A Hydroperoxy-epidioxide from the Autoxidation of a Hydroperoxide of Methyl Linolenate

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Summary Unlike autoxidation of linolenic acid or methyl linolenate which yields mixtures of hydroperoxy-epidioxides, sequential oxidation employing an enzymic first step followed by an autoxidation step can give rise to a single hydroperoxy-epidioxide

OXIDATION of unsaturated lipids containing more than two double bonds, *e.g.* methyl linolenate, can proceed readily with the incorporation of two molecules of oxygen to yield hydroperoxy-epidioxides. Previous investigations[1,2] using the fatty acid or its ester as the starting material resulted in complex mixtures of hydroperoxy-epidioxides. Whereas methods are available for the analysis of the mixtures of hydroperoxides formed[3] and for the preparation of a single hydroperoxide,[4] procedures for the preparation and analysis of individual hydroperoxy-epidioxides have not hitherto been devised. A method has been developed in this study whereby a single hydroperoxide isomer derived from the enzymic oxidation of linolenic acid was autoxidised to yield a hydroperoxy-epidioxide which was purified and analysed by h.p.l.c.

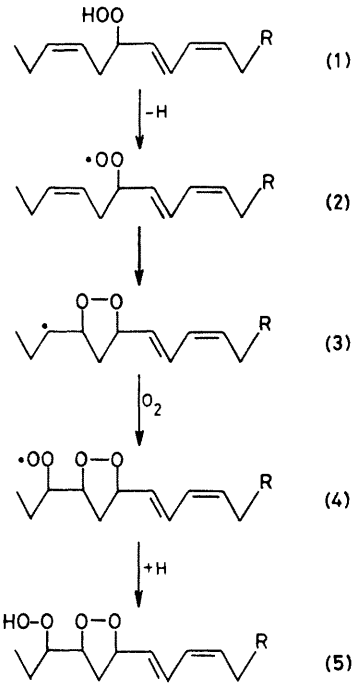
The hydroperoxide (**1**) (isomeric purity 97%; 5·7 μmol ml^{-1} in hexane) prepared by methylation (CH_2N_2) of the hydroperoxyacid obtained from the lipoxygenase-catalysed oxidation[4] of linolenic acid was heated (40 °C) in air for 96 h to yield a mixture containing (**1**) (25·4%), (**5**) (31·5%), and a mixture of polar products (43·0%). Preparative h.p.l.c.[3] yielded (**5**) (λ_{max} 233 nm; ν_{max} 952, 988, and 1745 cm^{-1}; *cis–trans* conjugated diene and COOR; peroxide to diene ratio[3] 1·71). Reduction ($NaBH_4$ followed by Pt/H_2) of (**5**) yielded methyl 13,15,16-trihydroxystearate {mass spectrum of tri-Me_3Si derivative after g.c. contained m/e = 131 ($EtCHOSiMe_3$) and 315 ($Me_3SiOCH[CH_2]_{11}CO_2Me$}. Spin decoupling experiments permitted the assignment of nine ^{1}H multiplets (Table) in the 360 MHz spectrum of (**5**) ($CDCl_3$) and confirmed the *cis*-9 ($J_{9,10}$ 10 Hz) and the *trans*-11 ($J_{11,12}$ 15 Hz) double bonds and the presence of the 5-membered cyclic peroxide ring.

TABLE. ^{1}H N.m.r. spectrum (360 MHz) of (**5**).

δ	Multiplicity	J/Hz	Assignment
2·47	octet[a]	12, 8, 5	14-H_A
2·84	octet[a]	12, 8, 9	14-H_B
4·15	octet[a]	9, 7, 3	16-H
4·49	octet[a]	9, 7, 5	15-H
4·80	q[b]	8, 8, 8	13-H
5·55	m[c]	10	9-H
5·62	q	15, 8	12-H
6·01	br t[b]	11, 10	10-H
6·67	q	15, 11	11-H

[a] Partially resolved. [b] Apparent. [c] Unresolved.

Formation of (**5**) from (**1**) is consistent with the mechanism outlined in the Scheme in which H-abstraction from (**1**) by (**4**) constitutes a propagation step of a chain. The steps involved resemble those of autoxidation but with the incorporation of a cyclisation step (**2**) → (**3**). Cyclisation of peroxyl radicals has been used to produce prostaglandin-like products[5] and the formation of peroxyl radicals from unsaturated fatty acid hydroperoxides has been implicated



R = $[CH_2]_7CO_2Me$

SCHEME

by the exchange of the peroxidic oxygen atoms with molecular oxygen in the rearrangement of the hydroperoxides.[6] In the formation of (**5**) from (**1**), the intermediacy of the peroxyl radical (**2**) was indicated by the enrichment in ^{18}O (26·1 atom %) in the oxygen atom at C-13 in (**5**) when the reaction was carried out in $^{18}O_2$ (70·4 atom %).

The formation of the mixture of hydroperoxy-epidioxides by the autoxidation of methyl linolenate has been assumed to proceed *via* the cyclisation of the peroxy radical (**2**) formed during autoxidation.[1] The isolation of a hydroperoxy-epidioxide from a hydroperoxide represents a new approach and is based on the previous observation[6] that hydroperoxides give rise to peroxyl radicals which undergo further radical chain reactions. By carrying out the two oxygenation reactions as distinct steps, each with a single starting material, a hydroperoxy-epidioxide has been obtained to enable detailed examination of its properties.

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