

## Formation of Hydroperoxy Bis-epidioxides in Sensitized Photo-oxidized Methyl Linolenate

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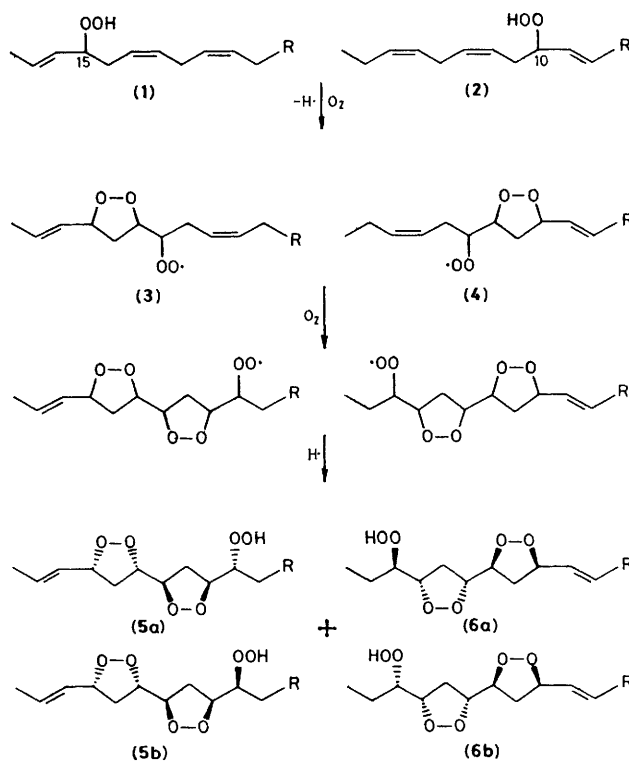
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Unique 10- and 15-hydroperoxides formed by sensitized photo-oxidation of methyl linolenate undergo serial 1,3-cyclization to produce hydroperoxy bis-epidioxides that may be separated into four diastereoisomeric pairs of enantiomers.

Although the uneven isomeric composition of methyl linolenate hydroperoxides has been known for a long time,<sup>1</sup> only recently has direct evidence been obtained to explain this distribution of isomers that does not conform to the classical free-radical autoxidation mechanism.<sup>2</sup> The tendency of the 12- and 13-hydroperoxide isomers to cyclize was first suggested to explain their significantly lower concentrations than the 9- and 16-hydroperoxide isomers in autoxidized methyl linolenate.<sup>2-4</sup> The formation of cyclic hydroperoxy epidioxides has now been firmly established as an important process in the autoxidation of methyl linolenate,<sup>5,6</sup> and in the sensitized photo-oxidation of methyl linoleate.<sup>7</sup> These cyclic oxidation products have received much attention recently because they may play a biological role as prostaglandin-related endoperoxides.<sup>4,8</sup> The 12- and 13-hydroperoxides in autoxidized linolenate and 10- and 12-hydroperoxides in sensitized photo-oxidized linoleate produce the homoallylic radicals required for 1,3-cyclization into the 5-membered epidioxides.<sup>5-7,9</sup> We now report that 1,3-cyclization of the 10- and 15-hydroperoxides of sensitized photo-oxidized methyl linolenate provides a pathway for both mono- and bis-epidioxides (Scheme 1). This type of serial cyclization has been suggested in the autoxidation of lipids on the basis of model studies with a 1,5-diene system producing 6-membered bis-cyclic peroxides.<sup>10</sup>

When methyl linolenate was photo-oxidized at 0 °C in methylene dichloride solution in the presence of Methylene Blue as sensitizer (40 to 45% conversion), the external 9- and 16-hydroperoxide isomers were found in significantly larger concentration than the internal 10-, 12-, 13-, and 15-hydroperoxides.<sup>11</sup> The hydroperoxy epidioxides isolated (in yields of 8 to 11% of oxidation products) by silicic acid chromatography were identified by g.l.c.-mass spectrometry (after hydrogenation to the triols followed by trimethylsilylation<sup>4</sup>) and <sup>1</sup>H n.m.r. spectroscopy.<sup>5</sup> The major portion (97%) of these hydroperoxy epidioxides comprised those derived from cyclization of the 12- and 13-hydroperoxides (9-hydroperoxy-10,12-epidioxo-13,15-diene and 16-hydroperoxy-13,15-epidioxo-9,11-diene), also formed in autoxidized methyl linolenate.<sup>5</sup> The hydroperoxy epidioxides from free radicals (3) and (4) derived from the corresponding 10- and 15-hydroperoxides (1) and (2) were formed in only minor amounts (3%) because they tend to cyclize again to form the hydroperoxy bis-epidioxides (5) and (6) (Scheme 1).

The diastereoisomers (5a), (6a), (5b), and (6b) (isolated in yields of 3 to 4% of oxidation products) were separated in increasing order of polarity by h.p.l.c. (10 μm microporous silica column, eluting with hexane-methylene dichloride-ethyl acetate, 5:3:1, v:v:v). Hydrogenation of (5) and (6) yielded, respectively, methyl 9,10,12,13,15- and 10,12,13,15,16-pentahydroxystearate (g.l.c.-mass spectrometry of the trimethylsilyl derivatives). The hydroxy-derivatives of (5) and (6) produced by Ph<sub>3</sub>P reduction cleaved under g.l.c.-mass spectrometry conditions (after trimethylsilylation) to yield fragments due to α-cleavage at the O-Me<sub>3</sub>Si-bearing carbon atom and bicyclic fragments. Studies of the <sup>1</sup>H n.m.r. spectra



Scheme 1. R = [CH<sub>2</sub>]<sub>n</sub>CO<sub>2</sub>Me.

for each isomer (5a), (5b), (6a), and (6b) are consistent with those reported for hydroperoxy monoepidioxides from methyl linoleate<sup>7</sup> and linolenate<sup>5,6</sup> (Table 1). Their stereochemistry is based on previous n.m.r. studies<sup>7,9</sup> showing that the more polar diastereoisomeric mono-epidioxides produce on hydrogenation the all-*R* trihydroxystearate. Positional isomers could be distinguished by the absorption due to the vinylic methyl group ( $\delta$  1.73) in (5a) and (5b), and due to the methyl  $\beta$  to CH-OOH ( $\delta$  1.05) in (6a) and (6b). The diastereoisomers† (5a)-(5b) and (6a)-(6b) could be differentiated by the shifts of the protons on carbon atoms bearing the hydroperoxy groups ( $\delta$  3.88 and 4.17) and the hydroperoxy protons ( $\delta$  8.73 and 9.20). The coupling constant for the olefinic protons on C-16 (5a, 5b) and C-9 (6a, 6b) confirmed *i.r.* evidence ( $\nu_{\max}$  920 cm<sup>-1</sup>) for isolated *trans* unsaturation. The stereochemical relationship between the hydroperoxide group in (5) and (6) relative to the adjacent epidioxide could be established by the n.m.r. data (Table 1) and retention volume order relative to the monoepidioxides identified in sensitized photo-oxidized methyl linoleate.<sup>7</sup> The relative ring stereo-

† Structures (5a), (5b), (6a), and (6b) each consist of pairs of enantiomers and only one isomer is shown.

**Table 1.**  $^1\text{H}$  (90 MHz) spectra of (5) and (6).

$\delta$	J/Hz	Multiplicity	Assignment	
			(5a), (5b)	(6a), (6b)
9.20 (5a), (6a)		s	OOH	OOH
8.73 (5b), (6b)		s	OOH	OOH
5.88	15.1, 6.2	dd	16-H	9-H
5.35		m	17-H	8-H
4.47		m	10,12,13,15-H	
4.17 (5a), (6a)		m	9-H	16-H
3.88 (5b), (6b)		m	9-H	16-H
2.1—2.8		m	11,14-H	11,14-H
1.73 (5a), (5b)	5.8, 1.5	dd	18-H	
1.05 (6a), (6b)		t		18-H

chemistry was only deduced, however, on steric grounds and from examination of models.

Although serial cyclization has been proposed as a general process for polyolefin autoxidation in natural lipids,<sup>10</sup> the required precursors to bis-epidioxides (Scheme 1) are only present in sensitized photo-oxidized but *not* in autoxidized methyl linolenate. Methyl arachidonate (eicosa-5,8,11,14-tetraenoate) and other fatty esters containing more than three double bonds, however, produce internal isomeric hydroperoxides,<sup>12,13</sup> on autoxidation, that would be expected to undergo serial cyclization.

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