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Chemistry of carotenoid oxidation and free radical reactions*†

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Abstract: When oxygenic photosynthesis evolved, one of the key functions of carotenoids was to protect aerobic photosynthetic organisms against destruction by photodynamic sensitization. Aerobic photosynthesis would not exist without the coevolution of carotenoids alongside the chlorophylls. As carotenoids are abundant in nature, in many fruits and vegetables, they are able to react with excited states of appropriate energy and quench them, and they can react with free radicals according to their reactivity, redox potentials, and X—H bond energies. This report concerns the bimolecular reactions of carotenoids with oxygen species, such as $^3O_2$, $^1O_2$, $HO^{\cdot}$, $HOO^{\cdot}$, $O_2^{\cdot-}$, etc.

INTRODUCTION

An important function of carotenoids is to intercept the chlorophyll triplet state, in order to prevent the formation of singlet oxygen, or to quench the singlet oxygen $^1O_2(^1\Delta_g)$ molecule directly. The discovery by Foote and Denny [1] that carotenoids quench singlet oxygen was essential to the understanding of protective efficiency. But, carotenoids are also reactive towards the non-excited oxygen ground state $^3O_2(^3\Sigma_g)$. This interaction is not well understood and is mostly carried out in the presence of radical initiators [2]. It consists of a complex sequence of free radical reactions which may build up a chain reaction. Surprisingly, the initiated autocatalytic oxidation of β-carotene and the autoxidation of substrates such as tetralin or methyl linoleate are dependent on oxygen pressure and, at elevated oxygen pressures, β-carotene and related compounds may act as pro-oxidants, because the oxidation rates at oxygen pressures of 150 Torr and higher with tetralin or 760 Torr or higher with methyl linoleate have minimum values at a β-carotene concentration of approximately 5×10^{-4} M [3]. The rates of peroxyl radical addition to β-carotene have been measured using pulse radiolysis of carbon tetrachloride to produce $ROO^{\cdot}$ radicals. The trichloromethylperoxyl radical, $CCl_3OO^{\cdot}$, bleaches β-carotene with a rate constant of 1.5×10^9/Ms, indicating the high reactivity of this peroxyl radical [4]. Carotenoids do not only add radicals or react with them in other ways, they are themselves prone to one-electron oxidations or reductions to form radical cations or anions [5].

THE SINGLET OXYGEN AND CAROTENOID INTERACTION

Polyenes and carotenoids are the best known of the compounds that quench $^1O_2(^1\Delta_g)$ by efficient energy transfer: $^1O_2 + {}^1Q \rightarrow {}^3O_2 + {}^3Q$ [5,6]. A large number of modified, synthetic analogues and derivatives have been synthesized in order to prepare even better quenchers than the natural carotenoids β-carotene, canthaxanthin or astaxanthin. Although in these cases the triplet energy of the polyene is the determinant,

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colour can give an indication of the quenching efficiency: deeply coloured, magenta, purple and blue polyenes are excellent quenchers at the diffusion limit [7,8].

It is believed that C_{40} carotenoids quench singlet oxygen through a very efficient energy transfer process approaching the diffusion-controlled limit [5,9–11]. The lowest excited states that are meaningful in energy transfer studies are S_1, S_2 and T_1 of β-carotene. Despite the importance of the carotenes, rather little is known of their key photochemical properties. In particular, the energy levels of the lowest singlet and the lowest triplet states of most carotenoids are not well established. Recently, the lowest triplet energy level of all-*trans*-β-carotene has been directly measured as 88 ± 3 kJ/mol from the weak phosphorescence observed using a sensitive Fourier transform based interferometer [12]. The assignment places the lowest triplet energy of β-carotene just below that of singlet oxygen $^1O_2(^1\Delta_g)$ (94 kJ/mol). Such an assignment is consistent with the second-order rate constant for energy transfer of 1.3×10^{10}/M/s [12]. The mechanism of bimolecular reactions between quenchers and singlet oxygen has provoked intense debate [5–7]. The quenching process can be physical (the quencher enters a vibrational or an electronic excited state) or chemical (the quencher combines with oxygen or is oxidized by oxygen) in nature. Physical quenching may occur via triplet energy transfer or by simple catalysis of the singlet oxygen ($^1O_2$) → ground state oxygen ($^3O_2$) transition via spin–orbit coupling. Charge transfer interactions are important during the quenching process. In many reactions, intermediates are involved, e.g. exciplexes, diradicals or zwitterions. Rate constants for quenching can be as high as 10^{10}–10^{11}/M/s (diffusion-controlled reactions), but are generally significantly less, 10^4–10^9/M/s. A more detailed definition of the mechanism

$$^1O_2 + {}^1Q \rightarrow {}^3O_2 + {}^3Q \quad \text{and} \quad {}^3Q \rightarrow {}^1Q + \text{heat}$$

is shown in Fig. 1 [11].

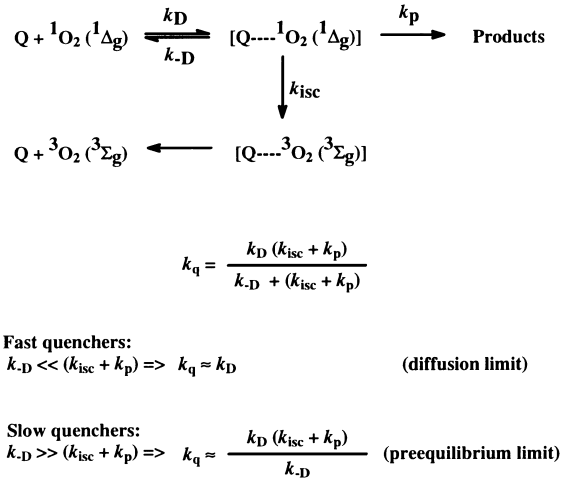

Fig. 1 Mechanism and kinetics for the reactions of $^1O_2$ with a quencher Q.

The plot of ln k_q for β-carotene versus the reciprocal temperature $1/T$ reveals clearly two linear regions corresponding at high and low temperature to activation energies of 4.18 and 7.10 kJ/mol, respectively. The change in slope in the region of -10 °C reflects progression from the diffusion limit to the pre-equilibrium limit. The relatively weak binding within the intermediate complex results in a balance between ΔH° and $\Delta H^\#$ for energy transfer which is just positive [11].

Carotenoids possibly display an interesting analogy to disulfides which yield thiol sulfinate and thiol sulfonates with singlet oxygen. Disulfides have the unique capability of deactivating $^1O_2$ by two different physical quenching mechanisms [13]. The total quenching may be described by several reactions. An estimate suggests the following proportions: chemical quenching, 4.0%; physical quenching, 0.2% (via a charge transfer complex); and physical quenching (via persulfoxide), 95.8%.

A recent density functional theory (DFT) approach of the singlet oxygen–carotene interaction unveiled

some surprising similarities to the otherwise different disulfides [9]. It was found that the main energy pathway is determined by an almost barrierless energy transfer $^1O_2 + {}^1Q \rightarrow {}^3O_2 + {}^3Q$ and $^3Q \rightarrow {}^1Q +$ heat. This is depicted in Fig. 2.

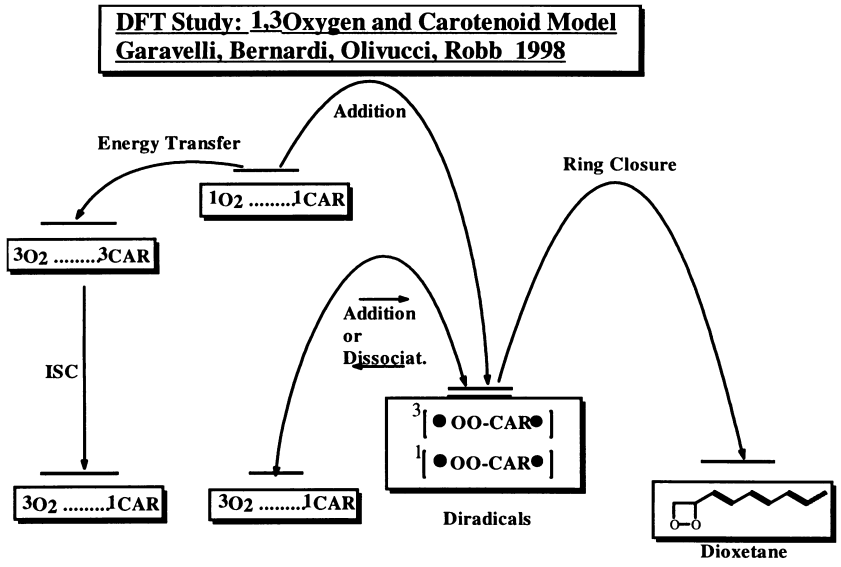

Fig. 2 Density functional theory (DFT) study of singlet oxygen–carotene showing the main reaction pathways: energy transfer, addition, ring closure, dissociation of diradicals and the $^3O_2$ addition to the polyene [9].

There are several notable results. In addition to the very fast energy transfer, there are concomitant low energy barrier reactions leading to diradicals that can undergo ring closure to 1,2-dioxetane products or lead, after intersystem crossing, to the regeneration of the carotenoid via dissociation. This catalytic singlet oxygen quenching seems to be at least competitive with respect to oxidation and therefore constitutes a second mechanism of physical quenching in analogy to disulfides. The reverse reaction, the addition of $^3O_2$ to carotenoids, is discussed below.

When the triplet energy of the carotenoid is about 12 kJ/mol lower than the donor singlet oxygen energy, the energy transfer rate is about the diffusion-controlled rate. When the triplet energy of the carotenoid is above the donor level, energy transfer becomes inefficient, and this deficiency can be supplied as an activation energy: $\Delta(\log\ k)/\Delta E = -1/(2.303RT)$. This has been demonstrated recently using a broad spectrum of light and deeply coloured carotenoids and is shown in Fig. 3 [8,14].

As singlet oxygen is a highly reactive form of oxygen, it is not surprising that concomitant chemical reactions consume sensitive carotenoids. Indeed, several products, such as β-ionone, diverse apo-carotenals and a 5,8-endo-peroxide, have been identified from β-carotene. Formation of these products, shown in Fig. 4, was dependent on the presence of the photosensitizer [14].

THE ONE-ELECTRON OXIDATION OF CAROTENOIDS

Pulse radiolysis and other methods were used to generate the radical ions of carotenoids and retinoids [5,16]. The radicals all absorb to the red side of the ground state absorption bands: β-carotene at 900 nm in CCl_4 [17]. Cation radicals can be produced in different ways in order to investigate their properties, but it is certainly of interest as to how they can be formed under conditions that are typical of anti- or pro-oxidative reaction sequences and strategies. Oxidants, among them the oxidizing radicals, are top candidates for achieving this electron abstraction. $NO_2^{\cdot}$, $CCl_3^{\cdot}$, $Br_2^{\cdot -}$, $CCl_3O_2^{\cdot}$ and $O_2^{\cdot -}$ are all reduced by β-carotene [18,19]. The inspection of thermodynamic data (Fig. 5) clearly shows that triplet oxygen is not a useful one-electron oxidant towards most carotenoids: the redox potential of β-carotene is around +650–780 mV (vs. normal hydrogen electrode, NHE) [18,22]. Important electron transfer processes

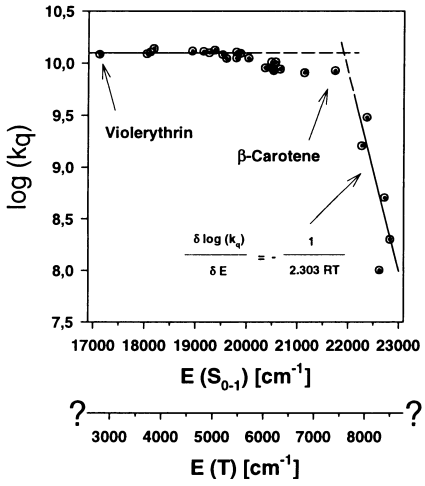

Fig. 3 Plot of singlet oxygen quenching constant log k_q vs. the singlet excitation energy for the $1^1A_g \rightarrow 1^1B_u$ ($S_0 \rightarrow S_2$) transition of carotenoids. A tentative abscissa is given for the predominantly unknown triplet energies: the $E(T)$ value of β-carotene was used for adjustment. From the slope of the onset, $\Delta(\log k)/\Delta E = -1/(2.303RT)$, it can be concluded that there is a proportionality of E(T) to E(S) [8].

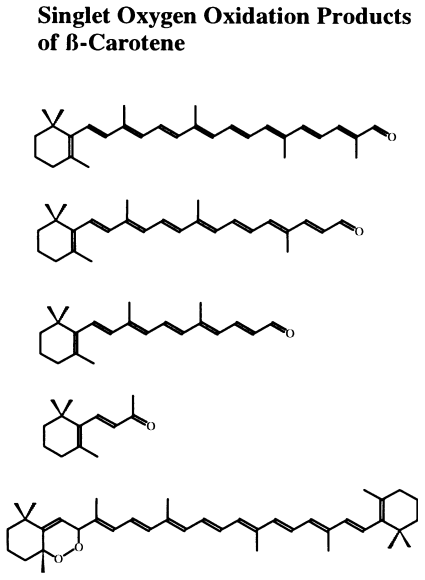

Fig. 4 Products derived from β-carotene and singlet oxygen generated by illuminating a mixture of β-carotene/rose bengal/oxygen in toluene/methanol [14].

therefore are [17]:

$$\beta\text{-car} + CCl_3O_2^{\bullet} \rightarrow \beta\text{-car}^{\bullet+} + CCl_3O_2^{-}$$

$$\beta\text{-car} + O_2^{\bullet-} + 2H^+ \rightarrow \beta\text{-car}^{\bullet+} + H_2O_2$$

Carotenoids participate in many cases in both electron abstraction and addition reactions. Sulfonyl radicals yield with β-carotene the radical cation and the addition product in a 3:1 distribution [18]. Figure 6 gives an approximate prognosis of the presumed product distribution.

The species Car, Car^{2+} and $Car^{\cdot+}$ coexist in a comproportionation equilibrium, and the lifetimes of these radical cations can therefore be assessed [18]. It was found that the greater the number of keto groups present at the end of the chromophore, the more the equilibrium favoured $Car^{\cdot+}$. Canthaxanthin$^{\cdot+}$ was therefore longer lived than β-carotene$^{\cdot+}$.

Redox-Potentials

$^1O_2 + e = O_2^{\bullet -}$	+ 0.64 V
$^3O_2 + e = O_2^{\bullet -}$	− 0.33 V
$O_2^{\bullet -} + e + 2H^+ = H_2O_2$	+ 0.89 V
$H_2O_2 + e + H^+ = OH^\bullet + H_2O$	+ 0.38 V
$OH^\bullet + e + H^+ = H_2O$	+ 2.33 V
$CH_3OO^\bullet + e + H^+ = CH_3OOH$	+ 1.05 V

Fig. 5 Redox potentials of reactive oxygen species vs. NHE as reference electrode [5,20,21].

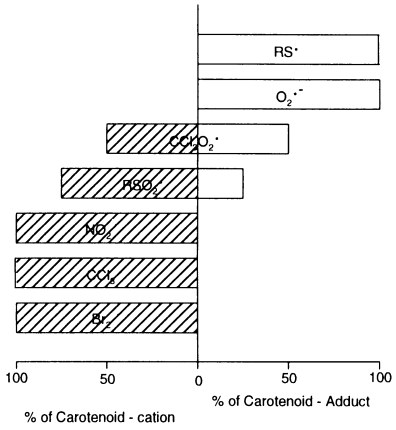

Fig. 6 Initial product distribution in reactions between β-carotene and oxidizing radicals. Radicals were generated by pulse radiolysis and the product distribution was estimated by spectrophotometry [18].

Solid state photoelectron spectroscopy is an appropriate method to obtain threshold energies eΦth (i.e. the lowest ionization energies) for carotenoids in the solid state. These ionization threshold energies will therefore satisfactorily reflect one-electron donor abilities of carotenoids in solid or semi-solid matrices. Furthermore, it is of interest to see that such solid state properties correlate satisfactorily with the redox potentials recorded by means of cyclic voltammetry. These values are true indicators of the one-electron donor ability of carotenoids either in the well solvating environment of a solvent or in the polarizable environment of the solid amorphous or crystalline state [23]. A correlation of threshold energies and oxidation potentials is displayed in Fig. 7. This functional dependence can be exploited to estimate the unknown oxidation potentials of some important keto-carotenoids with excellent antioxidative properties.

The ranking of activity with the stable radical cation $ABTS^{\cdot +}$ ($E_0 = 680$ mV vs. NHE [18]) is a useful but restricted method because the standard potential is rather low (cf. Fig. 7 with a value $E_0 = 460$ mV vs. Ag/AgCl). Carotenoid radical cations can be reduced by α-, β- and γ-tocopherol, whereas δ-tocopherol radical can be reduced by lycopene and β-carotene [24]. β-$Carotene^{\cdot -}$ transfers an electron to oxygen, but the reverse reaction is not observed, whereas lycopene undergoes reversible electron transfer with $O_2^{\cdot -}$ [25]. According to HAM/3 calculations, the electronegativity of lycopene is more positive due to the more planar geometry. It is interesting to compare astaxanthin's low reducing ability (Fig. 7) with the fact that $CCl_3OO^\cdot$ does not form a radical cation directly with astaxanthin, as with the other carotenoids, but yields first an addition radical which decays to give the cation radical [26].

THE RADICAL-INDUCED OXIDATION OF CAROTENOIDS

The thermal oxidation of β-carotene can be investigated in the presence and absence of radical starters such as AIBN or AMVN. The self-initiated autoxidation of β-carotene and the induced oxidation are both autocatalytic and inhibited by tocopherol. There are two possible pathways for the non-radical-induced interaction of $^3O_2$ with β-carotene: (1) the intermediate in the thermal *cis–trans*-isomerization,

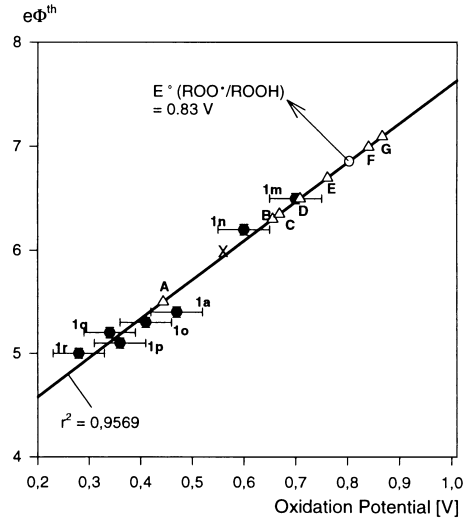

Fig. 7 This correlation of threshold energies eΦ/eV vs. oxidation potentials I/V is an extended version of that published recently [23]. Black circles represent compounds where both I/V and eΦ/eV have been recorded. Triangles indicate compounds where eΦ/eV is experimentally known, and I/V has been calculated using the regression function: eΦ/eV = 3.78 × I/V + 3.82. Compound X is β-carotene; the value I/V = 0.54 V [22] has been adjusted to the reference electrode used here: Ag/AgCl, 0.222 V (CH_2Cl_2). In addition, the redox potential of $CH_3OO^{\cdot}$ is indicated to assess the oxo-carotenoids. Compounds [23]: 1 m, β-C_{26}-carotene 0.7 V; 1 n, β-C_{30}-carotene 0.6 V; 1 a, β-carotene 0.47 V; 1 o, 0.41 V; 1 q, 0.34 V; 1 p, 0.36 V; 1 r, 0.28 V; A, lycopene 5.5 eV, 0.44 V; B, astacene 6.3 eV, 0.65 V; C, isonorcanthaxanthin 6.3 eV, 0.67 V; D, norcanthaxanthin 6.5 eV, 0.71 V; E, astaxanthin 6.7 eV, 0.76 V; F, isonorastaxanthin 7.0 eV, 0.84 V; G, violerythrin 7.1 eV, 0.87 V.

main oxidation products (stable/primary product)	position in carotenoid
Epoxides	in 5,6- or 5',6'-position in 5,8- or 5',8'-position in 15,15'-position
Diepoxides	in 5,6- and 5',6' –position in 5,8- and 5',8' -position
Apocarotenoids	Apo-8'-one Apo-10'-al Apo-15 = Retinal Apo-9 = ß-Jonon Apo-7 = ß-Cyclocitral
Epoxi-Apocarotenoid	5,6-Epoxy-ß-jonon
4-Oxo-compounds	Apo-9-one Apo-10-al Apo-11-al Apo-13-one
minor oxidation products(secondary products)	
Alcohols Semi-ß-carotene (one ring opened -diketo) ß-Caroteneolide (lactone) ß-Carotenone (two rings opened -tetraketo)	

Fig. 8 Oxidative degradation of β-carotene [2,27].

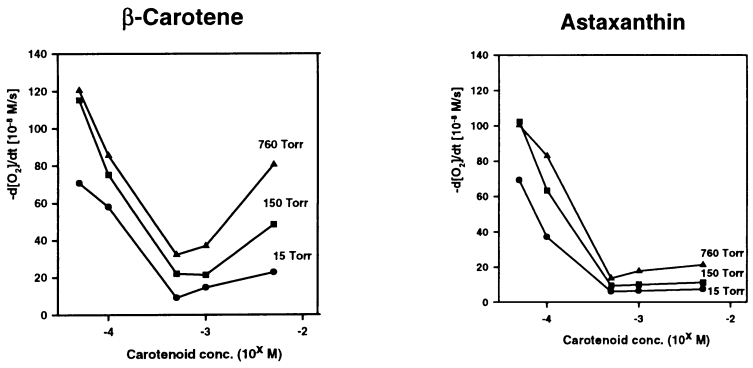

Fig. 9 Rate of oxidation for varying carotenoid concentrations and partial pressures of oxygen: (a) β-carotene; (b) astaxanthin [31].

'*Doering's Diradical*', captures oxygen and leads to a plethora of products such as epoxides, aldehydes, ketones, peroxides and other minor side-products [27]; or (2) the addition of oxygen takes a reaction channel according to Fig. 2, where the addition to an undisturbed carotene with calculated to require about 18 kcal/mol. The experimental value of $E_a = 16$ kcal/mol is in good agreement [28]. The product spectrum for this process is depicted in Fig. 8.

When the oxidation was induced using radical initiators or peracids, additional products can be formed: aldehydes and ketones from oxidized methyl groups, chain-located dihydro-oxepins and dihydrofurans [29].

The mechanism of substrate (lipid, cumene, tetralin, etc.) peroxidation is a chain reaction that provides a steady supply of free radicals. It may be represented as [2–5,18]:

Initiation: AIBN or AMVN $\rightarrow R^{\bullet}$

Propagation: $R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$ $ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet}$

Termination: $ROO^{\bullet} + ROO^{\bullet} \rightarrow$ non-radical products

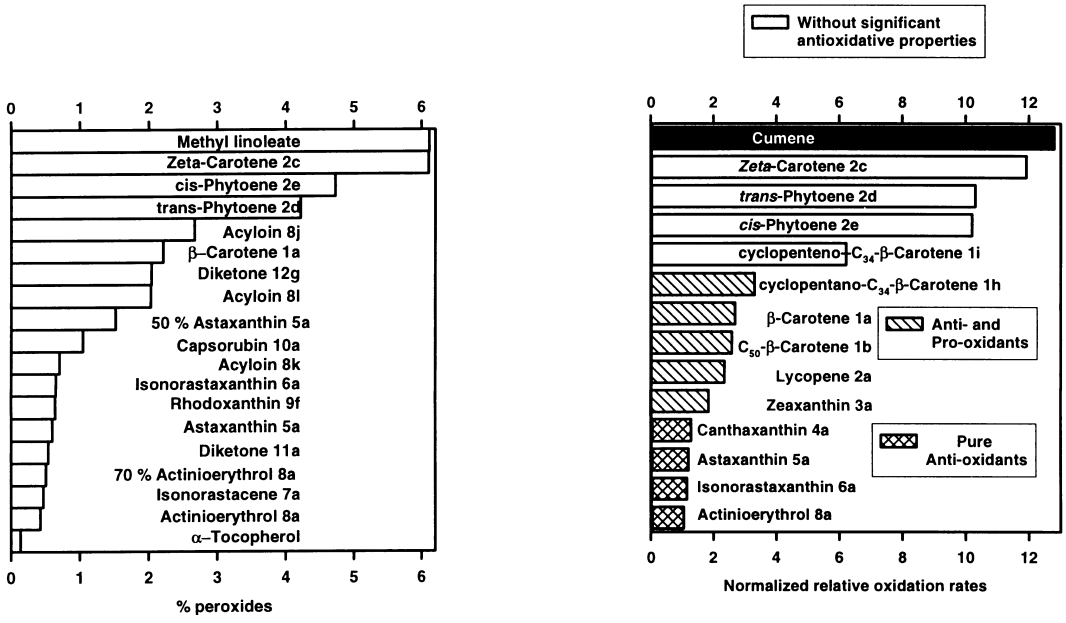

Fig. 10 Left: peroxide formation of pure methyl linoleate and in the presence of antioxidants; 50% astaxanthin indicates that the standard concentration of 7.7×10^{-4} M is reduced by 50%. Right: normalized (with respect to actinioerythrol = 1) rates of oxidation at $p(O_2) = 150$ Torr.

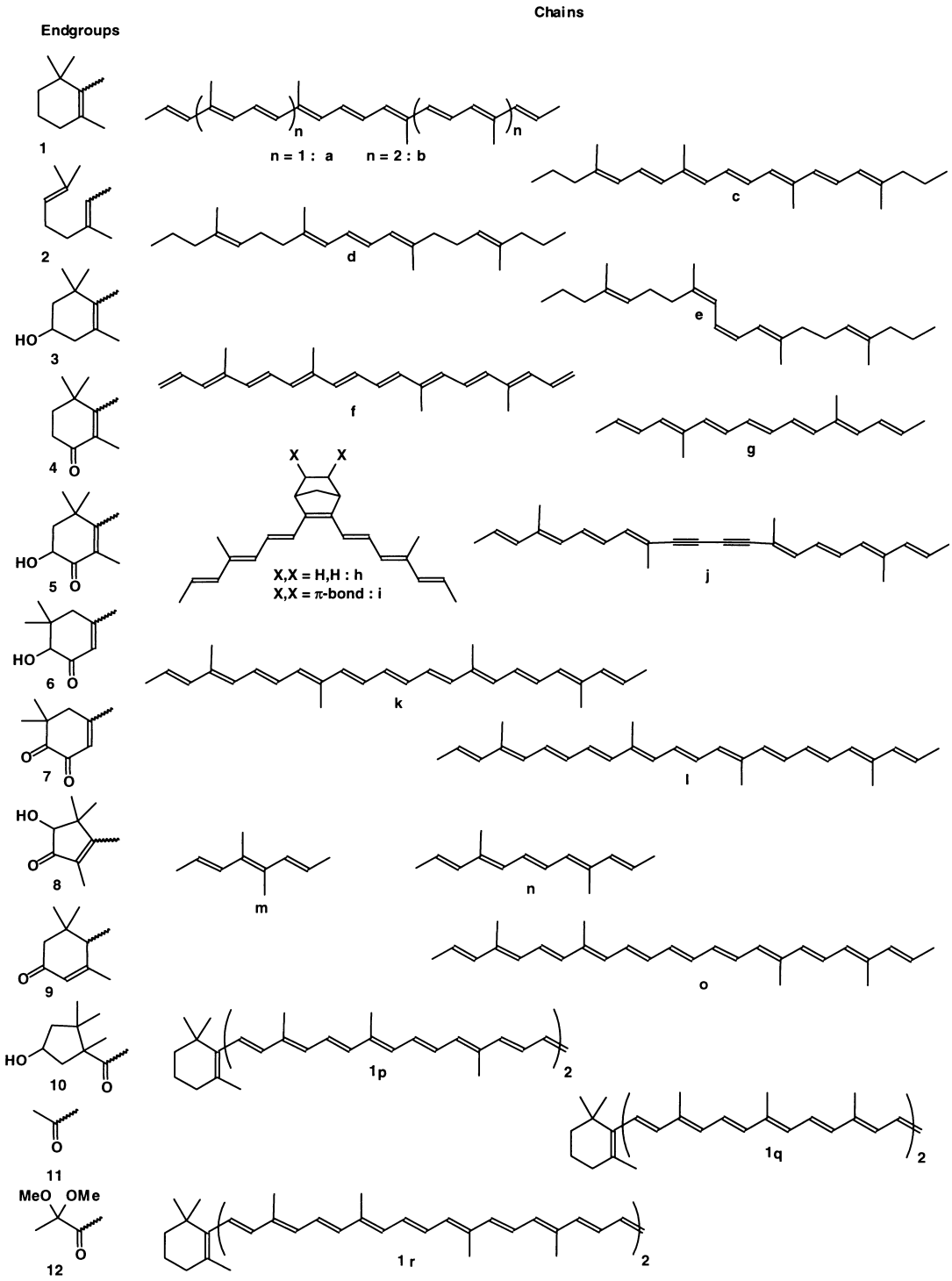

Fig. 11 Structure of the investigated carotenoids and related compounds.

Since the first propagation step is reversible, $ROO^{\cdot} \rightarrow R^{\cdot} + O_2$, in particular for resonance-stabilized radicals, the oxygen partial pressure is of importance. If the rate of oxidation ($-d[O_2]/dt$ in M/s) of a substrate is plotted against the concentration of β-carotene using varying oxygen partial pressures, the diagram in Fig. 9(a) is obtained [3]. The investigated carotenoids (Figs 10 and 11) can be divided into three classes. The first group contains molecules with very little antioxidative capability, and these are therefore

not of interest here. The second class comprises compounds with good antioxidative but also pro-oxidative properties (β-carotene, see Fig. 9(a)). The third class consists of carotenoids which react as strong antioxidants and without any pro-oxidative nature. All of these contain conjugated oxo-functions within their end groups. The results for astaxanthin as model compound are presented in Fig. 9(b). These results can be compared with the antioxidative effect on the autoxidation of methyl linoleate [30,32] and Fig. 10.

The reasons for the increasing antioxidative ability and decreasing pro-oxidative properties with increasing number of oxo and hydroxy functional groups are probably: (1) an unchanged tendency to form radical addition products; (2) a higher stability of the radical addition products owing to an extended π-system and increased electronegativity of the substituents; (3) a considerable reluctance to form radical cations because of more positive redox potentials (Fig. 7); and (4) a less pronounced tendency to enter the equilibrium with $^3O_2$ and to form the peroxyl diradical.

These results lead to the suggestion that the different chemical anti- and pro-oxidant behaviour of the carotenoids is caused by the different structure of their end groups, their chain length (minor importance) and the number and position of methyl groups. β-Carotene is able to react as a hydrocarbon with active allylic hydrogen atoms that can be removed by radicals. On the other hand, β-carotene also binds to peroxyl radicals. Both processes combine to produce reactions with the concomitant formation of epoxides and carbonyl compounds. It is possible to develop a sequence of radical abstraction and oxygen addition reactions as well as cleavage reactions resulting in a radical chain reaction. In contrast, astaxanthin, isonorastacene and actinioerythrol do not enter these pathways or do so much more slowly.

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ABBREVIATIONS

AIBN = α,α'-azo-bis-(isobutyronitrile)

AMVN = 2,2-azo-bis-(2,4-dimethylvaleronitrile)

ABTS = 2,2′-azino-bis-(3-ethyl-benzthiazoline-6-sulfonate)diammonium salt