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Identification of products from canthaxanthin oxidation

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ABSTRACT

Canthaxanthin is a carotenoid that lacks pro-vitamin A activity but is known to have antioxidant activity. The products of its oxidation in oxygen were found to be mainly substituted apo-carotenals and apo-carotenones. The product profile resembles that obtained in the oxidation of β-carotene, except that with canthaxanthin these products are the 4-oxo-β-apo-carotenals and 4-oxo-β-apo-carotenones. Epoxides and diepoxides were clearly identified from β-carotene oxidation but in contrast, with canthaxanthin, apart from 5,6-epoxy-canthaxanthin, which was detected at the early stage of oxidation and minor quantities of 5,6-epoxy-β-ionone and 5,6-epoxy-4-oxo-β-apo-11-carotenal, no other epoxides were detected. The identities of these products lead us to suggest that the mechanism of canthaxanthin oxidation bears significant similarity to that of β-carotene.

1. Introduction

Canthaxanthin (**1**, Chart 1) is a carotenoid of the xanthophyll family as it contains ketonic oxygens at positions 4 and 4′ of its cyclohexene rings. The presence of these carbonyl oxygens means that canthaxanthin lacks pro-vitamin A activity. It is, however, known to have antioxidant activity (Mayne & Parker, 1989; Surai, 2012; Venugopalan et al., 2013). Canthaxanthin has been reported to show antitumour activity, inducing apoptosis in cancer cells, as well as being effective in the treatment of photosensitivity by preventing cellular damage that arises from the interaction of porphyrins and light (Chew, Park, Wong, & Wong, 1999; Mathews-Roth, 1982, 1993; Palozza et al., 1998). Canthaxanthin is added to food, but it has been reported that the intake of foods with added canthaxanthin can lead to canthaxanthin retinopathy, retinal dystrophy and aplastic anemia (Bluhm, Branch, Johnston, & Stein, 1990; Daicker, Schiedt, Adnet, & Bermond, 1987; Hennekes, 1986; McGuiness & Beaumont, 1985; Oosterhuis, Remky, Nijinan, Craandijk, & de Wolff, 1989; Sujak, 2009). Canthaxanthin is also used as a skin tanning agent in cosmetics (Baker & Gunther, 2004; Lober, 1985).

Even with such diverse uses, and its reported antioxidant and anti-tumour activities, the oxidation products of canthaxanthin have not been characterised. Our objectives, therefore, were to investigate its interaction with oxygen, to identify its degradation products and to propose a reasonable mechanism for its degradation. Study of the air oxidation of β-carotene, which has a closely related structure, has already been reported (Mordi et al., 1991, 1993). Recognition of how the xanthophyll ketonic functional group might or might not influence oxidation product type and distribution was of particular interest in this investigation. By broadening the scope to include a xanthophyll structure we aimed to gain a more comprehensive understanding of the oxidative degradation modes of the carotenoid family of food additives.

2. Materials and method

Canthaxanthin (Lilley) was first purified by SiO_2 chromatography with dichloromethane as the eluting solvent. The first two coloured components (yellow and orange) collected (300 ml) were discarded on the assumption that they contained impurities. The methodology adopted was similar to that used in air oxidation

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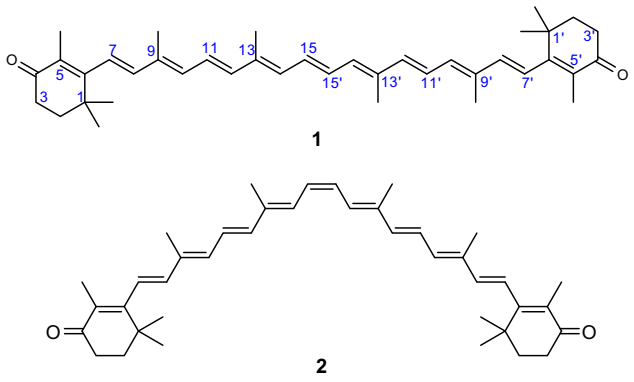


Chart 1. Structures of all-*trans* canthaxanthin (**1**) with numbering system and 15,15′-*cis*-canthaxanthin (**2**).

studies of β-carotene (Mordi et al., 1991, 1993). Oxygen was bubbled through a solution of this purified canthaxanthin (0.1052 g) dissolved in benzene (250 ml) in the dark at 25 °C. Samples were withdrawn at timed intervals and analysed with a variety of techniques, including C, H, O microanalyses, IR, UV, GC–MS(EI), HPLC–UV and HPLC–MS(CI). FTIR spectra were obtained on a Bomem MB 100 Fourier transform spectrometer equipped with a deuterated triglycine sulphate detector. Analytical conditions were as follows: HPLC was performed with a Perkin Elmer series 410 LC pump equipped with an LC 235 Diode Array detector. Reversed phase HPLC was carried out with a Spherisorb S5 ODS2 5μ column (250 × 4.6 mm), developed with a quaternary solvent system of water/methanol/acetonitrile/dichloromethane at a flow rate of 1 ml/min, and monitored at 450, 340, 300 and 280 nm. The initial solvent mixture was water/methanol (90/10), which was programmed to 100% acetonitrile for 5 min and then dichloromethane/acetonitrile (80/20) and finally to 100% acetonitrile. 10 μl aliquots of samples in benzene were usually injected onto the column. HPLC–MS was performed on a Hewlett–Packard 5988A-1090 system operated in the "thermospray" mode; a similar column was employed. The GC–MS data was obtained with a Finnigan MAT INCOS 50 instrument, with 70 eV EI ionisation. The GC was a Hewlett Packard 5890A fitted with a fused silica capillary column (25 cm × 0.2 mm) coated with cross-linked phenylmethyl silicone. The products reported here were identified from their mass spectra, UV spectra and retention times (t_R).

3. Results and discussion

A complex array of products was obtained and a typical HPLC chromatogram is shown in Fig. 1.

Major and some minor components of canthaxanthin oxidation were identified from their UV spectra which were obtained in the range 280–600 nm and from comparisons of retention times obtained by LC–MS and GC–MS chromatograms. The products so far identified and confirmed by these methods are shown in Chart 2. The mass spectra of the smaller shoulders on many of these peaks had the same molecular ions as their adjacent main products and their UV–Vis spectra were also similar. It was deemed that these minor peaks in the chromatograms are probably *cis* isomers of the adjacent components. The numbers assigned to peaks in Fig. 1 correspond to the structure numbers shown in Chart 2. It was observed during the HPLC analysis of the 4 h oxidation sample that the peak for canthaxanthin had resolved into two peaks with t_R of 33.9 and 33.7 min. The peak with t_R = 33.9 min (λ_{max} = 465 nm) was identified as all-*trans*-canthaxanthin (**1**) and the peak at t_R = 33.7 min (λ_{max} = 461 and 362 nm) was identified as 15,15′-*cis*-canthaxanthin (**2**). Taking into account the different solvents, the λ_{max} values for these compounds are in agreement with those reported by Gansser and Zechmeister (1957) (λ_{max} = 480, $\varepsilon = 11.1 \times 10^{-4}$ for all-*trans* and λ_{max} = 471, $\varepsilon = 7.9 \times 10^{-4}$; 366, $\varepsilon = 5.3 \times 10^{-4}$ for the 15,15′-*cis* isomer).

This canthaxanthin isomerisation was monitored by HPLC at 450 and 340 nm. It was found that, under controlled conditions of nitrogen atmosphere, the *cis*/*trans* isomer ratio was initially constant at 0.153. However, on following the oxidation process over a 6-h period the *cis*/*trans* isomer ratio increased from 0.252 to 0.442 after 30 min and then began to drop until it reached a steady value of 0.175 after 3 h oxidation.

The HPLC chromatogram of the oxidation products showed a set of peaks with retention times between 26 and 32 min (see Fig. 1). The MS analyses disclosed that these peaks all had molecular ions at m/z = 580, which suggested that they may be epoxides of canthaxanthin (i.e. compound **3** and *E*/*Z* isomers, Chart 2).

The amounts of these components decreased completely into the noise on the chromatogram after long oxidation times. Of course, uncertainty as to whether these were the 5,6-epoxy or 5,8-epoxy isomers remains. Apart from these, no other epoxy compound was identified from the HPLC or LC–MS analyses. However, two other minor epoxy components (**9** and **12**) were identified by GC–MS analysis. If other epoxides were formed in the reaction, they were in too small quantities to be picked up by our instrumentation.

The products of the canthaxanthin oxidations were also studied by GC–MS. Fig. 2 (below) illustrates the complex character of the GC–MS TIC chromatogram of the mixture after 174 h of oxidation. This technique enabled us to identify and confirm the presence of di-carbonyl compounds **5**, **6**, **8**, **10** and **11**, in addition to the epoxides **9** and **12** mentioned above (see Chart 2 for structures). Other minor components, which have not been characterised, were also present. As suggested above, the smaller shoulder peaks adjacent to many of the larger ones (Fig. 2b) are probably *E*/*Z*-isomers because they had identical molecular ions. It should be noted that the minor, uncharacterised components with t_R between 0 and 10 min were not present in the 72 h and earlier product mixtures. It is probable that these are secondary products of oxidation derived from the major initial components.

A plot of $\log_{10}(t_R)$ from the GC–MS chromatograms versus carbon number for the known di-carbonyl compounds was a smooth curve (see Fig. S1 in the Supplementary Material, full symbols). Interpolation from this curve enabled us to identify 4-oxo-β-apo-15-carotenal (**10**) and 5,6-epoxy-4-oxo-β-apo-13-carotenone (**13** derived from **5**) as part of the product mixture because their retention times fitted well on the smooth curve (dotted symbols in Fig. S1).

The FT-IR spectra of the canthaxanthin oxidation in a closed system were monitored between 3500 and 940 cm^{-1} by withdrawing samples at timed intervals. In agreement with other workers, development of a sharp absorption band at 2235 cm^{-1}, which increased continuously as the oxidation time increased, was observed. We attribute this absorption band to carbon dioxide production (Mordi et al., 1993). Estimation of the total carbonyl, from the 1775 to 1640 cm^{-1} bands in the oxidation product mixture, showed that these components increased as the time of oxidation increased but leveled off after about 120 h (see Fig. S2 in the Supplementary Material).

Elemental analyses of 10-ml samples withdrawn at timed intervals were also carried out and the results are shown in Table 1. The proportions of C and H decreased as the oxidation proceeded up to 72 h (cols. 2 and 3). In the same time period the proportion of oxygen in the reaction mixture increased (col. 4). It was evident that the proportion of oxygen increased as reaction proceeded and this is in accord with expectation. Interestingly however, after 72 h, a

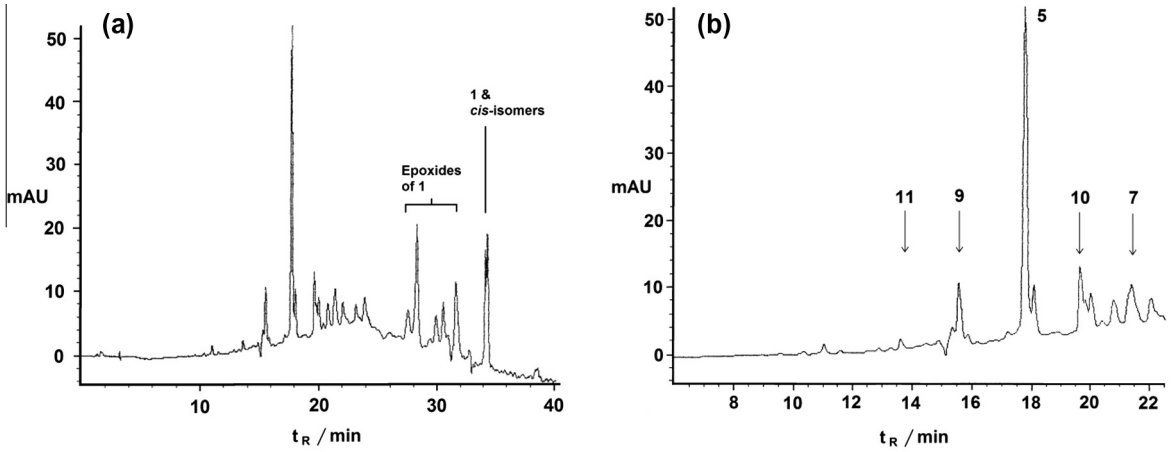


Fig. 1. (a) The HPLC chromatogram, monitored at 340 nm, of the complex mixture from a 72 h canthaxanthin oxidation: (b) an expanded section from t_R 10 to 25 min. The numbers assigned to peaks correspond to the structure numbers shown in Chart 2.

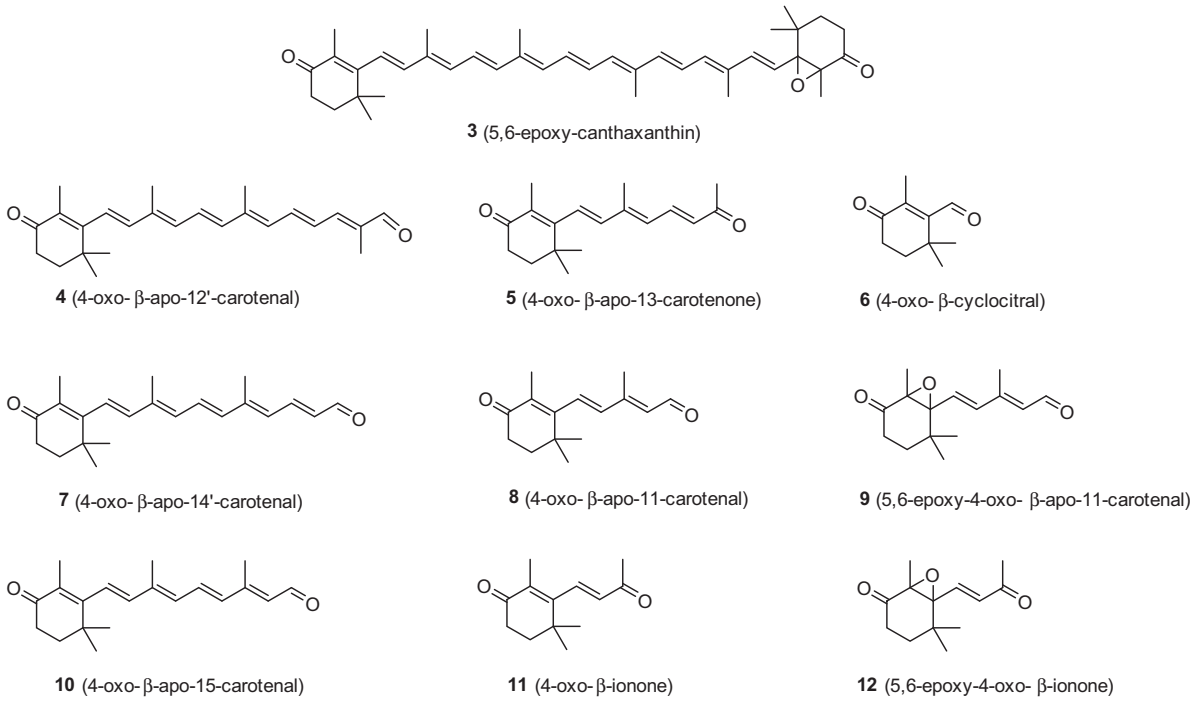


Chart 2. Structures of some identified products of canthaxanthin oxidation.

reversal in the proportion of oxygen was observed. This is not to say that oxygen was no longer consumed, or that the products were losing oxygen. Probably oligomeric products were then being formed with a lower relative oxygen content and the CO_2 eliminated at the same time was swept from the mixture by the oxygen stream.

The results presented above indicated that canthaxanthin oxidation with molecular oxygen produced mainly di-carbonyl compounds, that is the 4-oxo-β-apo-carotenals and 4-oxo-β-apo-carotenones, that resulted from cleavage of the polyene chain. HPLC and GC analyses also showed that isomerisation of the all-*trans* reactant to the *cis*-isomer **2** took place. Analogous isomerisations of other carotenoids have been reported previously by several workers (Doering & Kitagawa, 1991; Doering & Sarma, 1992; Gansser & Zechmeister, 1957; Mordi et al., 1993). The identities of the oxidation products pointed to unselective bond breaking at various sites along the canthaxanthin chain. No alcohols or keto-alcohols were detected at any stage in the oxidations. It is evident, therefore, that negligible contributions are made by peroxyl/peroxyl termination processes:

$$RR'CHOO^{\cdot} + RR'CHOO^{\cdot} \rightarrow RR'CHOH + RR'C{=}O + O_2$$

Peroxidations of many lipid components involve abstraction of allylic H-atoms by peroxyl radicals with production of hydroperoxides (Yin, Xu, & Porter, 2011). The six branching Me groups in canthaxanthin constitute allylic sites; but the absence of products from functionalisation of these groups rules out H-atom abstraction as a major process. Epoxides were much less important products than was the case for β-carotene oxidation. Epoxide **3** was found as a minor component in the early stages and it probably degraded to epoxides **9** and **12**. Formation of epoxide **3** can be accounted for as the result of peroxyl radical addition to C-5 leading to generation of the extensively delocalised radical **13** (see Scheme 1). An intramolecular homolytic substitution (S_Hi) then

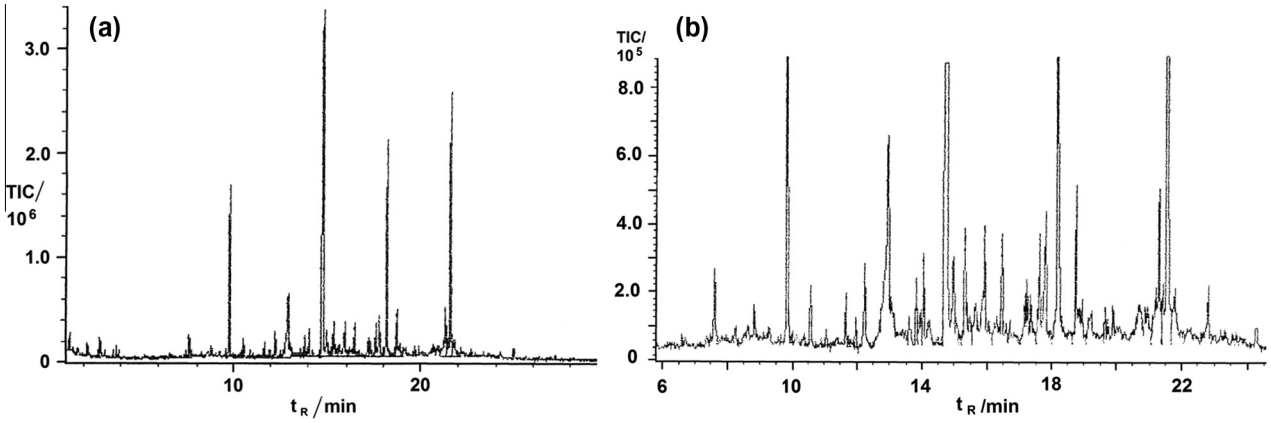


Fig. 2. (a) GC–MS TIC of canthaxanthin oxidation products after 174 h and (b) an expanded section between t_R 6 and 24 min (numbered peaks correspond to compounds in Chart 2).

Table 1
Elemental composition of aliquots withdrawn at timed intervals during canthaxanthin oxidation.

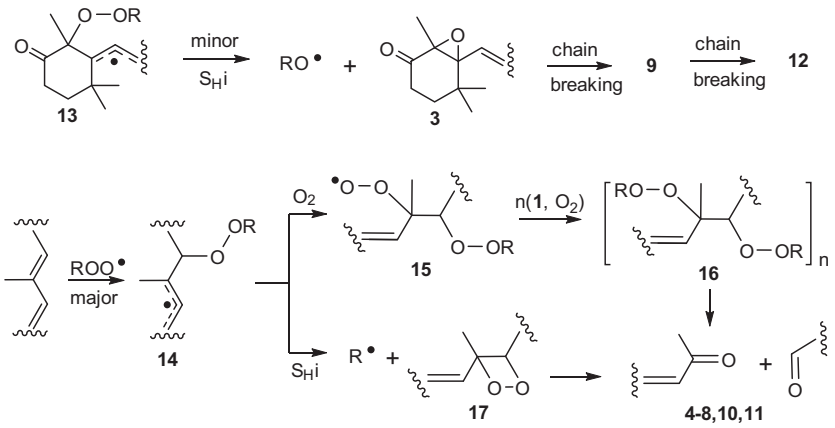
Oxidation time/h	Element (%)		
	C	H	O
0	85.11	9.22	5.67
1	80.75	8.84	10.41
4	80.31	9.20	10.49
20	73.80	8.49	17.71
48	68.30	8.57	23.13
72	65.75	8.63	25.62
144	69.57	9.66	20.77

converts **13** to epoxide **3** which subsequently degrades as shown. The presence of the adjacent C-4 carbonyl function in canthaxanthin may well inhibit peroxyl radical attack at C-5; thus accounting for the comparative paucity of epoxides. The 15,15′-epoxide was important in β-carotene oxidation but none of the analogous epoxide was detected with canthaxanthin. Peroxyl radical addition to C-15 or C-15′ of canthaxanthin would generate radicals with less electron delocalisation and hence less stabilisation. This, and/or rapid conversion of this epoxide to the 4-oxo-carotenals and carotenones, may account for the absence of the 15,15′-epoxide.

The main oxidation led to formation of the di-carbonyl 4-oxo-β-apo-carotenals and 4-oxo-β-apo-carotenones. Two routes are possible to account for this. Peroxyl radicals will readily add to **1** to generate delocalised C-centred radicals **14** (Scheme 1). The latter will couple with oxygen to produce peroxyl species **15**. Then repetition of the addition and coupling steps leads to oligomeric peroxides **16**. That the latter unzip to afford carbonyls analogous the dicarbonyls **4**–**8** and **10**, **11** is well documented (Bartlett & Banvali, 1991; Mayo, 1968; Mordi et al., 1993). Alternatively, the peroxyl radical adducts **14** could ring close with formation of dioxetanes **17** and delocalised canthaxanthyl-derived radicals $R^{\cdot}$. Such dioxetanes would be very fragile and would dissociate rapidly to afford two carbonyl components.

4. Conclusions

We have found that during oxidation of canthaxanthin with oxygen, degradation takes place rapidly, probably by a mechanism broadly similar to that of β-carotene. As expected, this is a radical-based process and its rapidity means that when canthaxanthin is used as a food additive for colouring, or for medication, its effectiveness will be improved by formulation with radical inhibitors, such as tocopherols or other hindered phenols. Isomerisation of all-*trans* to the 15,15′-*cis*-isomer takes place, probably followed by attack of oxygen and cleavage of the polyene chain. 5,6-Epoxide formation was less important than in β-carotene oxidation. It is likely this results from the proximity of the C-4 carbonyl group that is immediately adjacent to the putative 5,6-epoxidation site. Possibly the electron-withdrawing character of the 4-oxo group slows the epoxidation step. This appears to be the chief effect of the xanthophyll ketonic functional group on the overall



Scheme 1. Proposed mechanisms for canthaxanthin oxidation by air.

oxidation process. The major products were 4-oxo-β-apocarotenals and 4-oxo-β-apocarotenones whose biological functions in mammals are unknown. They may resemble the related β-apocarotenals and β-apocarotenones that are non-toxic and are known to modulate retinoid receptors. Activity against cell proliferation, tumours, and tumourigenic viruses has also been demonstrated.

Conflict of interest

Raphael C. Mordi declares that he has no conflict of interest. John C. Walton declares that he has no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

Not applicable.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.11.053.

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