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Exploratory Study of β-Carotene Autoxidation

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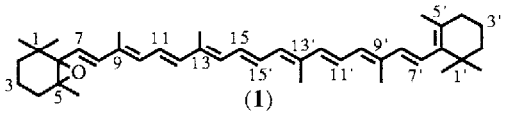
Abstract The main products in the early stages of β-carotene autoxidation were epoxides, β-ionone, β-apo-13-carotenone, retinal, and related carbonyl compounds, in the final mixture short chain carbonyl compounds predominated

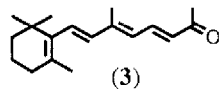
Carotenoids are naturally occuring pigments which owe their brilliant colours to the presence of extensively conjugated polyene chains Many carotenoids are biologically active For example, certain carotenoids (most notably, β-carotene) are sources of retinol (vitamin A), carotenoids protect plants from photosensitised oxidative damage[1], probably by deactivating singlet oxygen[2], epidemiological evidence indicates that carotenoid intake correlates inversely with the incidence of some types of cancer[3]; carotenoids have been shown to retard the development of some experimentally induced animal tumors[4], and finally, carotenoids have antioxidant properties at the low oxygen pressures found in tissues[5]. Unfortunately, carotenoids are highly reactive towards molecular oxygen and may therefore be quite rapidly degraded in foodstuffs during storage even at reduced temperatures. The products of such oxidative degradation of carotenoids and their potential physiological activities have, nevertheless, received remarkably little attention.

In the present work, we have examined the products formed during the self-initiated autoxidation of β-carotene These have not previously been separated by HPLC, nor adequately characterised Classic work, involving product separation by column chromatography/TLC and examination by UV spectroscopy, had indicated that only β-carotene epoxides were formed[6,7]

Purified β-carotene was allowed to react with molecular oxygen at 30 °C in the dark in benzene and in tetrachloromethane[8], and aliquots were withdrawn at timed intervals and analysed[9] by UV, GC/MS(EI), HPLC/UV, HPLC/MS(CI), and NMR. Satisfactory separation of the complex reaction mixture was achieved by reverse phase HPLC using a Spherisorb S5 ODS 2 column Products were tentatively identified from their mass and UV spectra and identification was confirmed by comparison of retention times, and other parameters, with authentic materials By these procedures the following compounds have been identified as products which are formed within the first few hours of oxidation: 5,6-epoxy-β-carotene (**1**), retinal (**2**),



(1)



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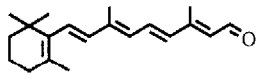

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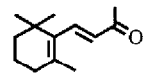

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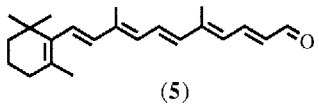
β-apo-13-carotenone (**3**), β-ionone (**4**), β-apo-14'-carotenal (**5**), β-ionylidene acetaldehyde (**6**), 5,6,5',6'-diepoxy-β-carotene, 5,8-epoxy-β-carotene (**7**) and 5,8,5',8'-diepoxy-β-carotene.



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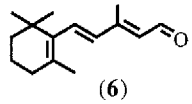

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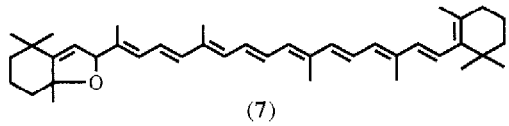
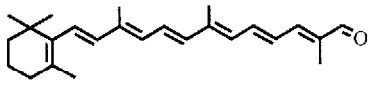


(7)

The product mixture became even more complex as the oxidation progressed. β-Apo-12'-carotenal (**8**) was identified, as well as a number of shorter-chain mono- and di-oxygenated compounds including, β-cyclocitral (**9**), 2,2,6-trimethylcyclohexanone (**10**), dihydroactinidiolide (**11**), 5,6-epoxy-β-ionone (**12**) and 5,8-epoxy-β-ionone (**13**).



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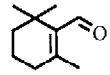

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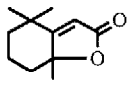

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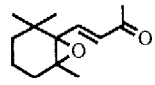

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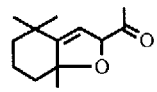


(13)

A substantial number of minor products were also formed but were not identified. After ca. 24 h all the β-carotene had been consumed[10] but oxygen uptake continued for another 24 h. In the later stages of the oxidation the longer-chain products, including the epoxides (**1**) and (**7**) and the carbonyls (**2**), (**3**), (**5**), (**6**) and (**8**), were consumed. The major components of the 48 h mixture were (**4**) and (**12**). Some of the short chain compounds including (**6**), (**10**), (**11**), and (**13**) were reported by Onyewu et al.[11] to be major products in the thermal degradation of β-carotene in glycerol under nitrogen.

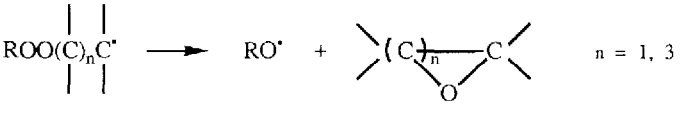
Epoxides and carbonyl compounds were formed even at the earliest stages of the oxidation. Epoxide

formation appears to have occured mainly or entirely in the end rings. The extensive series of carbonyl compounds formed indicates that oxidative scission of β-carotene can occur at a variety of sites along the chain (as has been suggested previously[12])

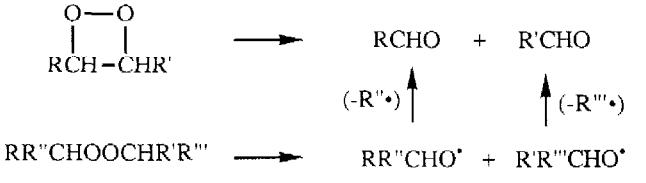
From a mechanistic viewpoint, the absence of certain classes of products can be as revealing as the presence of other classes Thus, no hydroperoxides or peroxides were detectable by HPLC (although such materials *may* have decomposed during analysis). The alcohols retinol and β-ionol were not formed in measurable yields at any stage in the oxidation and if other alcohols were formed they can only have been very minor products. This absence of alcohols indicates that the carbonyl compounds were not formed by the peroxyl/peroxyl termination reaction[13].

$$RR'CHOO^{\bullet} + RR'CHOO^{\bullet} \longrightarrow RR'CHOH + RR'C{=}O + O_2$$

Lipid peroxidation principally involves the abstraction of allylic and bis-allylic hydrogen atoms by peroxyl radicals[14]. In sharp contrast, the main processes in the autoxidation of β-carotene appeared not to give products in which an allylic position had become functionalized. This implies that oxidative addition to the conjugated system (followed by scission) is strongly favoured over abstraction. While it would be premature to formulate a complete mechanism for autoxidation of β-carotene at this time we do suggest that this is a peroxyl radical mediated reaction. Furthermore, we propose that the formation of 3- and 5-membered cyclic ethers occurs by the well-precedented[15] S_Hi reactions,



and that the carbonyl compounds are formed by the thermal decomposition of unstable cyclic and/or acyclic peroxides, e.g.,



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8. Typical procedure: molecular oxygen was bubbled through benzene (50 ml) containing purified β-carotene (587 mg) at 30 °C in the dark. Aliquots were withdrawn at intervals and analysed by the techniques described[9]. Reactions were also carried out in tetrachloromethane under an atmosphere of oxygen.
9. Analytical conditions: GC/MS(EI) was carried out on a Hewlett Packard GC HP 5890A coupled to a Finnigan MAT INCOS 50 MS in the electon impact mode (70 eV). The column was a Hewlett Packard fused silica capillary column (cross linked 50% phenylmethyl silicone 25 m x 0.2 mm). HPLC was performed on a Perkin Elmer series 410 LC pump equipped with an LC 235 Diode Array detector. Reversed phase HPLC on a Spherisorb S5 ODS 2 column (250 x 4.6 mm), developed with a quaternary solvent system of water/methanol/acetonitrile /dichloromethane (linear gradient) at a flow rate of 1 ml/min, and monitored at 350 nm and 250 nm. LC/MS was performed on a Hewlett-Packard 5988A-1090 system operated in the "thermospray" mode; a similar column was employed.
10. β-Carotene consumption was monitored by FT-IR observation of the 960 - 975 cm^{-1} absorption.
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