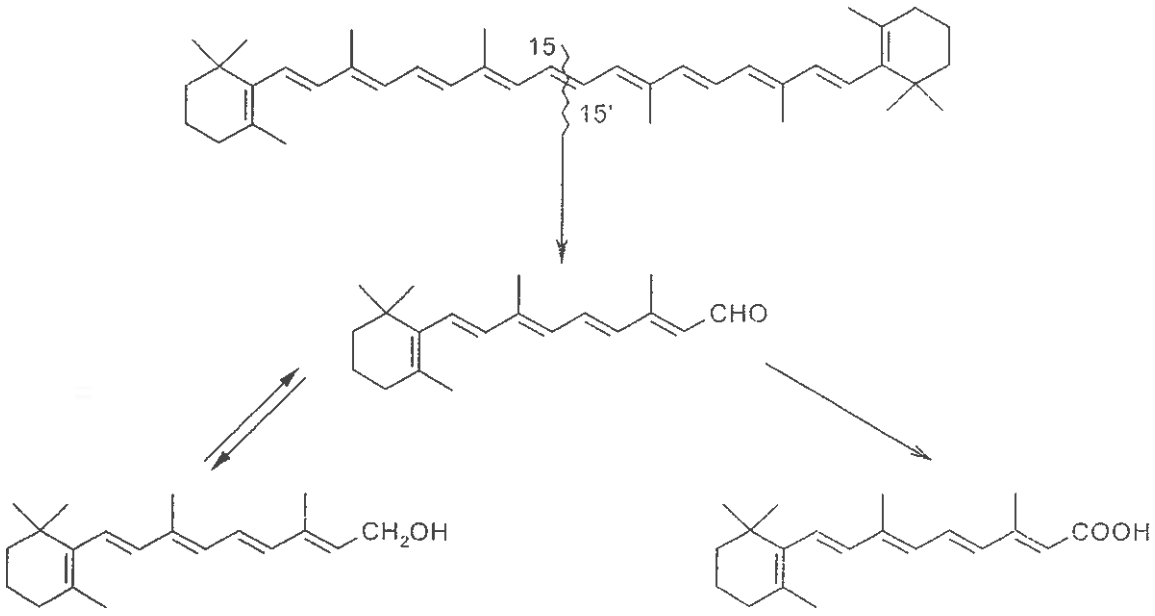
Carotenoids

Volume 4: Natural Functions

Edited by G. Britton
S. Liaaen-Jensen
H. Pfander



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Carotenoids
Volume 4: Natural Functions

Chapter 7

Carotenoid Radicals and Radical Ions

Ali El-Agamey and David J McGarvey

A. Introduction

1. Definitions

Various types of carotenoid-derived ions, radicals and radical ions are referred to in this Chapter and elsewhere in this Volume.The different species are defined below and their relationship to the parent carotenoid is illustrated by the example of β-carotene (**3**, $C_{40}H_{56}$).

Carotenoid radical anion ($CAR^{\bullet -}$)

$$C_{40}H_{56} + e^- \longrightarrow C_{40}H_{56}^{\bullet -}$$

Carotenoid radical cation ($CAR^{\bullet +}$)

$$C_{40}H_{56} - e^- \longrightarrow C_{40}H_{56}^{\bullet +}$$

(Carotenoid – H) cation ($^{\#}CAR^{+}$)

$$C_{40}H_{56} - H^{\bullet} - e^- \longrightarrow C_{40}H_{55}^{+}$$

Carotenoid dication (CAR^{2+})

$$C_{40}H_{56}^{\bullet+} - e^- \longrightarrow C_{40}H_{56}^{2+}$$

Carotenoid neutral radical ($CAR^{\bullet}$ or $^{\#}CAR^{\bullet}$)

$$C_{40}H_{56} - H^{\bullet} \longrightarrow C_{40}H_{55}^{\bullet}$$

$$C_{40}H_{56}^{\bullet+} - H^{+} \longrightarrow C_{40}H_{55}^{\bullet}$$

$$C_{40}H_{55}^{+} + e^- \longrightarrow C_{40}H_{55}^{\bullet}$$

2. The roles of carotenoid radicals

Carotenoids are susceptible to oxidation but relatively resistant to reduction. Consequently, carotenoid radicals can arise from their reductive quenching of excited states and from free-radical scavenging. The widespread natural occurrence of carotenoids in oxidizing and photo-oxidizing environments reflects these properties. Carotenoid radicals are often produced as intermediates in oxidizing environments, although the extent to which such species arise in biological systems under oxidative stress is largely unknown, as are any biological effects these species may have. The associations between the incidence of certain degenerative diseases and dietary intake of carotenoids (*Volume 5*) may be linked to the ability of carotenoids to scavenge free radicals, but the significance of this remains unclear.

Carotenoids have been shown to inhibit free-radical mediated oxidation of oxidizable substrates [1-6]. However, the oxidation chemistry of carotenoids is complex, as reflected in the variety of carotenoid oxidation products that are produced and the dependence of the product profile on the structure of the carotenoid under study and the nature of the oxidizing environment [7-11]. One of the main challenges in the study of the chemistry of carotenoid oxidation is to characterize fully the pathways from initial free-radical attack to oxidation products and subsequently to reconcile these data with the antioxidant/pro-oxidant properties of the carotenoids [8].

Carotenoid radicals also arise in photosynthetic systems (see *Chapter 14*). A specific example is the generation in photosystem II of carotenoid radical cations ($CAR^{\bullet+}$) [12-15], possibly formed by reaction of the carotenoid with the most strongly oxidizing species in photosystem II, $P^{\bullet+}_{680}$. This reaction (Scheme 1) has been proposed to be part of a sequential electron transfer from cytochrome b_{559} (cyt b_{559}) to $P^{\bullet+}_{680}$ *via* accessory chlorophylls (chl_z) and carotenoids (CAR) [12].

$$\text{cyt } b_{559} \longrightarrow chl_z \longrightarrow CAR \longrightarrow P_{680}$$

Scheme 1

Carotenoid radical cations have also been observed in the light-harvesting complex 2 (LH2), from *Rhodobacter sphaeroides* into which different carotenoids were incorporated [16]. The generation of $CAR^{\bullet+}$ is attributed to electron transfer from the S_2 excited state of the carotenoid to bacteriochlorophyll *a* (BChl) to form $CAR^{\bullet+}$ and $BChl^{\bullet-}$ (Scheme 2).

$$^{*}CAR\ (S_2) + BChl \longrightarrow [^{*}CAR\ (S_2)...\ BChl] \longrightarrow CAR^{\bullet+} + BChl^{\bullet-}$$

Scheme 2

Carotenoids can scavenge oxidizing free radicals *via* at least three primary reactions (equations 1-3), namely electron transfer, addition and hydrogen atom transfer [17,18].

$$CAR + R^{\bullet} \longrightarrow CAR^{\bullet+} + R^{-} \quad \text{Electron transfer} \quad (1)$$

$$CAR + R^{\bullet} \longrightarrow CAR^{\bullet} + RH \quad \text{Hydrogen atom transfer} \quad (2)$$

$$CAR + R^{\bullet} \longrightarrow RCAR^{\bullet} \quad \text{Addition} \quad (3)$$

However, secondary reactions are possible that can lead to different carotenoid radicals from the one formed in the initial step.

Progress has been made in understanding the free-radical chemistry of carotenoids, although there are many questions that remain unanswered. Carotenoid radicals are commonly detected *via* their UV/Vis/NIR optical absorption. Neutral radicals generally absorb in the same spectral region as the parent compound, though at somewhat longer wavelength. Radical anions and radical cations absorb at much longer wavelength (by several hundred nm), λ_{max} commonly being in the NIR region in the range 800-1100 nm (see Fig. 1, *Chapter 8*). Radical cations generally absorb at somewhat longer wavelength than the corresponding radical anion. The spectra of all carotenoid radicals show little or no fine structure, and the λ_{max} are strongly influenced by solvent/medium and experimental conditions (see Table 1). One area where there has been relatively little activity, however, is in the use of theoretical calculations to predict radical structures and physicochemical properties. Such work could prove invaluable in aiding assignment of the various carotenoid radicals that can be observed *via* UV/Vis/NIR spectrophotometric measurements.

It is therefore important that:

i) The routes by which carotenoid radicals are formed are understood.

ii) Carotenoid radicals are characterized structurally and spectroscopically.

iii) The chemistry of carotenoid radicals, in terms of the rates and mechanisms of intra-molecular and intermolecular chemical reactions, is fully understood.

iv) The pathways from carotenoid radical intermediates to final oxidation products are fully elucidated.

B. Radical Ions

1. Formation and detection of carotenoid radical ions

a) Pulse radiolysis

In pulse radiolysis, the sample is irradiated by a pulsed beam of high-energy electrons. The energy is absorbed by the solvent, resulting in the production of excited states and radicals of the solvent molecules. Scavenging/quenching of these solvent species by the solute(s) results in formation of solute excited states and radicals, which are normally monitored *via* UV/Vis/NIR absorption spectroscopy. The radiation chemistry is not the same for all solvents, so different solute-derived transient species are obtained in different media, although the use of various chemical additives allows some selectivity in terms of the solute transient species produced.

In media of low polarity, such as argon-saturated hexane [19,20] and benzene [17,20,21], carotenoid radical ions can be generated *via* scavenging of solvent radical cations and electrons (Schemes 3 and 4).

$$C_6H_{14} \rightsquigarrow C_6H_{14}^{\bullet+} + e^-$$
$$e^- + CAR \longrightarrow CAR^{\bullet-}$$
$$C_6H_{14}^{\bullet+} + CAR \longrightarrow CAR^{\bullet+} + C_6H_{14}$$

Scheme 3

$$C_6H_6 \rightsquigarrow C_6H_6^{\bullet+} + e^-$$
$$e^- + CAR \longrightarrow CAR^{\bullet-}$$
$$C_6H_6^{\bullet+} + CAR \longrightarrow C_6H_6 + CAR^{\bullet+}$$

Scheme 4

In the presence of oxygen, only $CAR^{\bullet+}$ is observed, due to scavenging of the electron and $CAR^{\bullet-}$ by oxygen.

In more polar solvents, *e.g.* argon-saturated methanol [19,20], only carotenoid radical anions ($CAR^{\bullet-}$) are generated by the reaction of the solvated electron with carotenoids (Scheme 5).

$$CH_3OH \rightsquigarrow {}^{\bullet}CH_2OH + e^-_{MeOH}$$
$$e^-_{MeOH} + CAR \longrightarrow CAR^{\bullet-}$$

Scheme 5

In aqueous environments [20,22,23], both oxidizing species ($^{\bullet}OH$) and reducing species (e^-_{aq} and $H^{\bullet}$) are formed (equation 4) in the radiolysis of water. In argon-saturated solution, a reducing environment is afforded by the use of additives such as formate ion (HCO_2^-), which reacts with $^{\bullet}OH$ to form $CO_2^{\bullet-}$. Under such conditions $CAR^{\bullet-}$ may be generated (Scheme 6).

$$H_2O \rightsquigarrow e^-_{aq} + {}^{\bullet}OH + H^{\bullet} + H_2 + H_2O_2 \qquad (4)$$

$$^{\bullet}OH + HCO_2^- \longrightarrow H_2O + CO_2^{\bullet-}$$
$$e^-_{aq} + CAR \longrightarrow CAR^{\bullet-}$$

Scheme 6

An oxidizing environment is afforded by saturation of the solution with N_2O, which scavenges e^-_{aq} leading to the formation of the highly oxidizing hydroxyl radical ($^{\bullet}OH$). Milder oxidizing species may be formed by reaction of hydroxyl radical with halide ions such as bromide, leading to formation of $Br_2^{\bullet-}$ and generation of $CAR^{\bullet+}$ (Scheme 7).

$$e^-_{aq} + H_2O + N_2O \longrightarrow N_2 + OH^- + {}^{\bullet}OH$$
$$^{\bullet}OH + 2Br^- \longrightarrow OH^- + Br_2^{\bullet-}$$
$$Br_2^{\bullet-} + CAR \longrightarrow CAR^{\bullet+} + 2Br^-$$

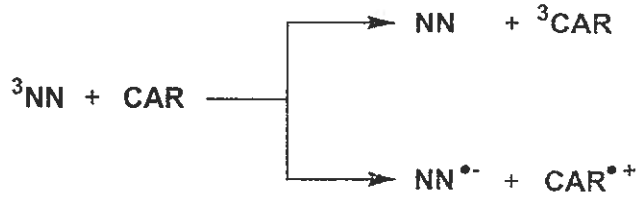
Scheme 7

b) Laser flash photolysis

Laser flash photolysis involves irradiation of the sample with a pulse (usually nanoseconds) of monochromatic laser light. This technique has been used extensively for generating free radicals [24-29] (see also section **D**) and excited states of carotenoids. There are no reports of the use of laser flash photolysis for the generation of carotenoid radical anions, howeyer.

Carotenoid radical cations ($CAR^{\bullet+}$) can be formed *via* reductive quenching of the triplet excited states of certain photosensitizers. For example, $CAR^{\bullet+}$ is produced from the reaction of CAR with the triplet excited state of toluidine blue, a reaction which involves competitive

energy-transfer and electron-transfer quenching pathways [30]. Similar behaviour is observed for the reaction of CAR with triplet excited nitronaphthalene (^{3}NN) [31] (Scheme 8).



Scheme 8

c) Chemical methods

The carotenoid radical cation $CAR^{\bullet+}$ can be produced by the reactions of carotenoids with strong oxidizing agents. For example, reaction of carotenoids with $FeCl_3$ in CH_2Cl_2 gives rise to the formation of carotenoid radical cations and dications ($CAR^{\bullet+}$ and CAR^{2+} respectively) (equation 5) [32]. $CAR^{\bullet+}$ is also produced from the reactions of carotenoids with quinones in dichloromethane or with iodine (equation 6; *cf Chapter 8*) [33,34]. More recent studies of the latter reaction and characterization of the complex as $CAR.I_4$ are described in *Chapter 8*.

$$CAR \xrightarrow{FeCl_3} CAR^{2+} \underset{}{\overset{CAR}{\rightleftharpoons}} 2\,CAR^{\bullet+} \qquad (5)$$

$$CAR + 3\,I_2 \rightleftharpoons 2\left(CAR^{\bullet+}\ldots I_3^-\right) \qquad (6)$$

Reactions of carotenoids with Metal-MCM-41 mesoporous molecular sieves [35-38], where the metal is Fe^{3+}, Cu^{2+}, Ni^{2+} or Al^{3+}, also give rise to the corresponding $CAR^{\bullet+}$.

d) Electrochemical methods

Electrochemical methods [32,39-48] have been used to generate and investigate carotenoid radical cations, carotenoid radical anions [49] and carotenoid neutral radicals (Section **B**.3).

2. Structural and spectroscopic properties of carotenoid radical ions

a) Vis/NIR spectroscopy

An extensive list of the λ_{max} and molar absorption coefficient (ε) data of the radical ions $CAR^{\bullet+}$ and $CAR^{\bullet-}$ for a range of carotenoids with different chromophore lengths in different solvents is given in Table 1.

Table 1. The λ_{max} and molar absorption coefficients (ε) of the radical anions ($CAR^{\bullet-}$) and radical cations ($CAR^{\bullet+}$) of some carotenoids and related compounds in different solvents [50].

Compound	cdb[a]	Spectroscopic data. Solvent: λ_{max} (ε x 10^{-5}) [Ref] Parent[b]	Radical anion	Radical cation
β-Cyclocitral (*1*)	1		M: ~315 [51] T: 370 [51]	
β-Ionone (*2*)	2		M: 350 [51] T: 380 [51] H: 385 [52] AM: 300 (0.12) [52]	H: 375 [52] DCE: 385 [51]
β-Ionylideneacetaldehyde (*3*)	3		H: 460 [52] AM: 350 (0.24) [52]	H: 470 [52]
(15*Z*)-Phytoene (**44**)	3	H: 286	H: 470 [19]	H: 470 [19]
(all-*E*)-Phytoene (**44**)	3	H: 286	H: 460 [19]	H: 460 [19]
C_{17}-Aldehyde (*4*)	4		M: 395 [51]	A: 515 [51]
Retinol (*5*)	5	E: 326	M: 370 [53] I: 370 [53]	A: 585 [54] DCE: 600 [54] H: 600 [54] TX: 590 [55]
Retinal (*6*)	5	E: 370	A: 495 [56] H: 580 [56] M: 445 [56] E: 458 [56] I: 460 [53] T: 530 [51] AM: 405 (0.79) [52] ACN:520 [56] BZN: 550 [56] B: 570 [56] TX10:460 [56] CTAB: 448 [56]	A: 585 [54] DCE: 595 [54] H: 590 [52] ACN: 585 [56] BZN: 595 [56] B: 600 [56] TX: 590 [55]
Retinal *n*-butylamine Schiff base (*7*)	5		M: 430 [53] I: 435 [53]	A: 615 [54] H: 635 [54]
Retinyl acetate (*8*)	5		M: 390 [53] I: 390 [53]	A: 580 [54] DCE: 595 [54] H: 590 (1.0) [54,57]
Retinoic acid (*9*)	5		H: 510 [57] M: 480 (1.2) [53,57] I: 505 [53]	A: 575 [54] DCE: 585 [54] H: 590 [54] M: 590 (0.7) [57] TX: 590 [55]
Methyl retinoate (*10*)	5		M: 480 [53] I: 510 [53]	A: 575 [54] DCE: 585 [54] H: 590 [54]
14′-Apo-β-caroten-14′-al (**513**)	6		M: 465 [51]	A: 640 [51]
C_{24}-Aldehyde (*11*)	7		M: 490 [51]	A: 700 [51]

Table 1 continued

Compound	cdb[a]	Spectroscopic data. Solvent: λ_{max} (ε x 10^{-5}) [Ref] Parent[b]	Radical anion	Radical cation
ζ-Carotene (**38**)	7	E: 399		M: 740 [24]
7,7′-Dihydro-β-carotene (**49**)	8	H: 402 [21]	H: 785 [19] M: 705 [19]	H: 830 [19] M: 770 [24] TX: 770 [58]
Heptaprcno-β-carotene (*12*)	9	H: 414 [21]	H: 785 [19] M: 705 [19] TX: 760 [23]	H: 915 [19] M: 820 [24] TX: 850 (0.37) [22]
Violaxanthin (**259**)	9	E: 440		E: 830 [59]
8′-Apo-β-caroten-8′-al (**482**)	9	E: 452	H: 840 [52] M: 555 [51] B: 820 (3.41) [60] AM: 500 (0.94) [52] T: 725 [51] TX: 590-600 [62] TX40: 590 [51]	H: 890 [22] M: 820 [21] B: 880 [17] C: 855 [28] DCM: 848 [61]
Lutein (**133**)	10	E: 445	H: 855 [23] B: 870 (3.320) [60] TX: 840 [23]	H: 973 [22] B: 950 [17] M: 865 [21] E: 880 [59] C: 940 [28] TX: 900 (0.67) [22]
β-Carotene (**3**)	11	E: 450	H: 880 (4.42) [63] B: 880 (3.27) [60] M: 800 [19] Cy: 900 (3.24) [63] TX10: 865 [64] Mix 3: 850 [65]	H: 1040 (2.18) [19,63] B: 1020 (1.24) [17,21] M: 900 [24] E: 910 (1.30) [30] Cy: 1050 (2.02) [63] C: 1000 [26] DCM: 970 [32] DMSO: 942 (0.16) [66] TX: 936 (0.87) [22] Mix1: 920 [67] Mix2: 910 (0.94) [68] ME: 840 [69]
(15*Z*)-β-Carotene (**3**)	11		H: 900 (2.51) [63] M: 820 [63]	H: 1050 (1.54) [63]
Lycopene (**31**)	11	E: 472	H: 950 (4.1) [19,63] B: 950 (3.71) [60] M: 870 [19]	H: 1070 (3.25) [19,63] B: 1050 (1.58) [17,21] M: 950 [21] C: 1040 [28] TX Mix: 970 [58]
Zeaxanthin (**119**)	11	E: 450	H: 900 [23] B: 880 (2.78) [60] M: 820 [60] TX: 861 [23]	H: 1040 [22] B: 1000 [17] M: 910 [21] E: 890 [30] C: 980 [28] TX: 936 (0.41) [22]

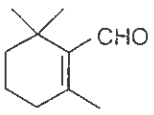
Table 1 continued

Compound	cdb[a]	Spectroscopic data. Solvent: λ_{max} (ε x 10^{-5}) [Ref] Parent[b]	Radical anion	Radical cation
(*meso*)-Zeaxanthin (**120**)	11	E: 450	B: 900 (2.48) [60]	B: 1020 [21] H: 1040 [21] M: 900 [21]
Canthaxanthin (**380**)	11	E: 474	H: 1150 [19] B: ≥1100 [60] M: 600-610 [62] TX: ~720 (0.21) [62]	H: 960 [19] B: 940 [17] M: 840 [21] C: 900 [28] DCM: 887 [32] TX: 862 (0.67) [22]
Astaxanthin (**406**)	11	E: 478	H: 1120 [70] B: ≥1100 [60] M: 610-620 [62] TX: ~720 (0.53) [62]	H: 940 [22] B: 920 [17] M: 840 [21] TX: 875 (0.30) [22]
Torularhodinaldehyde (**271**)	13	E: 507	H: 1130 [52] AM: 600 [52]	H: 1130 [52]
Decapreno-β-carotene (*13*)	15	P: 495 [19]	H: 1130 [19] M: 1010 [19]	H: 1250 [19]
Dodecapreno-β-carotene (*14*)	19	P: 534 [19]	H: 1355 [19] M: 1140 [19]	H: 1480 [19]

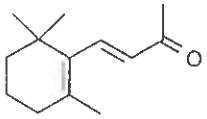
Solvents: A, acetone; ACN, acetonitrile; B, benzene; C, chloroform; E, ethanol; M, methanol; P, petroleum ether; BZN, benzonitrile; Cy, cyclohexane; H, hexane; DCE, 1, 2-dichloroethane; DCM, dichloromethane; AM, alkaline methanol (methanol containing 0.01 M NaOH); I, isopropyl alcohol; T, tetrahydrofuran; TX, 2% (w/v) aq. Triton X-100; TX10, 10 mM aq. Triton X-100; TX40, 40mM aq. Triton X-100; TX Mix, 3% (w/v) Triton X-405 and 1% (w/v) Triton X-100; Mix1, di-*t*-butylperoxide/cyclohexane (7:3 v/v); Mix2, *t*-butanol/water (1:1 v/v); Mix3, ethanol/water (86:14 v/v); CTAB, 18mM cetyltrimethylammonium bromide; ME, a microemulsion of sodium lauryl sulphate (3.23% w/v), cyclohexane (75%v/v), water (6.45% v/v) and pentan-1-ol (15.32% v/v).

[a] cdb is the number of conjugated double bonds (excluding carbonyl groups where relevant).

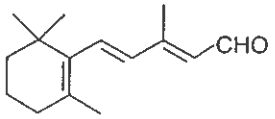
[b] The parent carotenoid. λ_{max} values are taken from *Vol. 1B, Chapter 2*, unless otherwise stated.



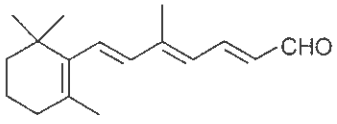
(*1*) β-cyclocitral



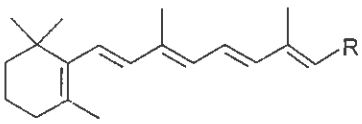
(*2*) β-ionone



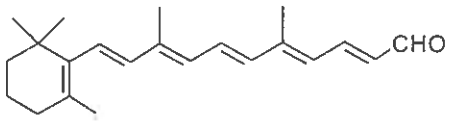
(*3*) β-ionylideneacetaldehyde



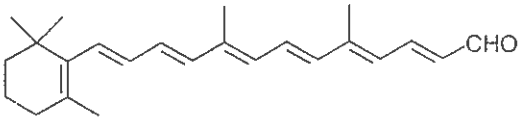
(*4*) 'C_{17}-aldehyde'



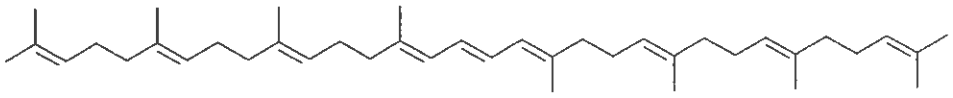
(*5*) R = CH_2OH: retinol
(*6*) R = CHO: retinal
(*7*) R = $CH{=}N(CH_2)_3CH_3$: retinal *n*-butylamine Schiff base
(*8*) R = CH_2OCOCH_3: retinyl acetate
(*9*) R = COOH: retinoic acid
(*10*) R = $COOCH_3$: methyl retinoate



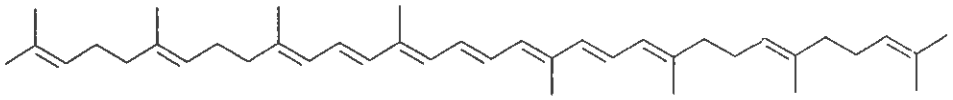
(513) 14'-apo-β-caroten-14'-al



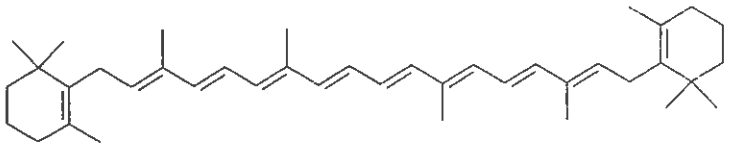
(11) 'C_{24}-aldehyde'



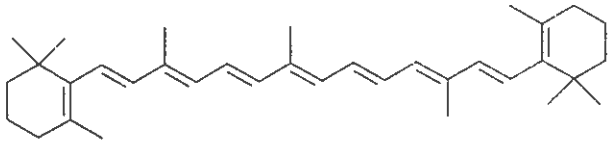
(44) phytoene



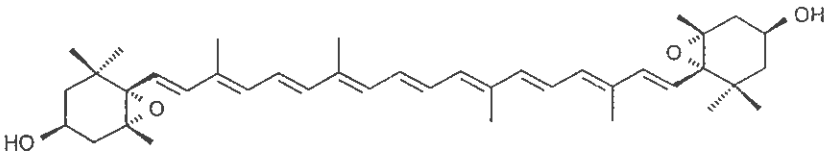
(42) phytofluene



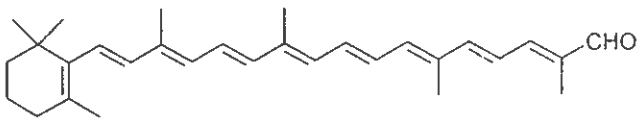
(49) 7,7'-dihydro-β-carotene (77DH)



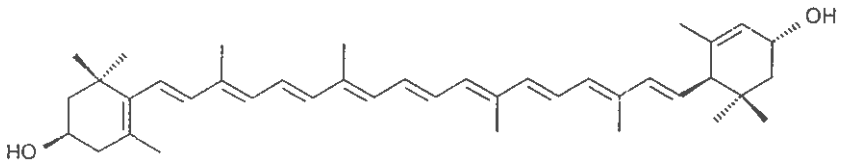
(12) heptapreno-β-carotene



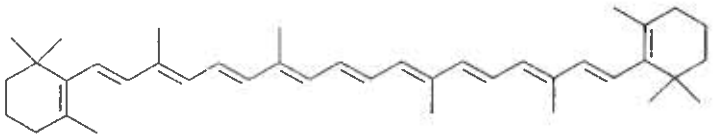
(259) violaxanthin



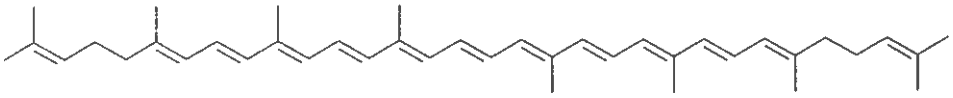
(482) 8'-apo-β-caroten-8'-al



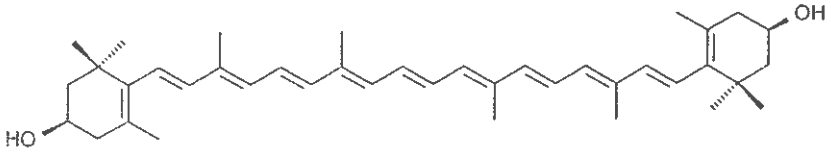
(**133**) lutein



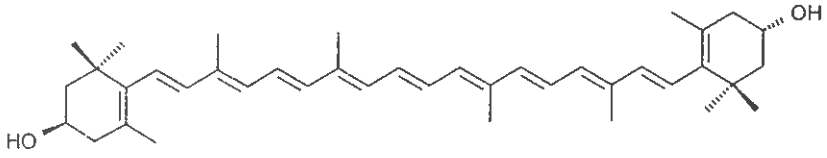
(**3**) β-carotene



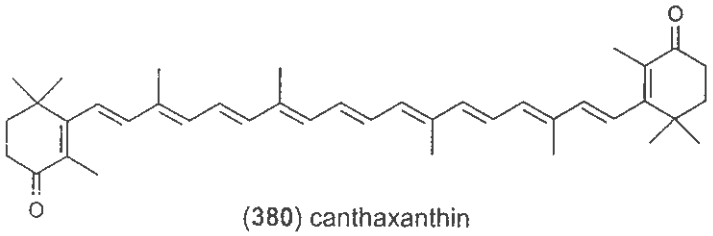
(**31**) lycopene



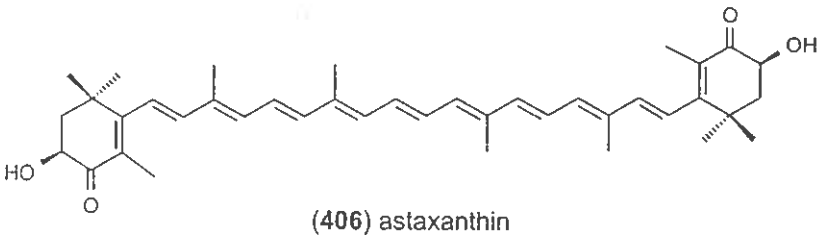
(**119**) zeaxanthin



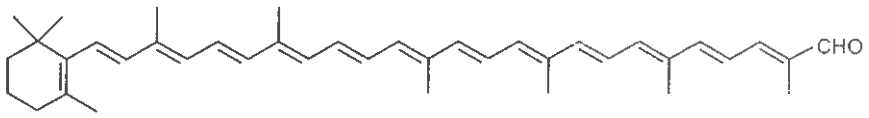
(**120**) *meso*-zeaxanthin



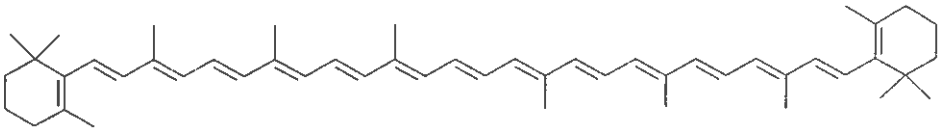
(**380**) canthaxanthin



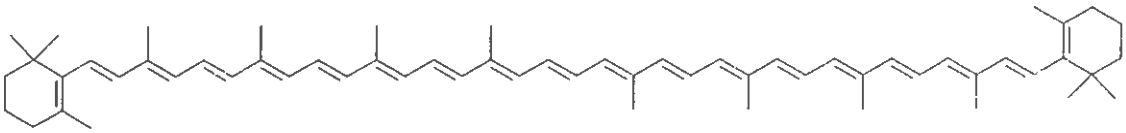
(**406**) astaxanthin



(**271**) torularhodinaldehyde



(*13*) decapreno-β-carotene



(*14*) dodecapreno-β-carotene

Generally, the λ_{max} of $CAR^{\bullet+}$ is at longer wavelength than that of $CAR^{\bullet-}$ [19] and, as the number of double bonds increases, the λ_{max} of $CAR^{\bullet+}$ and $CAR^{\bullet-}$ increases [52]. The spectral band positions for the radical ions are strongly dependent on solvent. The data show that the λ_{max} of $CAR^{\bullet-}$ is blue-shifted (to shorter wavelength) on changing the solvent from hexane to methanol. This is attributed to a less uniform charge distribution in the ground state of $CAR^{\bullet-}$ than in the excited state. Therefore, in methanol, the ground state is stabilized relative to the excited state [19].

More recently, theoretical and experimental work [71] has revealed the presence of an additional weak absorption band for polyene radical cations at longer wavelengths, attributed to the $D_0 \rightarrow D_1$ transition ('D' signifies 'doublet-state' in the same way that 'S' and 'T' signify 'singlet-state' and 'triplet-state' respectively). The more familiar intense band at shorter wavelengths is assigned to the $D_0 \rightarrow D_2$ transition (Table 2).

Table 2. The λ_{max} and molar absorption coefficients of the $D_0 \rightarrow D_1$ and $D_0 \rightarrow D_2$ transitions of $CAR^{\bullet+}$ in CH_2Cl_2 [32,72].

$CAR^{\bullet+}$	λ_{max} of $D_0 \rightarrow D_1$ / nm (log ε)	λ_{max} of $D_0 \rightarrow D_2$ / nm (log ε)
β-$CAR^{\bullet+}$	1425 (3.3)	970 (4.8)
$CAN^{\bullet+}$	1310 (4.6)	887 (5.4)

Abbreviations. β-CAR: β-carotene (**3**); CAN: canthaxanthin (**380**)

b) Electron paramagnetic resonance (EPR)

Vis/NIR spectra are used extensively for characterizing radical ions, but the presence of unpaired electrons is not proved by this method only. The absorption of carotenoid cations that have no unpaired electrons occurs within the same spectral region [73] (see *Chapter 8*). Electron paramagnetic resonance, which measures transitions between spin states of an unpaired electron, is a general method for demonstrating the presence of unpaired electrons [74] and is thus used as a tool for identifying and studying carotenoid radicals, including triplet states [34,42,44,75-84]. Most spectra are recorded in first-derivative form, as illustrated by the representative EPR spectrum of the radical cation of β-carotene (**3**), shown in Fig. 1. The single signal spectrum is characteristic of carotenoid radicals [86]. The spin properties of the electron (g-factor, hyperfine coupling) are influenced by nearby nuclei with non-zero nuclear spin, allowing structural information to be deduced.

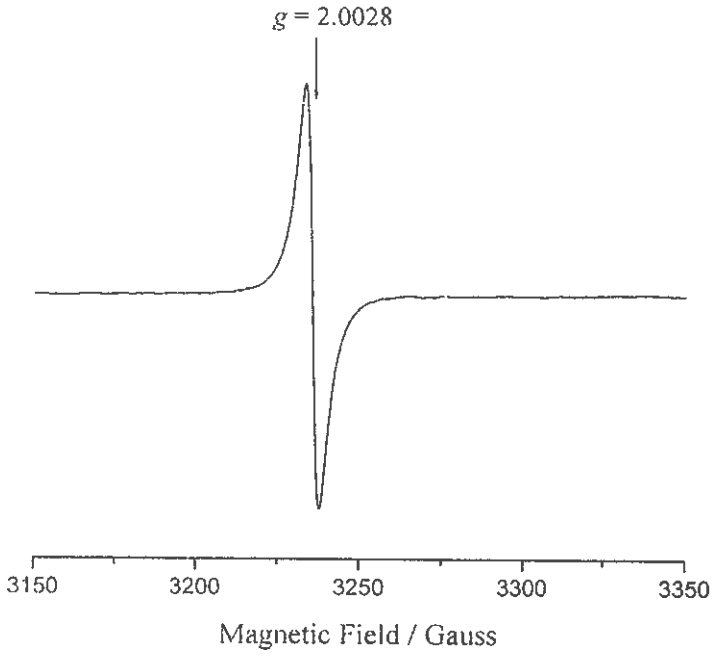


Fig. 1. EPR spectrum, measured at 207K, of β-carotene radical cation $CAR^{\bullet+}$ generated by treatment of β-carotene with antimony trichloride [85].

c) Electron nuclear double resonance (ENDOR)

The more recent development, ENDOR, which employs both nuclear magnetic and electron spin resonance, is used in modern studies and gives greatly enhanced high-resolution EPR spectra. Pulsed ENDOR spectra of the radical cations of β-carotene (**3**) and canthaxanthin (**380**) have been reported [79,82].

d) Resonance Raman spectroscopy

Resonance Raman spectroscopy [31,43] of $CAR^{\bullet+}$, together with AM1 [42] and gas-phase B3LYP [87] calculations, suggest the delocalization of the unpaired electron density over the entire carbon backbone. Also, earlier calculations [42,52] and resonance Raman studies [31,43] indicated that the bond lengths in $CAR^{\bullet+}$ and $CAR^{\bullet-}$ have intermediate values between the single-bond and double-bond values of parent carotenoids (see also *Chapter 8*).

3. Reduction (redox) potentials for carotenoid radical ions

Most redox potential data for carotenoids [44-48,61,88] have been determined by electrochemical methods [47,49]. Reduction potentials [in CH_2Cl_2, *versus* the saturated calomel electrode (SCE)] involving carotenoid radical cations and neutral radicals are given in Table 3. The more positive the E° value, the more strongly oxidizing is the species on the left-hand-side of the reduction half-reaction. In relative terms, a low E° value means that it is easier, in principle, to oxidize the species on the right-hand-side of the reduction half-reaction to form the species on the left-hand side.

In the equations given in Scheme 9, $CAR^{\bullet+}$, CAR^{2+}, $^{\#}CAR^{+}$ and $^{\#}CAR^{\bullet}$ represent respectively the carotenoid radical cation, carotenoid dication, [carotenoid – H] cation and carotenoid neutral radical (see Section A.1)..

$$CAR^{\bullet+} + e^- \overset{E^\circ_1}{\rightleftharpoons} CAR$$

$$CAR^{2+} + e^- \overset{E^\circ_2}{\rightleftharpoons} CAR^{\bullet+}$$

$$^{\#}CAR^{+} + e^- \overset{E^\circ_3}{\rightleftharpoons} {}^{\#}CAR^{\bullet}$$

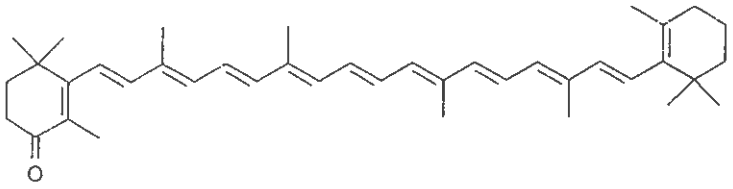
Scheme 9

Moreover, reactive species such as CAR^{2+} and $CAR^{\bullet+}$ can undergo various reactions (see equations 7-9).

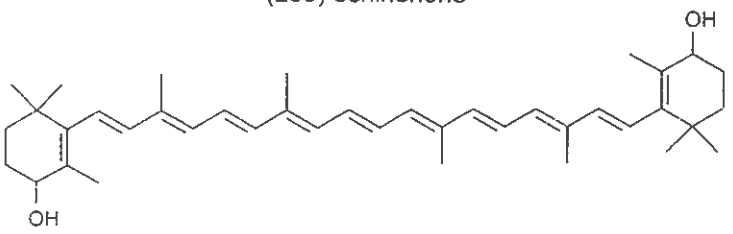
$$CAR^{2+} + CAR \rightleftharpoons 2\,CAR^{\bullet+} \quad (7)$$

$$CAR^{2+} \rightleftharpoons {}^{\#}CAR^{+} + H^+ \quad (8)$$

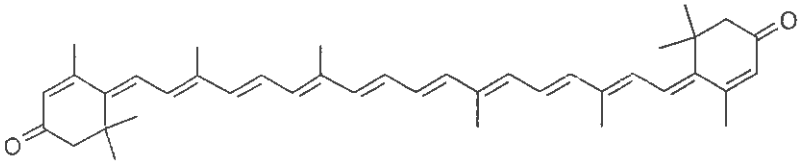
$$CAR^{\bullet+} \rightleftharpoons {}^{\#}CAR^{\bullet} + H^+ \quad (9)$$



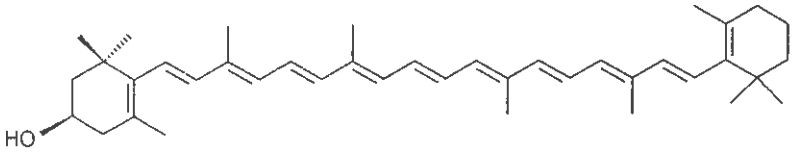
(**283**) echinenone



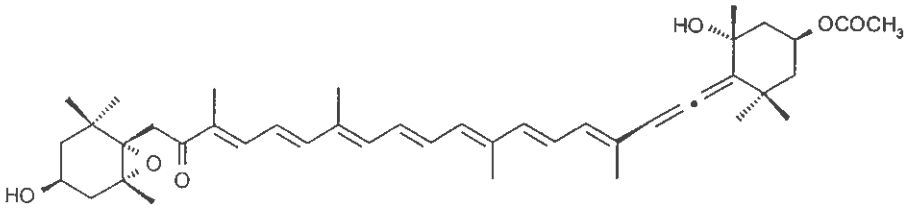
(**129**) isozeaxanthin



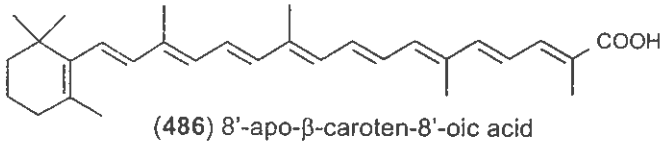
(**424**) rhodoxanthin



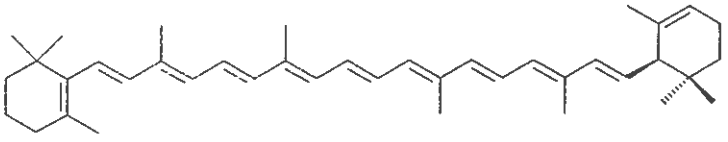
(**55**) β-cryptoxanthin



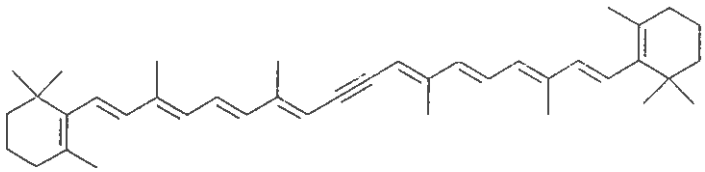
(**369**) fucoxanthin



(**486**) 8'-apo-β-caroten-8'-oic acid



(**7**) α-carotene



(*15*) 15,15'-didehydro-β-carotene

Table 3. Reduction potentials (E°_1) *versus* saturated calomel electrode (SCE) (± 2 mV, unless otherwise stated) for the carotenoid radicals as described in Scheme 9 [47]; in CH_2Cl_2 unless otherwise stated[a].

Carotenoid	E°_1 (mV)	E°_2 (mV)	E°_3 (mV)	Ref
β-Carotene (**3**)	567 ± 4	-	-	[45]
	540	545	35	[46]
	530	560	45	[44]
	510[a]			[49]
Canthaxanthin (**380**)	689	894	264	[46]
	705	945	250	[44]
Echinenone (**283**)	590	690	110	[44]
Isozeaxanthin (**129**)	570	550	80	[44]
Rhodoxanthin (**424**)	655	900	250	[44]
8'-Apo-β-caroten-8'-al (**482**)	720 ± 10	865 ± 25	75 ± 25	[61]
β-Cryptoxanthin (**55**)	560	570	60	[47]
Zeaxanthin (**119**)	571 ± 11	-	-	[45]
	530	550	90	[47]
Fucoxanthin (**369**)	790	820	240	[47]
8'-Apo-β-caroten-8'-oic acid (**486**)	686 ± 4	846 ± 8	263 ± 30	[47]
α-Carotene (**7**)	596 ± 4	623 ± 10	66 ± 16	[47]
	570[a]			[49]
Lycopene (**31**)	507 ± 6	524 ± 6	51 ± 28	[47]
	480[a]			[49]
Violaxanthin (**259**)	681 ± 14	-	-	[45]
15,15'-Didehydro-β-carotene (*15*)	763	766	240	[88]
	680[a]			[49]

[a]In acetonitrile/benzene (2:1) containing 0.027 M Et_4NClO_4.

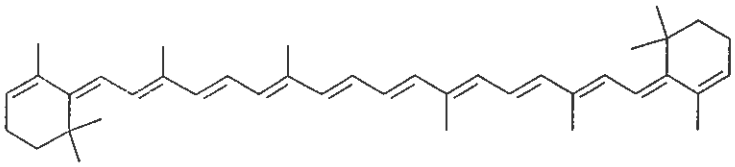
Pulse radiolysis has been used to determine the reduction potentials of a variety of carotenoids in micelles by monitoring the reversible electron transfer between tryptophan radical cation ($TrpH^{\bullet+}$) and the carotenoid at different pH values. Since the reduction potentials of $TrpH^{\bullet+}$/ TrpH at different pH values are known, the reduction potentials of $CAR^{\bullet+}$/ CAR can be estimated (Table 4) [15,89].

Table 4. Reduction potentials (E°_1) (± 25 mV) *versus* SCE for carotenoid radical cations in micelles (converted by adding 242 mV to the original data [*versus* normal hydrogen electrode (NHE)] [15,89].

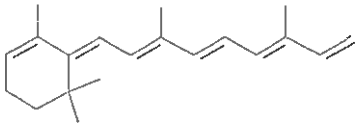
Carotenoid	Micelle	E°_1 (mV)
β-Carotene (3)	TX-100[a]	818
β-Carotene (3)	TX-405/ TX-100[b]	786
Canthaxanthin (380)	TX-100	799
Zeaxanthin (119)	TX-100	789
β-Cryptoxanthin (55)	TX-100	786 [90]
Astaxanthin (406)	TX-100	788
Lycopene (31)	TX-405/ TX-100[b]	738

[a]TX-100: 2% (w/v) aqueous Triton X-100.

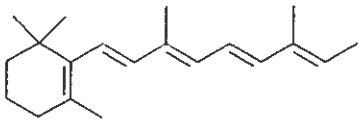
[b]TX-405/ TX-100: 3% (w/v) Triton X-405 and 1% (w/v) Triton X-100.



(*48*) *retro*dehydro-β-carotene



(*16*) anhydrovitamin A



(17) axerophtene

The one-electron reduction potentials (*v.* SCE) of β-carotene (**3**), lycopene (**31**), α-carotene (**7**), neo-A-*retro*dehydro-β-carotene (**48**) 15,15'-didehydro-β-carotene (*15*), anhydrovitamin A (*16*) and axerophtene (*17*) (in dimethylformamide/benzene (2:1, v/v) containing 0.027 M tetracthyl ammonium iodide) have been reported as -1.68, -1.65, -1.70, -1.65, -1.69, -1.97 and -2.32 V, respectively [49]. The relative order of reduction potentials for β-carotene and lycopene is in agreement with the observed reaction of β-carotene$^{\bullet -}$ with lycopene in hexane ($k \sim 1.4 \times 10^{10}$ M^{-1} s^{-1}) and benzene ($k \sim 6.2 \times 10^{9}$ M^{-1} s^{-1}) (equation 10) [21].

$$\beta\text{-CAR}^{\bullet -} + \text{LYCO} \longrightarrow \beta\text{-CAR} + \text{LYCO}^{\bullet -} \qquad (10)$$

C. Carotenoid Neutral Radicals

1. Formation and detection of carotenoid neutral radicals

There are several potential routes to the formation of carotenoid neutral radicals, including radical addition to the polyene backbone (giving a carotenoid addition radical), allylic hydrogen abstraction and de-protonation/protonation of radical ions (equations 11 and 12). Carotenoid addition radicals and other neutral radicals appear to absorb light in a similar spectral region to the parent carotenoid and, in some cases, there is a spectrally resolved absorption band on the red edge (long wavelength) of the parent carotenoid absorption [24,27,51-53,56,68,91,92]. These experimental observations are consistent with theoretical ZINDO/S calculations [93] of the absorption maxima of carotenoid neutral radicals, which are predicted to absorb close to the region where the parent carotenoid absorbs.

An early report [50] showed that the radical anions of carbonyl-containing carotenoids can be protonated in methanol to form the corresponding α-hydroxy radical derivatives (equation 11). In alkaline methanolic solutions, the reaction shifts toward the left and only carotenoid radical anions were observed. Similar observations were reported in different protic solvents and in micelles [51,53,56]. For example, the λ_{max} of the corresponding α-hydroxy radical derivatives of astaxanthin (**406**), canthaxanthin (**380**), 8'-apo-β-caroten-8'-al (**482**) and retinal (*6*) in aqueous Triton X-100 are 570, 580, 510 and 405 nm, respectively [56,62].

(11)

(12)

When carotenoid addition radicals were first observed spectroscopically, the assignment at the time was uncertain. The reaction of glutathione thiyl free radicals ($GS^{\bullet}$) with retinol (*5*) was studied in aqueous methanol [91] and a strongly absorbing species was observed (λ_{max} 380 nm, $\varepsilon_{380\ nm} = 4.0 \times 10^4\ M^{-1}\ cm^{-1}$) that was attributed to either retinol$^{\bullet+}$ or [GS-retinol]$^{\bullet}$. However, retinol$^{\bullet+}$ is known to absorb around 585 nm [54] so the species absorbing at 380 nm is almost certainly [GS-retinol]$^{\bullet}$ (equation 13).

(13)

Addition radicals were shown to be formed by reaction of β-carotene (**3**) with thiyl radicals and sulphonyl radicals (which also yield carotenoid radical cations under the experimental conditions used) [68,92]. This work was subsequently extended to the study of other

carotenoids [astaxanthin (**406**), canthaxanthin (**380**), zeaxanthin (**119**), lutein (**133**), lycopene (**31**)] [94].

The *retro*-carotenoid 7,7'-dihydro-β-carotene (7,8-dihydro-8,7'-*retro*-β,β-carotene, **49**) is distinctive in that addition radicals derived from it display exceptionally intense absorption bands on the red edge of the parent absorption [24,27]. The spectral profile of addition radicals derived from 7,7'-dihydro-β-carotene is not strongly affected by the nature of the scavenged radical, although the chemical properties of the addition radicals vary considerably (see Section **E**.2).

D. Unidentified Carotenoid Radicals

The reactions of various radicals, including $CCl_3O_2^{\bullet}$, acylperoxyl radicals, sulphonyl radicals and phenoxyl radicals, with different carotenoids in polar environments leads to formation of carotenoid radicals that absorb in the near infrared, but at shorter wavelengths than the corresponding radical cations [22,25,26,28,67,94]. The decay of these carotenoid radical species appears to lead to formation of $CAR^{\bullet+}$ [22]. Speculation surrounding the assignment of these NIR absorption features initially included carotenoid radical adducts and other neutral radicals [22,25,26,28,67,95] and ion pairs [28,29,94]. Subsequent work on carotenoid addition radicals in polar and apolar environments, however, appears to preclude the assignment of these species as carotenoid neutral radicals, which would absorb in the visible region close to the absorption of the parent carotenoid [27,52,56,68,91]. More recently, in the light of evidence that these carotenoid radical species are not formed directly, but from ionic dissociation of carotenoid addition radicals, the possibility that these species are geometrical (*i.e. cis-trans*) isomers of the carotenoid radical cation has been suggested [24].

E. Reaction of Carotenoids with Oxidizing Free Radicals

1. Factors that influence the mechanism of reactions of free radicals with carotenoids

The mode of reaction of carotenoids with free radicals [96-100] is dependent upon the nature of the free radical (see sections **E**.3.a and **E**.3.c), solvent polarity and also the nature of the carotenoid *i.e.* number of conjugated double bonds, presence and type of oxygen functions (see section **E**.3.d).

2. Free-radical scavenging mechanisms in environments of low polarity

The reactions of carotenoids with oxidizing free radicals in polar environments often proceeds *via* electron transfer leading to formation of the carotenoid radical cation. In apolar environments, however, this is generally not possible, as charge separation is not supported in such environments. Given that carotenoids are likely to be found in apolar environments within biological systems, it is surprising that comparatively few studies of free-radical reactions with carotenoids in apolar environments have been carried out. Nevertheless, even in apolar environments, the reactions of carotenoids with certain free radicals are extremely rapid [24,101]. Thus, the reaction of carotenoids with phenylthiyl and acylperoxyl radicals proceeds *via* radical addition [24,27,101] with rate constants in the region of 10^9 $M^{-1}s^{-1}$. Alternative scavenging mechanisms such as H-atom abstraction have been ruled out on the basis that the reactions of different radicals (*e.g.* acylperoxyl and phenylthiyl radicals) with 7,7'-dihydro-β-carotene (**49**) lead to carotenoid neutral radicals with very similar spectra, but very different kinetic behaviour [24,27]; reaction with acylperoxyl radicals is 100-1000 times more rapid. Less reactive peroxyl radicals such as benzylperoxyl radical ($C_6H_5CH_2OO^{\bullet}$) also react with carotenoids in apolar environments, probably by radical addition but at a much lower rate [102]. Recent EPR studies of the reaction of $NO_2^{\bullet}$ with β-carotene in chloroform [103] suggest an addition mechanism.

3. Free-radical scavenging mechanisms in polar and heterogeneous environments

In polar and heterogeneous environments (*e.g.* micelles and microemulsions), the mechanisms by which carotenoids scavenge free radicals are more complex and more varied. The behaviour with different classes of free radical is summarized below.

a) Thiyl radicals ($RS^{\bullet}$)

In polar (and low polarity) solvents, thiyl radicals react with carotenoids [27,68,94] exclusively *via* radical addition (see equation 13 and Scheme 14) and there is no evidence for formation of $CAR^{\bullet+}$ in these reactions. This reflects the relatively low reduction potentials of these radicals (*e.g.* for 2-mercaptoethanol thiyl radical, $E^{\circ} \sim 0.78$ V *versus* NHE) [104] compared with, for example, acylperoxyl radicals ($E^{\circ} \sim 1.12$ V *versus* NHE) [105].

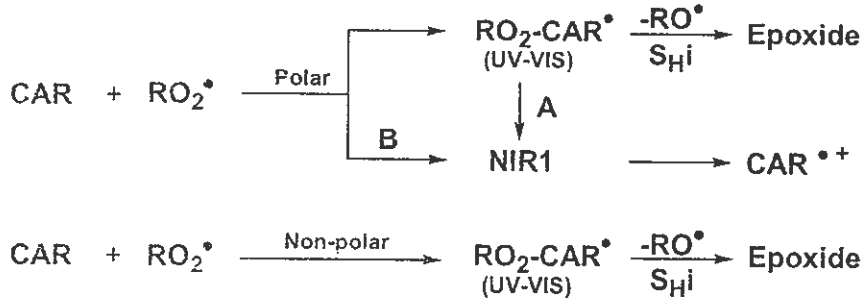
b) Sulphonyl radicals ($RSO_2^{\bullet}$)

The reactions of $RSO_2^{\bullet}$ with carotenoids [68,94] in polar environments (*t*-butanol/water mixtures) appear to proceed *via* radical addition (RSO_2-$CAR^{\bullet}$) and electron transfer to

produce the carotenoid radical cation ($CAR^{\bullet+}$). However, formation of $CAR^{\bullet+}$ is preceded (at least in part) by formation of an intermediate species that absorbs at shorter wavelengths, and the possibility that both these species are preceded by the addition radical cannot be rigorously excluded. The reactions of sulphonyl radicals with carotenoids in apolar environments have not been reported.

c) Peroxyl radicals ($RO_2^{\bullet}$)

From studies of the reactions of acylperoxyl radicals with carotenoids in polar environments there is some evidence that radical addition is the initial step and that the addition radical (RO_2-$CAR^{\bullet}$) subsequently undergoes an ionic dissociation *via* an intermediate (NIR1) to form $CAR^{\bullet+}$ in a consecutive process (Pathway A in Scheme 10) [24]. This process probably operates in competition with the unimolecular S_Hi process that prevails in environments of low polarity. Whether this pathway operates more generally remains unresolved and direct formation of NIR1 (Pathway B in Scheme 10) cannot be excluded [22]. Also, for less strongly oxidizing peroxyl radicals (*e.g.* alkylperoxyl radicals), the formation of $CAR^{\bullet+}$ may not be feasible thermodynamically when the respective redox potentials are taken into account [106].



Scheme 10

d) Phenoxyl radicals ($PhO^{\bullet}$)

In di-*t*-butyl peroxide/benzene (70:30), $PhO^{\bullet}$ reacts with β-carotene (**3**) to form the carotenoid radical cation *via* a NIR1 species [25]. It is possible that addition precedes NIR1, but further work is required to establish whether this is so. For canthaxanthin (**380**), the reaction with $PhO^{\bullet}$ is very much slower than that with β-carotene, and no reaction was observed at all with astaxanthin (**406**), possibly reflecting the higher reduction potentials of the xanthophylls [25]. The reactions of phenoxyl and alkoxyl radicals with carotenoids in low polarity environments have not been studied.

e) Other radicals

The reactions of carotenoids with $CCl_3^{\bullet}$ [22], $NO_2^{\bullet}$ [68,92,94,107,108] and with $Br_2^{\bullet-}$ and $(SCN)_2^{\bullet-}$ in polar environments [109,110] appear to proceed by direct electron transfer to produce $CAR^{\bullet+}$. There is no evidence for the formation of addition radicals or NIR1 species.

F. Reactions of Carotenoid Radicals

1. Carotenoid radical cations ($CAR^{\bullet+}$)

a) Reactions with nucleophiles

The presence of the positive charge means that carotenoid radical cations may be susceptible to nucleophilic attack. These reactions have been observed for the radical cations of shorter carotenoid-like polyenes such as retinoids. The rate constants for the reactions of these radical cations with water, triethylamine and bromide ion in acetone are shown in Table 5. From this, it is clear that the reaction rate decreases as the length of the conjugated polyene chain increases [51,54].

Table 5. The rate constants k_q (± 20%) for the reaction of the radical cations of some retinoids and related compounds with nucleophiles [water, triethylamine (TEA), bromide ion] in oxygen-saturated acetone [51,54].

Compound	cdb[a]	k_q/ M^{-1} s^{-1}		
		Water/10^5	TEA/10^8	Br^-/10^9
C_{17}-Aldehyde (*4*)	4	1.9	21	56
Retinal (*6*)	5	0.84	1.8	53
Retinol (*5*)	5	1.9	0.27	41
Retinyl acetate (*8*)	5	1.3	0.39	31
Retinoic acid (*9*)	5	3.5	1.9	39
Methyl retinoate (*10*)	5	1.6	1.2	37
Retinal Schiff base (*7*)	5	1.5	0.39	31
14′-Apo-β-caroten-14′-al (**513**)	6	0.45	0.56	41
8′-Apo-β-caroten-8′-al (**482**)	9	< 0.1	0.056	0.12

[a] cdb is the number of conjugated double bonds (carbonyl group not included).

In the absence of any other reactants, carotenoid radical cations disproportionate to give carotenoid dication and the parent carotenoid (equation 14) [32]. The equilibrium constants for these reactions have been compiled [88].

$$2CAR^{\bullet+} \rightleftharpoons CAR + CAR^{2+} \qquad (14)$$

b) De-protonation

The equilibrium constants (K'_{dp}) and forward rate constants (k'_f) for the de-protonation of carotenoid radical cations (equation 15) in dichloromethane have been reported [88].

$$CAR^{\bullet+} \rightleftharpoons {}^{\#}CAR^{\bullet} + H^{+} \qquad (15)$$

c) Reactions with amino acids and peptides

Various carotenoid radical cations are able to oxidize both tyrosine (TyrOH) and cysteine (CySH) ($k \sim 10^4$ and 10^6 M^{-1} s^{-1} respectively) (equations 16 and 17) [15,58,89]. Also, some carotenoid radical cations react reversibly with tryptophan (TrpH) to form the tryptophan radical cation (equation 18) in a pH-dependent process [15,89].

$$CAR^{\bullet+} + TyrOH \longrightarrow CAR + TyrO^{\bullet} + H^{+} \qquad (16)$$

$$CAR^{\bullet+} + CysH \longrightarrow CAR + Cys^{\bullet} + H^{+} \qquad (17)$$

$$CAR^{\bullet+} + TrpH \rightleftharpoons CAR + Trp^{\bullet+} + H^{+} \qquad (18)$$

d) Reactions with porphyrins

Some porphyrin derivatives (POR) such as chlorophyll *a* can be oxidized by $CAR^{\bullet+}$ (equation 19) [111-113].

$$CAR^{\bullet+} + POR \longrightarrow CAR + POR^{\bullet+} \qquad (19)$$

e) Reactions with other carotenoids

The relative reduction potentials of a variety of carotenoid radical cations have been established by monitoring the reaction of the radical cation of one carotenoid ($CAR1^{\bullet+}$) with another carotenoid (CAR2) in benzene (equation 20 and Table 6) [17]. For example, astaxanthin$^{\bullet+}$ can react with lycopene to form lycopene$^{\bullet+}$, therefore the reduction potential of astaxanthin$^{\bullet+}$ ($E°_{ASTA^{\bullet+}/ASTA}$) is greater than that of lycopene ($E°_{LYCO^{\bullet+}/LYCO}$, equation 21). By studying various pairs of carotenoids, the relative reduction potentials of $CAR^{\bullet+}$/ CAR were established [17]. This study reveals that $E°_{ASTA^{\bullet+}/ASTA} > E°_{APO^{\bullet+}/APO} > E°_{CAN^{\bullet+}/CAN} > E°_{LUT^{\bullet+}/LUT} > E°_{ZEA^{\bullet+}/ZEA} \approx E°_{MZEA^{\bullet+}/MZEA} > E°_{\beta\text{-}CAR^{\bullet+}/\beta\text{-}CAR} > E°_{LYCO^{\bullet+}/LYCO}$. The relative order of the reduction potentials is similar to the order reported in other studies [25,47,114,115] (see Table 6 for abbreviations).

$$CAR1^{\bullet+} + CAR2 \longrightarrow CAR1 + CAR2^{\bullet+} \quad (20)$$

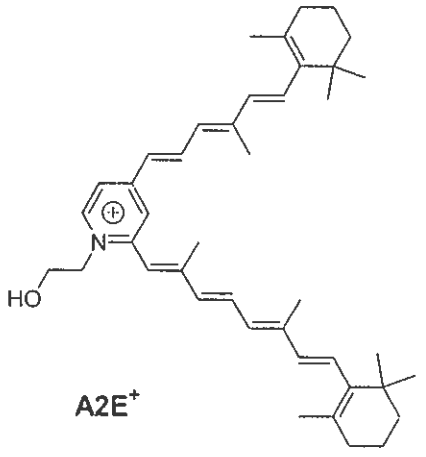
$$ASTA^{\bullet+} + LYCO \longrightarrow ASTA + LYCO^{\bullet+} \quad (21)$$

Table 6. Rate constants k_q (± 10%) for electron transfer between a carotenoid (CAR2) and a carotenoid radical cation ($CAR1^{\bullet+}$) (Equation 20) [17].

	$k_q / 10^9\ M^{-1} s^{-1}$		
$CAR^{\bullet+}$	Lycopene (31)	β-Carotene (3)	Zeaxanthin (119)
$ASTA^{\bullet+}$	9	8	5
$APO^{\bullet+}$	11	6	8
$CAN^{\bullet+}$	8	5	< 1
$LUT^{\bullet+}$	5	<1	<1
$MZEA^{\bullet+}$	7.8 [21]	< 1 [21]	-
$ZEA^{\bullet+}$	7	< 1	-

Abbreviations. ZEA: zeaxanthin (**119**); ASTA: astaxanthin (**406**); APO: 8'-apo-β-caroten-8'-al (**482**); CAN: canthaxanthin (**380**); LUT: lutein (**133**); MZEA: (*meso*)-zeaxanthin (**120**).

Recently [106], the reactivity of the radical cation of the retinoid product $A2E^+$ (Scheme 11) with carotenoids has been investigated. Oxidation of $A2E^+$ by $Br_2^{\bullet-}$ in 2% (w/v) aqueous Triton X-100 (Scheme 11) gives $A2E^{\bullet2+}$ (λ_{max} = 590 nm; ε_{590} = 8400 ± 500 $M^{-1}\ cm^{-1}$), which subsequently reacts with β-carotene to form β-carotene$^{\bullet+}$ ($k \sim 2 \times 10^9\ M^{-1}\ s^{-1}$).



$$A2E^+ + Br_2^{\bullet-} \longrightarrow A2E^{\bullet2+} + 2Br^-$$

$$A2E^{\bullet2+} + \beta\text{–CAR} \longrightarrow \beta\text{–CAR}^{\bullet+} + A2E^+$$

Scheme 11

f) Association with the parent carotenoid

Radical cations of retinal (*6*), retinoic acid (*9*) and methyl retinoate (*10*) in acetone have been observed to undergo association with the parent compound at high concentration [117]. This behaviour has also been observed for the radical cations of canthaxanthin (380) in CH_2Cl_2 [118] and β-carotene (3) in DMSO ($k = 2.5 \times 10^7\ M^{-1}\ s^{-1}$) [66,119] (equation 22). Carotenoid

radical cation dimer ($CAR_2^{\bullet+}$) can deprotonate to give the carotenoid radical dimer ($^{\#}CAR_2^{\bullet}$) (equation 23) [118].

$$CAR^{\bullet+} + CAR \rightleftharpoons CAR_2^{\bullet+} \quad (22)$$

$$CAR_2^{\bullet+} \rightleftharpoons {}^{\#}CAR_2^{\bullet} + H^+ \quad (23)$$

g) Reactions with oxygen

In fast, time-resolved studies of carotenoid radical cations (*e.g.* by pulse radiolysis), no evidence has been found for reaction with oxygen on the timescales employed [63]. In a recent investigation [120], however, mechanistic arguments were presented to suggest that carotenoid radical cations do react with oxygen to form $CARO_2^{\bullet+}$ (Scheme 12).

$$CAR^{\bullet+} + O_2 \longrightarrow CARO_2^{\bullet+}$$

$$CARO_2^{\bullet+} + CAR \text{ (or } Fe^{2+}) \rightleftharpoons CARO_2 + CAR^{\bullet+} \text{ (or } Fe^{3+})$$

Scheme 12

h) Miscellaneous reactions

In aqueous medium [2% (w/v) Triton X-100], carotenoid radical cations $CAR^{\bullet+}$ can be quenched by dopamelanin ($k \sim 10^6\ M^{-1}\ s^{-1}$) [121], cysteinyldopamelanin ($k \sim 10^6\ M^{-1}\ s^{-1}$) [121], trolox ($k \sim 10^8\ M^{-1}\ s^{-1}$) [58], uric acid ($k \sim 10^7\ M^{-1}\ s^{-1}$) [58] and ferulic acid ($k \sim 10^6\ M^{-1}\ s^{-1}$) [58]. Moreover, carotenoid radical cations react with ascorbic acid (AscH, equation 24) *via* electron-transfer reactions {$k \sim 10^8$-10^9, $\sim 10^7$-10^8 and $\sim 10^7\ M^{-1}\ s^{-1}$ in methanol [21,122], 2% (w/v) Triton X-100 [55,58,122] and unilamellar dipalmitoylphosphatidylcholine (DPPC) vesicles [109], respectively}. Also, some tocopherols (TOH), *e.g.* α-tocopherol, are able to reduce various carotenoid radical cations [29,115] (equation 25).

$$CAR^{\bullet+} + AscH \longrightarrow CAR + Asc^{\bullet} + H^+ \quad (24)$$

$$CAR^{\bullet+} + TOH \longrightarrow CAR + TO^{\bullet} + H^+ \quad (25)$$

2. Carotenoid radical anions ($CAR^{\bullet-}$)

a) Reactions with oxygen

Carotenoid radical anions are readily scavenged by oxygen *via* an electron-transfer process to produce superoxide ion, $O_2^{\bullet-}$ (equation 26) [123]. The rate constant of this reaction decreases as the carotenoid chain length increases (Table 7).

$$CAR^{\bullet-} + O_2 \longrightarrow CAR + O_2^{\bullet-} \qquad (26)$$

Table 7. Rate constants k_q for the reaction of the radical anions ($CAR^{\bullet-}$) of some carotenoids with oxygen in hexane (Equation 26) [123].

Carotenoid	cdb[a]	$k_q / 10^8\ M^{-1}\ s^{-1}$
β-Carotene (**3**)	11	25 ± 5
(15*Z*)-β-Carotene (**3**)	11	20 ± 5
(9*Z*)-β-Carotene (**3**)	11	20 ± 5
Lycopene (**31**)	11	2 ± 1
Decapreno-β-carotene (*13*)	15	1 ± 0.5

[a] cdb is the number of conjugated double bonds.

Also, the rate constant of the reaction of O_2 with $A2E^{\bullet}$ ($\lambda_{max} \sim 500$ nm, $\varepsilon_{500} = 25100 \pm 1200\ M^{-1}\ cm^{-1}$), formed by reduction of $A2E^{+}$ by $NAD^{\bullet}$ or $CO_2^{\bullet-}$ in 2% (w/v) aqueous Triton X-100, has been determined as $k \sim 3 \pm 1 \times 10^8\ M^{-1}\ s^{-1}$ [116].

b) Reactions with porphyrins

Carotenoid radical anions react very efficiently ($\sim 10^9$-$10^{10}\ M^{-1}\ s^{-1}$) with porphyrins (POR) *via* electron transfer (Table 8, equation 27) [111-113].

$$CAR^{\bullet-} + POR \longrightarrow CAR + POR^{\bullet-} \qquad (27)$$

Table 8. Rate constants k_q for the reaction of the radical anion ($CAR^{\bullet-}$) of some carotenoids with porphyrins in hexane (Equation 27) [111].

	$k_q / 10^{10}\ M^{-1}\ s^{-1}$		
Carotenoid	Chlorophyll a	Phaeophytin a	Phaeophytin b
Decapreno-β-carotene (*13*)	0.54	0.61	1.14
Lycopene (**31**)	0.70	0.99	1.49
β-Carotene (**3**)	0.85	1.93	2.45

c) Other carotenoids

A similar approach to that described in Section **F**.1.e has allowed the relative reduction potentials of a variety of carotenoids to be established by monitoring the reaction of carotenoid radical cation ($CAR1^{\bullet+}$) with another carotenoid (CAR2) in hexane and benzene (equation 28) [60]. For example, β-$CAR^{\bullet+}$ can react with LYCO to form $LYCO^{\bullet+}$, therefore the reduction potential of LYCO ($E^0_{LYCO/LYCO^{\bullet+}}$) is greater than $E^0_{\beta\text{-}CAR/\beta\text{-}CAR^{\bullet+}}$ (equation 29). By repeating the same reaction with different pairs of carotenoids, the relative reduction potentials of $CAR/CAR^{\bullet+}$ have been established [63]. In hexane, the order is $E^0_{DECA/DECA^{\bullet+}} > E^0_{LYCO/LYCO^{\bullet+}} > E^0_{\beta\text{-}CAR/\beta\text{-}CAR^{\bullet+}} > E^0_{HEPT/HEPT^{\bullet+}}$. In benzene, the order is $E^0_{ASTA/ASTA^{\bullet+}} > E^0_{CAN/CAN^{\bullet+}} \approx E^0_{APO/APO^{\bullet+}} > E^0_{LYCO/LYCO^{\bullet+}} > E^0_{LUT/LUT^{\bullet+}} \approx E^0_{\beta\text{-}CAR/\beta\text{-}CAR^{\bullet+}} > E^0_{ZEA/ZEA^{\bullet+}}$. The rate constants for the reaction of $CAR^{\bullet+}$ with oxygen and porphyrins, in hexane, are in agreement with the observed reduction potential order (see Tables 7 and 8) [111-113,123]. Also, the rate constants for the reaction of $CAR1^{\bullet+}$ with CAR2 in hexane and benzene have been estimated (Tables 9 and 10) [60].

$$CAR1^{\bullet+} + CAR2 \longrightarrow CAR1 + CAR2^{\bullet+} \quad (28)$$

$$\beta\text{-}CAR^{\bullet+} + LYCO \longrightarrow \beta\text{-}CAR + LYCO^{\bullet+} \quad (29)$$

Table 9. Rate constants k_q (±10 %) for electron transfer between a carotenoid (CAR2) and a carotenoid radical anion ($CAR1^{\bullet+}$) in argon-saturated hexane (Equation 28) [60].

	k_q/ 10^9 M^{-1} s^{-1}		
$CAR^{\bullet+}$	Decapreno-β-carotene (*13*)	Lycopene (31)	β-Carotene (3)
$HEPT^{\bullet+}$	63	12	20
β-$CAR^{\bullet+}$	11	14	-

Abbreviations: β-CAR: β-carotene (3); HEPT: heptapreno-β-carotene (*12*).

Table 10. Rate constants k_q (± 10%) for electron transfer between between a carotenoid (CAR2) and a carotenoid radical anion ($CAR1^{\bullet+}$) in argon-saturated benzene (Equation 28) [60].

	k_q/ 10^9 M^{-1} s^{-1}					
$CAR^{\bullet+}$	Astaxanthin (406)	Canthaxanthin (380)	8'-Apo-β-caroten-8'-al (482)	Lycopene (31)	Lutein (133)	β-Carotene (3)
$ZEA^{\bullet+}$	15	15	10	3.0	3.8	3.7
β-$CAR^{\bullet+}$	14	7.7	13	6.2	≤ 0.5	
$LUT^{\bullet+}$	13	7.5	10	2.5		
$LYCO^{\bullet+}$	12	10	10			
$APO^{\bullet+}$	1.1	≤ 0.2				
$CAN^{\bullet+}$	1.9					

Abbreviations. ZEA: zeaxanthin (**119**); β-CAR: β-carotene (**3**); LUT: lutein (**133**); LYCO: lycopene (**31**); APO: 8'-apo-β-caroten-8'-al (**482**); CAN: canthaxanthin (**380**).

3. Carotenoid neutral radicals

a) Unimolecular fragmentation reactions

Unimolecular fragmentation reactions have been observed for addition radicals derived from reactions of 77DH (**49**) with acylperoxyl radicals in non-polar environments [24,124]. These radicals absorb strongly in the 450-470 nm region and the relatively fast first order decay is attributed to an S_Hi mechanism leading to epoxide formation (see Scheme 10). The direct observation of similar processes for other carotenoids was precluded by kinetic and/or spectroscopic resolution factors.

b) Ionic dissociation

This type of reaction may be relevant to the behaviour of carotenoid addition radicals when these encounter the lipid/aqueous interface in biological systems. Good evidence for this type of reaction has been found for carotenoid addition radicals derived from reaction with acylperoxyl radicals in polar environments (Scheme 13) [24]. In these systems, the addition radical RO_2-CAR$^\bullet$ that is formed initially decays to the carotenoid radical cation *via* an intermediate (NIR1). Addition radicals derived from reactions of carotenoids with other peroxyl radicals (*e.g.* $CCl_3O_2^\bullet$) or phenoxyl radicals may undergo similar reactions in polar environments.

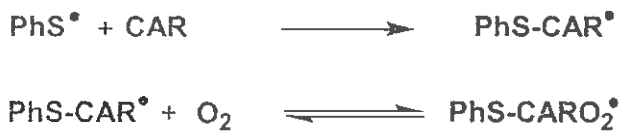
$$RO_2^\bullet + CAR \longrightarrow RO_2\text{-}CAR^\bullet \longrightarrow NIR1 \longrightarrow RO_2^{\bullet-} + CAR^{\bullet+}$$

Scheme 13

c) Reactions with oxygen

The reversible reaction of carotenoid neutral radicals with oxygen has been proposed to explain the dependence of the antioxidant behaviour of carotenoids on oxygen concentration [125]. However, it is only recently [27] that such reactions have been directly observed in real time *via* laser flash photolysis. Carotenoid addition radicals derived from the reaction of phenylthiyl radicals (PhS$^\bullet$) with 7,7'-dihydro-β-carotene (**49**) and β-carotene (**3**) (*i.e.* PhS-77DH$^\bullet$ and PhS-β-CAR$^\bullet$) (Scheme 14) are exceptionally long-lived (tens of milliseconds) and such radicals are ideal candidates for detecting any reaction with oxygen. These radicals are observed to react reversibly with O_2 probably *via* formation of carotenoid peroxyl radicals (PhS-CARO$_2^\bullet$) (Scheme 14) [27]. The rate constants for oxygen addition to PhS-77DH$^\bullet$ and PhS-β-CAR$^\bullet$ are $(4.3 \pm 0.07) \times 10^4$ and $(0.64 \pm 0.09) \times 10^4$ M^{-1} s^{-1}, respectively, and these

data suggest that the oxygen addition rate constant decreases as the conjugated chain length increases. Recently, this has been confirmed in a study extended to other carotenoids with shorter chromophores, e.g. the heptaene ζ-carotene (**38**). The results are presented in Table 11 [126].



Scheme 14

Table 11. Rate constants k_q for the reaction of carotenoid neutral radical (PhS-CAR•) with oxygen (Scheme 14) and the λ_{max} of PhS-CAR• in benzene [126].

Carotenoid	cdb[a]	$k_q / 10^9\ M^{-1}\ s^{-1}$	λ_{max} (nm)
ζ-Carotene (**38**)	7	3.44 ± 0.4	455
7,7'-Dihydro-β-carotene (**49**)	8	4.3 ± 0.07	470
Heptapreno-β-carotene (*12*)	9	3.5 ± 1.5	500
β-Carotene (**3**)	11	0.64 ± 0.09	540
Lycopene (**31**)	11	0.32 ± 0.03	545
Zeaxanthin (**119**)	11	1.1 ± 0.5	540

[a] cdb is the number of conjugated double bonds.

G. Antioxidant and Pro-oxidant Properties

Carotenoids are commonly included amongst the lipid-soluble components of the vast array of dietary antioxidants. Working towards an understanding of the impact of carotenoids on biological systems under oxidative stress requires a detailed understanding of their free radical chemistry, ideally within environments that mimic biological environments as closely as possible. The role of carotenoids in free-radical processes within lipophilic environments and at lipid/water interfaces has been the subject of much work [22,58,109,121,122,127] but many of the mechanistic and kinetic details of the processes involved remain to be resolved. Figure 2 illustrates some of the free-radical processes that may be relevant for the participation of carotenoids in peroxyl radical mediated oxidation within a biological system (see *Volume 5, Chapter 12*).

Based on the results of recent studies of free-radical scavenging by carotenoids in apolar media [24,124] the mechanism by which carotenoids scavenge peroxyl radicals in the lipophilic compartment within the cell is likely to be *via* a radical addition reaction (Scheme 10, Fig. 2). Ionic dissociation of the addition radical is possible at the interface between the lipophilic and hydrophilic compartments and gives rise to the carotenoid radical cation.

Evidence for ionic dissociation reactions of addition radicals derived from the addition of acylperoxyl radicals to 7,7'-dihydro-β-carotene (**49**) in polar solvents has recently been reported [24]. Although there were some differences in interpretation at the time of publication, the kinetics of carotenoid radical cation formation in the scavenging of acylperoxyl radicals in polar solvents may be taken to suggest a preceding ionic dissocation process [24].

Moreover, there is the possibility of epoxide formation from ROO-CAR$^{\bullet}$ in competition with ionic dissociation as well as addition of oxygen to ROO-CAR$^{\bullet}$ to produce a carotenoid-derived peroxyl radical ROO-CARO$_2^{\bullet}$ (Fig. 2). It is only recently that the rate constants for oxygen addition to carotenoid neutral radicals have been reported. It has been shown that the rate constant of the oxygen addition reaction displays a moderate dependence on the chain length of the carotenoid [27,126]. In the literature, there are numerous reports concerning the influence of oxygen concentration on the antioxidant properties of carotenoids [1,3,5,6,27,96,125,128]. This influence is attributed to the reversible addition of oxygen to ROO-CAR$^{\bullet}$ to form ROO-CARO$_2^{\bullet}$ (Fig. 2). At low oxygen concentrations, the equilibrium is positioned toward ROO-CAR$^{\bullet}$, whilst at high oxygen concentrations the equilibrium is positioned toward the ROO-CARO$_2^{\bullet}$, a peroxyl radical that may contribute to the propagation of the lipid peroxidation process and hence inhibit the antioxidant potency of the carotenoid.

There are many factors, besides the oxygen concentration, that may influence the antioxidant properties of carotenoids. The structure of the carotenoid can play a major role in its orientation within biological membranes and thereby on its ability to scavenge free radicals. For example, zeaxanthin (**119**) is able to scavenge both lipid and aqueous phase radicals since it spans the lipid membrane in a way that brings it into contact with both aqueous and lipid media. β-Carotene (**3**), however, is only able to scavenge lipid phase radicals due to its limited exposure to aqueous media [18,129,130]. Carotenoid concentration can influence antioxidant properties; decreased antioxidant effects are attributed to carotenoid aggregation [18,131].

Moreover, the presence of other antioxidants can reduce the damage induced by harmful species that are formed by the reactions of free radicals with carotenoids. For example, CAR$^{\bullet+}$, generated from free radical oxidation of the carotenoid, can oxidize amino acids (equations 16-18) [15,89,95]. Should such reactions occur *in vivo*, they may lead to structural modifications within proteins, with consequent effects upon the functions of the proteins [15,89]. The long lifetimes of carotenoid radical cations, as observed in micelles and liposomes [58,109,122], increase the chance of their interaction with biological molecules. However, in the presence of other antioxidants such as ascorbic acid (AscH) and vitamin E (TOH), CAR$^{\bullet+}$ can be recycled (equations 24 and 25) [18,29,115,122].

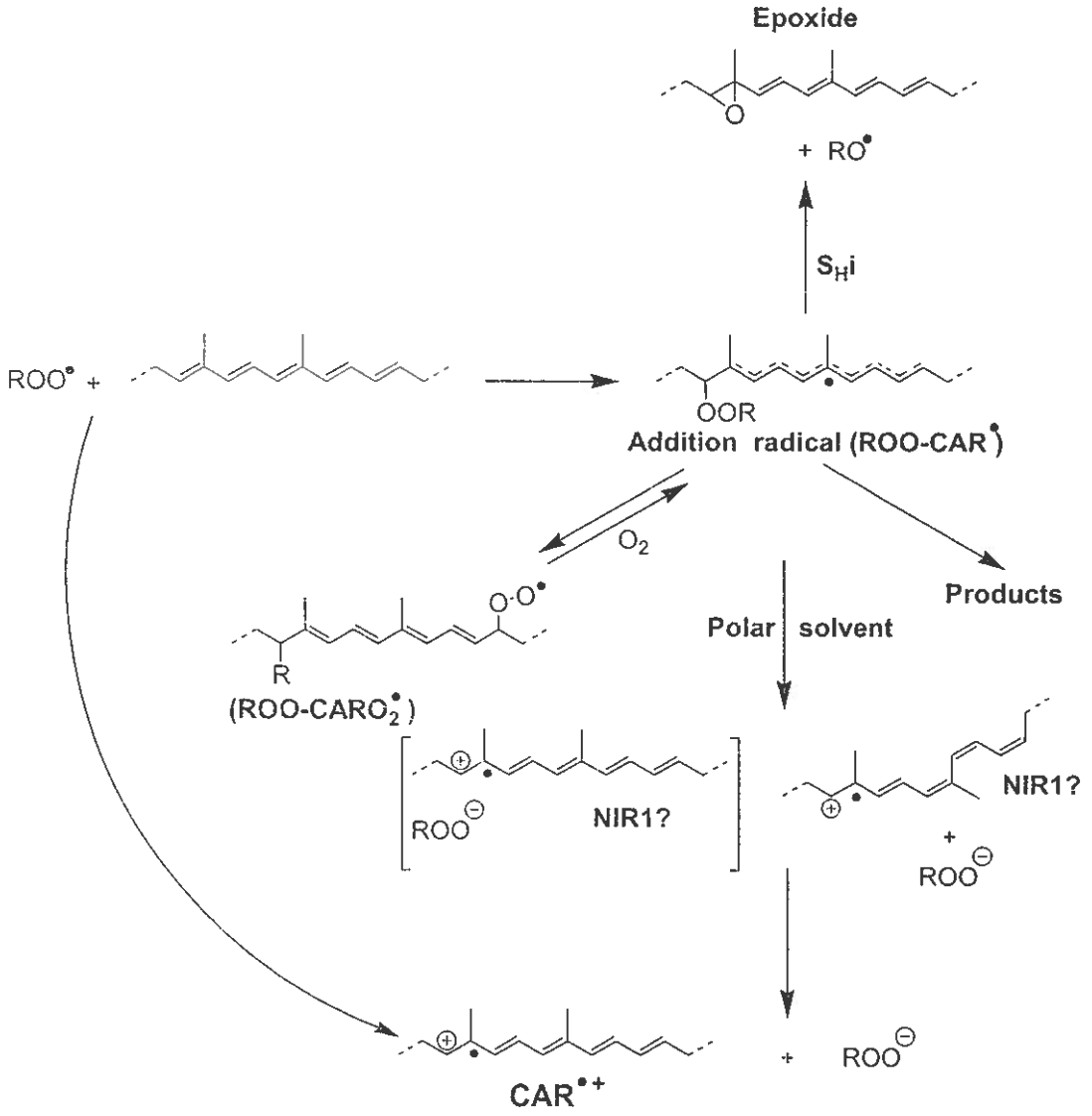
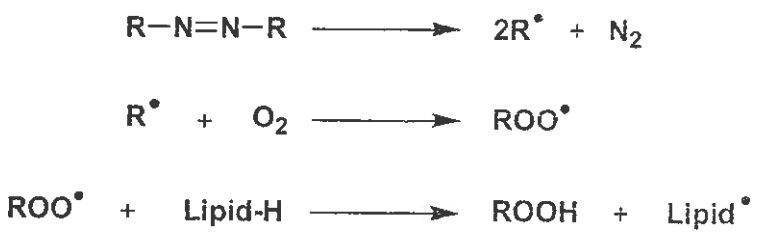


Fig. 2. Scheme summarizing the reactions of a carotenoid polyene chain with peroxyl radicals and the subsequent reactions of the addition radical product. NIR1: an uncharacterized transient intermediate detected by its NIR absorption properties. The significance of these reactions for the antioxidant properties of carotenoids in biological systems is discussed in *Vol. 5, Chapter 12*.

To conclude this section, it is important to discuss the use of azo-compounds as free radical initiators since many studies have employed these compounds to investigate the antioxidant properties of carotenoids [1,3,4,6,7,10,11,125,129,132]. Scheme 15 illustrates the mechanism for generation of free radicals by use of azo-initiators such as 2,2'-azobis-(2,4-dimethylvaleronitrile) (AMVN) and 2,2'-azobis-(2-amidinopropane) dihydrochloride (AAPH) [133]. When the temperature is raised, azo-initiators undergo fragmentation reactions to give alkyl free radicals ($R^{\bullet}$). In the presence of oxygen, these react with oxygen ($k \sim 10^{9} M^{-1} s^{-1}$) to form alkylperoxyl radicals ($RO_2^{\bullet}$), which can initiate lipid peroxidation. In Scheme 15, Lipid-H represents peroxidizable lipid.



Scheme 15

H. Conclusion

The influence of the structural characteristics of the carotenoid on the propensity to participate in the free-radical processes discussed above is an area that requires further work and may shed some light on the differences in antioxidant behaviour between the hydrocarbon carotenes and the xanthophylls, for example. In addition, the behaviour, in heterogeneous systems, of carotenoid radicals derived from reaction with radicals other than peroxyl radicals is an area that warrants further study.

Future work concerned with an understanding of the free-radical chemistry of carotenoids necessitates an integrated approach that combines direct observations of individual reactions with product analyses and theoretical calculations. The potential of such an approach has been shown [93] and this work has contributed significantly to our understanding of the electrochemical, spectroscopic and acid-base properties of carotenoid radicals and radical ions, as well as other carotenoid species. A similar approach is required in order to interpret fully the results obtained from fast time-resolved studies of short-lived carotenoid radical intermediates.

With regard to the interpretation of antioxidant/pro-oxidant behaviour of carotenoids, the influence of the carotenoid oxidation products is an area that requires further study [134-137]. In addition, the system used to investigate the antioxidant activity of carotenoids has a significant effect on the evaluation. Therefore, such an evaluation must be assessed after conducting many experiments *in vitro* and *in vivo*, exploring the effectiveness in a range of different environments [138].

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Carotenoids
Volume 4: Natural Functions

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The *Carotenoids* book series provides detailed accounts of the fundamental chemistry of carotenoids and the basic methods used in carotenoid research, and critical discussions of the biochemistry, functions and applications of these important compounds.

Volume 4 and its companion, *Volume 5*, deal with the functions of carotenoids in all kinds of living organisms and the actions of carotenoids in human nutrition and health. The material presented in the earlier Volumes is all relevant to studies of biological functions and actions. In particular, biological studies must be supported by a rigorous analytical base. The various analytical procedures described in *Volumes 1A* and *1B*, supplemented by the data for individual compounds given in the *Carotenoids Handbook*, must be understood and applied correctly, whether they are being used for quantitative analysis, identification or in complex studies of carotenoids *in situ*.

In the first part of *Volume 4*, the structural features that are most important for determining the properties and hence the biological roles of carotenoids are emphasized. The overall molecular geometry (size, three-dimensional shape, presence of functional groups) is vital to ensure that the carotenoid fits into cellular, sub-cellular and molecular structures in the correct location and orientation to allow it to function efficiently. Specific interactions with other molecules, *e.g.* to form aggregates or complexes with proteins, strongly influence the properties of a carotenoid *in vivo* and are thus also crucial to functioning. The extended delocalized π-electron system that characterizes the central part of the structure gives the carotenoids their peculiar photochemical properties and reactivity towards oxidizing agents and free radicals. This treatment provides a foundation for the description of the main functions of carotenoids and their breakdown products in the second part of *Volume 4* and in *Volume 5*. Topics covered in *Volume 4* include various aspects of the roles of carotenoids in colour and colouration, photosynthesis and other photofunctions, and protection. The formation and roles of carotenoid metabolites and breakdown products as perfume/aroma compounds and as vitamin A are also outlined; the latter is dealt with in more detail in *Volume 5*, which provides a comprehensive discussion of carotenoids in human health and nutrition.

Biologists now are not only discovering new phenomena but are striving to elucidate details of the underlying mechanisms that explain their observations. Chemistry is moving in new directions relevant to studies *in vivo* and new techniques are being developed to investigate structural details and interactions and to detect and interpret changes on an ever shorter timescale. *Volumes 4* and *5* thus point the way to the future of carotenoid research by highlighting the importance of interdisciplinary approaches to study these complex and sophisticated systems.

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