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Letter

Mild oxidative cleavage of *β*,*β*-carotene by dioxygen induced by a ruthenium porphyrin catalyst: characterization of products and of some possible intermediates†

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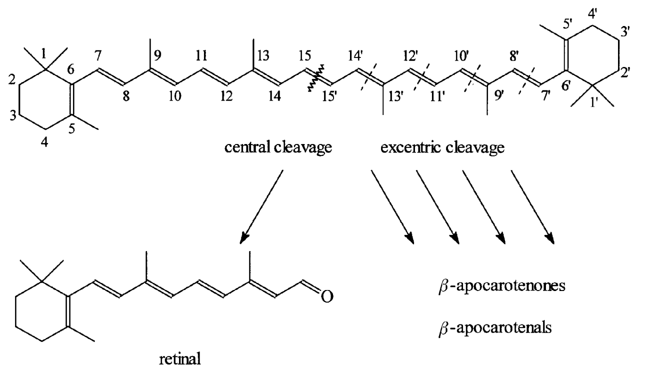
Mild oxidative cleavage of *β*,*β*-carotene by dioxygen is induced by a ruthenium tetramesitylporphyrin catalyst, and it leads to the full possible range of *β*-apocarotenals and *β*-apocarotenones. The slow reaction kinetics allow the sequence of events leading to double bond cleavage over a period of 24 h to be monitored by HPLC-DAD and HPLC-MS.

The beneficial effects of fruits and vegetables in the prevention of certain degenerative diseases such as cancers have been related to their carotenoid content.[1] Since these effects may be due to the intact pigments and/or to their metabolites, there is strong current interest in the catabolism of *β*,*β*-carotene and other carotenoids. Two distinct oxidative pathways (Scheme 1) are generally accepted for the catabolisation of *β*,*β*-carotene: (1) central cleavage by *β*,*β*-carotene-15,15′-dioxygenase producing retinal, and (2) excentric cleavage producing a series of shorter-chain compounds (*β*-apocarotenals, *β*-apocarotenones) with a terminal aldehyde or ketone function. Recently, Woggon *et al.*[2] have described a central cleavage mimic using a model catalytic system that exhibits a 15,15′-regioselectivity of about 40% in the oxidative cleavage of *β*,*β*-carotene by *tert*-butylhydroperoxide (TBHP). Herein we report on the excentric cleavage of *β*,*β*-carotene using molecular oxygen as oxidant and a ruthenium tetramesitylporphyrin



Scheme 1 The two distinct oxidation pathways for the catabolism of *β*,*β*-carotene, (top).

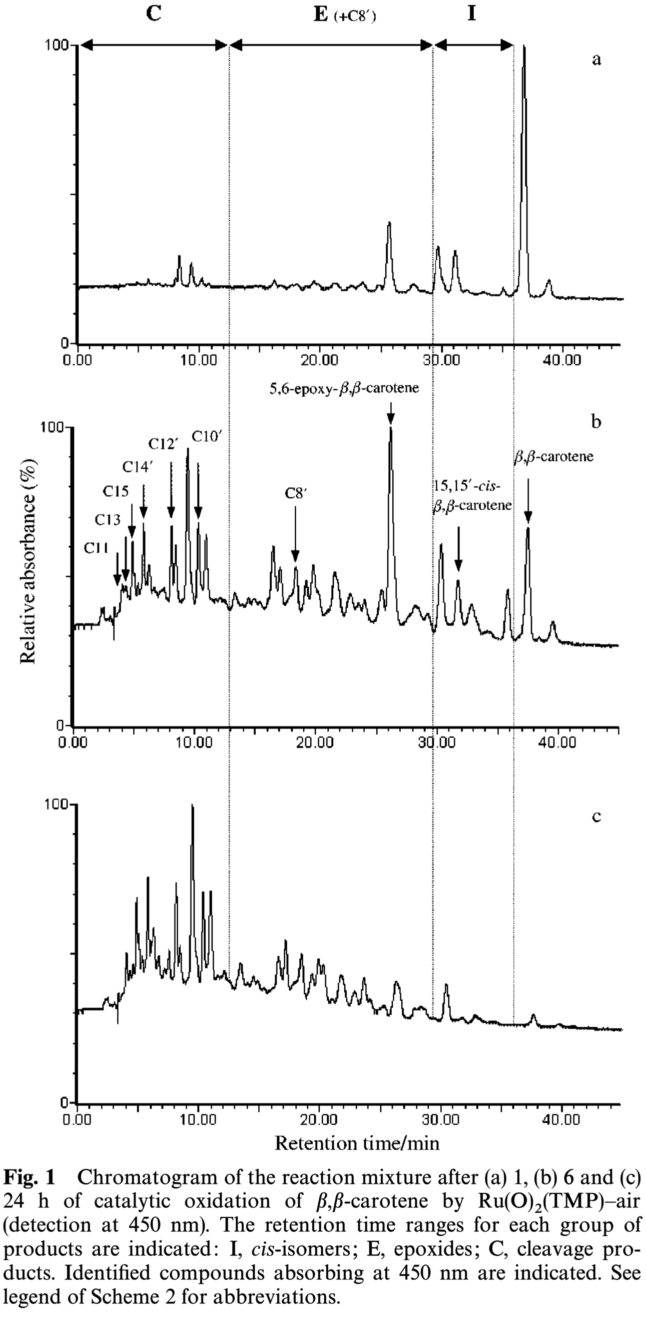


Fig. 1 Chromatogram of the reaction mixture after (a) 1, (b) 6 and (c) 24 h of catalytic oxidation of *β*,*β*-carotene by $Ru(O)_2(TMP)$–air (detection at 450 nm). The retention time ranges for each group of products are indicated: I, *cis*-isomers; E, epoxides; C, cleavage products. Identified compounds absorbing at 450 nm are indicated. See legend of Scheme 2 for abbreviations.

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† Electronic supplementary information (ESI) available: full experimental details and UV-visible spectra of 15,15′-*cis*-*β*,*β*-carotene and *β*,*β*-carotene-5,6-epoxide. See http://www.rsc.org/suppdata/nj/b0/b006975m/

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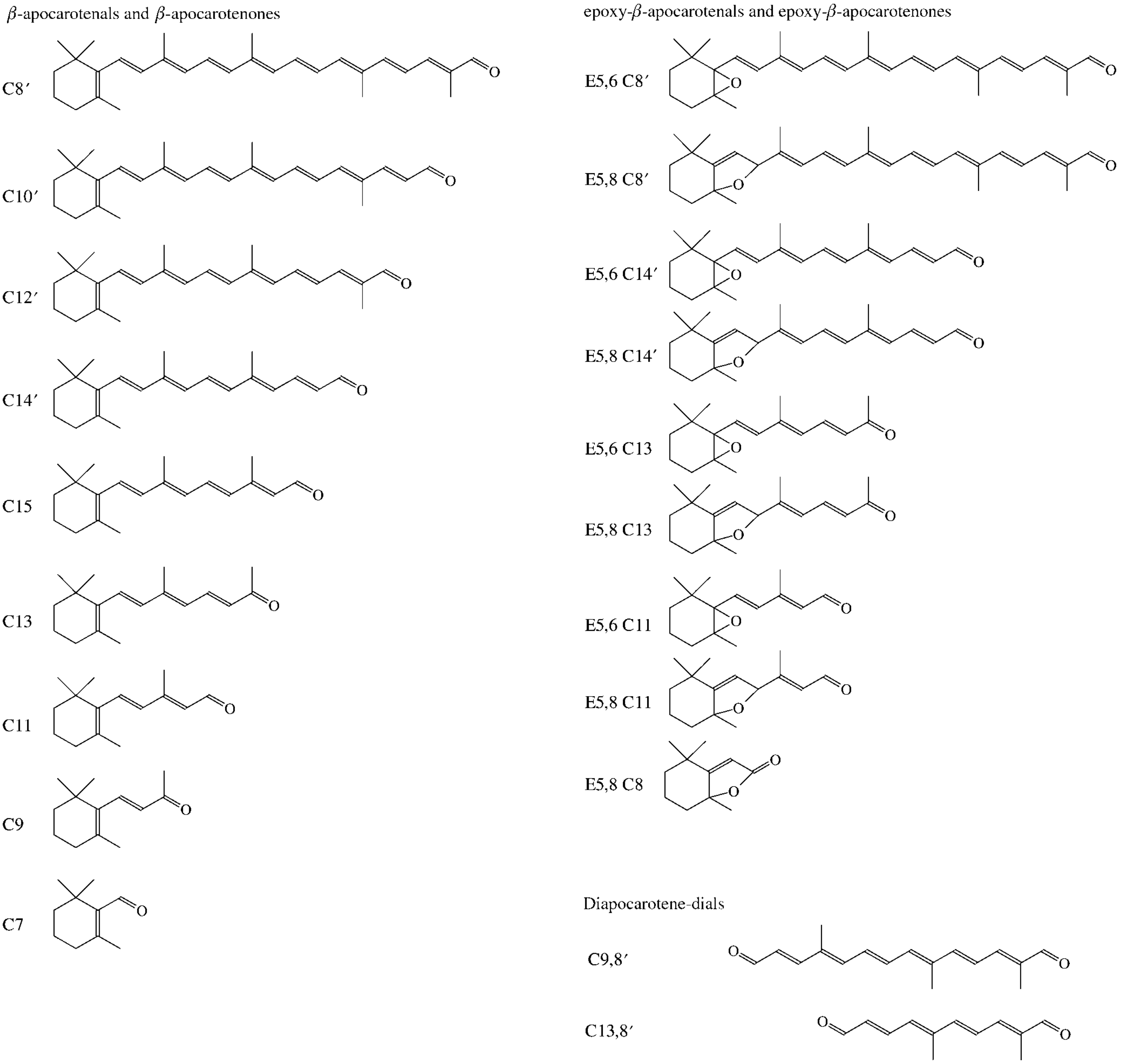
catalyst and on the identification of the various oxidation products using HPLC-DAD (diode array detector) and HPLC-MS. With these analytical techniques, it has been possible for the first time to monitor the sequence of events leading to the cleavage of β,β-carotene.

The ability of ruthenium tetramesitylporphyrin complexes to catalyse oxygen atom transfer from dioxygen to various alkenes in benzene solution is well documented.[3–5] Addition of 2 equiv. of *meta*-chloroperbenzoic acid, MCPBA, to carbonyl(5,10,15,20-tetramesitylporphyrinato)ruthenium(II), Ru(CO)(TMP), generates *in situ* the *trans*-dioxo(5,10,15,20-tetramesitylporphyrinato)ruthenium(VI), $Ru(O)_2(TMP)$, active species, which effects alkene epoxidation and is regenerated by subsequent reaction with atmospheric oxygen.[3] The behaviour of β,β-carotene as a substrate of this catalytic system was examined under the conditions previously described.[6] All experiments were carried out under dim light to avoid photoinduced isomerisation and/or degradation of β,β-carotene. The evolution of the reaction mixture was followed over 24 h with HPLC-DAD and HPLC-MS. Suitable controls confirmed the stability of β,β-carotene toward the components of the catalytic system [Ru(CO)(TMP), MCPBA] taken separately over the same period. Gentle catalytic oxidation was observed and numerous products were detectable at 450 nm after 1 h. The chromatographic profile changed with time until almost complete disappearance of β,β-carotene after 24 h; the chromatograms obtained after 1, 6 and 24 h are illustrated in Fig. 1. Plausible structures for the various products separated by HPLC were assigned on the basis of mass spectral data and comparison of UV-visible spectral characteristics with compounds described in the literature. The products derived from β,β-carotene could be classified into



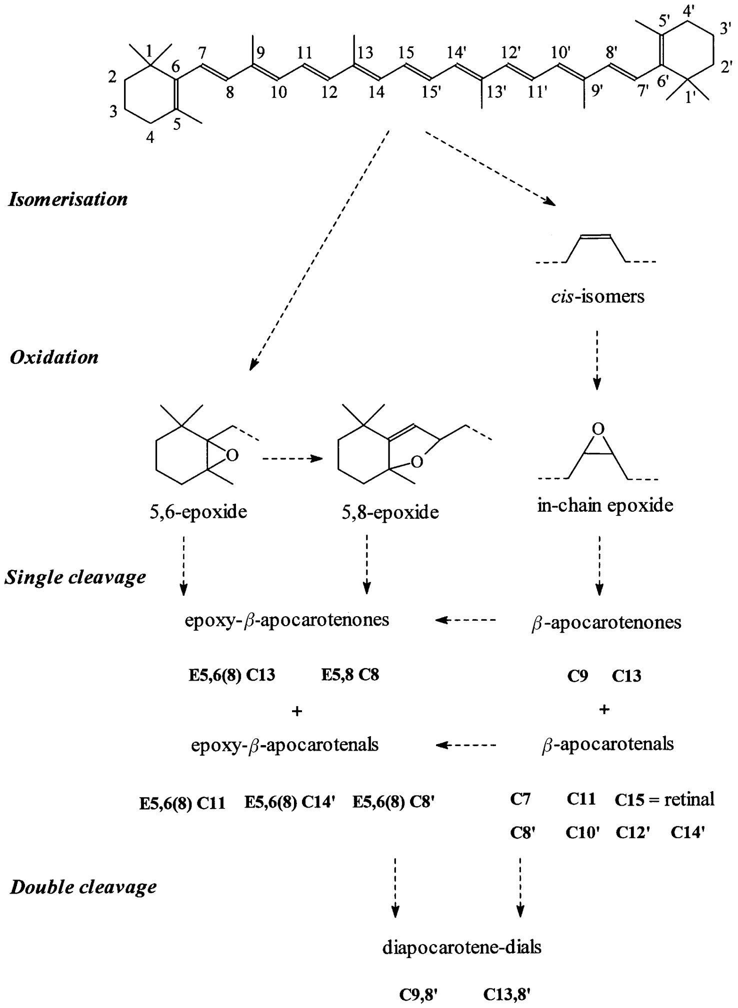
Scheme 2 Structures of the cleavage products detected upon catalytic oxygenation of β,β-carotene by $Ru(O)_2(TMP)$–air. Abbreviations: C*n*, product of single cleavage at position *n*; E5,6(8) C*n*, cleavage product at position *n* also containing an epoxy group at 5,6 (or 5,8); C*n*,*n*′, product of double cleavage at positions *n* and *n*′.

three groups: *cis*-isomers (I), epoxides (E) and cleavage products (C). The structures of the detected products are summarised in Scheme 2.

Examination of the relative chromatographic peak heights after 1, 6 and 24 h gave hints on the possible oxidation pathways. The most abundant products detected after 1 h were *β,β*-carotene *cis*-isomers (among them the 15,15′-*cis*-isomer) and epoxides (mainly the 5,6-, but also in-chain epoxides); low-abundance cleavage products (*β*-apocarotenals and *β*-apocarotenones) were also seen. Epoxides, as well as cleavage compounds, became more abundant after 6 h (see Fig. 1). After 24 h, the *β,β*-carotene substrate was almost completely consumed, *cis*-isomers and epoxides, mainly the 5,6-epoxide, were less abundant whereas cleavage compounds became relatively more abundant. These observations suggest that *cis*-isomers and in-chain epoxides of *β,β*-carotene are potential precursors of the C*n* cleavage products (see Scheme 2) in this catalytic system. A *cis*-olefin is known to be at least 10 times more reactive than the *trans*-isomer in a competitive oxidation by $Ru(O)_2(TMP)$[3] and *cis* double bonds located on the 9, 13 and 15 positions of the unsaturated chain of *β,β*-carotene are thermodynamically favoured.[7] Thus, isomerisation followed by epoxidation and subsequent cleavage might be prevalent at these positions.

Products of double oxidation were also detected. In addition to their terminal carbonyl group, epoxy-*β*-apocarotenones and epoxy-*β*-apocarotenals E5,6(8) C*n* (see Scheme 2) contain either a 5,6-epoxy or a so-called 5,8-epoxy group[7] (a 5-membered cyclic ether). Both types of products can derive from 5,6-epoxy-*β,β*-carotene by cleavage and/or epoxide-furanoid rearrangement.[8] Finally the diapocarotene dials C*n,n′* are likely derived from the cleavage of in-chain epoxy-*β*-apocarotenals. Possible filiations between the detected compounds are summarised in Scheme 3.

In conclusion, this work has shown that mild oxidative cleavage of *β,β*-carotene by dioxygen is induced by a ruthenium tetramesitylporphyrin catalyst. The complete range of *β*-apocarotenals and *β*-apocarotenones that can possibly be formed from *β,β*-carotene have been detected, as well as several other cleavage compounds and possible intermediates. The slow reaction kinetics allowed the sequence of events leading to double bond cleavage over a period of 24 h to be monitored by HPLC-DAD and HPLC-MS. Plausible filiations between the various products of this catalytic system are suggested on the basis of these sequential steps. The ruthenium tetramesitylporphyrin catalyst appears to be a good mimic of the putative enzyme that effects excentric cleavage of *β,β*-carotene by dioxygen *in vivo*. Further investigations are in progress on this and other metalloporphyrin systems, in order to understand the mechanisms of this oxidative cleavage, including a possible metal-[9] or acid-catalysed[10] *trans–cis* isomerisation prior to epoxidation.



Scheme 3 Possible filiations between cleavage products derived from *β,β*-carotene upon catalytic oxygenation by $Ru(O)_2(TMP)$–air. See legend of Scheme 2 for abbreviations.

Acknowledgements

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