

## The Origin and Structures of Dimeric Fatty Acids from the Anaerobic Reaction between Soya-bean Lipoxygenase, Linoleic Acid and its Hydroperoxide

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In an anaerobic system soya-bean lipoxygenase catalyses in the presence of linoleic acid and L-13-hydroperoxyoctadeca-*cis*-9-*trans*-11-dienoic acid the formation of dimeric fatty acids and of carbonyl compounds. The analogous reaction does not take place when D-9-hydroperoxyoctadeca-*trans*-10-*cis*-12-dienoic acid is used instead of the 13-hydroperoxy isomer. Non-oxygenated dimers stem directly from linoleic acid and have C<sub>(11)</sub>-C<sub>(13)</sub> or -C<sub>(9)</sub> and C<sub>(13)</sub>-C<sub>(13)</sub> or -C<sub>(9)</sub> linkages. Dimers that contain oxygen originate from linoleic acid and linoleic acid hydroperoxide. It is most likely that the oxygen is present in epoxy groups.

As reported previously (Garssen *et al.*, 1971), soya-bean lipoxygenase (EC 1.13.1.13) catalyses *in vitro* a chain-cleavage reaction of L-13-hydroperoxyoctadeca-*cis*-9-*trans*-11-dienoic acid in the presence of linoleic acid at pH 9, provided that molecular oxygen is excluded. This cleavage reaction leads to the formation of 13-oxotrideca-*cis*(*trans*)-9-*trans*-11-dienoic acid and *n*-pentane, whereas simultaneously several fatty acid dimers and 13-oxo-octadeca-9,11-dienoic acid are formed.

The present study deals with the characterization of the dimeric products. In addition, the origin of the dimer constituents was investigated by means of incubations with [1-<sup>14</sup>C]linoleic acid or its enzymically prepared hydroperoxides.

### Materials and Methods

#### Materials

[1-<sup>14</sup>C]Linoleic acid (57 mCi/mmol) was purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Linoleic acid (purity >99%) was a gift from the Unilever Research Laboratories, Vlaardingen/Duiven, The Netherlands. Soya-bean lipoxygenase (activity 8000 units/mg) was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A., or isolated from soya beans. Subsequent purification resulted in an electrophoretically homogeneous protein with a pH optimum of 9. The linoleic acid hydroperoxides were prepared enzymically (Garssen *et al.*, 1971). Soya-bean lipoxygenase (Nutritional Biochemicals Corp.) was used for the preparation of the L-13-hydroperoxylinoleic acid; the lipoxygenase isolated from maize (*Zea mays*) germ was used to prepare the D-9-hydroperoxy isomer (Veldink *et al.*, 1972).

The hydroperoxide concentration was determined spectrophotometrically, by using  $\epsilon_{234}$  25000 litre·mol<sup>-1</sup>·cm<sup>-1</sup> (Johnston *et al.*, 1961). All reagents were of analytical-grade quality.

#### Incubation procedure

In a vessel provided with a seal cap, linoleic acid (0.36 mmol) and linoleic acid hydroperoxide (0.18 mmol) were dissolved as their sodium salts in 100 ml of 0.2 M-sodium borate buffer, pH 9.0. The solution was flushed with H<sub>2</sub> until a very low oxygen concentration was reached (0-5  $\mu$ M; checked with a Clark oxygen electrode). Then 30 mg of lipoxygenase (Nutritional Biochemicals Corp.) in 0.4 ml of de-aerated buffer was injected through the seal cap, and during incubation at 25°C a blanket of H<sub>2</sub> was maintained above the incubation mixture.

#### Isolation and detection of reaction products

After 1 h the products were recovered by acidification of the reaction mixture and extraction with diethyl ether. The washed extracts were dried over Na<sub>2</sub>SO<sub>4</sub> and the ether was evaporated *in vacuo*. The fatty acids were methylated with CH<sub>2</sub>N<sub>2</sub> and separated on t.l.c. plates of silica gel G (0.3 mm layer) with solvent systems containing *n*-hexane and diethyl ether in different proportions as indicated in the text. After t.l.c. the methyl esters were located by spraying the edges of the plates with 5% (w/v) phosphomolybdic acid in 96% (v/v) ethanol followed by heating at 110°C for 10 min. Silica gel G impregnated with AgNO<sub>3</sub> (30%, w/w) was used for further separation; the compounds were detected under u.v. light after the plate had been sprayed with 0.2%

2,7-dichlorofluorescein in 96% (v/v) ethanol. After this t.l.c. the distribution of  $^{14}\text{C}$ -labelled compounds was obtained by scanning (Berthold Dünnschicht-scanner II, provided with an electronic integrator). Samples of the isolated radioactive compounds in ethanol were dried in a stream of  $\text{N}_2$ , and then 16 ml of scintillation fluid that contained 2,5-diphenyloxazole (0.5%, w/v) and 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene (0.03%, w/v) in toluene was added. Radioactivity was measured with a Packard Tri-Carb liquid-scintillation spectrometer. The relative counting efficiency was 90%.

#### Identification of reaction products

U.v. spectra were recorded with a Unicam SP.800B or a Zeiss PMQ-2 spectrophotometer. I.r. spectra of the products as pure liquids were obtained with an Infracan H900 instrument. Proton-magnetic-resonance (p.m.r.) spectra were registered with a Varian HA-100 spectrometer with FT-100 Fourier-transform accessory.

The 70eV-mass spectra were recorded with an AEI-MS 9 spectrometer, provided with a direct insertion probe, at an ion-chamber temperature of 80–100°C.

Ester determinations were performed as described by Renkonen (1961).

Reductive ozonolysis (Pryde *et al.*, 1962) was followed by the conversion of the carbonyl products into the dinitrophenylhydrazones. Dinitrophenylhydrazones were separated by the method of Meijboom (1966) and determined spectrophotometrically at 357 nm in chloroform.

## Results

#### *Anaerobic incubation of linoleic acid together with L-13-hydroperoxylinoleic acid*

After t.l.c. of the fatty acid methyl esters in *n*-hexane–diethyl ether (3:2, v/v), in addition to the carbonyl compounds previously described, two bands were detected in the region more polar than that of the methyl ester of unchanged linoleic acid. These were collected together and rechromatographed in *n*-hexane–diethyl ether (4:1, v/v), which gave a better resolution into fractions  $\text{D}_1$  ( $R_F$  0.50) and  $\text{D}_2$  ( $R_F$  0.38) (cf. methyl linoleate:  $R_F$  0.63). A further purification of the components was obtained by t.l.c. on  $\text{AgNO}_3$ -impregnated silica gel in *n*-hexane–diethyl ether (7:3, v/v). Fraction  $\text{D}_1$  separated into components  $\text{D}_{1.1}$  ( $R_F$  0.50), and  $\text{D}_{1.2}$  ( $R_F$  0.23), and fraction  $\text{D}_2$  gave components  $\text{D}_{2.1}$  ( $R_F$  0.46) and  $\text{D}_{2.2}$  ( $R_F$  0.35). U.v. spectra in ethanol gave strong absorption maxima at 241 nm (component  $\text{D}_{1.1}$ ), 236 nm (component  $\text{D}_{1.2}$ ), 236 nm (component  $\text{D}_{2.1}$ )

and 235 nm (component  $\text{D}_{2.2}$ ). No shift was observed with *n*-hexane as a solvent, which points to the presence of conjugated diene systems. After hydrogenation in methanol with a platinum catalyst ( $\text{PtO}_2$ ) the u.v. absorption in the 230–240 nm range disappeared. Mass spectra showed pronounced molecular ion peaks at  $m/e$  586 (components  $\text{D}_{1.1}$  and  $\text{D}_{1.2}$ ) and  $m/e$  602 (components  $\text{D}_{2.1}$  and  $\text{D}_{2.2}$ ), which shifted to  $m/e$  594 and 608 respectively after hydrogenation.

**Component  $\text{D}_{1.2}$ .** The mass spectrum of the hydrogenated component  $\text{D}_{1.2}$  (above  $m/e$  250) and  $m/e$  values of the characteristic fragment ions are shown in Fig. 1 and Table 1. This component showed a fragmentation that is characteristic for a dibasic acid methyl ester. From the intensities of the peaks characteristic for the branching position of the two  $\text{C}_{18}$  chains it is concluded that the compound with a  $\text{C}_{(11)}\text{--}\text{C}_{(13)}$  link is present in excess over a compound with a  $\text{C}_{(11)}\text{--}\text{C}_{(9)}$  link. The partial structure (I) is proposed for component  $\text{D}_{1.2}$ , account being taken of the p.m.r. data as summarized in Table 2. The quartet at  $\delta$ 3.20 (1 proton) is consistent with the proton on the  $\text{C}_{(11)}$  position. There is clear evidence for a *cis*–*trans* conjugated diene system in view of the coupling constants for  $J_{c,d}$  (10.5 Hz) and  $J_{c',e}$  (14.5 Hz), which are characteristic for coupling across *cis* and *trans* double bonds respectively. A spectrum recorded in  $[\text{D}_6]\text{benzene}$  suggested that the *trans* double bond is adjacent to the branching with an apparent doublet of doublets ( $J_{d',c}$  9 Hz and  $J_{c',e}$  15 Hz) at  $\delta$ 5.60 emerging from the complex absorption from the other remaining olefinic protons. No conclusion could be drawn about the stereoconfiguration of the isolated double bonds. I.r. absorptions at 720 and  $3010\text{cm}^{-1}$  revealed the *cis* configuration of the isolated double bonds. Moreover, the presence of a *cis*–*trans* conjugated diene system ( $985$  and  $950\text{cm}^{-1}$ ) was confirmed.

Component  $\text{D}_{1.2}$  was subjected to reductive ozonolysis followed by conversion of the degradation products into the dinitrophenylhydrazones. After separation the dinitrophenylhydrazones of hexanal and methyl 9-oxo-nonanoate were found. Their molar ratio was found to be 1:1.5 by spectrophotometry. These data are consistent with a mixture of  $\text{C}_{(11)}\text{--}\text{C}_{(13)}$ -linked dimer and a smaller amount of  $\text{C}_{(11)}\text{--}\text{C}_{(9)}$ -linked isomer. The polyfunctional dinitrophenylhydrazones that remained at the starting line were not further examined.

**Component  $\text{D}_{1.1}$ .** From the mass spectrum of the hydrogenated component  $\text{D}_{1.1}$  (Fig. 1 and Table 1) it was concluded that the main type of branching involves a  $\text{C}_{(13)}\text{--}\text{C}_{(13)}$  linkage and that to a minor degree a  $\text{C}_{(13)}\text{--}\text{C}_{(9)}$ -linked compound is present. The p.m.r. data for component  $\text{D}_{1.1}$  (Table 2), namely coupling constants of 14.5 Hz for  $J_{h,f}$  and 10.5 Hz for  $J_{f,g}$ , clearly indicate that all olefinic protons present are in a *cis*–*trans* conjugated system (II). It was not

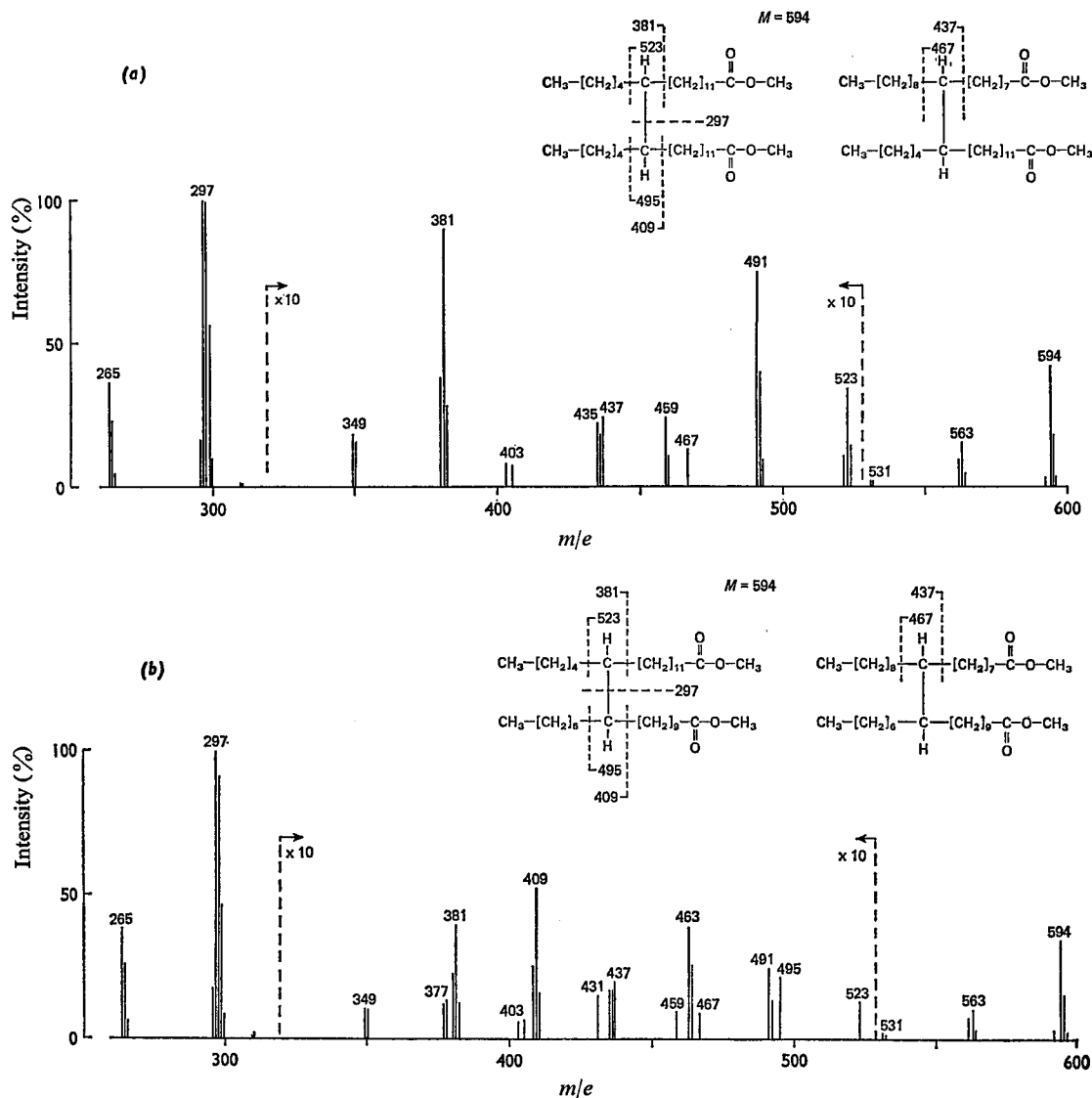


Fig. 1. Mass spectra of the methyl esters of the hydrogenated dimeric fatty acids  $D_{1.1}$  (a) and  $D_{1.2}$  (b)

possible to interpret the complex for protons (*f*), although, however, their pattern was clearly different from that for the corresponding protons in methyl octadeca-*cis*-9-*trans*-11-dienoate. It is most likely that the branching points at  $C_{(13)}$  or  $C_{(9)}$  are adjacent to the double-bond systems. Their exact position could not be derived from the available p.m.r. data. I.r. data for component  $D_{1.1}$  confirmed the presence of a *cis*-*trans* conjugated diene system ( $985$  and  $950\text{cm}^{-1}$ ). After reductive ozonolysis the component

$D_{1.1}$  dimer gave the dinitrophenylhydrazones of hexanal and methyl 9-oxononanoate in the molar ratio 1:1.5.

**Components  $D_{2.1}$  and  $D_{2.2}$ .** The mass spectra of components  $D_{2.1}$  and  $D_{2.2}$  could not be fully elucidated. The observed loss of water in the fragmentation pathway and the molecular ion peaks, which were 16 mass units higher than those for components  $D_{1.1}$  and  $D_{1.2}$ , are indicative of dimers containing an extra oxygen atom. The observed loss of

Table 1. Characteristic fragment ions in the mass spectra of the methyl esters of the hydrogenated dimeric fatty acids  $D_{1.1}$  and  $D_{1.2}$ 

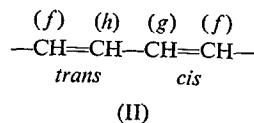
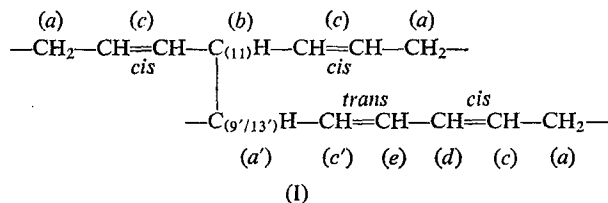
Proposed ion	m/e		
	Ion	$-\text{CH}_3\text{OH}$	$-2 \times \text{CH}_3\text{OH}$
$M$	594	562	
$M-\text{CH}_3\text{O}$	563	531	
$M-\text{C}_5\text{H}_{11}$	523	491	459
$M-\text{C}_7\text{H}_{15}$	495	463	431
$M-\text{C}_9\text{H}_{19}$	467	435	403
$M-\text{C}_7\text{H}_{14}\text{CO}_2\text{CH}_3$	437	405	
$M-\text{C}_9\text{H}_{18}\text{CO}_2\text{CH}_3$	409	377	
$M-\text{C}_{11}\text{H}_{22}\text{CO}_2\text{CH}_3$	381	349	
$\frac{1}{2}M$	297	265	

Table 2. Relevant chemical shifts and coupling constants of the methyl esters of fatty acids  $D_{1.1}$  and  $D_{1.2}$ 

P.m.r. spectra were obtained in carbon tetrachloride with tetramethylsilane as internal standard.

Component	Proton*	Chemical shift ( $\delta$ ) (p.p.m.)	Pattern	Coupling constants ( $J$ ) (Hz)	Integral
$D_{1.1}$	$\text{H}_{(f)}$	5.15-5.45	Complex		4
	$\text{H}_{(g)}$	5.89		$J_{g,h}$ 10.5	2
	$\text{H}_{(h)}$	6.17		$J_{f,g}$ 10.5 $J_{h,f}$ 14.5	2
$D_{1.2}$	$\text{H}_{(a)}$	1.90-2.25	Complex		Approx. 7
	$\text{H}_{(b)}$	3.20	Approx. quartet (~8)		
	$\text{H}_{(c)}$	5.05-5.40	Complex		
	$\text{H}_{(d)}$	5.85		$J_{c,d}$ 10.5	1
	$\text{H}_{(e)}$	6.13		$J_{d,e}$ 11.0 $J_{c'e}$ 14.5	1

\* Notations refer to formulae (I) and (II).



$\text{CH}_3\text{O}$ ,  $\text{CH}_3\text{OH}$  and  $\text{CH}_3\text{O}+\text{CH}_3\text{OH}$  is characteristic for a dibasic acid methyl ester. From i.r. spectra, which were nearly identical, no evidence was found for ether or hydroxy groups; however an absorption band at  $885\text{cm}^{-1}$  probably represents a *trans*-epoxy group. In addition a *cis-trans* conjugated diene system and an isolated *trans* double bond ( $975\text{cm}^{-1}$ ) were noticed. P.m.r. spectra from carbon tetrachloride solutions showed the normal absorptions of fatty acid methyl esters. The complexity of the patterns of the olefinic protons (approx. 6) suggests

that the components are mixtures of isomers. Broad absorptions in components  $D_{2.1}$  and  $D_{2.2}$  at  $\delta 2.42$  and  $\delta 2.47$  (approx. 2 protons) respectively are consistent with the protons of an isolated *trans*-epoxy group. Also, for component  $D_{2.1}$  indications were found for a *trans*-epoxy group next to a double bond. Two absorptions at lower field position, i.e. at  $\delta 2.58$  (complex pattern) and at  $\delta 2.87$  (doublet-split doublet), were assigned to the protons of this epoxy group. The coupling constant of 2 Hz observed in the latter absorption is a typical value for a *trans*-epoxy coupling.

Although structures of the epoxy dimers have not so far been fully elucidated, it seems reasonable to propose the partial structures (III) and (IV).

Molar extinction coefficients of the dimeric fractions are given in Table 3. The high  $\epsilon$  value for component  $D_{1,1}$  points to the presence of two conjugated diene systems in the molecule.

The following experiments were conducted to trace the origin of the dimeric products. Linoleic acid (24.4 mg), 20  $\mu\text{Ci}$  of  $[1-^{14}\text{C}]$ linoleic acid and 13.5 mg of linoleic acid hydroperoxides were incubated anaerobically in 20 ml of 0.2M-sodium borate buffer, pH9.0, with 4.8 mg of purified soya-bean lipoxygenase at 25°C for 60 min. The products were isolated and separated as their methyl esters by t.l.c. and scanned for radioactivity (Fig. 2a). Besides unchanged methyl linoleate only the dimers proved to be labelled; their radioactivity amounted to 30% of the total. Sub-fractions were obtained as described above. From the

specific radioactivities (Table 4) it was concluded that components  $D_{1,1}$  and  $D_{1,2}$  stem entirely from the linoleic acid whereas the dimers that contain oxygen arise from one molecule of linoleic acid and one molecule of linoleic acid hydroperoxide. A cross-check was obtained by incubating 6.2 mg of enzymically prepared  $[1-^{14}\text{C}]$ linoleic acid hydroperoxides ( $4.1 \times 10^5$  c.p.m./ $\mu\text{mol}$ ) and 12 mg of unlabelled linoleic acid together with 2.6 mg of purified lipoxygenase under anaerobic conditions in 15 ml of the borate buffer, pH9.0, for 60 min. The products were isolated and separated as their methyl esters. The distribution of radioactivity is shown in Fig. 2(b). Now the dimer zone  $D_1$  contained no radioactivity. Zones A and C represent the esters of unchanged linoleic acid and linoleic acid hydroperoxides respectively; zones B and E contain the 13-oxo-octadeca-9,11-dienoic acid and 13-oxo-trideca-9,11-dienoic acid methyl esters respectively. The structure of the polar product (zone F) has not yet been elucidated. In addition to zone  $D_2$ , the zones C, B, E and F also contain radioactivity. Fraction  $D_2$  was separated into components  $D_{2,1}$  and

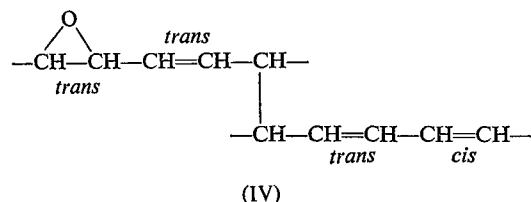
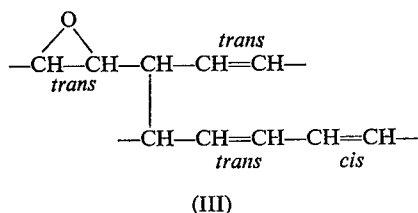


Table 3. Molar extinction coefficients of dimeric fatty acid methyl esters calculated by relating extinction values to ester values

Methyl stearate was used as a standard. Values are given as means  $\pm$  S.E.M. for the numbers of determinations shown in parentheses.

Dimer component	$10^{-3} \epsilon$ (litre $\cdot$ mol $^{-1}$ $\cdot$ cm $^{-1}$ )
$D_{1,1}$	$51 \pm 7$ (3)
$D_{1,2}$	$30 \pm 4$ (4)
$D_{2,1}$	$29 \pm 4$ (3)
$D_{2,2}$	$26 \pm 3$ (3)

Table 4. Specific radioactivities of starting materials and of dimeric fatty acids (methyl esters) isolated from the incubation experiments (a) and (b)

Specific radioactivities were calculated from scintillation counting and extinction values. Values are given as means  $\pm$  range for two determinations

Starting material	Product	$10^{-5} \times$ Specific radioactivity (c.p.m./ $\mu\text{mol}$ )
(a) $[1-^{14}\text{C}]$ Linoleic acid		$1.8 \pm 0.2$
	$D_{1,1}$	$3.7 \pm 0.6$
	$D_{1,2}$	$3.3 \pm 0.5$
	$D_{2,1}$	$1.8 \pm 0.3$
	$D_{2,2}$	$2.4 \pm 0.4$
(b) Hydroperoxy $[1-^{14}\text{C}]$ linoleic acid		$4.2 \pm 0.4$
	$D_{2,1}$	$4.5 \pm 0.7$
	$D_{2,2}$	$5.9 \pm 0.9$

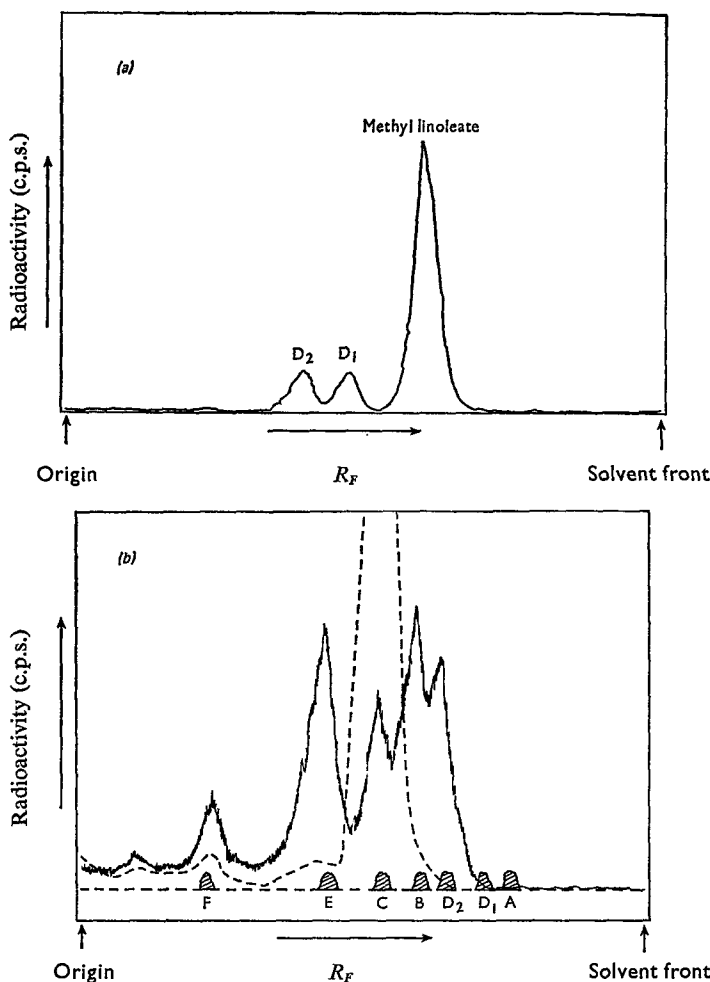


Fig. 2. Radioactivity scanning diagrams after t.l.c. (silica gel G) of the methylated reaction products from anaerobic incubations

(a) Incubation of soya-bean lipoxygenase, [ $1-^{14}\text{C}$ ]linoleic acid and 13-hydroperoxylinoleic acid. The solvent system was *n*-hexane-diethyl ether (3:1, v/v). (b) Incubation of soya-bean lipoxygenase, linoleic acid and 13-hydroperoxy[ $1-^{14}\text{C}$ ]linoleic acid. The solvent system was *n*-hexane-diethyl ether (3:2, v/v). ----, Sample before the addition of enzyme. The bands A-F were located by spraying with phosphomolybdic acid.

$D_{2,2}$ , the specific radioactivities of which (Table 4) show that they stem from hydroperoxylinoleic acid and linoleic acid.

#### Anaerobic incubations of linoleic acid together with D-9-hydroperoxylinoleic acid

Incubations under anaerobic conditions (Garssen *et al.*, 1971) were performed with the hydroperoxides prepared with maize-germ lipoxygenase (yielding

approx. 85% of the D-9-isomer and 15% of the L-13-isomer) together with linoleic acid and purified soya-bean lipoxygenase at pH 10.0. Surprisingly the 9-isomer did not give rise to the formation of oxodiene compounds. After incubations on a preparative scale virtually all the 9-hydroperoxy isomer was recovered unchanged. Only products arising from the conversion of the concomitant 13-isomer were found. Evidently the D-9-hydroperoxylinoleic acid is not converted under these anaerobic conditions.



## Discussion

The anaerobic formation of dimeric fatty acids and of carbonyl compounds from linoleic acid and 13-hydroperoxylinoleic acid is catalysed by soya-bean lipoxygenase; heat-denatured enzyme (100°C for 3 min) does not give any conversion into these products.

The fact that both a normal substrate and its product participate in this enzyme-dependent reaction suggests binding of the hydroperoxide to the enzyme, perhaps at a special 'product site'. Another indication for a specific interaction with the enzyme is the observation that the isomeric 9-hydroperoxylinoleic acid does not give rise to an analogous reaction. Smith & Lands (1972) have presented a kinetic model for the aerobic lipoxygenase catalysis in which they presume distinct product and substrate sites on the enzyme.

In the aerobic reaction of soya-bean lipoxygenase with linoleic acid it is highly probable that, in analogy to the finding by Hamberg & Samuelsson (1967) with bis-homo- $\gamma$ -linolenic acid as a substrate, the rate-determining step is the stereospecific abstraction of hydrogen from C<sub>(11)</sub>. The enzyme may hold the hydrogen atom, resulting in a radical state of the lipoxygenase.

Our observation that dimeric fatty acids consisting of two linoleic acid residues or of a linoleic acid and a hydroperoxide moiety are formed suggests the participation of linoleic acid radicals, and thus it is reasonable to assume that the abstraction of hydrogen at C<sub>(11)</sub> is also the initial step in the anaerobic reaction. In the absence of oxygen the hydroperoxide would then react with the [enzyme-H]-radical-[linoleic acid]-radical complex.

Since there is still no clue about the nature of the active centre, we visualize this as indicated in Scheme 1, which should be considered as a tentative model. The character of X and Y is unknown. It cannot be excluded that X-Y represents a disulphide bridge. The return of the enzyme into its native form is a prerequisite to account for the catalytic nature of the conversion of the hydroperoxide (approx. 80% conversion). Tentative pathways may be designed proceeding either through an alkoxy (a) or a peroxy (b) radical, which result from the reaction between 13-hydroperoxylinoleic acid and the enzyme-radical complex: (a) in a radical displacement at the O-O bond of 13-hydroperoxylinoleic acid (LOOH) an alkoxy radical (LO $\cdot$ ) and water may be formed, regenerating the enzyme at the same time; (b) by abstraction of an H radical from LOOH the enzyme passes into the reduced state; the peroxy radical (LOO $\cdot$ ), after a rearrangement, gives rise to chain fission; the new radicals then regenerate the enzyme. Both possibilities are summarized in Scheme 1. Formation of non-oxygenated fatty acids (LL) can be

brought about by coupling between an enzymically formed and a non-enzymically formed linoleic acid radical in close proximity to the enzyme. The latter radical could be created by a pentyl radical (or other radical splitting products), which removes a C<sub>(11)</sub> hydrogen atom from an intact linoleic acid molecule in a non-stereospecific way. The dimers with a C<sub>(11)</sub> linkage to C<sub>(13)</sub> or C<sub>(9)</sub> must have been produced in a synchronous process, whereas the formation of the oxygen-containing dimers (LLO) would result from reaction of an enzymically formed linoleic acid radical with a rearranged alkoxy or peroxy radical. Whether linoleic acid alkoxy or peroxy radicals are key intermediates in this anaerobic reaction remains to be established. With regard to this question suitable labelling experiments may give more information. If, for example, L-[11-<sup>3</sup>H]linoleic acid is used as the substrate, 13-oxotridecadienoic acid will be found unlabelled if the proposed alkoxy pathway is correct. If, however, the reaction proceeds via peroxy radicals, as outlined in Scheme 1, at least part of the abstracted <sup>3</sup>H label should be reincorporated in the formation of these products, provided that no exchange takes place of the enzyme-bound <sup>3</sup>H radical with water. Moreover, more information will be gained into the mechanism of the formation of LL dimer. The outcome of these experiments as well as those with D-[11-<sup>3</sup>H]linoleic acid might also provide a better understanding of the mechanism of the enzymic hydroperoxidation *per se*.

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