

Evidence for a Lack of Reactivity of Carotenoid Addition Radicals towards Oxygen: A Laser Flash Photolysis Study of the Reactions of Carotenoids with Acylperoxyl Radicals in Polar and Non-polar Solvents

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Abstract: In this paper, we report the results of a laser flash photolysis study of the reactions of a range of carotenoids with acylperoxyl radicals in polar and nonpolar solvents. The results show, for the first time, that carotenoid addition radicals do not react with oxygen to form carotenoid peroxy radicals; an observation which is of significance in relation to antioxidant/pro-oxidant properties of carotenoids. Acylperoxyl radicals, generated by photolysis of ketone precursors in oxygenated solvents, display high reactivity toward carotenoids in both polar and nonpolar solvents, but the nature of the carotenoid radicals formed is dependent on solvent polarity. In hexane, acylperoxyl radicals react with carotenoids with rate constants in the region of $10^9 \text{ M}^{-1} \text{ s}^{-1}$ and give rise to transient absorption changes in the visible region that are attributed to the formation of addition radicals. All of the carotenoids show bleaching in the region of ground-state absorption and, with the exception of 7,7'-dihydro- β -carotene (77DH), no distinct absorption features due to addition radicals are observed beyond the ground state absorption region. For 77DH, the addition radical displays an absorption band that is spectrally resolved from the parent carotenoid absorption. The rate of decay of the 77DH addition radical is unaffected by oxygen in the concentration range 10^{-4} – 10^{-2} M , suggesting that these resonance-stabilized carbon-centered radicals are not scavenged by oxygen. At low incident laser intensities, the 77DH addition radical decay kinetics are 1st order with $k_1 \approx 4 \times 10^3 \text{ s}^{-1}$ at room temperature. The 1st order decay is attributed to an intramolecular cyclization process, which is supported by the substantial negative entropies of activation obtained from measurements of the decay rate constants for different 77DH addition radicals as a function of temperature. No transient absorption features are observed in the red or near-infrared regions in hexane for any of the carotenoids studied. In polar solvents such as methanol, acylperoxyl radicals also react with carotenoids with rate constants in the region of $10^9 \text{ M}^{-1} \text{ s}^{-1}$, but give rise to transient absorption changes in both the visible and the red/near-infrared regions, where it is evident that there are two distinct species. For 77DH, the addition radical absorption around 450 nm is still evident, although its kinetic behavior differs from its behavior in hexane. For 77DH and ζ -carotene (ζ -CAR) the spectral and kinetic resolution of the various absorption bands simplifies kinetic analysis. The kinetic evidence suggests that addition radical formation precedes formation of the two near-infrared absorbing species, and that the kinetics of the addition radical decay match the kinetics of formation of the first of these species (NIR1, absorbing at shorter wavelengths). The decay of NIR1 leads to NIR2, which is attributed to the carotenoid radical cation. The solvent dielectric constant dependence of the relative amounts of NIR1 and NIR2 formed leads us to speculate that NIR1 is an ion-pair. However, an alternative assignment for NIR1 is an isomer of the radical cation. The results, in terms of the pattern of reactivity the carotenoids display and of the properties of the carotenoid radicals formed, are discussed in relation to the antioxidant/pro-oxidant properties of carotenoids.

Introduction

Epidemiological studies have revealed that a high intake of fruits and vegetables is associated with reduced incidence of certain cancers and other diseases.^{1–6} A possible contributory factor is that various substances present in fruit and vegetables,

including carotenoids (see Figure 1), inhibit the onset of disease by virtue of their free radical scavenging antioxidant properties.^{7,8} However, recent intervention studies involving β -carotene

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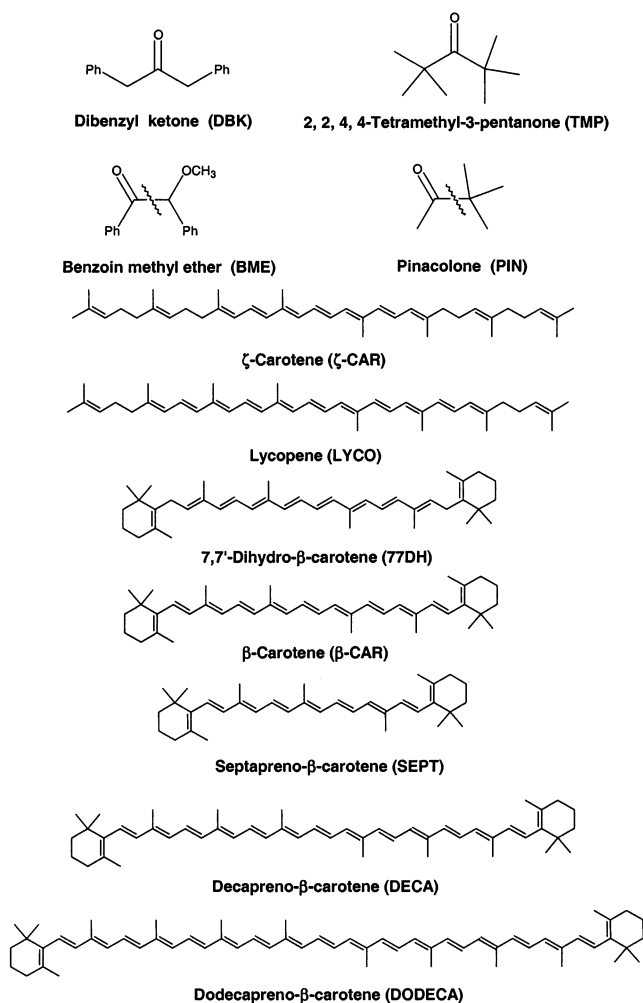


Figure 1. Structures of carotenoids and ketones (showing the position of α -cleavage for unsymmetrical ketones).

supplementation (in combination with α -tocopherol⁹ or retinol¹⁰) showed increased incidence of lung cancer among smokers suggesting possible adverse effects of β -carotene that may be associated with pro-oxidant behavior.^{11–14}

The reactions of free radicals with carotenoids and the properties of carotenoid free radicals are thus of widespread interest because of their potential roles in biological systems, and in particular their antioxidant/pro-oxidant properties. One important (but little studied) reaction of significance in relation to pro-oxidant effects of carotenoids is the reaction of oxygen with carotenoid addition radicals (e.g., ROOCAR \cdot) to form carotenoid peroxy radicals (ROOCAROO \cdot)^{8a} (see eqs 4 and 5). Such reactions have not been studied systematically and we now show for the first time that carotenoid addition radicals do not react with oxygen and thus the observed pro-oxidant effects of carotenoids must be attributable to some other mechanism.

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Much remains to be done to fully understand the mechanisms of the reactions of free radicals with carotenoids and of the properties of carotenoid radicals. A recent review^{8a} article presents a scenario that shows carotenoids react with free radicals by addition and/or electron-transfer reactions (see eqs 1 and 3), the reaction channel distribution varying with the nature of the reacting free radical. However, the characteristics of the medium in which the reaction takes place are not taken into consideration. In this paper, we show for the first time that for a specific free radical–carotenoid reaction system, the solvent polarity has a pronounced effect on the overall reaction mechanism. Furthermore, in polar solvents where both addition radicals and carotenoid radical cations are formed, we present kinetic evidence that the carotenoid radical cation is not formed directly by reaction with acylperoxy radicals, but rather it is formed from an initially formed addition radical that dissociates into ions.

Autoxidation processes, such as lipid peroxidation, are associated with free radical chain reactions that involve peroxy radicals (ROO \cdot). Chain breaking antioxidants (AH) may impede such processes by rapidly and efficiently scavenging such free radicals (often by H-atom transfer). However, the ability to scavenge free radicals is not in itself a sufficient prerequisite for an antioxidant. The resulting antioxidant derived radical (A \cdot) must not be capable of propagating the chain reaction, meaning that it will not undergo H-atom abstraction reactions or react with oxygen to form another peroxy radical.^{15,16} Of course, antioxidant activity depends on numerous other factors among which are its concentration and cellular distribution.

Thus, the antioxidant/pro-oxidant properties of carotenoids are not only dependent on how rapidly they scavenge different types of free radicals but also on the mode of reaction and consequently, the properties of the resulting carotenoid radicals.^{8a,17,18}

Three overall reaction channels may be envisaged; electron transfer, addition and hydrogen abstraction^{19–21} (eqs 1–3)



The relative importance of the three reaction channels will depend on a number of factors including the nature of the reacting free radical and the structural features of the carotenoid^{17,20} and, in biological systems, its location and orientation within the membrane.²⁰

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The fate of the different carotenoid radical species is important^{8a,22} in consideration of antioxidant/pro-oxidant properties and thus their propensity to propagate oxidation. Carotenoid radical cations have been studied extensively and do not appear to react with molecular oxygen.²³ However, the assignment (see below) and fate of the neutral carotenoid radicals, derived respectively from allylic H-atom abstraction (CAR[•]) and radical addition (ROO-CAR[•]), is less clear. To establish whether such neutral carotenoid radicals react with oxygen to yield carotenoid peroxy radicals (equations 4 and 5), with potential chain-carrying pro-oxidant properties, is critical to the development of our understanding of the antioxidant/pro-oxidant behavior of carotenoids^{8a} (extrapolation of rate constant data for β -fragmentation in lipid peroxy radicals would suggest the reverse of reaction 4 will be very rapid²⁵)



Burton and Ingold²² in a seminal paper suggested that β -carotene reacts with peroxy radicals via an addition reaction. They presented evidence that β -carotene does function as a very effective chain-breaking antioxidant, but that under conditions of high oxygen pressures (particularly at high β -carotene concentrations) it exhibits pro-oxidant behavior, possibly due to autoxidative processes.

Most carbon-centered radicals react rapidly with oxygen to form peroxy radicals.²⁴ However, where the carbon-centered radical is resonance stabilized the reaction is reversible^{22,25} (reaction 6)



The resolution of the factors that govern reactivity of carbon-centered radicals toward oxygen remains an active area of research.^{26,27}

There have been numerous studies of the reactions of carotenoids with free radicals using both direct^{17,28–37} and indirect methods.^{38–45} The use of laser flash photolysis (LFP) and pulse radiolysis (PR) techniques, usually coupled with

kinetic absorption spectroscopy, enables the measurement of rate constants for the reactions of carotenoids with many free radicals, although assignment of the transient absorption bands to specific carotenoid radicals, and hence the mechanistic interpretation of the reactions observed, is necessarily subject to speculation in some cases. Evidence from product analysis studies implicates the involvement of carotenoid addition radicals^{38,39,42,44,45} and CAR^{•39,41} as intermediates in peroxidation systems.

A variety of transient UV–vis–NIR absorption features arise in LFP and PR studies of free radical reactions with carotenoids^{17,28,30,32,33,36,46}, the profile of observed bands being dependent on the nature of the carotenoid, the reacting free radical and the characteristics of the medium (homogeneous, heterogeneous, polar, nonpolar, presence or absence of oxygen) in which the reaction is studied. Although the absorption spectra of carotenoid radical cations are quite well characterized,⁴⁷ a degree of uncertainty continues to surround the assignment of other carotenoid radical absorption bands. These absorption bands include visible and NIR absorption bands (reported for a number of systems^{17,28,30,32,36,46} and peaking at shorter wavelengths than the corresponding radical cation absorption). For example, Willson et al.⁴⁸ reported the transient absorption spectra resulting from the reaction of β -carotene with CCl₃O₂[•] in 50:50 aqueous *tert*-butyl alcohol solution. They observed two NIR absorption bands at ~780 nm and ~920 nm, the latter of which was ascribed to the radical cation, whereas the former was not assigned. Similar observations have been reported for the reactions of other free radicals with various carotenoids.^{28–32,49–51} In contrast, the reactions of carotenoids with RS[•] in an alcohol/water mixture do not give rise to NIR absorption bands but do give rise to UV–vis absorption changes in the same wavelength region as the parent carotenoid.^{17,36,46,52} The species that gives rise to the UV–vis absorption was proposed to be an addition radical [RS–CAR][•], which decays by second-order kinetics.^{17,46,52} Reactions of phenylthiyl (PhS[•]),³³ benzylperoxy,³⁷ acetylperoxy³⁵ and propionylperoxy³⁵ radicals with carotenoids in benzene also lead to the formation of a proposed addition radical.

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Everett et al.^{17,52} studied the reaction of β -carotene with $\text{CH}_3\text{-CH}_2\text{SO}_2^{\bullet}$ in a $\sim 50:50$ *tert*-butyl alcohol/water, where they observed transient absorption in the NIR attributed to $\text{CAR}^{+\bullet}$ and UV-vis absorption features attributed to an addition radical. However, the reaction of $\text{CH}_3\text{SO}_2^{\bullet}$ with other carotenoids in 60:40 *tert*-butyl alcohol/water showed two NIR absorption bands as well as the addition radical.⁴⁶

The reactions of carotenoids with CCl_3^{\bullet} ,²⁸ $\text{O}_2^{\bullet-}$,^{20,53} ArO_2^{\bullet} ,^{20,54} and NO_2^{\bullet} ,^{17,46,52,55,56} in polar solvents and with $\text{Br}_2^{\bullet-}$ and $(\text{SCN})_2^{\bullet-}$ in liposomes⁵⁷ or aqueous TX-100⁵⁸ appear to produce only $\text{CAR}^{+\bullet}$.

In this paper, we report the reaction of carotenoids with reactive acylperoxyl radicals derived from the α -cleavage products of carbonyl compounds. These peroxy radicals are clearly more reactive than the peroxy radicals that arise during lipid peroxidation (although acylperoxyl radicals are known to be involved in the autoxidation of carotenoids³⁸). The high reactivity of these radicals leads to rapid reactions in both polar and nonpolar solvents and this makes the reactions convenient for study by laser flash photolysis techniques, allowing different carotenoid radicals to be prepared and studied using comparatively low laser intensities ($\sim 1\text{--}2$ mJ per pulse).

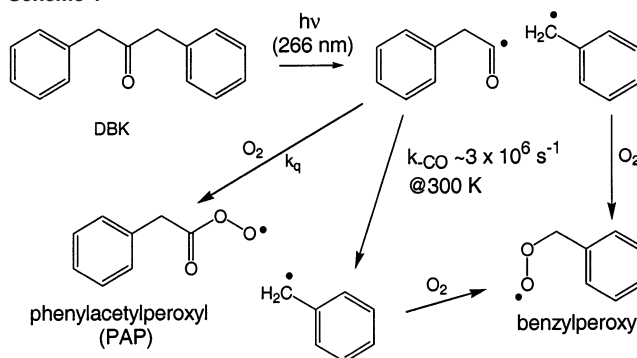
α -Cleavage of ketones produces two different types of radicals (acyl and alkyl). Each of these radicals reacts rapidly with O_2 (approaching diffusion-control) to form the corresponding acylperoxyl and alkylperoxyl radicals. However, we have previously demonstrated that acylperoxyl radicals are very much more reactive than alkylperoxyl radicals to the extent that the former is solely responsible for the observed reactivity toward carotenoids on our time scales.⁵⁹ A feature of the systems that we have employed as peroxy radical precursors is the flexibility in variation of solvent polarity and oxygen concentration, thus allowing investigation of the influence of these variables on the reaction channel distribution and the reactivity of the carotenoid radicals toward oxygen. The structures of the carbonyl precursors and the carotenoids used in this study are shown in Figure 1.

Experimental Section

Materials. Methanol, hexane, 1-pentanol, 1-decanol, benzene, (Aldrich, HPLC grade, or Riedel-de Haën, chromatographic grade) ethanol, and 1-butanol (BDH, HPLC grade) were used as received. Dibenzyl ketone (DBK), 2, 2, 4, 4-tetramethyl-3-pentanone (TMP) and pinacolone (PIN) were from Aldrich and were used as received. Benzoin methyl ether (BME) from Lancaster and linoleic acid (Sigma, 99%) were used as received. All of the carotenoids were supplied by Hoffmann-La Roche Ltd and used as received. Oxygen (1%, 5%, 50% (balance nitrogen) and 100%) and argon were supplied by the British Oxygen Company (BOC).

Instrumentation. The details of the laser flash photolysis system have been described previously.⁶⁰ Unless otherwise stated, 266 and 355

Scheme 1



nm laser energies were in the range $0.4\text{--}1$ mJ pulse⁻¹ with a beam diameter of ~ 4 mm. Quartz sample cells (2 mm excitation path length \times 10 mm monitoring path length) fitted with vacuum taps (where necessary) were employed for the laser flash photolysis measurements. If necessary, (for example, during the acquisition of transient absorption spectra) fresh solution was introduced into the sample cell following each exposure to the laser. For temperature dependence studies, a quartz temperature cell (excitation and monitoring path lengths of 4 and 10 mm respectively) with a water-circulating jacket coupled to a Pye Unicam cell temperature controller was used.

Results and Discussion

(a) Reactions between Acylperoxyl Radicals and Carotenoids in Hexane. Laser photolysis (266 nm and/or 355 nm) of hexane solutions of the acyl radical precursors (DBK, TMP, PIN, and BME) in the presence or absence of oxygen does not give rise to any transient absorption features in the vis or NIR. In addition, no transient absorption features are observed following laser photolysis (266 nm and/or 355 nm) of argon-saturated hexane solutions of the acyl radical precursors in the presence of each of the carotenoids studied. These observations suggest that under our experimental conditions the carotenoids are unreactive toward the carbon-centered radicals generated.

The principal processes in the solution phase photolysis of DBK in the presence of oxygen are summarized in Scheme 1. DBK undergoes rapid intersystem crossing from the excited singlet state producing the triplet state ($\tau_T = 0.1$ ns), which undergoes α -cleavage to give benzyl and phenylacetyl radicals. Benzyl radical undergoes oxygen addition giving benzylperoxyl radical, while phenylacetyl radical undergoes decarbonylation⁶¹ in competition with oxygen addition to give phenylacetylperoxyl (PAP) radical.

77DH (Figure 1) is a retro carotenoid with eight conjugated double bonds. A feature of the UV-vis absorption spectrum of this carotenoid is the highly resolved vibrational structure within the $S_0\text{--}S_2$ absorption band. Following laser (266 nm) photolysis of DBK ($A @ 266 \text{ nm} = 0.2\text{--}0.4$ in a 2 mm cell) in air-equilibrated hexane containing 77DH ($\sim 10^{-5}$ M), a strong visible absorption around 455 nm (Figure 2) attributed to an addition radical (PAP-77DH[•]) is observed to grow-in on microsecond time scales at the concentrations of 77DH used.

Similar transient absorption features have been detected for the reaction of carotenoids with thiyl or sulfonyl radicals^{17,46}

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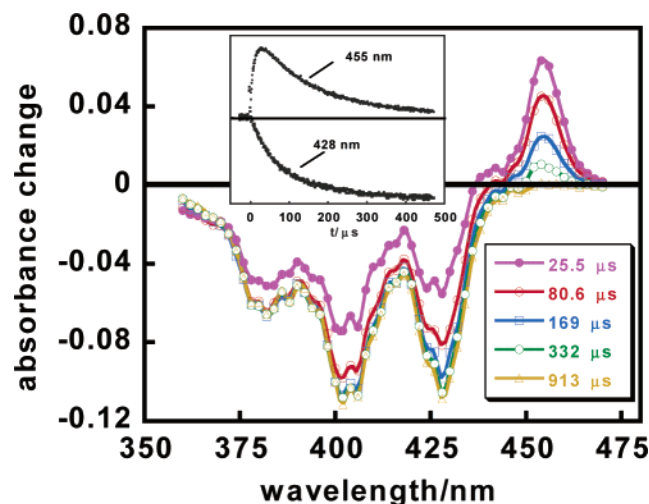


Figure 2. Transient spectra obtained following LFP (266 nm) of a solution of DBK (Abs at 266 nm \approx 0.4 in a 0.2 cm cell) and 77DH (1.5×10^{-5} M) in hexane (air-saturated, LE \approx 1 mJ). Inset shows transient absorption profiles at 428 and 455 nm.

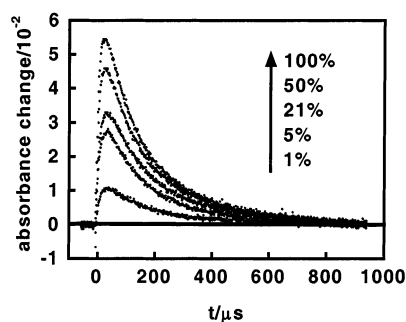


Figure 3. Oxygen concentration dependence of the transient absorption amplitude at 455 nm arising from the reaction of 77DH (1.2×10^{-5} M) with PAP following 266 nm photolysis of DBK (Abs at 266 nm \approx 0.4 in a 0.2 cm cell) in hexane (LE \approx 1 mJ).

in *tert*-butyl alcohol/water mixtures. The possibility that the 455 nm transient absorption is due to the 77DH triplet excited state is excluded on the basis that 3 77DH has an absorption maximum at 475 nm^{60,62} and is quenched by oxygen with a rate constant of $\sim 6 \times 10^9$ M⁻¹ s⁻¹ (measured by triplet sensitization using β -ionone in hexane in the absence and presence of 1.5×10^{-4} M oxygen). No transient absorption features were observed in the longer wavelength region of the visible spectrum or in the NIR region. The amplitude of the transient absorption signal exhibits a strong dependence on oxygen concentration at oxygen concentrations less than $\sim 10^{-2}$ M, although the kinetics (monoexponential) of the formation and decay of this species are unaffected by oxygen concentration in the range 10^{-4} – 10^{-2} M (Figure 3).

This oxygen concentration dependence is similar to the behavior we reported previously⁵⁹ for the ABTS^{•+} absorption signal following 266 nm laser photolysis of DBK in methanol containing ABTS²⁻, and suggests that formation of the oxidizing peroxy radical (via oxygen addition) is competitive with another fast sub-microsecond process, namely decarbonylation. The observed oxygen concentration dependence of the 455 nm transient absorption amplitude strongly suggests that the ob-

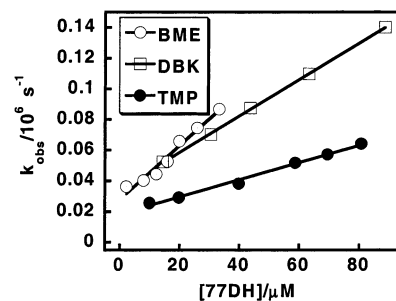


Figure 4. Plots of observed pseudo-first-order rate constants (k_{obs}) for formation of the 77DH addition radical at 455 nm from reaction of 77DH with different acylperoxy radicals (Abs at the excitation wavelength \sim 0.1–0.4 in a 0.2 cm cell) versus the concentration of 77DH in air-saturated hexane solution (LE \approx 0.5 mJ).

served reaction is due solely to reaction of 77DH with PAP in agreement with our previous observations for ABTS²⁻ in methanol.

The formation of the addition radical absorption at 455 nm follows pseudo 1st order kinetics and from the linear dependence of the observed rate constant on the 77DH concentration, a value of $1.2 \pm 0.2 \times 10^9$ M⁻¹ s⁻¹ (see Figure 4) was obtained for the rate constant for reaction of PAP with 77DH. A comparable value for this rate constant was obtained by monitoring the bleaching of the 77DH absorption at 365 nm.

The reactions of 77DH with other acylperoxy radicals in hexane are also rapid and give rise to similar transient absorption spectra as observed for PAP. Of course, the oxygen concentration dependence of the 455 nm transient absorption amplitudes depends on the rates of decarbonylation for the different acyl/aroxy radical precursors.⁶³ The variation in the rate constants (283–288 K) for reaction of 77DH with acylperoxy radicals follows the same order (BZP (1.8 ± 0.2) $\times 10^9$ > PAP (1.2 ± 0.2) $\times 10^9$ > PVP (0.6 ± 0.1) $\times 10^9$) observed for reaction of ABTS²⁻ with acylperoxy radicals in methanol, which we reported previously⁵⁹ (see Figure 4). The nonzero intercepts of the plots in Figure 4 give an indication of the intrinsic lifetimes (~ 30 μ s) of the peroxy radicals under these experimental conditions.

Under our experimental conditions the decay of the 455 nm transient absorption is exponential with a rate constant of $\sim 4 \times 10^3$ s⁻¹ at room temperature that is independent of oxygen concentration (see Figures 3 and 5, and eq 7) in the range 10^{-4} – 10^{-2} M (at higher incident laser intensities, the decay kinetics are best described as concurrent 1st and 2nd order). The decay of the transient absorption at 455 nm is accompanied by increased bleaching in the region of ground state absorption (Figure 2), although around 365 nm it is clear that the addition radical and its decay product(s) has similar absorption coefficients. In fact, the kinetic profiles vary considerably (see inset of Figure 2) across the region of ground-state absorption according to the relative values of the absorption coefficients of 77DH, the addition radical and its decay product(s). For example, the kinetic profile at 428 nm is consistent with ϵ (77DH) \approx ϵ (addition radical) > ϵ (product(s))—see inset of Figure 2.

The apparent lack of reactivity of these addition radicals toward oxygen suggests that the reported pro-oxidant behavior

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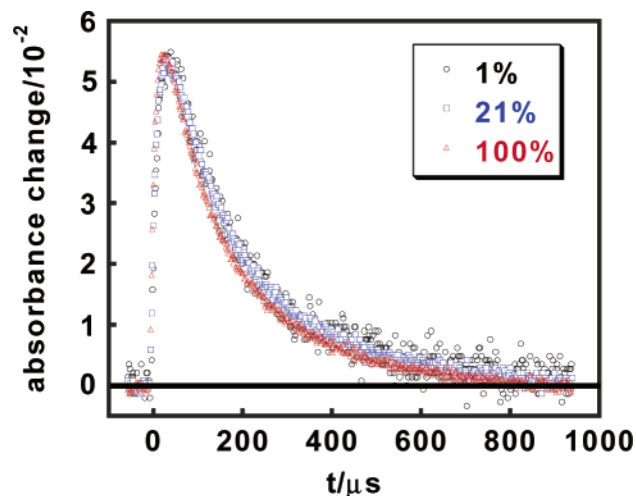
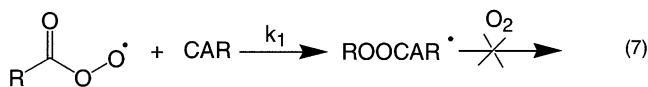


Figure 5. Normalized transient absorption profiles at 455 nm arising from the reaction of 77DH (1.2×10^{-5} M) with PAP following 266 nm photolysis of DBK (Abs at 266 nm ≈ 0.4 in a 0.2 cm cell) in hexane at different oxygen concentrations (LE ≈ 1 mJ).



exhibited by some carotenoids at high oxygen concentrations may not be due to chain propagation by carotenoid peroxy radicals, as has been proposed,^{12,22,53,64,65} but to some other mechanism.

The decay of the addition radical is also unaffected by the presence of H-atom donors such as linoleic acid (LA) up to concentrations of 0.08 M. This mirrors the observations of Everett et al.¹⁷ of a lack of reactivity toward hydrogen donors for the addition radical resulting from reaction of β -CAR with thyl radicals in 60:40 *tert*-butyl alcohol/water mixtures.

PAP reacts with both 77DH and LA and thus the rate constant for the reaction between PAP and LA can be determined by competition kinetics using 77DH as a reference^{59,66} (eq 8)

$$k_{\text{obs}} = k_1 + k_2[77\text{DH}] + k_3[\text{LA}] \quad (8)$$

Here, k_1 is the 1st order rate constant for the decay of PAP, k_2 is the rate constant for reaction of PAP with 77DH and k_3 is the rate constant for the reaction between PAP and LA. By using a constant concentration of 77DH, a plot of k_{obs} versus [LA] yields a straight line with a slope equal to k_3 . Using this method the rate constant for the reaction between the PAP and LA was determined to be $4.8 \pm 0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$. This value is comparable to the reported rate constants ($0.5, 3.9 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$)^{67,68} for the reaction of $\text{CCl}_3\text{O}_2^{\bullet}$ with LA. This comparison is based on the comparable reactivity of $\text{CCl}_3\text{O}_2^{\bullet}$ and acylperoxy radicals since their reactions with carotenoids exhibit comparable rate constants ($\sim 10^9 \text{ M}^{-1} \text{ s}^{-1}$) and they have similar reduction potentials (~ 1.1 V versus NHE).⁶⁹

It is possible that the addition radical reacts with the solvent via an H-atom abstraction process, because this would also give rise to 1st order kinetics. Consequently, the addition radical decay was studied in benzene, a non-hydrogen donor solvent, using BME as peroxy radical precursor and 355 nm excitation. In air-equilibrated benzene solution, the transient absorption is red-shifted from 455 to 465 nm. This red shift may be due to the increased refractive index (hence electronic polarizability) of benzene relative to hexane ($n_{\text{hexane}} = 1.375$, $n_{\text{benzene}} = 1.501$),⁷⁰ which is known to cause a red shift in the absorption spectra of carotenoids.⁷¹ A red shift is also observed in the absorption maximum of the UV-vis spectrum of 77DH from 402 nm in hexane to 411 nm in benzene. Interestingly the rate constant for the decay of the 465 nm transient absorption in benzene is larger than that for the 455 nm decay in hexane, by approximately a factor of 3–4 ($k(\text{benzene}) = \sim 15 \times 10^3 \text{ s}^{-1}$ at room temperature). This observation suggests that the addition radical does not react with hexane, but the reason for the increased rate constant for its decay in benzene is unclear at present.

The reactions between acylperoxy radicals and 77DH in hexane are proposed to proceed via addition of the peroxy radical to the polyene forming a resonance-stabilized carbon-centered radical (eq 3), as described previously by Burton and Ingold²² and others.^{38,39,42,44,45} The other possible reaction channels, electron transfer and H-atom abstraction, are respectively excluded on the basis that no evidence for 77DH^{•+} is observed ($\lambda_{\text{max}} = 830$ nm in hexane)⁴⁷ and that the decay of the transient absorption at 455 nm is 1st order. There is no obvious unimolecular decay channel for 77DH[•] and decay by second-order kinetics would be anticipated,¹⁷ as observed for thyl-carotenoid addition radicals which also do not possess an obvious unimolecular decay channel.

H-atom abstraction from carotenoids by alkylperoxy radicals to yield CAR[•] has been suggested previously.^{34,51} Also, the involvement of CAR[•] has been implicated from product analysis studies of the peroxy radical oxidation of β -CAR.^{39,72} Woodall et al.⁷² separated and identified the 4- and 4'-substituted β -CAR. The formation of these products indicates hydrogen abstraction from 4- or 4'-positions to form the corresponding β -CAR[•]. In addition, 4-nitro- β -carotene was identified in the cigarette smoke oxidation of β -CAR, implicating the same intermediate carotenoid radical (β -CAR[•]).⁴¹ We do not rule out H-atom abstraction as a reaction channel in reactions of carotenoids with peroxy radicals. However, we do not have any evidence that it is a significant contributory reaction pathway in the systems we have studied.

The decay at 455 nm for 77DH is attributed to an S_{H1} intramolecular cyclization^{38,44,45,73,74} with consequent elimination of an acyloxy radical (See Scheme 2). This proposal is supported by the substantial negative entropies of activation ($\Delta S^{\ddagger} \approx -80 \text{ J K}^{-1} \text{ mol}^{-1}$) determined from measurements of the decay rate constants for the different 77DH addition radicals as a function of temperature (Figures 6 and Table 1, also see the

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Scheme 2

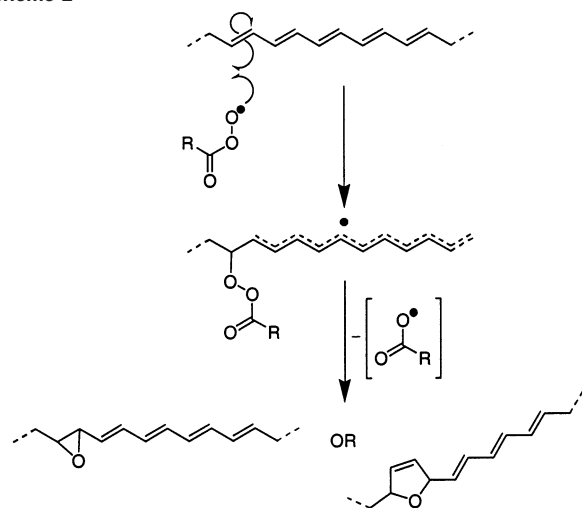


Table 1. Arrhenius Parameters, Standard Enthalpies and Entropies of Activation for the Decay of 77DH Addition Radicals Observed at 455 nm Following the Reaction of 77DH (1.2×10^{-5} M) with Different Acylperoxy Radicals in Air-Equilibrated Hexane (LE ≈ 0.5 mJ)

precursor	$\log(A/s^{-1})$	$E_a/kJ\ mol^{-1}$	$\Delta H^\ddagger/kJ\ mol^{-1}$	$\Delta S^\ddagger/J\ K^{-1}\ mol^{-1}$
TMP	8.7 ± 0.1	30.9 ± 0.7	28.2 ± 0.8	-87.9 ± 2.4
DBK	8.7 ± 0.2	29.2 ± 1.5	26.6 ± 1.5	-86.4 ± 4.5
BME	8.6 ± 0.1	28.5 ± 0.9	25.8 ± 0.9	-88.9 ± 2.8

Supporting Information). The unusually low preexponential factors ($\log A \approx 9$), reflected by the substantial negative activation entropies, suggests that cyclization is more likely to be via a five-membered rather than three-membered (leading to epoxide or cyclic ether formation) cyclic transition state (Scheme 2). An alternative explanation for the low $\log A$ values is the existence of a preequilibrium for the formation of the addition radical, in which case our $\log A$ values would be composite. However, the pre-equilibrium model predicts that the observed rate constant for the decay of the addition radical would be pseudo 1st order and thus affected by the 77DH concentration. We found no evidence for such a dependence.

The possibility that the acyloxy radical also reacts with the carotenoid was suggested by a referee. However, these acyloxy radicals undergo extremely rapid decarboxylation and thus are unlikely to react with carotenoids under our experimental conditions.⁷⁵

Certain addition radicals (or species assigned as addition radicals) resulting from reaction of carotenoids with other radicals do not decay by 1st order kinetics but decay exclusively by second-order kinetics.^{17,46} For example, the addition radical

resulting from reaction of carotenoids with thiyl radicals ($[RS\cdots CAR]^*$) decays by 2nd order kinetics.¹⁷ However, addition radicals resulting from reactions of carotenoids with peroxy radicals ($[CH_3C(O)O_2-CAR]^*$ and $[PhCH_2O_2-CAR]^*$) decay by first-order kinetics.^{35,37} There are various possibilities for peroxy radical addition to carotenoids, which may be distinguished as terminal addition or middle addition. Terminal addition is favored by a high electron density in the terminal region of the conjugated π system.^{42,43} However, middle addition may be favored by reduced steric hindrance.⁴²

Peroxy radical addition^{38,42,44,45,74,76} to carotenoids yields carotenoid addition radicals, which can eliminate RO^\bullet (S_H reaction) to form an epoxide or cyclic ether (Scheme 2). The driving force is the increased bond enthalpy of the C–O bond relative to the O–O bond (~ 340 and ~ 130 – 160 kJ mol⁻¹, respectively).^{70,77} From product analysis studies of β -CAR oxidation, 5,6-epoxy- β -carotene,^{38,40–43,45,65} 15,15'-epoxy- β -carotene,^{38,40,42} 5, 6, 5', 6'-diepoxy- β -carotene,^{38,43,45} 5, 8-epoxy- β -carotene,^{41,43,45} and 5, 8, 5', 8'-diepoxy- β -carotene^{43,45} have been identified. In addition,^{44,74} 5, 6-epoxy-retinoic acid has been isolated following peroxy radical oxidation of retinoic acid. Also, it has been proposed⁷⁶ that the peroxy radical oxidation of canthaxanthin in benzene follows a similar mechanism. However, following elimination of RO^\bullet , a canthaxanthin alkoxide radical undergoes a number of rearrangements leading to a mixture of epoxy and carbonyl compounds. The general mechanism of peroxy radical addition to olefins in the liquid^{73,77} or gas phases⁷⁸ parallels that proposed for peroxy radical oxidation of carotenoids. Furthermore, epoxidation of olefins by acylperoxy and aroylperoxy radicals proceeds via addition reactions.⁷⁹

In a recent paper, Mortensen³⁷ reported the results of a study of the reaction of β -carotene (β -CAR) and canthaxanthin with benzylperoxy radical, formed by photolysis of di-*tert*-butyl peroxide in air-equilibrated benzene containing toluene (10%). A lower limit of $\sim 10^6$ M⁻¹ s⁻¹ for the rate constant was reported and this is reflected by the relatively large incident laser intensities required to observe the reaction (26 mJ @ 297 nm), which far exceed the laser intensities employed by us (0.5–2 mJ). Indeed, it may be that the time profiles reported in Mortensen's paper for the bleaching of the carotenoid absorption simply reflects the decay kinetics of the benzylperoxy radicals (i.e., the carotenoid, as a consequence of its intense absorption, merely serves as a monitor for the peroxy radical decay) because the carotenoid concentrations used were around 10^{-5} M. We see no evidence for reaction of carotenoids with benzylperoxy radicals under our experimental conditions, which are characterized by low laser excitation energies (0.5–2 mJ) and low carotenoid concentrations ($\sim 10^{-5}$ M).

The transient absorption spectra obtained from reaction of PAP (and other acylperoxy radicals) with the other carotenoids in hexane do not exhibit an equivalent highly resolved absorption band, as observed for 77DH at 455 nm. For the other carot-

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(75) (a) A referee drew our attention to the possibility that acyloxy radicals may be responsible for some of the reactions with carotenoids that we observe in these systems (e.g. formation of CAR^{+}). However acyloxy radicals undergo very rapid decarboxylation reactions (e.g., k_{-CO_2} of benzoyloxy radical in $CCl_4 = 2 \times 10^9$ s⁻¹ at 297 K and k_{-CO_2} of *tert*-butyloxy, benzoyloxy and acetyloxy radicals in methanol = 11, 5 and $< 1.3 \times 10^9$ s⁻¹ at 293 K)^{75c, 75d} and they also react readily with the solvent (e.g., $k_{cyclohexane}$ for benzoyloxy radical = 1.4×10^6 M⁻¹ s⁻¹ at 297 K).^{75c} These reactions preclude any significant involvement of these radicals in the reactions we observe. (b) Chateaufeuf, J.; Luszyk, J.; Ingold, K. U. *J. Am. Chem. Soc.* **1988**, *110*, 2877. (c) Chateaufeuf, J.; Luszyk, J.; Ingold, K. U. *J. Am. Chem. Soc.* **1988**, *110*, 2886. (d) Hilborn, J. W.; Pincock, J. A. *J. Am. Chem. Soc.* **1991**, *113*, 2683.

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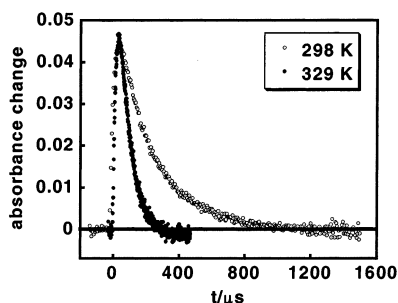


Figure 6. Normalized transient absorption profiles at 455 nm from the reaction of 77DH (1.2×10^{-5} M) with BZP following 355 nm photolysis of BME (Abs at 355 nm ≈ 0.1 in a 0.2 cm cell) in hexane at different temperatures (LE ≈ 0.5 mJ).

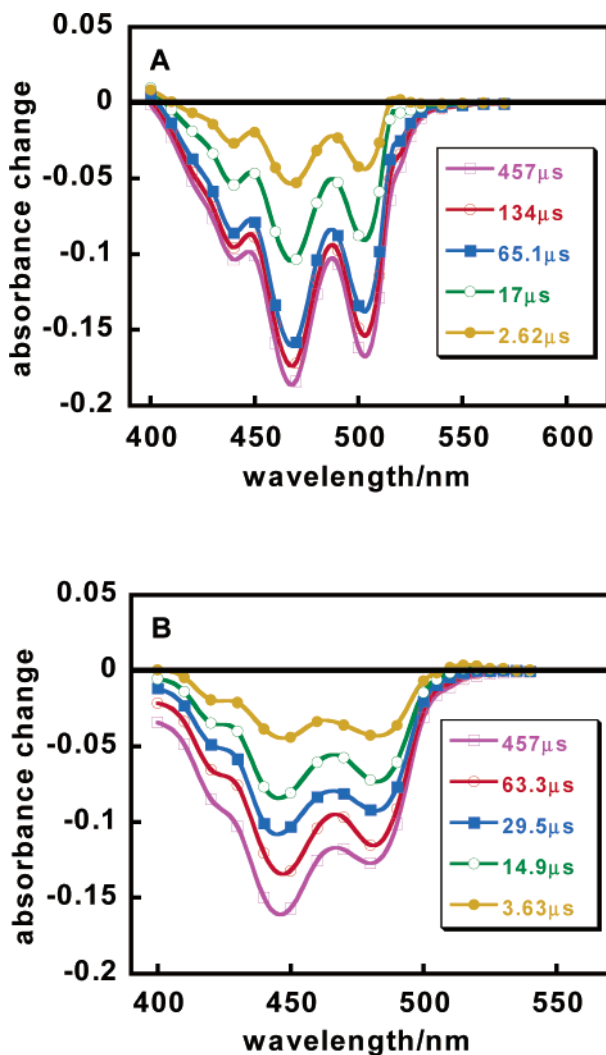


Figure 7. Transient spectra obtained following LFP (355 nm) of a solution of (A) LYCO (6×10^{-6} M) or (B) β -CAR (7.5×10^{-6} M) and BME (Abs at 355 nm ≈ 0.2 in a 0.2 cm cell) in hexane (air-saturated, LE ≈ 1.5 mJ).

enoids, β -CAR, SEPT, ζ -CAR, LYCO, DECA, and DODECA, the carotenoid radical intermediates and the products of their decay absorb in the same region as the parent carotenoid^{35,46} and no significant positive transient absorption features are observed at longer wavelengths than the parent carotenoid absorption. Instead, the other carotenoids display rapid ground-state bleaching (Figure 7) and, in the case of DECA and DODECA, positive and permanent absorption features at shorter wavelengths (see the Supporting Information).

The lack of observation of a resolved addition radical absorption band for the other carotenoids could reflect a situation whereby the decay rate constant of the addition radical substantially exceeds the rate constant for its formation. Under such conditions, the addition radical corresponds to a reactive intermediate in the context of the steady state approximation, and consequently will be more difficult to observe because of its low concentration. The positive absorption features observed for DECA and DODECA (at ~ 400 nm) correspond to final products because similar spectra are observed in conventional UV-vis spectra of degraded solutions following exposure to a number of laser pulses. Similar observations have been reported for the reaction of $\text{CCl}_3\text{O}_2^{\bullet}$ with β -CAR in a quaternary microemulsion system,²⁹ where a permanent product absorbing at 345 nm was observed and identified as retinol on the basis of HPLC analysis and fluorescence.

The lack of spectral resolution of the addition radical absorption for the other carotenoids makes it more difficult to monitor their decays. Nevertheless, the observed kinetics are unaffected by oxygen concentration in the range 10^{-4} – 10^{-2} M, suggesting that the carotenoid addition radicals formed by reaction of acylperoxyl radicals with ζ -CAR, SEPT, β -CAR, LYCO, DODECA, and DECA are also not reactive toward oxygen. Mortensen³⁵ in a study of the laser photolysis of biacetyl in benzene containing β -CAR and oxygen observed bleaching of the carotenoid parent absorption that was attributed to reaction of β -CAR with ACP radical formed by oxygen induced α -cleavage of biacetyl triplet state. Mortensen³⁵ reports a rate constant for reaction of ACP radical with β -CAR in benzene of $9.2 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$, which is of a similar order of magnitude to the rate constants determined by us for reactions of carotenoids with ACP radicals in hexane. Furthermore, the 1st order rate constant reported by Mortensen³⁵ for the decay of the addition radical ($1.35 \times 10^3 \text{ s}^{-1}$) resulting from reaction of ACP radical with β -CAR is of a similar magnitude to the values we observe.

It is known that carotenoids, especially hydrocarbon carotenoids, are found predominantly in lipophilic regions⁸⁰ (e.g., adipose tissues² and the interior of cell membranes^{11,81,82}). Therefore, if carotenoids act as antioxidants in vivo by scavenging free radicals then it is possible that addition reactions constitute the predominant reaction channel because of the nonpolar environment. The reactions of peroxy radicals with carotenoids in aqueous 2% Triton X-100 micelles (TX-100) appear to indicate that electron-transfer is the principal reaction pathway.⁸² However, such micellar environments are crude models of the environment within a cellular membrane and carotenoids may be distributed differently within the micelles in a way that allows some carotenoid molecules to be in the outer region. Consequently, they will be influenced by the bulk polar solvent.⁸²

(b) Reactions between Acylperoxyl Radicals and Carotenoids in Polar Solvents. Laser photolysis (266 nm and/or 355 nm) of methanol solutions of the acyl radical precursors (DBK, TMP, PIN, and BME) in the presence or absence of oxygen does not give rise to any transient absorption features in the vis or NIR. In addition, no transient absorption features are observed

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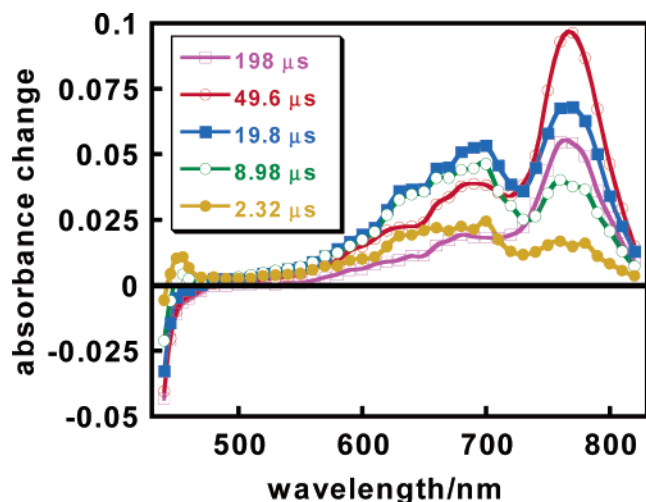


Figure 8. Transient spectra obtained following LFP (355 nm) of an air-equilibrated solution of BME (Abs at 355 nm ≈ 0.2 in a 0.2 cm cell) and 77DH (1.9×10^{-5} M) in methanol (LE ≈ 1.5 mJ).

following laser photolysis (266 nm and/or 355 nm) of argon-saturated methanol solutions of the acyl radical precursors in the presence of each of the carotenoids studied. These observations suggest that under our experimental conditions the carotenoids are unreactive toward the carbon-centered radicals generated.

Following laser photolysis of air-equilibrated methanol solutions of DBK (A @ 266 nm = 0.2–0.4 in a 2 mm cell) or BME (A @ 355 nm = 0.2–0.4 in a 2 mm cell) containing 77DH ($\sim 10^{-5}$ M) a visible absorption around 450 nm (Figure 8) is observed, as in hexane, and which is similarly assigned as an addition radical. However, the amplitude of the transient absorption at 450 nm is much reduced compared with the equivalent band in hexane. The smaller amplitude in methanol appears to be a consequence of an increased decay rate for the addition radical in this solvent. Indeed, the rapid grow-in, which is not observed at any other probe wavelengths, combined with the small signal size leads us to conclude that the rate constant for the addition radical decay (k_d) is comparable with, or exceeds, the pseudo 1st order rate constant ($k_q[77DH]$) for its formation in methanol. Under conditions such that $k_d > k_q[77DH]$, the decay kinetics of the addition radical are given by eq 9, which describes the temporal behavior of an intermediate in a consecutive reaction in which the decay rate exceeds the formation rate

$$[\text{ROO}77\text{DH}^*] \propto \frac{k_q[77\text{DH}]}{k_d} \exp(-k_q[77\text{DH}]t) \quad (9)$$

The rate constants for the reactions of carotenoids with acylperoxyl radicals in methanol were determined from the carotenoid concentration dependence of the observed 1st order rate constant for bleaching in the ground state absorption region. The rate constants obtained follow the same trend (i.e., BZP > PAP > PVP see Table 2) as reported previously for reactions of acylperoxyl radicals with ABTS²⁻ in methanol.⁵⁹ Furthermore, for each peroxyl radical the rate constants increase with the number of conjugated double bonds in the carotenoid (see Table 2).

In contrast to the behavior observed in hexane, the exponential decay of the 450 nm addition radical absorption signal for 77DH

Table 2. Rate Constants ($k_q/10^9 \text{ M}^{-1} \text{ s}^{-1}$) for Reaction between Different Carotenoids and Acylperoxyl Radicals in Methanol

peroxyl radical (source)	β -CAR	SEPT	77DH
PAP (DBK)	2.6 ± 0.2	1.0 ± 0.1	0.92 ± 0.03
PVP (TMP)	3.1 ± 0.4	1.1 ± 0.1	0.86 ± 0.07
$^{\bullet}\text{CCl}_3\text{O}_2$	2.1	1.9	
BZP (BME)	3.9 ± 0.3	2.1 ± 0.2	1.2 ± 0.1

^a In aqueous 2% Triton X-100.²⁸

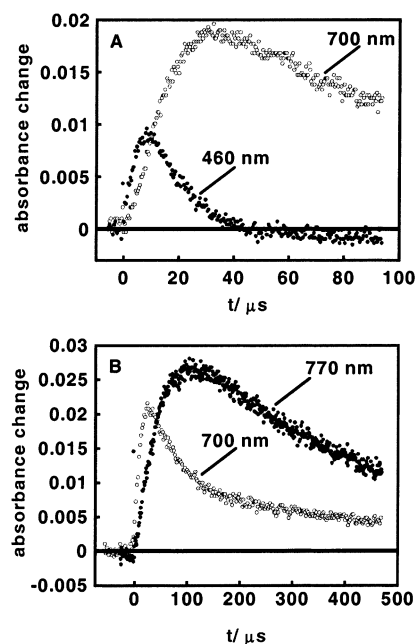


Figure 9. Transient absorption profiles at (A) 460 and 700 nm and (B) 700 and 770 nm, obtained following 355 nm photolysis of BME (Abs at 355 nm ≈ 0.2 in a 0.2 cm cell), in the presence of 77DH (1.4×10^{-5} M) in air-saturated ethanol solution (LE ≈ 1 mJ).

Table 3. Positions of the Absorption Bands of NIR1 and NIR2 (CAR⁺) for Different Carotenoids in Methanol

carotenoid	λ_{max} (NIR1)/nm	λ_{max} (NIR2 = CAR ⁺)/nm
77DH	700	770
ζ -CAR	660	740
SEPT	670	820
β -CAR	750	900

is accompanied by the growth of an absorption signal at ~ 690 – 700 nm (NIR1), the decay of which leads apparently to the growth of a further absorption signal at ~ 760 – 770 nm (NIR2, see Figure 9 and Table 3). The growth profile at 700 nm shows sigmoidal behavior at early times, which is indicative that this species is formed from an intermediate in a consecutive reaction (i.e. NIR1 corresponds to C in the sequence $A \rightarrow B \rightarrow C$, where B is the addition radical and A is the parent carotenoid). Similar growth profiles are observed for the NIR1 species of other carotenoids even though a resolved addition radical absorption band is not observed (see the Supporting Information).

These observations highlight the importance of the characteristics of the solvent medium in influencing the profile of carotenoid radical products. Within experimental error the decay of the transient absorption at 450 nm matches the growth of NIR1 and the decay of NIR1 matches the growth of NIR2, which subsequently decays over millisecond time scales. The

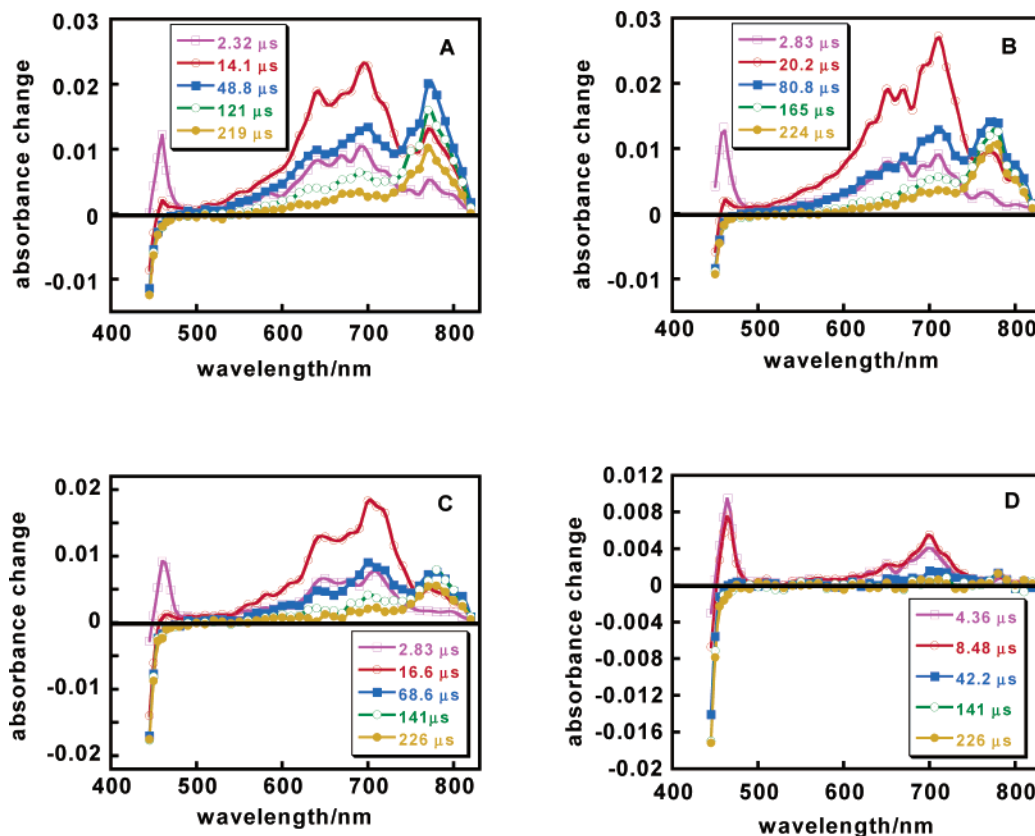
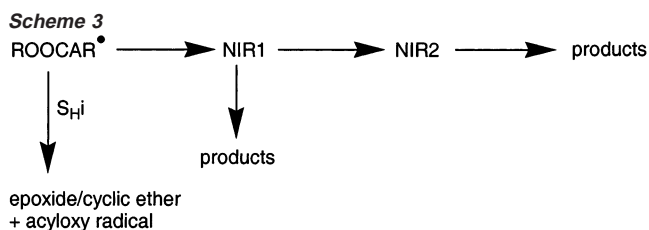


Figure 10. Transient absorption spectra obtained following LFP (355 nm) of air-equilibrated solutions of BME (Abs at 355 nm \approx 0.2 in a 0.2 cm cell) and 77DH (1.4×10^{-5} M) in (A) ethanol, (B) 1-butanol, (C) 1-pentanol and (D) 1-decanol (LE \approx 3–3.3 mJ).

temporal behavior of the transient absorption signals throughout the VIS and NIR regions are unaffected by oxygen concentration in the range 10^{-4} – 10^{-2} M. Similar results were obtained for SEPT and β -CAR in methanol (DECA and DODECA were not studied due to their poor solubility in this solvent), although the absorption bands due to NIR1 and NIR2 are less well resolved than for 77DH and ζ -CAR (see below).

The appearance of two NIR absorption peaks has been observed previously in studies of the reactions of carotenoids with phenoxy radicals in a relatively polar solvent mixture (70/30 v/v di-*tert*-butyl peroxide/benzene (or hexane) containing 1.75 M phenol)^{30,31} and with $\text{CCl}_3\text{O}_2^{\bullet}$ in *tert*-butyl alcohol/water mixtures,⁴⁸ micelles²⁸ and a quaternary microemulsion.²⁹ The absorption maxima of the NIR2 absorption bands of various carotenoids match the accepted absorption maxima of the corresponding carotenoid radical cations.⁴⁷ However, the assignment of the NIR1 absorption bands located at shorter wavelengths has been the subject of considerable uncertainty.^{32,49,51} Proposed assignments include a carotenoid radical formed by H-atom transfer to the scavenged radical,^{32,51} an ion-pair^{46,49,50} and an addition radical.^{28,30,31,49}

In an attempt to resolve some of the uncertainty surrounding the assignment of NIR1, we investigated the influence of solvent dielectric constant by studying the reactions of acylperoxy radicals with 77DH and ζ -CAR in a series of alcohols (methanol, ethanol, 1-butanol, 1-pentanol, and 1-decanol). The transient absorption spectra obtained are shown in Figure 10 (see Supporting Information for ζ -CAR), where it can be seen that a decrease in solvent dielectric constant has a pronounced effect on the relative transient absorption amplitudes of NIR1 and



NIR2. As the solvent polarity decreases the NIR2 (radical cation) absorption is suppressed relative to NIR1. In 1-decanol, NIR2 is not observed and for 77DH the absorption at \sim 450 nm due to the addition radical is larger than the absorption due to NIR1.

Solvent polarity is expected to have a strong effect on the yield of 77DH^{•+} since the activation energy for 77DH^{•+} formation will increase as solvent polarity decreases. Consequently, other decay pathways of NIR1 become more competitive.⁸³

Interestingly for 77DH (where the addition radical is spectrally resolved) the rate constants for the decay at 450 nm, the growth of NIR1, the decay of NIR1 and the growth of NIR2 do not vary markedly in the different polar solvents, which suggests that other parallel decay channels exist (Scheme 3) for the addition radical and NIR1, and that the decay channel distributions vary with solvent polarity to the extent that in hexane no absorptions due to NIR1 or NIR2 are observed at all.

The influence of solvent dielectric constant on the relative amount of NIR1 and NIR2 formed leads us to suggest that NIR1 is an ion-pair formed from ROOCAR[•] and composed of the carotenoid radical cation (CAR^{•+}) and a peroxy anion (ROO⁻),

as suggested previously by Mortensen and Skibsted.^{49,50} Similar dissociative processes involving neutral carbon-centered radicals dissociating to form a radical cation and a phosphate anion have been reported previously for 4'-DNA radicals.⁸⁴ Separation of the ion-pair into free ions becomes less favorable as the solvent dielectric constant decreases. An alternative possibility is that dissociation of the addition radical yields an isomer of the carotenoid radical cation and subsequent thermal isomerization yields the thermodynamically stable isomer (NIR2). Thermal isomerization of polyene radical cations has been observed previously following photoisomerization of all-trans polyene radical cations.⁸⁵ Interestingly, a transient absorption spectrum ($\lambda_{\max} \approx 600$ nm) assigned to an ion-pair formed by reaction of BZP (or ACP)⁸⁶ radical with N,N,N',N'-tetramethyl-*p*-phenylenediamine (TMPD), in ethyl acetate, diethyl ether and benzene⁷⁹ has been reported. However, the reduction potential of the TMPD radical cation ($E = -0.1$ V in acetonitrile versus the silver electrode)⁸⁷ is very much lower than for carotenoid radical cations, which may explain the observation of an ion-pair in benzene, a nonpolar solvent.

In summary, we propose that the reactions of acylperoxy radicals with 77DH in polar solvents proceed by a radical addition reaction followed by intramolecular cyclization via an $S_{\text{H}}1$ mechanism in competition with ion-pair formation (or the formation of an intermediate carotenoid radical cation isomer). One decay channel for NIR1 yields the carotenoid radical cation. No evidence was obtained for reaction of any of the transient carotenoid species with oxygen in the concentration range 10^{-4} – 10^{-2} M. For the other carotenoids, spectral and/or kinetic resolution factors preclude observation of distinct addition radical absorption features. However, it seems reasonable to conclude that the other carotenoids react with acylperoxy radicals in a manner similar to 77DH.

Articles summarizing the published data on carotenoid reactions with free radicals emphasize the relative importance of addition and electron-transfer scavenging^{8a,18} without explicit consideration of the characteristics of the medium in which the reactions have been studied. For example, a review of the studies of the reactions of $\text{CCl}_3\text{O}_2^{\bullet}$ with β -CAR suggest a 50:50 distribution of radical cation and carotenoid addition radical products. However, we argue that if this reaction were to be studied in a nonpolar solvent such as hexane then it is likely that no radical cation would be observed at all. We suggest that the nature of the medium in which the reaction takes place is, not surprisingly, important and has been overlooked to some extent in previous studies. Consequently, carotenoid reactions

with specified free radicals may very well follow a completely different course in a more or less polar solvent environment, as we have demonstrated in this paper for reactions of acylperoxy radicals with carotenoids. Of course, the extent to which the solvent environment can be varied is often a practical restriction. Nevertheless, statements concerning the mechanisms by which carotenoids react with free radicals should be qualified, given that experimental data is often only available for a polar^{17,30–32,46,49} or nonpolar^{33,35,37} environment. On the other hand, no NIR absorption features are observed in studies of the reactions of $\text{RS}^{\bullet 17,46}$ or 2-hydroxy-2-methylpropylperoxy radical³⁴ with carotenoids in polar solvents, emphasizing that the reaction mechanism depends also on the type of radical involved. Clearly, the reduction potential of the reacting radical is one of the important properties that can influence the reaction mechanism. For example, in *tert*-butyl alcohol/water (7:3, v/v), the 2-hydroxy-2-methylpropylperoxy radical does not react with β -CAR by electron transfer because its reduction potential³⁴ is too small.

Abbreviations. ABTS²⁻ = 2, 2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; TMPD = N,N,N',N'-tetramethyl-*p*-phenylenediamine; DBK = dibenzyl ketone; TMP = 2, 2, 4, 4-tetramethyl-3-pentanone; PIN = pinacolone; BME = benzoin methyl ether; ACP = acetylperoxy; BZP = benzoylperoxy; PAP = phenylacetylperoxy; PVP = pivaloylperoxy; β -CAR = β -carotene; 77DH = 7, 7'-dihydro- β -carotene; SEPT = septapreno- β -carotene; ζ -CAR = ζ -carotene; LYCO = lycopene; DODECA = dodecapreno- β -carotene; DECA = decapreno- β -carotene; CAR $^{\bullet}$ = carotenoid radical; NIR = near-infrared; TX-100 = Triton X-100 micelles; RS^{\bullet} = thiyl radical; LFP = laser flash photolysis; PR = pulse radiolysis; LA = linoleic acid; LE = laser energy.

Conclusion

The conclusions that arise from the work reported in this paper are as follows:

- (i) The decays of carotenoid addition radicals follow first-order kinetics and are not influenced by oxygen.
- (ii) The profile of carotenoid radical products formed is dependent on the characteristics (polarity) of the solvent medium.
- (iii) In nonpolar solvents, only addition radicals are formed.
- (iv) In polar solvents, addition radicals are formed which decay to ion-pairs (or carotenoid radical cation isomers) and subsequently to carotenoid radical cations.

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Supporting Information Available: Measurements of the decay rate constants for the different 77DH addition radicals as a function of temperature, positive and permanent absorption features at shorter wavelengths of DECA, DODECA, and growth profiles observed for the NIR1 species of other carotenoids and additional transient absorption spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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