteristic UV chromophore.

(2) Isolation of the Main Reaction Products. In order to identify the chemical structure of the main products of 12-keto-(9Z)-octadecenoic acid methyl ester oxygenation, a large-scale preparation (2-L reaction mixture, 200 μM substrate, 1 μM enzyme, 1-h incubation at room temperature under oxygen atmosphere) was performed and the products were analyzed by RP-HPLC (Figure 3). With the chromatogram recorded at 230 nm, two main products were detected. Product b, which shows the typical UV spectral properties of the incubation mixture (inset to Figure 3) coeluted with an authentic standard of 9,12-diketo-(10*E*)-octadecenoic acid. For product a, a UV absorbance maximum at 220 nm was found (inset to Figure 3). Quantification of the products prepared indicated a product a/product b ratio of about 1/2. Recording the chromatogram at 210 nm indicated that the reaction was not complete since substantial amounts of the substrate were recovered. After treatment of the reaction products with triphenylphosphine, the chromatogram looked very similar to that of the untreated mixture, suggesting the absence of hydroperoxy compounds which would have been reduced to the corresponding hydroxy derivatives. For detailed

Table I: ^{1}H NMR Spectroscopic Data for 12-keto-(9Z)-Octadecenoic Acid Methyl Ester and Its Main Oxygenation Products

substrate				product a				product b			
chemical shift (ppm)	multiplicity	protons at carbon	coupling constants (Hz)	chemical shift (ppm)	multiplicity	protons at carbon	coupling constants (Hz)	chemical shift (ppm)	multiplicity	protons at carbon	coupling constants (Hz)
0.9	t	18	$J_{17,18}$ = 6.0	–	–	–		0.9	t	18	$J_{17,18}$ = 6.2
1.2–1.4	m	3,4,5,6,7,		1.3–1.4	m	3,4,5,		1.3–1.4	m	3,4,5,6,7,	
1.4–1.7	m	8,14,15,		1.5–1.7	m	6,7,8		1.5–1.7	m	14,15,16,17,	
1.9–2.1	m	16,17									
2.3	t	2	$J_{2,3}$ = 7.0	2.3–2.4	m	2,9		2.3	t	2	$J_{2,3}$ = 7.0
2.4	t	13	$J_{13,14}$ = 7.0	–	–	–	–	–	–	–	–
–	–	–		–	–	–		2.6	t	8,13	J = 7.1
3.2	d	11	$J_{10,11}$ = 6.0	–	–	–		–	–	–	
3.6	s	0[a]		3.7	s	0[a]		3.7	s	0[a]	
5.4–5.7	m	9,10		–	–	–		–	–	–	
–	–	–		6.1	dd	11	$J_{10,11}$ = 15.6	–	–	–	
–	–	–		6.9	dt	10	$J_{9,10}$ = 8.1	6.9	s	10,11	
–	–	–		9.5	d	12	$J_{11,12}$ = 7.9	–	–	–	

[a] The carbon of the methyl group in the ester function is called C-0.

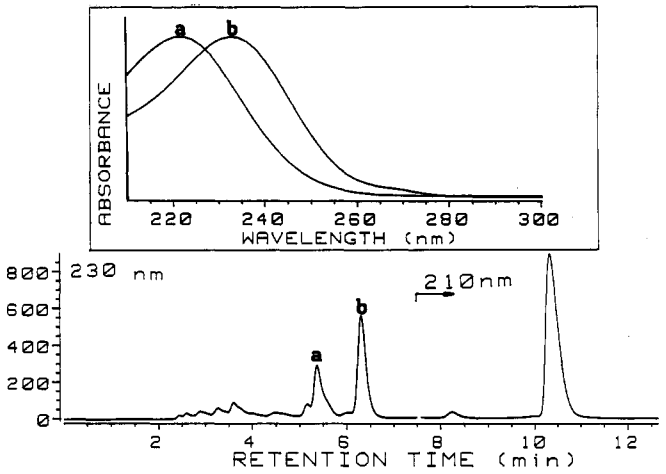


FIGURE 3: RP-HPLC of the products formed during the oxygenation of 12-keto-(9Z)-octadecenoic acid methyl ester. Product preparation and HPLC analysis were as described under Material and Methods. The chromatogram was recorded at 230 nm (0–7.5 min) and at 210 nm (7.5–12.5 min). Inset: UV spectrum recorded at the apex of peaks a and b.

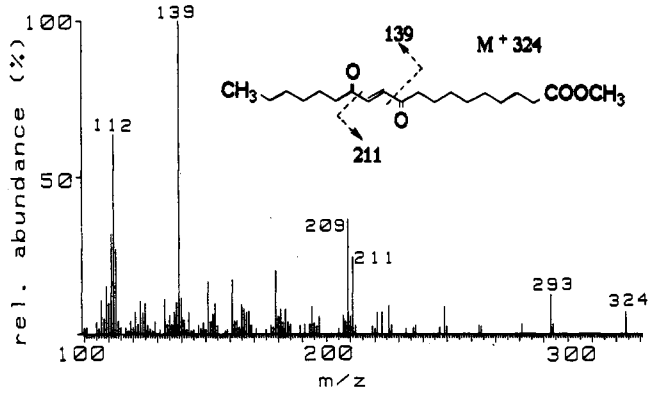


FIGURE 4: Mass spectrum of product b. Product b was isolated by RP-HPLC, repurified by SP-HPLC (*n*-hexane/2-propanol, 100/2 v/v/v), and an aliquot was injected to GC/MS analysis as described under Materials and Methods. Inset: fragmentation pattern explaining the most prominent ions observed.

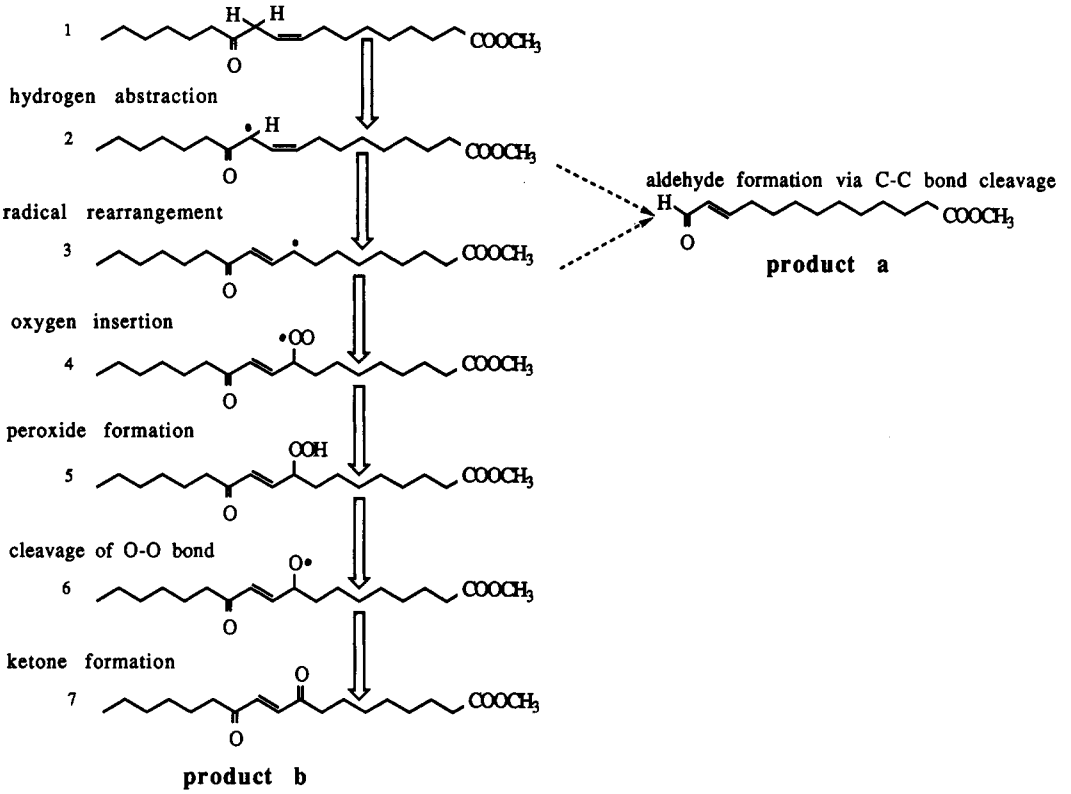
structure elucidation the products a and b, which were proven to be pure by SP-HPLC, were prepared by RP-HPLC.

(*3*) *Structure Elucidation of the Main Products.* The UV spectrum (λ_{max} at 231.5 nm in methanol) and the IR spectrum [a band of strong absorbance at 1739 cm^{-1} (absorbance of the ester carbonyl), bands of weak absorbance at 1688 cm^{-1} (carbonyl absorbance of α-β-unsaturated ketones) and at 1660 (C=C double bond)] of the purified product b suggest 1,4-diketo-2-monoene structure (Boyer et al., 1979). In the gas chromatogram of product b, a single compound chromatographing with a *C*-value of 24.4 was observed. The mass spectrum (Figure 4) with its ions at *m/z* 139 and 211 (α-cleavage) and those at *m/z* 324 (M^+) and 293 ($M^+ - 31$, loss of $CH_3O^\bullet$) suggest the structure of product b as 9,12-diketo-10-octadecenoic acid methyl ester. The prominent ion at *m/z* 112 represents the central unit remaining after McLafferty rearrangement [$^\bullet CH_3COCH{=}CHCOCH_3{}^\bullet$]. The mass spectrum of the hydrogenated compound with its ions of minor intensity at *m/z* 326 (M^+) and 295 ($M^+ - 31$, loss of $CH_3O^\bullet$), the α-cleavage fragments at either site of the keto groups at C-12 [*m/z* 241, $^\bullet CO(CH_2)_2CO(CH_2)_7COOCH_3$; *m/z* 113, $CH_3(CH_2)_2CO^\bullet$] and C-9 [*m/z* 169, $CH_3(CH_2)_5CO(CH_2)_2CO^\bullet$; *m/z* 185, $^\bullet CO(CH_2)_7COOCH_3$], and the McLafferty fragments at *m/z* 256 [$^\bullet CH_3CO(CH_2)_2CO(CH_2)_7COOCH_3$] and at *m/z* 184 [$^\bullet CH_3CO(CH_2)_7CO(CH_2)_5CH_3$] was also supportive of this structure. The mass spectra of both the native and hydrogenated compounds are similar to those reported in the literature for the corresponding free acids (Abbot et al. 1970).

In Figure 5 the ^{1}H NMR spectra of the substrate (part B) and product b (part A) are shown. The spectrum of the substrate was characterized by the signals of the olefinic protons at C-9 and C-10 (m at 5.4–5.7 ppm), by the signals of the protons at C-11 (d at 3.2 ppm), and by the signals of the protons at C-13 (t at 2.40 ppm). In the spectrum of reaction product b, the olefinic protons of the substrate are not present any more. Instead, a singlet, the integral of which indicates two protons, was detected at 6.9 ppm. This signal is due to the olefinic protons of the 1,4-diketo-(2*E*)-monoene structure (Boyer et al., 1970). The four protons at C-8 and C-13 are indicated by the triplet at 2.6 ppm. The data of the NMR analysis, which are summarized in Table I, are compatible with the structure of 9,12-diketo-10E-octadecenoic acid methyl ester. It is of particular interest that this compound is very similar to the product formed during the oxygenation of 10,13-epoxy-10,12-octadecadienoic acid methyl ester by the soybean lipoxygenase I (Boyer et al., 1979).

The UV spectrum of product a with its absorbance maximum at 219 nm looks similar to that of 2-butenal. Its infrared spectrum contains bands of strong absorbance at 1740 cm^{-1} (carbonyl function of the ester group) and 1697 cm^{-1} (carbonyl function of an aldehyde) and a band of weak absorbance at 1652 cm^{-1} (*E* double bond in conjugation to the aldehyde function). Unfortunately, in our hands the mass spectrum of this compound was not very informative. The ^{1}H NMR spectrum of product a (Table I) is characterized by a doublet

Scheme I: Proposed Reaction Sequence for the Oxygenation of 12-keto-(9*Z*)-Octadecenoic Acid Methyl Ester by the Soybean Lipoxygenase I[a]



[a] The dioxygenase step encompasses the formation of the products **1–5**, whereas compounds **6** and **7** are formed via the hydroperoxidase reaction. This scheme does not consider the fact that the abstractions of hydrogen and hydroxy radical presumably proceed in a thermodynamically favorable way as H^+ and OH^-, respectively, by a simultaneous valency change of the enzyme-bound iron (Schewe et al., 1986; Veldink et al., 1984).

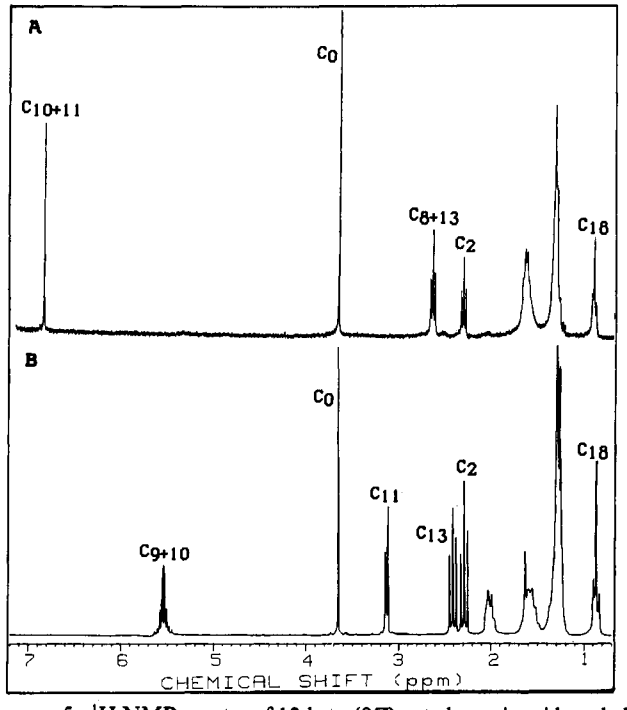


FIGURE 5: ^1H NMR spectra of 12-keto-(9*Z*)-octadecenoic acid methyl ester (B) and its main oxygenation product (A). ^1H NMR spectra were recorded with a Bruker WM-250 (B) and a Bruker AM-300 instrument (A) in $CDCl_3$ at 250 and 300 MHz, respectively.

at 9.5 ppm indicating an aldehyde proton (Glasgow et al., 1986), by a doublet of triplets at 6.9 ppm (one proton by integral), and a doublet of doublets at 6.1 ppm (one proton by integral) in the olefinic region. Decoupling experiments indicated that the signal at 6.1 ppm is coupled to the aldehyde proton whereas the proton at 6.9 ppm is coupled to the signal at 6.1 and to protons giving a signal at 2.3–2.4 ppm. The integral of the multiplets at 1.3–1.4 ppm, at 1.5–1.7 ppm, and at 2.3–2.4 ppm (Table I) indicates 16 protons, suggesting a chain of eight CH_2 groups. Comparison of the spectrum of product a with that of the substrate indicate the presence of the methyl ester protons in both compounds but the lack of the terminal CH_3 protons in product a (Table I). The coupling constant across the double bond C_{10}=C_{11} of 15.6 Hz indicates the trans geometry. Summarizing the data on structure analysis, one may conclude that product a is 12-oxo-(10*E*)-dodecenoic acid methyl ester.

Discussion

For a long time lipoxygenases have been believed to oxygenate only substrates containing at least one doubly allylic methylene group. The data presented here and the report on the oxygenation of furanoic fatty acid (Boyer et al., 1979) clearly indicate that lipoxygenases are also capable of oxygenating fatty acid derivatives not containing a doubly allylic methylene. It is of particular interest that the keto group in the 12-keto-(9*Z*)-octadecenoic acid methyl ester is absolutely necessary for the oxygenation. Despite the fact that the mechanism of the oxygenation of this substrate has not been studied in detail, it is proposed that the reaction sequence is initiated by a hydrogen removal from C-11 which is a "quasi-doubly allylic" one (Scheme I). A hydrogen removal from this carbon atom appears plausible since the unpaired electron in the fatty acid radical formed is delocalized in the π-electron system.

The biological relevance of the oxygenation reaction described remains to be investigated. It is well-known that in plants the combined action of lipoxygenase and hydroperoxide dehydrase on linolenic acid leads to the formation of an allene oxide (Brash et al., 1988; Hamberg, 1987), which is further

decomposed to 12-oxophytodienoic acid and α-ketols (Baertschi et al., 1988; Hamberg, 1988). Since α-ketols contain a "quasi-doubly allylic" methylene group, they may be further oxygenated by lipoxygenases. In fact, it has been shown recently that linoleic acid is converted by the partially purified maize lipoxygenase inter alia to 9,13-dihydroxy-12-keto-(10*E*)-octadecenoic acid. Despite the fact that the mechanism of the formation of this compound has not been studied, it might be possible that it is formed via lipoxygenase-catalyzed oxygenation of an α-ketol and subsequent reduction of the hydroperoxy intermediate (Albian et al., 1991). The biological importance of this metabolic pathway in plants is, however, still unclear.

In general, the primary products of the lipoxygenase reaction are hydroperoxy compounds (Schewe et al., 1986; Veldink & Vliegenthart, 1984). It is, however, well-known that under certain conditions the soybean lipoxygenase I catalyzes the secondary decomposition of hydroperoxy fatty acids (hydroperoxidase activity) forming inter alia keto compounds and short-chain fatty acid aldehydes (Garssen et al., 1971; Veldink & Vliegenthart, 1984). Other lipoxygenases such as isoenzyme I from peas (Kühn et al., 1991; Yoon & Klein, 1979) also exhibit a hydroperoxidase activity as indicated by the formation of 9- and 13-ketooctadecadienoic acids. Lipoxygenases have also been proposed to be involved in the formation of keto compounds (Fruteau deLaclos & Borgeat, 1987) and fatty acid aldehydes (Glasgow et al., 1986) by animal cells. During the formation of methyl 9,12-diketo-(11*E*)-octadecenoate reported here, the corresponding 9-hydroperoxy derivative as intermediate may be presumed. We tried to detect hydroperoxy compounds but failed to do so. Therefore, it might be concluded that the formation of the hydroperoxy intermediate is the rate-limiting step in the reaction sequence leading to the formation of methyl 9,12-diketo-(11*E*)-octadecenoate, whereas the secondary decomposition of the hydroperoxy intermediate is much faster, so that it does not accumulate. Product b was shown to be the only reaction product containing the characteristic UV spectrum of the reaction mixture. From the spectral changes of the incubation mixture we calculated the amount of product b formed [ϵ_{232} = 13 250 $(M\ cm)^{-1}$] and compared it with the oxygen uptake assayed. The results indicated that the formation of product b only accounted for about 15–20% of the oxygen consumption. This result is not surprising since the primary oxygenation product, the 9-hydroperoxy-12-keto-(10*E*)-octadecenoic acid methyl ester (**5** in Scheme I), is likely to be decomposed not only to the corresponding keto derivative (product b) but also to other products that were not detected by HPLC. In fact, earlier studies indicated that 13-HPODE is converted by the soybean lipoxygenase not only to the corresponding keto derivative but also to oxygenated fatty acid dimeres, epoxy hydroxy compounds, short-chain aldehydes, and volatile products (Veldink & Vliegenthart, 1984). Similar products may be formed during the decomposition of the 9-hydroperoxy-12-keto-(10*E*)-octadecenoic acid methyl ester but would escape detection by RP-HPLC because of their hydrophobicity (fatty acids dimeres) or because they do not contain a UV chromophore (epoxy hydroxy compounds).

The mechanism of the formation of 12-oxo-(10*E*)-dodecenoic acid, which is a side product of lipoxygenase-catalyzed oxygenation of 12-oxo-(9*Z*)-octadecenoic acid methyl ester, is difficult to explain. Short fatty acid aldehydes are formed in living cells by the secondary decomposition of hydroperoxy intermediates (Glasgow et al., 1986), which involves a radical-mediated cleavage of a C–C bond. Similarly, the 12-oxo-(10*E*)-dodecenoic acid reported here may be formed via cleavage of the C_{12}–C_{13} bond after enzyme-catalyzed hydrogen removal from C-11 but before oxygen insertion takes place (Scheme I).

It is of particular interest that the methyl ester of the 12-keto-(9*Z*)-octadecenoic acid is a better lipoxygenase substrate than the free acid. A similar behavior has been observed for 15-HETE, the methyl ester of which being more efficiently oxygenated than the free acid (Kühn et al., 1986). The mechanistic reason for this behavior has been proposed to be an inverse orientation of the substrate at the active site of the enzyme, which appears to be favored by methylation of the carboxylic group because of the increase in hydrophobicity. This increase in hydrophobicity renders the carboxylic tail of the substrate fitting better into the hydrophobic substrate binding region of the enzyme.

References

Abbot, G. G., Gunstone, F. D., & Hoyes, S. D. (1970) *Chem. Phys. Lipids 4*, 351.

Abian, J., Gelpi, E., & Pages, M. (1990) *Plant Physiol. 95*, 1277.

Baertschi, S. W., Ingram, C. D., Harris, T. M., & Brash, A. R. (1988) *Biochemistry 27*, 18.

Boyer, R. F., Litts, D., Kostishak, J., Wijesundera, R. C., & Gunstone, F. D. (1979) *Chem. Phys. Lipids 25*, 237.

Brash A. R., Baertschi S. W., Ingram C. D., & Harris T. M. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 3382.

Estabrook, R. W. (1967) *Methods Enzymol. 10*, 41.

Fruteau deLaclos, B., & Borgeat, P. (1987) *Biochim. Biophys. Acta 958*, 424.

Garssen, G. J., Vliegenthart, J. F. G., & Boldingh, J. (1971) *Biochem. J. 122*, 327.

Glasgow, W. C., Harris, T. M., & Brash, A. R. (1986) *J. Biol. Chem. 261*, 200.

Hamberg, M. (1987) *Biochim. Biophys. Acta 920*, 76.

Hamberg, M. (1988) *Biochem. Biophys. Res. Commun. 156*, 543.

Holman, R. T. (1947) *Arch. Biochem. Biophys. 15*, 403.

Ingram, C. D., & Brash, A. R. (1988) *Lipids 23*, 340.

Kühn, H., Schewe, T., & Rapoport, S. M. (1986) *Adv. Enzymol. Relat. Areas Mol. Biol. 58*, 273.

Kühn, H., Wiesner, R., Rathmann, J., & Schewe, T. (1991) *Eicosanoids 4*, 9.

Nichols, J., & Schipper, E. (1958) *J. Am. Chem. Soc. 80*, 5705.

van Os, C. P. A., Rijke-Schilder, G. P. M., van Halbeek, H., Verhagen, J., & Vliegenthart, J. F. G. (1981) *Biochim. Biophys. Acta 663*, 177.

Schewe, T., Rapoport, S. M., & Kühn, H. (1986) *Adv. Enzymol. Relat. Areas Mol. Biol. 58*, 191.

Veldink, G. A., & Vliegenthart, J. F. G. (1984) *Advances in Inorganic Biochemistry* (Eichhorn, G. L., & Marzilli, L. G., Eds.) Vol. 6, p 139, Elsevier, Amsterdam.

Wisanathan, T. S., & Cushley, R. J. (1981) *J. Biol. Chem. 256*, 7155.

Wiseman, J. S., & Nichols, J. S. (1988) *Biochem. Biophys. Res. Commun. 154*, 544.

Yoon, S., & Klein, B. (1979) *J. Agric. Food Chem. 27*, 955.