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One-electron transfer product of quinone addition to carotenoids EPR and optical absorption studies

Nikolai E. Polyakov [a], Valery V. Konovalov [a,1], Tatyana V. Leshina [a], Olga A. Luzina [b], Nariman F. Salakhutdinov [b], Tatyana A. Konovalova [c], Lowell D. Kispert [c,*]

[a] *Institute of Chemical Kinetics and Combustion, Novosibirsk, SB RAS 630090, Russia*
[b] *Institute of Organic Chemistry, Novosibirsk, SB RAS 630090, Russia*
[c] *Department of Chemistry, Box 870336, University of Alabama, Tuscaloosa, AL 35487-0336, USA*

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Abstract

It was shown by EPR and UV–VIS studies that one-electron transfer reactions between carotenoids (β-carotene, 8′-apo-β-caroten-8′-al, canthaxanthin) and quinones [2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), tetrachlorobenzoquinone (CA)] include the formation of a charge-transfer complex (CTC), which exists in equilibrium with an ion–radical pair ($Car^{\bullet+}\cdots Q^{\bullet-}$). UV spectra display a new absorption band in the near IR region (at 1030 nm) for the β-carotene–DDQ mixture which is assigned to a CTC. The absorption maximum of this band gradually shifts from 1030 nm to shorter wavelengths and finally corresponds to that of the β-carotene radical cation (1000 nm). A new absorption band at 340 nm, detected for all systems under study, is attributed to the product of quinone addition to carotenoid. The EPR spectra of the Car–DDQ mixture measured at 77 K when [Car] ≤ [DDQ] exhibit a structureless singlet line with $g = 2.0066 \pm 0.0002$ which is attributed to a CTC. Increasing the temperature gives rise to a new five-line signal with $g = 2.0052 \pm 0.0002$ and hyperfine coupling constant of 0.6 G due to the DDQ radical anion. At room temperature stable radicals (more than 24 h) with $g = 2.0049 \pm 0.0002$ and $\Delta H_{p-p} = 4.3$ G were detected. Electron nuclear double resonance (ENDOR) results indicate that these species contain both nitrogen and chlorine atoms. We attribute these signals to a carotenoid–quinone radical adduct. For chloranil paramagnetic species are generated only after irradiation. Both the carotenoid radical cation and the quinone radical anion were observed in this case. Analysis of the isolated reaction product made using the Beilstein test, ^{1}H-NMR and IR spectroscopies suggests that the major product is a carotenoid–hydroquinone ether. A mechanism of the Car–Q adduct formation is proposed.

Keywords: Carotenoids; Quinones; Charge-transfer complex; Radicals; EPR; ^{1}H-NMR; UV–VIS spectroscopy

1. Introduction

Carotenoids and quinones are intrinsic components of the reaction centers and the pigment–protein complexes in photosynthetic membranes [1–5]. The close association with chlorophylls (Chl) or bacteriochlorophylls (Bchl) and the extended π-electron system of carotenoids determines their active participation in an electron transfer in photosystem II (PS II). Studies of isolated PS II reaction centers have demonstrated that photoactivation of these centers leads to selective photooxidation and irreversible bleaching of β-carotene by electron transfer to the primary donor $P680^{\bullet+}$ to form the carotenoid radical cation ($Car^{\bullet+}$) [6]. Absorption changes due to the formation of carotenoid radical cations have been found upon light-excitation of chloroplasts at the PS II reaction center [7,8]. The EPR signal arising from a radical cation of β-carotene has been detected in PS II upon illumination at ≤20 K [9]. Pulse EPR methods have been employed to distinguish between $Car^{\bullet+}$ and $Chl^{\bullet+}$ generated in PS II during low temperature illumination [10].

Natural hydrophobic quinones such as plastoquinones and ubiquinones serve as terminal electron acceptors in the PS II reaction centers. It has been shown that these quinones can be reduced to radical anions when they are subjected to red light illumination in the presence of Bchl in dry acetone at low temperature [11]. An EPR signal due to an ubiquinone radical has been detected in a bacterial reaction center [12]. In PS I an EPR signal photoaccumulated under reducing conditions has been assigned to a phylloquinone radical anion [13].

* Corresponding author. Tel.: +1-205-348-8428; fax: +1-205-348-9104.
E-mail address: lkispert@bama.ua.edu (L.D. Kispert).
[1] Present address: Center for Materials for Information Technology, University of Alabama, P.O. Box 870209, Tuscaloosa, AL 35487, USA.

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Transient absorption signals have been recorded in cyanobacteria [14] and *Chlorella* [15]. Absorptions in the 370–400 nm range and the band at 480 nm have been attributed to phyllosemiqunone and carotenoid, respectively.

Because in the photosynthetic reaction centers the carotenoid molecule is in close vicinity of the quinone, it is of importance to determine an electron transfer mechanism and possible photoinduced reactions between carotenoids and quinones.

Gust and co-workers have reported a series of triads in which a porphyrin (P) is covalently linked to both a carotenoid (Car) and a quinone (Q). Photo-excitation of this model system resulted in a two-step electron transfer ultimately yielding a charge-separated state $Car^{\bullet+}$–P–$Q^{\bullet-}$ [16–18].

There have been several reports about possible electron transfer mechanisms which include intermediate complex formation between quinones and Chl, Bchl and bacteriopheophytin (Bph) [19–22]. The semiquinone radical anion has been produced by Chl or Bchl photosensitized electron transfer from solvent (ethanol) to quinone via a ternary complex as shown in the following reaction [19].

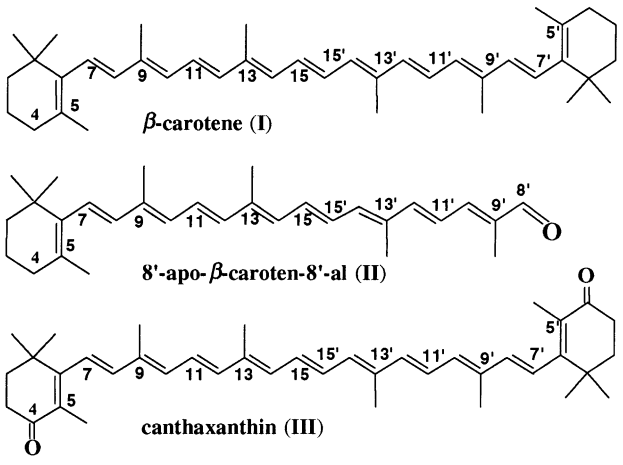
$$(\text{Chl–Q–EtOH})^* \rightarrow (\text{Chl–Q}^{\bullet-}\text{–EtOH}^{\bullet+})$$

In the present work electron-transfer reactions of three carotenoids (β-carotene, 8′-apo-β-caroten-8′-al and canthaxanthin) with strongly oxidizing quinones (DDQ and chloranil) were studied by optical and EPR spectroscopies in CH_2Cl_2, acetonitrile, benzene, and toluene solutions.

2. Experimental

2.1. Chemicals

β-Carotene (**I**) was supplied by Sigma, 8′-apo-β-caroten-8′-al (**II**) by Roche Vitamins and Fine Chemicals, canthaxanthin (**III**) by Fluka (Scheme 1). Purity of the carotenoids was checked by ^{1}H-NMR (360 MHz, $CDCl_3$) and TLC analyses. They were stored at −14°C in a desiccator containing drierite. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and tetrachlorobenzoquinone (chloranil, CA) were supplied by Aldrich. The solvents, CH_2Cl_2, toluene (Aldrich, anhydrous) and acetonitrile (Fisher, HPLC grade) were used as received.



Scheme 1. Structures of carotenoids I, II, III.

2.2. Optical measurements

A Xe/Hg lamp (1 kW) equipped with a Kratos monochromator was used as the light source. UV–VIS absorption spectra were recorded with a Shimadzu UV-1610 spectrometer equipped with a temperature control unit. All samples were freshly prepared and degassed with Ar. The reaction with DDQ was initiated by injection of a solution containing a known amount of DDQ into the spectrophotometer cell containing the carotenoid solution. Recording of the spectra was started a few seconds after mixing.

2.3. EPR measurements

EPR measurements were carried out with a X-band (9 GHz) Bruker ESP 300E EPR spectrometer, comprising a DICE electron nuclear double resonance (ENDOR) ESP 350 system and a temperature controller. Solutions (1 mM) of carotenoids and DDQ were prepared separately under a N_2 atmosphere. The reaction was initiated by mixing these two solutions, immediately cooling to 77 K, and recording the EPR spectra at 77 K.

2.4. Product isolation and analysis

The product of the β-carotene–DDQ reaction was prepared by addition of an equimolar amount of DDQ to the 5×10^{-3} M β-carotene solution in CH_2Cl_2. Both solutions were degassed with Ar. After 1 h incubation under Ar the solvent was removed using a vacuum rotary evaporator (20–30 mm), and the residue was separated by preparative TLC. The eluent was 2% diethyl ether in hexane. The yield of the major product after repeated purification by TLC was approximately 6%. The isolated product was characterized by the Beilstein test, ^{1}H-NMR and IR spectroscopy. The reaction products from carotenoids **II** and **III** could not be isolated because of their poor stability. Product analysis in these cases was made only by ^{1}H-NMR spectroscopy carried out during the reaction directly in the probe of a DPX-200 Bruker NMR spectrometer (200 MHz ^{1}H operating frequency). IR spectra were obtained using a Fourier–Bruker 22 spectrometer.

3. Results

3.1. Optical absorption measurements

UV–VIS spectra of the reaction mixture of the carotenoids (**I–III**) and DDQ showed a decrease of the carotenoid

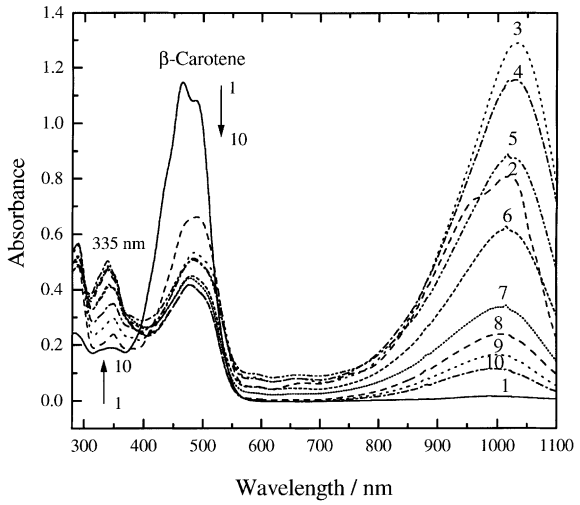


Fig. 1. UV–VIS spectra of the mixture of β-carotene (0.01 mM) and DDQ (0.01 mM) solutions in CH_2Cl_2 ($T = -40°C$): (1) β-carotene only; (2) just after mixing with DDQ; (3) after 2 min; (4) after 7 min; (5) after 15 min; (6) after 25 min; (7) after 50 min; (8) after 80 min; (9) after 120 min; (10) after 150 min.

absorption band (465–480 nm) and the appearance of two new bands. For the β-carotene (**I**)–DDQ mixture in CH_2Cl_2 ([Car] = [Q] = 0.01 mM), a near IR absorption (λ_{max} = 1030 nm), red-shifted compared to that of the β-carotene radical cation in this solvent (λ_{max} = 1000 nm) [23], was observed. Fig. 1 shows that with time this absorption band diminishes in intensity, and the maximum shifts from 1030 to 1000 nm. The decay rate of the absorption band at 1000 nm was found to depend on the quinone concentration. An increase of the quinone concentration leads to rapid decrease of this band (Fig. 2). The absorption spectrum of the **I**–DDQ mixture measured just after mixing also shows a shoulder near 950 nm (Fig. 1, curve 2, see Discussion section for assignment). When the quinone concentration is much smaller than that of the carotenoid [Car] > [Q], generation of only the carotenoid radical cation was observed in the near IR region.

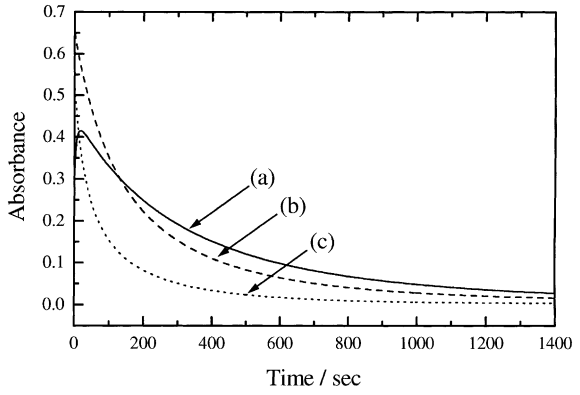


Fig. 2. The kinetics of the CTC decay for β-carotene (0.01 mM) in CH_2Cl_2 in the presence of (a) 0.01 mM DDQ; (b) 0.05 mM DDQ; (c) 0.5 mM DDQ.

New bands at 840 nm for 8′-apo-β-caroten-8′-al (**II**)–DDQ (Fig. 3a) and 890 nm for canthaxanthin (**III**)–DDQ (Fig. 3b) were detected after quinone addition. These bands correspond to the radical cations of **II** and **III** in CH_2Cl_2 [23]. The UV band with $\lambda_{max} = 340 \pm 5$ nm appeared for all carotenoids and gradually grew in intensity. Increasing the quinone concentration resulted in increased intensity of this band. Similar results were obtained in ACN solutions, but both the near IR and the near UV new bands were blue-shifted compared to those in CH_2Cl_2 solutions. When benzene or toluene was used as solvent, no evidence of carotenoid–quinone reaction was found.

Carotenoid–CA mixtures do not show any dark reaction. UV-absorption spectra of final products after photolysis of these systems show the same behavior as the carotenoid–DDQ mixture. While the absorption band of **III** almost dissapeared during the reaction and the products were formed with λ_{max} around 350 nm, only a small decrease of the absorption band of **II** was observed. This indicates no destruction of the **II** conjugated chain in reaction product. In all cases the quinone absorption band also decreased during the reaction, so that the quinones participate in the product formation.

3.2. EPR measurements

Reactions between carotenoids (**I**–**III**) and quinones resulted in relatively stable paramagnetic products. Freshly prepared Car–DDQ samples were frozen immediately and their EPR spectra were recorded at 77 K. The EPR spectrum obtained after mixing β-carotene with an excess of DDQ exhibited a structureless single line with $\Delta H_{p-p} = 6$ G and $g = 2.0066 \pm 0.0002$ (signal A) and another signal B with $g = 2.0026 \pm 0.0002$ which can be attributed to $Car^{\bullet+}$ [23] (Fig. 4a). Increasing the temperature to 180 K produced a resolved five-line hyperfine structure with $A_N = 0.6 \pm 0.03$ G and $g = 2.0052 \pm 0.0002$ (Fig. 4b), which is characteristic of the DDQ radical anion [24]. The intensity of the line with $g = 2.0066$ decreased with temperature increase, and finally the EPR spectrum exhibited only the five-line signal of the $DDQ^{\bullet-}$ (Fig. 4c). The $Car^{\bullet+}$ signal was not observed at higher temperatures. When the quinone concentration was smaller than the carotenoid concentration [Car] > [Q], the EPR spectrum at 77 K exhibited a single line with $g = 2.0042 \pm 0.0002$ and $\Delta H_{p-p} = 12.8$ G (Fig. 5, insert) that appeared to be a superposition of the $Car^{\bullet+}$ signal ($g = 2.0027$) and the $DDQ^{\bullet-}$ signal ($g = 2.0052$). With increasing temperature the g value of the signal increased (2.0044 ± 0.0002) and the line-width decreased (11.8 G) (Fig. 5a). After keeping the sample for 24 h at room temperature its EPR signal showed a single line with $g = 2.0049 \pm 0.0002$ and $\Delta H_{p-p} = 4.3$ G (Fig. 5b). To reveal the origin of this stable signal we carried out ENDOR measurements. The ENDOR spectrum of the stable radical product of the β-carotene–DDQ reaction (200 K) exhibited lines at 0.86, 1.72 and 3.4 MHz (Fig. 6). We assume that

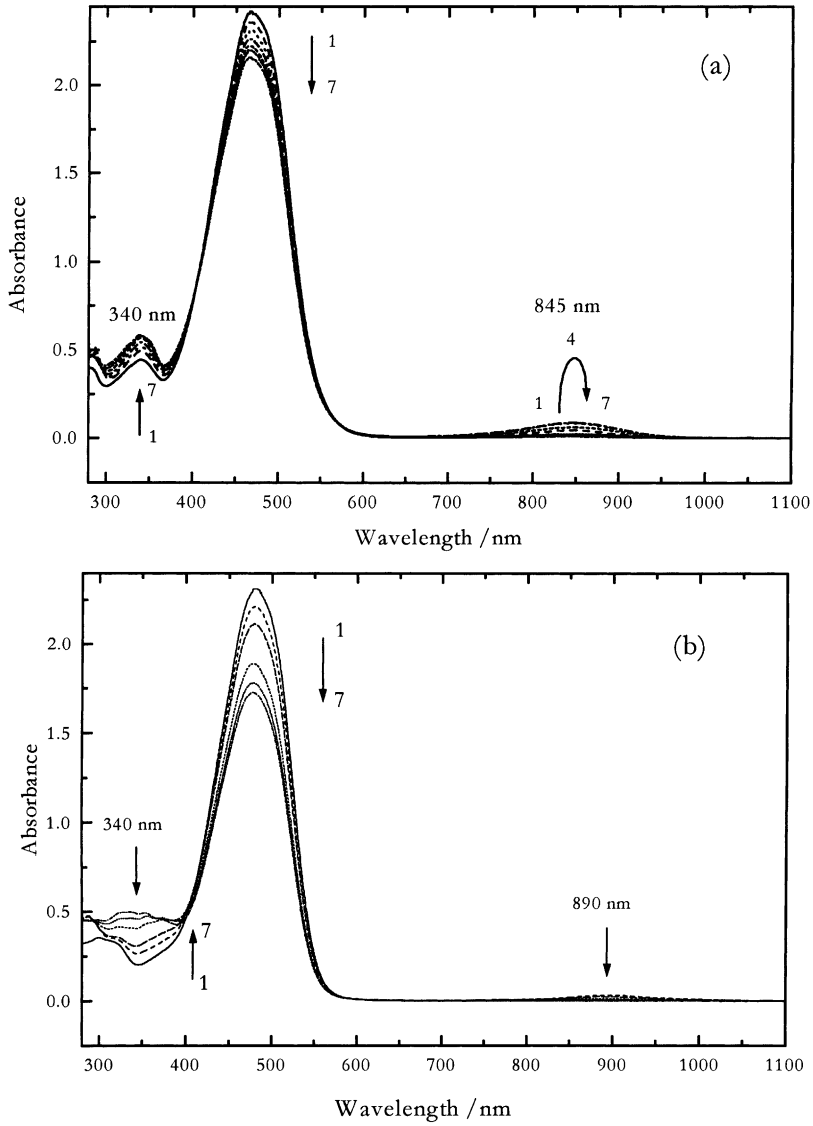


Fig. 3. UV–VIS spectra of the mixture of (a) 0.02 mM 8′-apo-β-caroten-8′-al and 0.02 mM DDQ solutions in CH_2Cl_2: (1) carotenoid only; (2) +DDQ; (3) after 2 min; (4) after 5 min; (5) after 10 min; (6) after 20 min; (7) after 60 min and (b) 0.02 mM canthaxanthin and 0.02 mM DDQ solutions in CH_2Cl_2: (1) carotenoid only; (2) +DDQ; (3) after 2 min; (4) after 5 min; (5) after 10 min; (6) after 20 min; (7) after 50 min.

both the nitrogen and the chlorine atoms of DDQ contribute to this spectrum. If the ^{14}N Larmor frequency, $\nu_N = 1.1$ MHz $< A_N/2$, the low-frequency ($\nu_{N^-} = 0.86$ MHz) and high-frequency ($\nu_{N^+} = 3.4$ MHz) nitrogen components are placed symmetrically around $A_N/2 = 1.72$ MHz and separated by $2\nu_N$. The chlorine atom with the ^{35}Cl Larmor frequency, $\nu_{Cl} = 1.3$ MHz, generally exhibits fairly small isotropic hyperfine couplings. Therefore, in this case the high and low frequency features are placed around ν_{Cl} and separated by $A_{Cl} = 0.86$ MHz. No proton ENDOR was detected.

For chloranil (CA), in contrast to DDQ, reaction with carotenoids (**I–III**) proceeds only after irradiation by light with $\lambda \leq 365$ nm. The carotenoid radical cation ($g = 2.0026 \pm 0.0002$) and the chloranil radical anion ($g = 2.0051 \pm 0.0002$) were detected upon irradiation of the Car–CA mixture in CH_2Cl_2 at 77 K (Fig. 7). No photo-induced electron transfer from carotenoid to solvent molecules resulting in generation of solvent radical anions was observed.

3.3. Product analysis

The β-carotene–DDQ adducts were isolated by preparative TLC. NMR analysis of the isolated product indicates that it is not a single compound (at least three new lines appear in the region of C(1)–CH_3 (1–1.2 ppm). The Beilstein test of the reaction products indicated the presence of halogen (chlorine) atoms. Because the eluent used for separation of the reaction product by TLC (2% diethyl ether

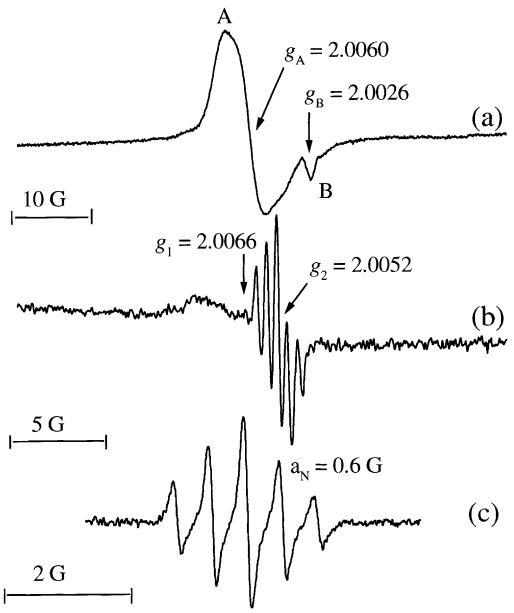


Fig. 4. EPR spectra of β-carotene (1 mM) and DDQ (1.5 mM) mixture in CH_2Cl_2 (a) at 77 K; (b) at 240 K and (c) at 280 K 24 h after mixing.

in hexane) did not contain any halogen atoms, we conclude that the isolated products contains a DDQ fragment.

The IR spectrum showed the absence of carbonyl groups and the presence of hydroxyl groups in the isolated

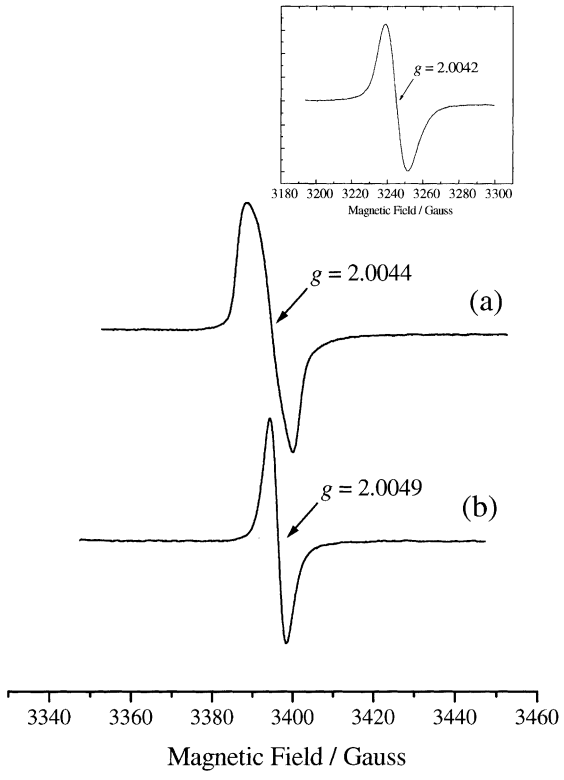


Fig. 5. EPR spectra of the mixture of β-carotene (3 mM) and DDQ (0.3 mM) in CH_2Cl_2: (insert) at 77 K; (a) after warming to RT and (b) at RT after 24 h.

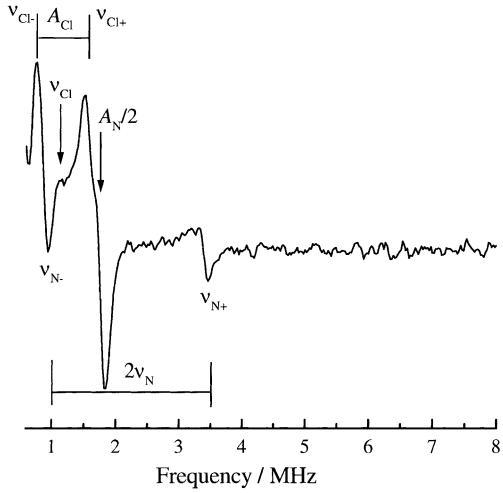


Fig. 6. ENDOR spectrum of β-carotene and DDQ mixture in CH_2Cl_2 measured at 200 K.

products. Admixture of water in the sample was minimized by means of TLC. The Fourier IR spectrometer used provides automatic subtraction of possible background signals. We, therefore, suggest that hydroxyl groups belong to the product which is a carotene–hydroquinone ether.

Unfortunately, we could not isolate the **II**–DDQ and **III**–DDQ reaction products by preparative TLC because of their poor stability. To determine the adduct structures in these cases we analyzed changes in the ^{1}H-NMR spectra during the reaction carried out directly in the NMR probe. The reaction mixture was prepared in CD_2Cl_2 with concentrations of 0.005 M for both reagents. The ^{1}H-NMR spectra of the initial carotenoids (**I**–**III**) show the presence of several characteristic groups of protons (see Figs. 8 and 9 for **II** and **III**): =CH– protons are between 6 and 7 ppm; CH_3 groups attached to the conjugated chain (C(9,9′)–CH_3 and C(13,13′)–CH_3) are near 2.0–1.9 ppm; the CH_3 group attached to the terminal double bond (C(5)–CH_3) is near 1.8–1.7 ppm; and the geminal CH_3 groups attached to C(1)

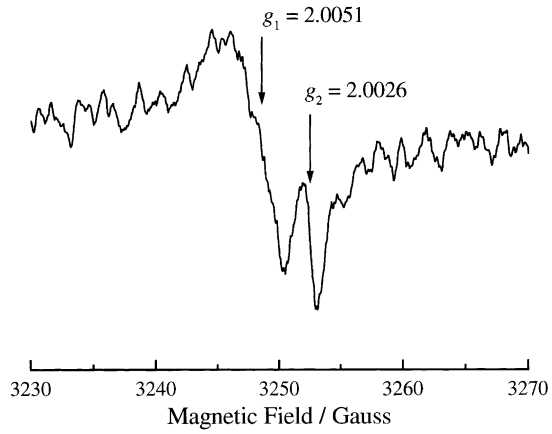


Fig. 7. EPR spectrum of β-carotene–CA mixture in CH_2Cl_2 irradiated at 77 K and recorded at 150 K.

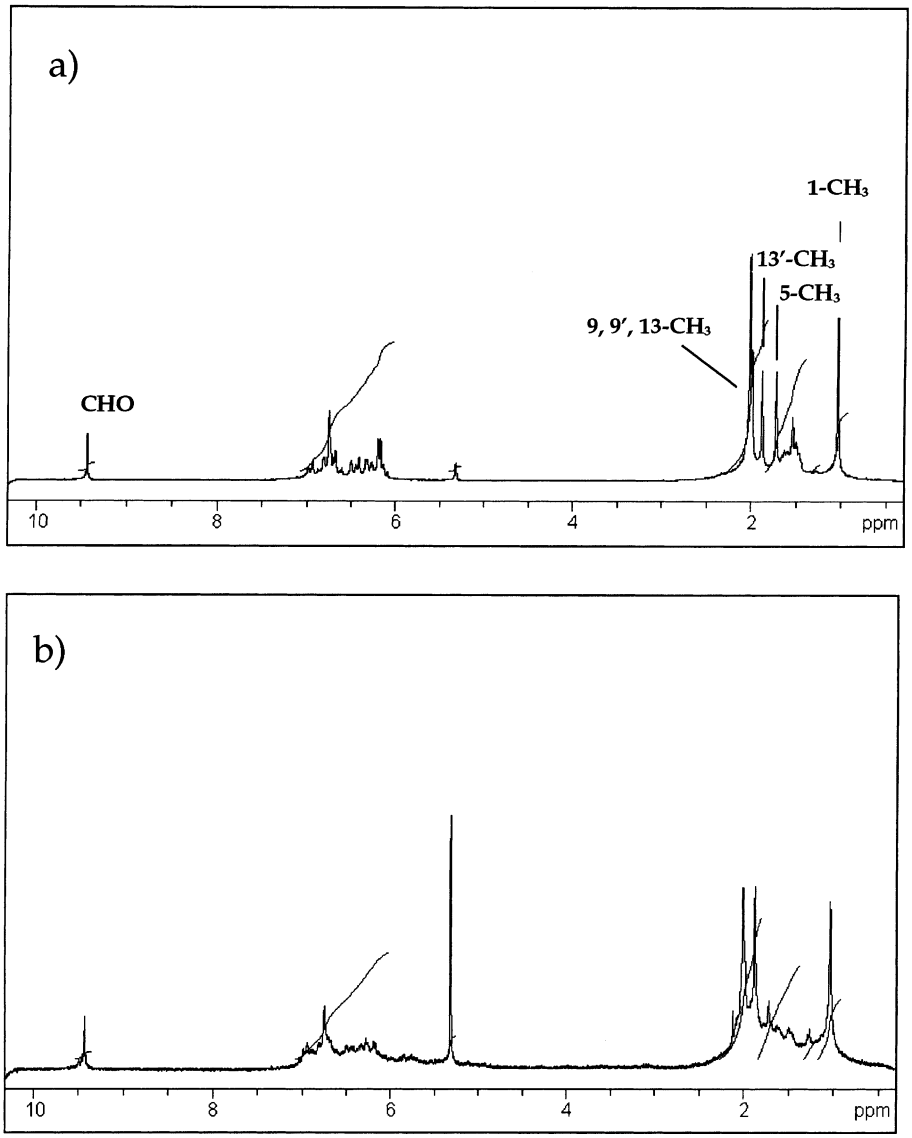


Fig. 8. ^{1}H-NMR (200 MHz) spectrum of the product of apo-carotenal/DDQ reaction in CD_2Cl_2 [Car] = [DDQ] (a) before mixing; (b) 5 h after mixing.

are near 1.0 ppm. The addition of DDQ to the carotenoid solutions leads to significant broadening of NMR signals. Some sharp lines available for analysis appeared only several hours later. We suggest that the broadening is due to the presence of long-lived paramagnetic species.

The ^{1}H-NMR spectrum of the **II**–DDQ adduct showed a shift of the C(5)–CH_3 group to lower magnetic field and no shifts of the C(9,9′)–CH_3 and the C(13,13′)–CH_3 NMR signals (Fig. 8). We, therefore, suggest addition of the semiquinone radical to the C(4) position of **II**. Some structureless lines appeared near 5.8 ppm which might be due to the –C<u>H</u>–QH proton.

Comparison of the ^{1}H-NMR spectra of isolated **I**–DDQ products and initial β-carotene also shows a shift of the C(3,4)–CH_2 and C(1,5)–CH_3 proton lines in the spectra of products to lower field (spectrum not shown). As for **II**, this might be the result of the presence of substitutent on the cyclohexene ring. Additional broad lines appeared near 5.8 and 5.6 ppm. The integral intensity of these groups are about 1/9 of the total integral intensity of the 5,9,13-CH_3 groups, so this indicates presence of CH group. On the basis of these results we suggest formation of the carotene–hydroquinone ether bonded via a ring carbon atom.

Because canthaxanthin (**III**) is substituted at the C(4) position, such an adduct cannot be formed. Indeed, in the ^{1}H-NMR spectrum no shifts of ring protons were observed, while the signals of the C(9)–CH_3 and the C(13)–CH_3 methyl protons shifted to higher magnetic field (Fig. 9). It is seen from comparison of the ratios of integral intensities of terminal (C(5)–CH_3) and C(9), C(13)–CH_3 groups attached to the conjugated chain of **III** and the product with DDQ that this ratio changes from 1/2 to 2. It points to the shift of C(9) and C (13) to higher field, as a result of loss of conjugation.

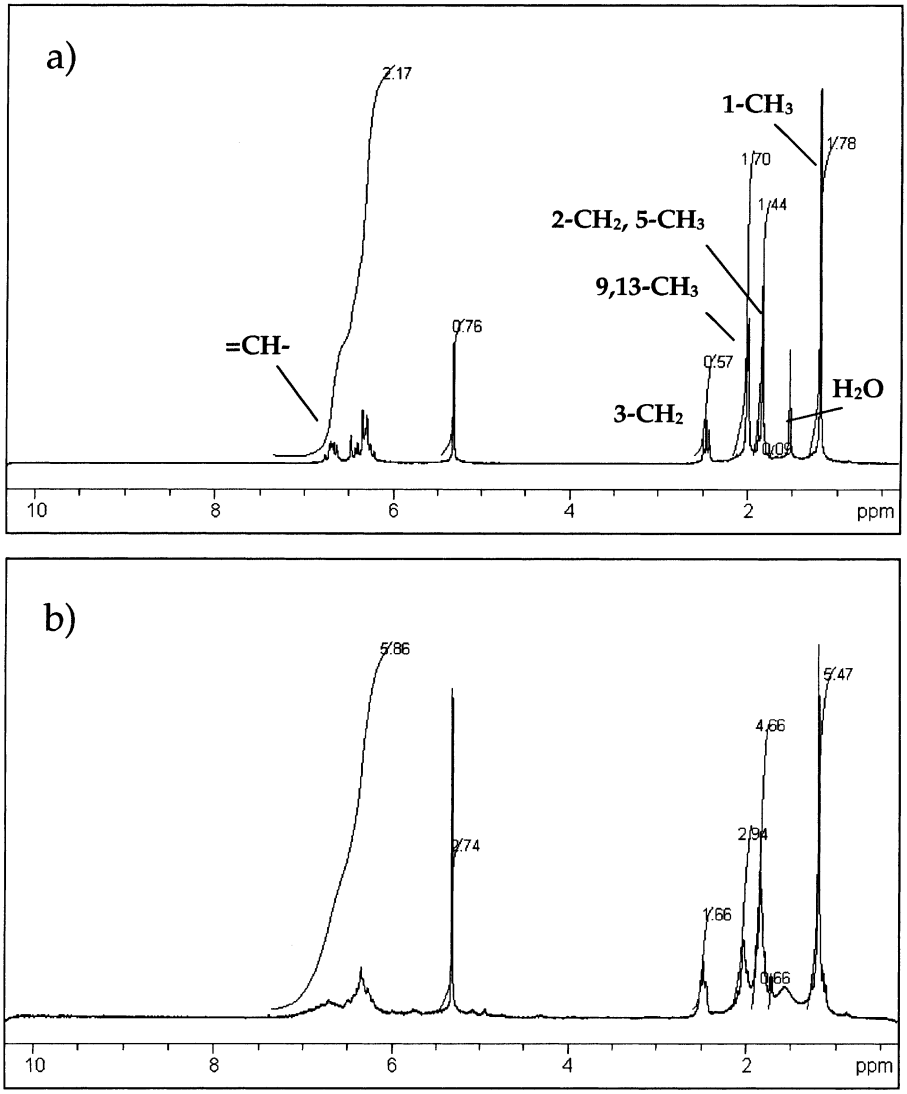


Fig. 9. ^{1}H-NMR (200 MHz) spectrum of the product of canthaxanthin/DDQ reaction in CD_2Cl_2 [Car] = [DDQ] (a) before mixing; (b) 5 h after mixing.

4. Discussion

It is known that the high electron affinity of quinones and the stability of their radical anions result in large numbers of charge-transfer complexes (CTCs) between quinones and effective electron donors [25]. The ability of quinones to form CTCs with compounds including polymeric bases has been reported [26,27]. Usually in a CTC the absorption band is shifted to longer wavelength. Therefore, the appearance of a new absorption band in the near IR at 1030 nm after mixing DDQ and β-carotene solutions can be attributed to a CTC [$Car^{\delta+}\cdots Q^{\delta-}$]. Decay of this complex resulted in shifting the absorption maximum from 1030 to 1000 nm which indicates the presence of the carotenoid radical cation. For carotenoids **II** and **III**, only the $Car^{\bullet+}$ absorption was detected. This is in accord with data obtained for carotenoid/iodine solutions in CH_2Cl_2 [28,29]. It has been demonstrated [28] that when β-carotene and iodine solutions are mixed, two new bands in the UV (290–360 nm) and the near IR (∼1000 nm) are observed. The absorptions in the UV were assigned to I_3^- ions while the band in the near IR was attributed to the CTC [Car $2I_2$], which exists in equilibrium with the ionic form [$Car^+\cdots I_3^-$]. Later [29] it was established that when solutions of β-carotene and iodine are mixed, in dilute iodine solutions at room temperature, the $Car^{\bullet+}$ was stabilized by I_3^- ions, while at 77 K and in highly concentrated iodine solutions CTCs were present. The absorption maximum of such a complex was red-shifted compared to the $Car^{\bullet+}$ and the *g*-value was larger than that of the $Car^{\bullet+}$. For canthaxanthin and 8′-apo-β-caroten-8′-al with I_2 only the $Car^{\bullet+}$ absorption bands were produced.

It is known that the ability to form CTCs depends on the oxidation potential of the donor and the electron affinity of the acceptor [30–32]. The likelihood of an electron transfer reaction follows from the free energy change ΔG involved

Table 1
Oxidation potentials (I_P) of carotenoids and electron affinities (E_A) of quinones

Compound	I_P (V)	E_A (eV)
β-Carotene	0.54[a]	
8′-Apo-caroten-8′-al	0.72[a]	
Canthaxanthin	0.69[a]	
DDQ		3.13[b]
Chloranil		2.49[b]

[a] [41].
[b] [29].

in the electron transfer:

$$\Delta G = I_P - E_A \frac{-e^2}{\varepsilon, r} \quad (1)$$

where I_P is the oxidation potential of the donor, E_A the electron affinity of the acceptor and $e^2/\varepsilon, r$ is the Coulombic term. The carotenoid oxidation potentials and the quinone electron affinities are given in Table 1. The free energy change ΔG estimated from Eq. (1) is near 0 eV for the **I**–DDQ complex; 0.2 eV for **II**–DDQ, **III**–DDQ; 0.5 eV for **I**–CA and 0.7 eV for **II**–CA, **III**–CA. Because of the lowest oxidation potential of β-carotene and the highest electron affinity of DDQ, the CTC of **I**–DDQ is the strongest and may be observed by UV–VIS spectroscopy. Similar results have been obtained for Chl–benzoquinone [21] and P–benzoquinone [22] electron transfer reactions. Because of the lower oxidation potential of pheophytin, exciplex ($P^{\delta+}$ $Q^{\delta-}$) formation was detected in this system, while for Chl only the $Chl^{\bullet+}$ and the $Q^{\bullet-}$ were observed. No reaction between carotenoids and quinones was observed in benzene and toluene because formation of the separate CTC is unlikely in nonpolar solvents [33]. Formation of complexes between the carotenoid or the quinone and the solvent itself will also be precluded in this case [34].

EPR measurements at 77 K ([DDQ] > [β-carotene]) showed the presence of a single unresolved line with $g = 2.0066$ which is greater than that of the β-carotene radical cation or the DDQ radical anion. According to the data obtained for polymer/quinone [27] and carotenoid/I_2 [29] complexes, these species can be attributed to the CTC between the carotenoid and quinone. Increasing the temperature resulted in the appearance of a quintet with $g = 2.0052$ which is due to the DDQ radical anion. Existence of a stable $Car^{\bullet+}$ species in Car–DDQ systems at temperatures >77 K was not supported by the EPR results. This is in accord with data reported for the reaction of quinones with polymeric compounds [27]. It has been demonstrated [27] that Q reacts with polymeric bases (PB) forming a polymer-bound radical anion PB–$Q^{\bullet-}$. Probably $Car^{\bullet+}$ disappears via reaction with quinone leaving $Q^{\bullet-}$ to be the only stable paramagnetic species.

$$Car^{\bullet+} + Q \rightarrow Car^{2+} + Q^{\bullet-} \quad (2)$$

Another possible mechanism of the carotenoid radical cation decay is the reaction with solvent molecules.

$$Car^{\bullet+} + \text{solvent} \rightarrow Car + \text{oxidized solvent} \quad (3)$$

However, no reactions with solvent were detected under our experimental conditions. An alternative to the solvent reaction which can also lead to an excess of $Q^{\bullet-}$ is the reaction with hydroquinone (H_2Q):

$$Car^{\bullet+} + H_2Q \rightarrow Car + Q^{\bullet-} + 2H^+ \quad (4)$$

The hydroquinone H_2Q in this reaction could arise from disproportionation:

$$2Q^{\bullet-} + 2H^+ \rightarrow Q + H_2Q \quad (5)$$

Fig. 1 also demonstrates the appearance of an additional absorption band near 950 nm immediately after starting the reaction. A similar absorption band has been observed by Hill et al. [35] in pulse radiolysis experiments of carotenoids in CCl_4 in the presence of air and was assigned to the adducts of carotenoids and $CCl_3OO^{\bullet}$ radicals. The authors also considered the possibility of dication formation and decided that this reaction was unlikely under their experimental conditions. In our case we cannot exclude the possibility of chemical generation of dications in the β-carotene–DDQ mixture. Chemical generation of dications in the carotenoid reaction with the weaker oxidant $FeCl_3$ has been reported [36]. Oxidation of β-carotene to the dication is expected since it has almost the same first and second oxidation potentials. β-Carotene dication can react with neutral carotenoid molecule and produce the $Car^{\bullet+}$ through the comproportionation reaction (previously deduced from electrochemical and EPR measurements) [23].

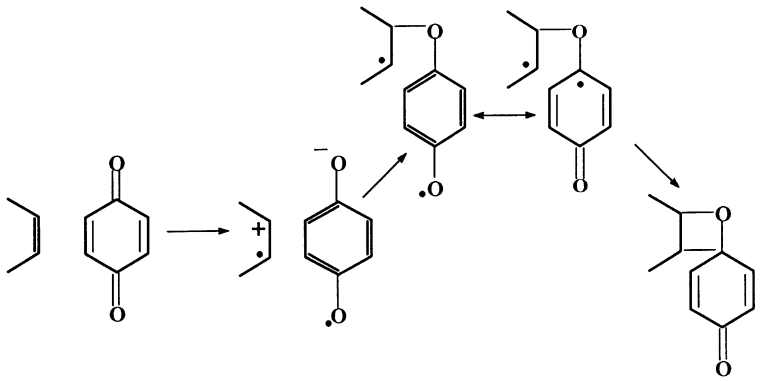
$$Car^{2+} + Car \rightleftharpoons 2Car^{\bullet+} \quad (6)$$

It is known that 1,4-quinones have maximum absorption corresponding to the $n\pi^*$ singlet–singlet transition in the same region as carotenoids (400–500 nm; ε, 20–100) [25]. Absorptions in the 370–400 nm range is due to semiquinone radicals [14,15]. The appearance of a new absorption in the UV region (340 nm) might be due to the product of quinone addition to carotenoid. Stable paramagnetic products were detected at room temperature. The ENDOR results indicate that this species contains both nitrogen and chlorine atoms. It could be the reaction product of DDQ and the carotenoid.

With strong electron acceptor and donor partners (like quinones and carotenoids) complete electron transfer may occur in the CTC. Such a complex exists in equilibrium with an ion radical pair. This ion pair can back transfer or give products P.

$$Car + Q \rightleftharpoons [Car^{\delta+} \cdots Q^{\delta-}] \rightleftharpoons (Car^{\bullet+} \cdots Q^{\bullet-}) \rightarrow P \quad (7)$$

Regarding the decay mechanism of the carotenoid–quinone donor–acceptor complex, two sites of attack on the carotenoid by quinone may be suggested. One is a double



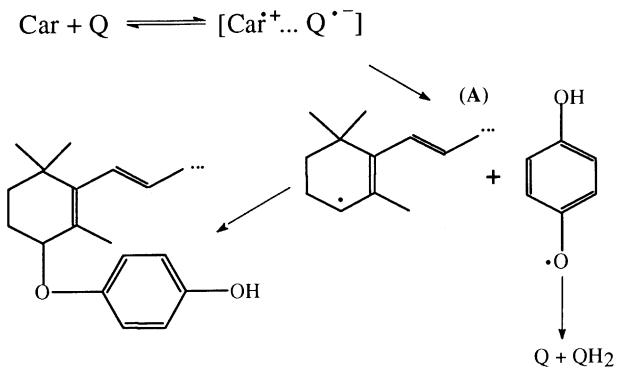
Scheme 2. Condensation of a carotenoid with quinones.

bond in the conjugated chain and another is the allylic 4-CH_2 group of the cyclohexene ring. The first mechanism evidently occurred with all carotenoids, but it is most obvious with **III**, because its C(4) position is occupied. The second mechanism takes place only with **I** and **II**.

The first process, the addition of a quinone carbonyl group to a polyene double bond, is well known as Paterno–Buchi reaction [37,38]. In this case also it is suggested that the first step in the reaction is an electron transfer producing the intermediate ion pair ($Car^{\bullet+}\cdots Q^{\bullet-}$) followed by formation of a biradical which transforms to the final products (see Scheme 2).

For β-carotene (**I**) and 8′-apo-β-caroten-8′-al (**II**) the C(4)–CH_2 position is the most likely site of the quinone attack. We suggest that the first step is also an electron transfer. Formation of the final products (carotenoid–hydroquinone ether) can proceed through an intermediate neutral radical pair. A possible mechanism of diamagnetic product formation via intermediate generation of carotenoid and quinone neutral radicals followed by their recombination is presented on Scheme 3.

Radical adduct detected by EPR study can be formed via recombination of Car^+ with $Q^{\bullet-}$. Car^+ can arise by deprotonation of carotenoid dication by the quinone. It is likely that at high quinone concentrations, [Q] > [Car], the formation



Scheme 3. Diamagnetic product formation.

of Car^{2+} by dehydrogenation of $Car^{\bullet+}$ involving quinone molecules (reaction 2) takes place. DDQ and chloranil are the most common dehydrogenating agents [39]. In contrast, when [Q] < [Car], reaction A (see Scheme 3) occurs. We suggest that the allylic C(4) position should easily undergo deprotonation. Proton abstraction from this position would result in the resonance-stabilized carbon-centered radical with unpaired electron density delocalized over the polyene chain, in accord with the conclusion of Woodall et al. [40].

Additional support of carotenoid–hydroquinone ether formation comes from analysis of NMR spectra of the final products (see Section 3). The ^{1}H-NMR spectra of the **I**–DDQ and **II**–DDQ adducts showed a shift of the C(5)–CH_3 and CH_2 groups of the ring to lower magnetic field and no shifts of the C(9,9′)–CH_3 and the C(13,13′)–CH_3 NMR signals. This points to the addition of semiquinone radical to the C(4) position of **I** and **II**. Some structureless lines appeared near 5.6–5.8 ppm which we assign to the –C<u>H</u>–QH protons. The same conclusion was made from the IR spectrum and the Beilstein test of the **I**–DDQ adduct.

In contrast to **I** and **II**, for canthaxanthin which has substituted C(4) positions, such an adduct cannot be formed. Indeed, in the ^{1}H-NMR spectrum no shifts of ring protons were observed while the signals of the C(9)–CH_3 and the C(13)–CH_3 methyl protons shifted to higher magnetic field. It means the destruction of the conjugated chain of carotenoid. We suggested Patterno–Buchi reaction gives major contribution in this case (see Scheme 2).

5. Conclusion

The above results indicate that electron transfer reactions of carotenoids (β-carotene, 8′-apo-β-caroten-8′-al, canthaxanthin) with strongly oxidizing quinones (2,3-dichloro-5,6-dicyano-1,4-benzoquinone, tetrachlorobenzoquinone) proceed through formation of CTCs which exist in equilibrium with ion–radical pairs. The rate of the CTC formation and decay depends on the oxidation potentials of carotenoids

and electron affinities of quinones. The strongest CTC was formed when β-carotene as electron donor and DDQ as electron acceptor were used. A new absorption band in the near IR region (1030 nm) was attributed to this complex. The EPR spectra of the Car–DDQ mixture measured at 77 K when [Car] ≤ [DDQ] exhibit a single line with $g = 2.0066 \pm 0.0002$ which is also attributed to a CTC. No EPR signal of the carotenoid radical cation was detected at higher temperatures. With increasing temperature a stable paramagnetic species with $g = 2.0049 \pm 0.0002$ and $\Delta H_{p-p} = 4.3$ G appeared. We assigned this signal to a carotenoid–quinone radical adduct.

Optical absorption studies show the formation of an absorption band in the near UV region (at 340 ± 5 nm). This absorption is attributed to the product of quinone addition to carotenoid. It is suggested that the major products of the interaction of carotenoids with quinones are carotenoid–hydroquinone ethers. For 8′-apo-β-caroten-8′-al and β-carotene the ether forms via the addition of semiquinone to the C(4) position of the cyclohexene ring of carotenoids. For canthaxanthin the reaction proceeds primarily via the addition of semiquinone to a double bond of the conjugated chain of carotenoids. In both cases the first step of the process is electron transfer with the formation of a radical ion pair.

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