Selective Formation of Dihydroperoxides in the α-Tocopherol Inhibited Autoxidation of Methyl Linolenate

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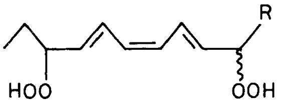
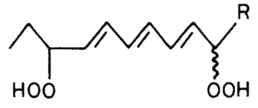
Autoxidation of methyl linolenate in the presence of α-tocopherol leads to the selective formation of 9,16-dihydroperoxides in addition to the four previously reported isomeric monohydroperoxides.

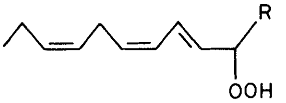
A selective effect of α-tocopherol in suppressing isomerisation and cyclisation reactions in linolenate[1] and arachidonate[2] autoxidations has been previously observed. A closer examination of the products from the α-tocopherol inhibited autoxidation of methyl linolenate revealed that there was a second more polar product formed in addition to mono-



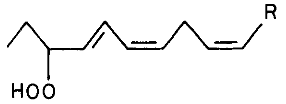
(1a,b)



(2a,b)



(3)



(4)

$R = [CH_2]_7CO_2Me$

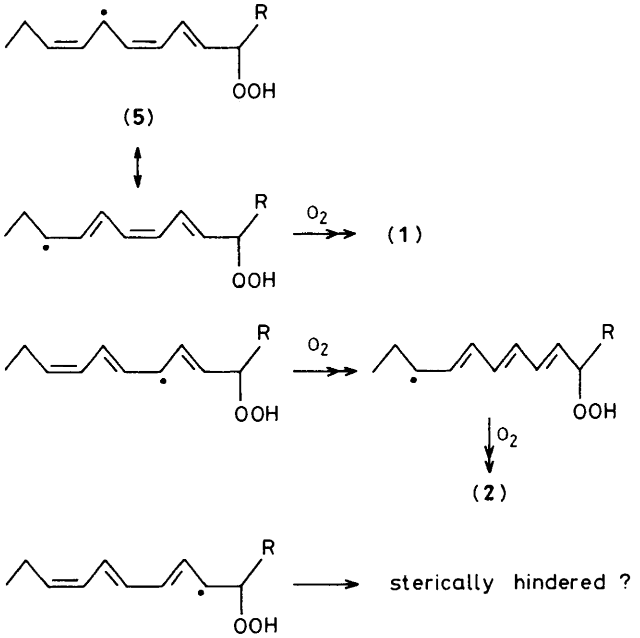
Table 1. ^{1}H N.m.r. spectra (300 MHz) δ values.

	9-H, 16-H	10-H, 15-H	11-H, 14-H	12-H, 13-H	-OOH
(1a)	4.40	5.68	6.76	6.06	7.83, 7.88
(1b)	4.40	5.69	6.77	6.06	7.83 (2H)
(2a)	4.36	5.66	6.30	6.30	7.76, 7.77
(2b)	4.36	5.66	6.29	6.29	7.74, 7.77

hydroperoxides. At the same time it was observed that in the monohydroperoxide fraction from these oxidations the proportions of 9- and 16-isomers were consistently less than those of the 12- and 13-isomers. This more polar product has now been identified as an isomeric mixture of 9,16-dihydroperoxides (**1**) and (**2**). The significance of this relatively new class of lipid oxidation product in both food and biological systems has yet to be investigated.

Methyl linolenate (either neat or as a 10% w : w solution in hexane) was autoxidised in oxygen in the presence of α-tocopherol (10% w : w). Oxidation of the neat liquid at 40 °C in the dark produced approximately 30% conversion into diene monohydroperoxide (as estimated by u.v. absorbance for conjugated diene at 234 nm, $\varepsilon = 25\,000$) after 600 h. Dihydroperoxides were isolated (in yields up to 14% of the oxidation products) by silicic acid chromatography followed by individual isomer separation by h.p.l.c. (25 × 1 cm 10 μm microporous silica column; eluting with hexane–ethanol, 97 : 3, v : v; flow rate 8 ml min^{-1}). Both diastereoisomers (**a**) and (**b**) of each product were well separated with retention times (**1a**) 11.4, (**1b**) 13.2, (**2a**) 14.7, and (**2b**) 17.2 min. U.v. spectra of the four isomers were virtually identical with λ_{max} 259, 268, and 278 nm (ε_{max} 34 000, 43 400, and 33 500, respectively). Products were characterised by ^{1}H n.m.r. spectroscopy (Table 1 gives pertinent data). The symmetry of the triene systems in both the *trans,cis,trans* and the all *trans* isomers is reflected in the occurrence of overlapping pairs of olefinic proton resonances, *i.e.* 9-H and 16-H, 10-H and 15-H, 11-H and 14-H, and 12-H and 13-H. The positions of oxygenation were determined by g.l.c.–mass spectrometry of trimethylsilyl derivatives of the corresponding dihydroxystearates obtained by catalytic hydrogenation.

Dihydroperoxides may be formed by further oxidation of either the 9- or 16-monohydroperoxides hence explaining the lower proportion of these two isomers in the accompanying monohydroperoxide fraction. Oxidation of the isolated 9-hydroperoxide of methyl linolenate[3] in the presence of α-tocopherol provided an even greater yield (*ca.* 20%) of exactly the same mixture of 9,16-dihydroperoxides as obtained from methyl linolenate autoxidised with α-tocopherol. Both the 9- and 16-monohydroperoxides (**3**) and (**4**) retain a *cis,cis*-penta-1,4-diene system which should make them more susceptible to further oxidation than the 12- and 13-isomers. Thus, for example, the initial step in the further free radical oxidation of the 9-hydroperoxide (**3**) would be abstraction of a hydrogen atom from C-14 to produce the conjugated radical (**5**). Various resonance stabilised forms of this radical could lead to different positional isomers by further reaction with oxygen (Scheme 1).



Scheme 1. $R = [CH_2]_7CO_2Me$.

Dihydroperoxides have been previously obtained by lipoxygenase catalysed oxidation of linolenic and arachidonic acids[4—7] and by photo-oxidation[8] and autoxidation[9] of methyl linolenate. The reported autoxidation products were conjugated diene 9,12- and 13,16-dihydroperoxides in addition to conjugated triene 9,16-dihydroperoxides. No information on the relative proportions of these products was provided.

In the α-tocopherol inhibited autoxidation of methyl linolenate or its 9-hydroperoxide only conjugated triene 9,16-dihydroperoxides were obtained. The four isomers were produced in essentially equal proportions representing in total about 14% of the oxidation products (the remainder being monohydroperoxides). The mechanism of this oxidation reaction is under further investigation.

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